

ANTIBACTERIAL, ANTITUMOR ACTIVITY AND PHYTOCHEMICAL STUDIES OF METHANOLIC EXTRACT OF (*CATHARANTHUS ROSEUS*) (LINN.) G. DON.

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Abstract

The therapeutic role of plant materials is getting more attention due to fewer or no side effects as compared to synthetic chemical based medicines. *Catharanthus roseus* (L.) G Don is an important medicinal plant belonging to apocynaceae family, which serves as a reservoir of various important phytochemicals. We have investigated the antibacterial, antitumor activity, and phytochemical study of methanol-based leaf extracts of *C. roseus*. The qualitative phytochemical analysis showed the presence of flavonoids, terpenoids, glycosides, anthraquinone, tannins, proteins, carbohydrates, saponins, and alkaloids in the methanol based leaf extract of *C. roseus*. Extract of *C. roseus* also displayed DPPH radical scavenging activity at all the tested concentrations. Antibacterial potential of *C. roseus* was evaluated against a few selected human pathogens viz., *M. luteus*, *S. aureus*, and *E. coli* in replicated experiments. The methanolic extract of *C. roseus* displayed maximum inhibition (17.7 ± 0.88 mm) and (17.3 ± 0.67 mm) against *M. luteus* and *E. coli* and minimum inhibition (13 ± 1.15 mm) against *S. aureus*. Methanolic leaf extract of *C. roseus* also displayed antitumor activity *In vitro*. The results have revealed that methanolic extract of *C. roseus* can be used as a source of potential antibacterial products.

Key words: Antibacterial, Antitumor, Secondary metabolites, *Catharanthus roseus*, Therapeutics.

Introduction

Medicinal plants are an important source of livelihoods of poor people all over the world and these conventional drugs served as the most reasonable and simply accessible supply of treatment within the health care system to different societies. Local inhabitants use plants for meditative functions (Hosseinzadeh *et al.*, 2015). World Health Organization (WHO) reported that 80% of the world's population rely on natural medicinal products for their primary healthcare (Farnsworth, 1990). Out of 32000 higher plant species, more than 10% are used in conventional medicines. Pakistan has a vast variety of medicinal plants which are used as natural products for maintaining human health and are used in various formulations in the treatment of various ailments and diseases caused by microbial agents (Prance, 2001).

Medicinal plants also serve as antimicrobial agents and these plants are used as natural drugs (Srivastava *et al.*, 1996). All most all the plant parts are used in the formulation of natural drugs and these plant parts including, leaves, stems, flowers, fruits, twigs, and roots are also sold in local markets which are used in the manufacturing of herbal medicines. Various researchers have reported the antimicrobial (Oyetayo, 2008; Lee *et al.*, 2004; Jayawardana *et al.*, 2015), anti-inflammatory (Guo *et al.*, 2014; Gupta *et al.*, 2019), anti-allergic (Cota *et al.*, 2013), antidiarrheal (Tradtrantip *et al.*, 2014), acquired immunodeficiency syndrome (AIDS) (Liu *et al.*, 2005) and anti-severe acute respiratory syndrome (SARS) (Zhang *et al.*, 2020) potential of herbal medicines.

Various microbes attack humans, plants, domestic and wild animals and serve as severely pathogenic in many of the cases. These microbes continue to mutate and result in emerging diseases in their target hosts. *Staphylococcus aureus* can cause different infections ranging from minor skin infections to more severe

ailments such as endocarditis, bacteremia, sepsis, and osteomyelitis (Jenkins *et al.*, 2015). This bacterium also causes many other ailments which include cellulitis, keratitis, osteomyelitis, septic arthritis, and mastitis in animals (Balaban *et al.*, 2000). *Micrococcus luteus* is a gram-positive coccus bacterium that is mild in virulence however, it can turn into a severe pathogen in patients with weak immunity (Dürst *et al.*, 1991). It can cause various diseases including skin infection (Sharma *et al.*, 2012), prosthetic valve endocarditis (PVE) (Dürst *et al.*, 1991), and can be foodborne. *E. coli* cause the most common bacterial infections in Humans and also results in gastroenteritis, urinary tract infections, and neonatal meningitis (Chaman *et al.*, 2013), and also causes cholecystitis, bacteremia, cholangitis, neonatal meningitis, and pneumonia.

Medicinal plants are used to treat microbial infections as these naturally occurring medicinal plants possess strong antimicrobial potential (Nwonuma *et al.*, 2019; Nwonuma *et al.*, 2020). Like other medicinal plants, *Catharanthus roseus* possess antibacterial, antifungal, and antiviral potential and is reported to contain different kinds of alkaloids and chemotherapeutic molecules that possess anti-cancer potential (Chaman *et al.*, 2013) and also displayed antifungal, antiviral, and antihelminthic potential (Gajalakshmi *et al.*, 2013). The plant also possesses antihypertensive and antispasmodic properties due to the presence of alkaloids (Kumari & Gupta, 2013). *Catharanthus roseus* is distributed in tropical and subtropical domains of the world and it is commercially grown in many countries for its medicinal importance. The drugs extracted from *C. roseus* are sold in the biggest markets around the world. Various studies have highlighted the antimicrobial potential (Chaman *et al.*, 2013; Patil & Ghosh, 2010; Pham *et al.*, 2019) and therapeutic (Das & Sharangi, 2017) and anticancer applications of *C. roseus* (Retna & Ethalsa, 2013).

Microbial agents develop resistance result the failure of chemotherapies and antibiotic potential of medicinal plants. This resistance development exhibited by the pathogens led the researchers to screen more medicinal plants to break the resistance and to explore the strong antibiotic potential of medicinal plants. The majority of the medicinal plants have been exposed well to possess antimicrobial potential but a vast majority of the medicinal plants have not been studied well to for their antimicrobial and chemotherapeutic potential. Keeping in view the importance of pathogenic bacterial agents and the potential of medicinal plants, this study was conducted to explore the antibacterial and antitumor potential of methanolic extract of *C. roseus*. The objectives of this research were to carry our qualitative phytochemical analysis of *C. roseus* extract, to conduct DPPH Radical Scavenging assay, to test the antibacterial and antitumor activates.

Materials and Methods

Collection of plant materials: The fully mature plant samples of *Catharanthus roseus* L. were collected from the Govt. College Women University Sialkot Pakistan (32.4945° N, 74.5229° E). The plant *Catharanthus roseus* L. belongs to the phylum Magnoliopsida, order Gentianales, and family Apocynaceae, and is commonly known as *Madagascar periwinkle* (Mp). The collected plant samples were washed using sterilized distilled water and excess water was removed from the plant samples using filter paper before these plants were used for extraction.

Extract preparation: The methanolic extracts of *C. roseus* were prepared by following the procedure previously reported by Govindasamy & Srinivasan, (2012). In brief, the leaves were detached from the plants, thoroughly washed with tap water to remove any contamination followed by washing with the sterilized distilled water. The leaves samples were shade dried for four days to remove the excess water contents and dried leaves were crushed to a fine powder using an electric blender (Osaka). The leaves powder was taken in a conical flask and methanol as solvent was added to it in the ratio of 1:50 and the mouth of the flask was closed with a rubber cork. The flask was then placed on the orbital shaker for two days and filtration was carried out using Whatman No.1 filter paper. The obtained filtrate was concentrated with the help of a rotary evaporator and both the powder and filtrate were stored in the refrigerator in airtight jars for further analysis.

Qualitative phytochemical analysis

In order to confirm metabolites in the plant samples, phytochemical analysis was performed for methanol soluble fraction as per method previously published by Roopashree *et al.*, (2008) while the screening and identification of bioactive chemicals in *Madagascar periwinkle* were carried out with the extracts using the standard method previously reported by Harborne, (1973).

Test for saponin (foam test): The presence of saponin in the tested plant samples was determined by following the methodology adopted by Devmurari, (2010). In particular, 20ml distilled water was added to 1ml solution of the plant extract in a graduated cylinder and was shaken for 15 min. The formation of stable foam confirmed the positive test results.

Test for flavonoids: Flavonoids were detected in the tested sample by adopting the procedure as previously described by Gul *et al.*, (2017). For this, 2ml of 2.0% sodium hydroxide mixture was added with crude plant leaf extract and dark yellow color was developed. In this mixture, 2 drops of diluted acid were added and color change from yellow to colorless confirmed the positive test results.

Test for alkaloids: Alkaloids were detected by following the procedure adopted by Devmurari, (2010). In brief, 1 mg of extract was mixed with 5 ml of 1.5% (v/v) HCl and filtered. The obtained filtrate was used in alkaloids testing. To test alkaloids, few drops of Mayer's reagent were added to 2mg of extract and the development of white or pale yellow precipitates indicated the alkaloids. In another test, 2mg of methanolic extract was mixed with 1.5% (v/v) of HCl and a few drops of Wagner's reagent were added. The development of yellow or brown precipitates confirmed the alkaloids in the tested samples.

Test for protein: The presence of protein in the test sample was determined by the Xanthoproteic test. In this study, 1ml of the test solution was taken while another test tube containing distilled water only was taken as control. 1ml of conc. Nitric acid (HNO_3) was added to all test tubes and mixed thoroughly. The solution was cooled under tap water. 2ml of 40% NaOH was added to all test tubes. Color change from yellow to orange confirmed the positive test results.

Test for tannins: A gelatin test was performed to detect the tannins in the samples. In this test, the extract was mixed with 5ml of 1% gelatin solution that contain Sodium chloride (NaCl). The development of white precipitates indicated the positive test results.

Test for terpenoids: Terpenoids in the tested samples were detected by following the procedures adopted by Das *et al.*, (2014). In Salkowski's test, 5ml of the extract was mixed with 2ml chloroform and 3ml con. H_2SO_4 was added to form a layer. Reddish-brown coloration confirmed the terpenoids.

Test for carbohydrates: In this test, the extract was melted in 5ml distilled water and filtered. 1ml of the filtrate was treated with Molisch reagent (10% alcoholic α -naphthol). To this, 0.2ml of H_2SO_4 was added. The development of purple to violet rings confirmed the positive test results. In another procedure, equal volumes of Fehling's A (Cupric sulfate pentahydrate in distilled water) and Fehling's B (Potassium sodium tartrate tetrahydrate and NaOH in distilled water) were mixed with few drops of the test sample and boiled. The

development of brick red precipitates confirmed the positive test results. The test results were also verified by mixing the tested sample with Benedict's reagent. The development of yellow or orange color confirmed the positive test results for carbohydrates.

Test for glycoside: Glycoside was confirmed by Legal test as previously described by (Yadav *et al.*, 2017). In this test, the plant extract was added to dilute Hydrochloric acid (HCl) followed by treatment with Legal's Reagent. The formation of pink to red color confirmed the glycoside in the samples.

Test for anthraquinones: Anthraquinones were detected by adopting the procedure as explained by Gul *et al.*, (2017). In particular, 0.5g of the plant extract was shaken in 10ml of benzene and then filtered. The filtrate was then added in 5ml of 10% NH₃ and was shaken. The development of pink, red, or violet color indicated the anthraquinones in the tested samples.

DPPH radical scavenging assay: Test was performed by following the previously published method of Braca *et al.*, (2001) using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. In this test, 0.1mM solution of DPPH in methanol was prepared. 1 ml of this solution was added to 3ml of prepared concentrations (1000ppm, 500ppm, 250ppm) of plant extracts in methanol. After shaking vigorously, the mixture was left at room temperature for 30 minutes. Absorbance was measured at 517nm spectrophotometrically. Ascorbic acid was used as a reference standard and the experiment was carried out in triplicate. A curve was drawn with % DPPH scavenged against the concentrations of the standard antioxidant (Trolox). The capability to scavenging the DPPH free radical was calculated by the equation (Yen *et al.*, 1994).

$$\% \text{ of radical scavenging activity} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100 \%$$

Abs control = Absorbance of Methanol solution

Abs sample = Absorbance of extract and ascorbic acid

Antimicrobial and anti-tumor assay

Bacterial strains: A total of three different bacterial strains *Microccou luteus*, *Staphylococcus aureus*, and *Escherichia coli* were selected to study the antibacterial potential of phytochemical extracts of *C. roseus* while the pure culture of *Agrobacterium tumefaciens* was used for the antitumor assay. All these bacterial strains were collected from Nawaz Sharif Medical College, University of Gujrat Pakistan.

Bacterial inoculum preparation: All three collected bacterial isolates were aseptically streaked on sterilized Nutrient agar (NA) medium (5g peptone; 5g NaCl; 1.5g peptone B; 1.5g yeast extract; 15g agar; 1000ml distilled water; pH 7.4 ± 2) containing Petri plates and incubated at 28 ± 2°C for 24 hours. Pure bacterial cultures were then inoculated in the test tubes containing Luria Broth (LB) medium and incubated at 28 ± 2°C for 24 hr on a

shaker. Every bacterial suspension in LB medium was compared with 0.5 McFarland standard solutions (1.5 × 10⁸ CFU/ml) and bacterial concentration was maintained at 10⁸ CFU/ml.

Antibacterial and anti-tumor assays

Agar well diffusion assay: The antibacterial activity of methanol-based extracts of *Madagascar periwinkle* was evaluated against three bacterial strains viz., *Microccou luteus*, *Staphylococcus aureus*, and *Escherichia coli* by agar well diffusion method of Daoud *et al.*, (2015) in repeated experiments. In brief, 1ml of the fresh bacterial inoculum prepared in LB medium was poured into the center of the sterilized Petri plate (9cm) using a pipette. Molten cooled sterilized NA medium was aseptically poured into the Petri Plates, mixed well, and left for media solidification. Upon solidification, wells were made using sterilized cork borer (6mm) into Petri plate containing bacterial inoculum. Then, 100µl extract was aseptically added to these wells in all the Petri plates. The plates containing only bacterial inoculum were kept as negative control while ampicillin was kept as a standard drug in positive control. Plates were parafilmmed and incubated at 37°C for 24hrs. Each treatment was replicated three times and the zone of inhibition was measured from each of the Petri plates after the incubation.

Antitumor potato disc bioassay: Antitumor activity of the methanolic extract was tested by following the procedure reported by Coker *et al.*, (2003). For this assay, the Culture of *A. tumefaciens* was maintained on Luria Bertani (LB) medium at 30°C for 24 hr for pure colony development. The pure bacterial colony was aseptically streaked into LB broth medium and incubated at 30°C for 48 hr. After incubation, bacterial suspension was transferred into 10 ml sterilized phosphate buffer saline using a sterilized loop, and bacterial concentration was maintained to 1 × 10⁸ CFU/ml. Red-skinned healthy potatoes were surface disinfected by washing under running tap water, peeled, and immersed in 10% Clorox (NaClO) for 20 min. Each side of the potato was removed for getting a smooth surface by placing it on a paper towel, and then small discs (0.5cm thick) were cut aseptically using a sterilized cork borer. These discs were dipped in 20% Clorox for 15 min followed by dipping in sterile distilled water. 400µl of the bacterial culture was uniformly spread on the Petri plates containing solidify PDA medium. Sterilized potato discs were overlaid with 50µl of methanolic plant extract and were placed aseptically on the bacterial inoculated Petri plates. Camptothecin was used as positive control and three replications were maintained for each treatment. Plates were parafilmmed and incubated at 28 ± 2°C for 20 days. After incubation, potato discs were treated with Lugol's solution for 30 min. The tumor-induced by *A. tumefaciens* did not take up the dye color, and appear creamy to orange while, the starch on staining with the Lugol's solution shows dark blue to brown color.

Statistical analysis: All the experiments were performed in a completely randomized design (CRD) with three replications for each treatment. Means were subjected to ANOVA test and were analyzed by the least significant difference test (LSD) at $p < 0.05$ probability value.

Results

Identification of Plant: *Catharanthus roseus* is a member of Apocynaceae family. It is also grown for its flowers and is also known as *Madagascar periwinkle* (Mp). It is one of the best-studied medicinal plants. It is a self-pollinated, deciduous herb and has a woody base. Leaf-blade was 2 to 4 inches; the upper leaf surface was dark green while the lower leaf surface was lighter green. All these characters were confirmed by following the nomenclature and taxonomic key which was previously followed by (Kaushik et al., 2017).

Qualitative phytochemical analysis: The Phytochemical analysis for methanolic extracts of *Catharanthus roseus* (*Madagascar periwinkle*) was carried out (Table 1). The tested plant extract displayed a positive response for flavonoids, terpenoids, and glycosides while the presence of anthraquinone was not detected. Tannins and proteins were also detected in the methanolic leaf extract of *Madagascar periwinkle*. The Molisch reagent, Fehling's reagent, and Benedict's reagent were used in three different tests to confine the presence of carbohydrates in the tested methanolic extract of *Madagascar periwinkle* and all the test results were found positive for the carbohydrate presence. The foam test also confirmed saponins in the extract while the presence of alkaloids was also detected positive in two tests using Mayer's reagent and Wagner's reagent.

DPPH radical scavenging assay: The effect of antioxidants on DPPH is given in Fig. 1. The antioxidant activity of methanol leaf extract of *Madagascar periwinkle* at different concentrations 250ppm, 500ppm, and 1000ppm was checked by DPPH free radical assay against the standard ascorbic acid.

The scavenging activity was found at 1000ppm than the other two concentrations. The scavenging activity was observed minimum at 500ppm concentration and moderate at 250ppm.

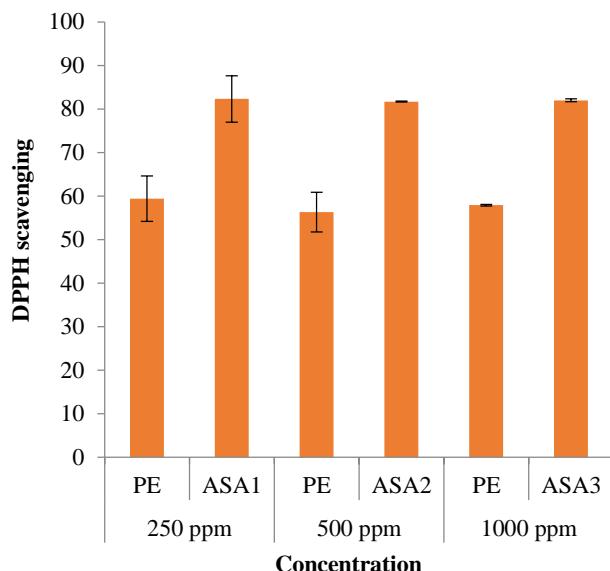


Fig. 1. DPPH radical scavenging activities of methanolic leaf extracts of *Catharanthus roseus* (*Madagascar periwinkle*).

Antibacterial activity of *Catharanthus roseus*: The methanolic based leaf extracts of *Catharanthus roseus* were tested for their antibacterial potential against three human infecting bacterial strains viz., *Stayphlococcus aureus*, *Micrococcus luteus*, and *Escherichia coli* in the disc diffusion assay (Figs. 2 and 3) against the standard antibiotic Ampicillin. Leaf extract of *C. roseus* showed a varied level of antibacterial response against all the targeted bacterial strains *In vitro*. The methanolic extract of *C. roseus* inhibited the colony growth of bacterial isolates. Maximum inhibition 17.7 ± 0.88 mm and 17.3 ± 0.67 mm was produced against *M. luteus* and *E. coli* while inhibition zone 13 ± 1.15 mm was observed against *S. aureus* against the standard antibiotic with a zone of inhibition 15.3 ± 0.33 mm.

Table 1. Preliminary screening of methanolic extracts of *Catharanthus roseus* (*Madagascar periwinkle*) plant leaves.

Sr. No.	Constituent	Chemical	Mp response
1.	Flavonoids	Sodium hydroxide (NaOH) + Dil. acid + Extract	+ve
2.	Terpenoids	Extract + Chloroform + Con. Sulphuric acid (H_2SO_4)	+ve
3.	Glycosides	Legal's Reagent	+ve
4.	Anthraquinine	Extract + Benzene + Ammonia (NH_3)	-ve
5.	Tannins	Gelatin solution containing Sodium chloride (NaCl)	+ve
6.	Proteins	Con. Nitric acid (HNO_3) and Sodium hydroxide (NaOH)	+ve
		Molisch reagent	+ve
7.	Carbohydrates	Fehling's reagent	+ve
		Benedict's reagent	+ve
8.	Saponins	Foam	+ve
9.	Alkaloids	Mayer's reagent	+ve
		Wagner's reagent	+ve

Key: Mp = *Madagascar periwinkle*; +ve = Positive test result; -ve = Negative test results

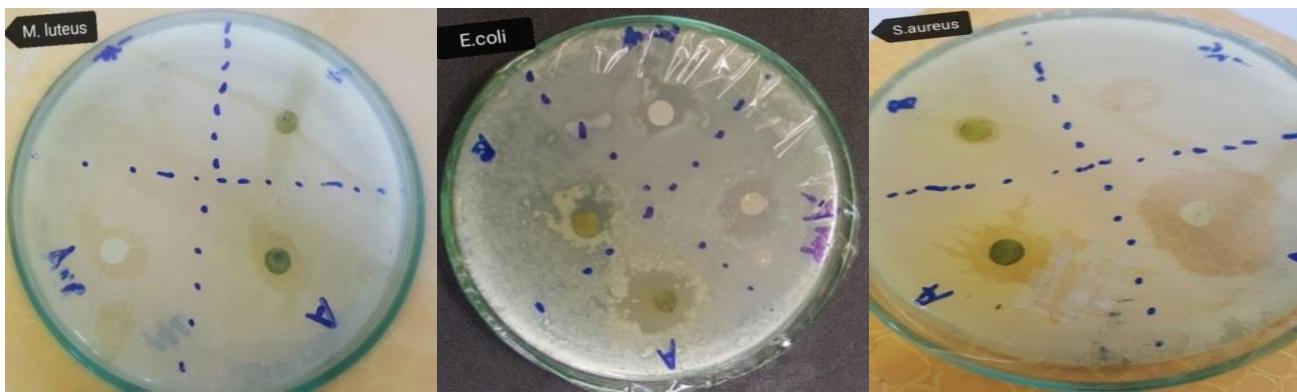


Fig. 2. Antibacterial potential of methanolic leaf extracts of *Catharanthus roseus* against *Staphylococcus aureus*, *Micrococcus luteus* and *Escherichia coli*.

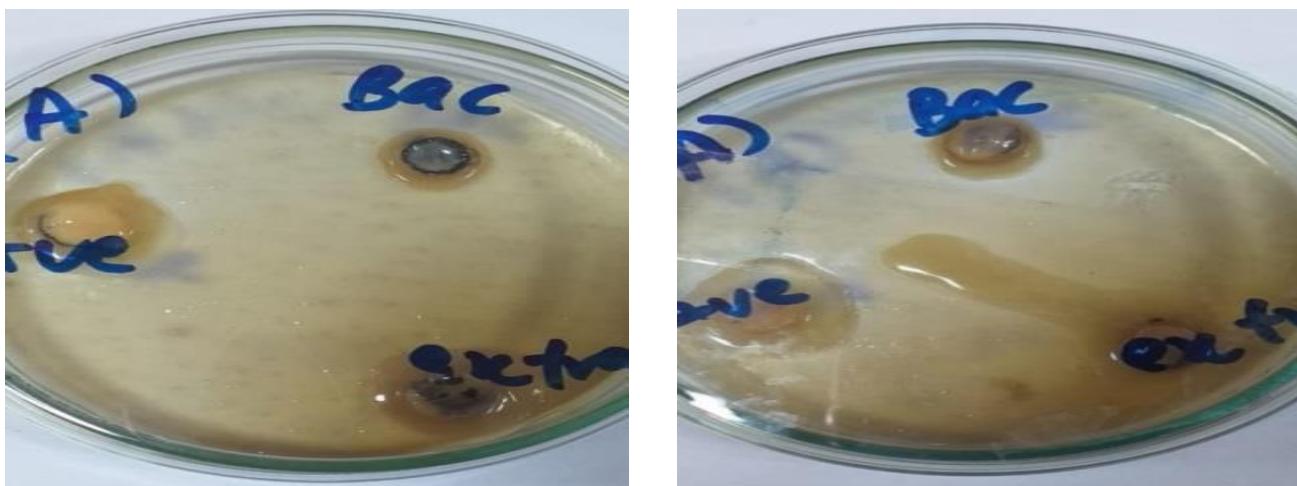


Fig. 4. Antitumor activities of methanolic leaf extract of *Catharanthus roseus* against *A. tumefaciens*.

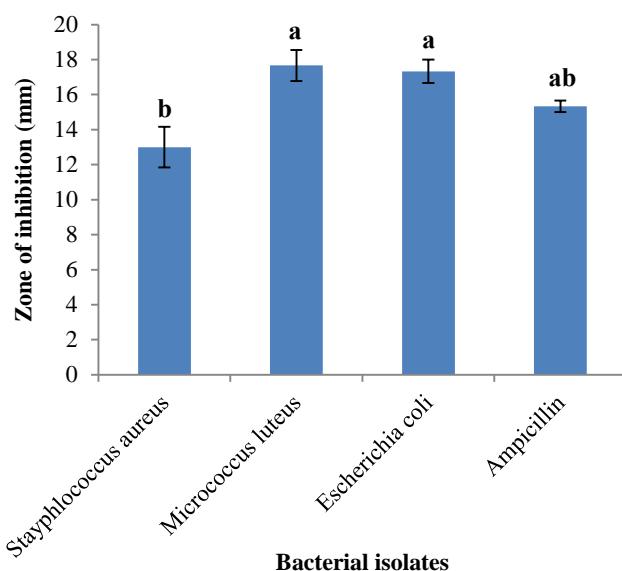


Fig. 3. Antibacterial potential of methanolic leaf extracts of *Catharanthus roseus* against bacterial strains.

*Antibacterial potential of plant extract was tested by agar well diffusion methods *In vitro*. Bacterial cultures were added to Petri plates and were mixed with sterilized NA medium. Upon media solidification, 100 μ l of plant extract was added to the wells. Petri plates were parafilmmed and

incubated at 37°C for 24 hr. Data on the zone of inhibition was recorded from each Petri plate and analyzed statistically. A graph was made by using the mean values of three replications from each treatment. Bars on the graphs showed standard error. Bars sharing the common letter showed non-significant among the treatments.

Antitumor bioassay: Antitumor potential of methanolic extract of *C. roseus* evaluated against *A. tumefaciens* in potato disc bioassay (Fig. 4). The collected results have shown the antitumor potential of methanolic extract of *C. roseus* against *A. tumefaciens* *In vitro*. The results were comparable to the positive control (Camptothecin). No tumor cells were observed in the potato discs treated with the methanolic extracts of *C. roseus*.

Discussion

Medicinal plants are extensively used in the therapeutical health care system and a number of life-saving natural drugs have been derived and extensively investigated. A large variety of plants have been explored to possess a variety of secondary metabolites that are used against disease causative microorganisms (EL-Kamali & EL-Amir, 2010). These medicinal plants occupy a central place in the natural health care system and about 80% of the world's populations around the globe rely on the use of various plants and plant-derived natural products to cure ailments of various kinds.

Catharanthus roseus L. an ornamental shrub, which is known as *Madagascar periwinkle* (Mp) belongs Apocynaceae family and it grows up to 30–100cm in height (Tiong *et al.*, 2013). It is originated from Madagascar and is pantropically distributed around the world due to its potential ability to survive under various ecological conditions (Van Bergen & Snoeijer, 1996). The Catharanthus genus consists of 8 plant species of annual or perennial shrubs and herbs, including *C. ovalis*, *C. trichophyllum*, *C. longifolius*, *C. coriaceus*, *C. lanceous*, *C. scitulus*, *C. pusillus* and *C. roseus* (Kumar *et al.*, 2007). In our studies, plant samples of *Catharanthus roseus* L. were collected from the Govt. College Women University Sialkot Pakistan. Collected plants were identified based on typical plant characters and were compared with the plant descriptions presented by (Kumar *et al.*, 2007; Kaushik *et al.*, 2017).

Methanol based plant extracts were prepared and subjected to qualitative phytochemical analysis. Methanolic plant extracts of *C. roseus* showed positive test results for flavonoids, terpenoids, glycosides, anthraquinone, tannins, proteins, carbohydrates, saponins, and alkaloids. Various studies have supported the presence of important phytochemicals in the *C. roseus*. In a previous study of Kabesh *et al.*, (2015) on phytochemical screening, confirmed alkaloids, phenol, saponins, and protein. Our findings are also endorsed by the research of (Fa *et al.*, 2019; Patharajan & BalaAbirami, 2014; Kabesh *et al.*, 2015; Govindasamy & Srinivasan, 2012). These secondary metabolites are known for their strong biological properties which include antimicrobial, antifungal, antitumor, and anti-inflammatory activities. Our research study also detected Flavonoids in the methanolic extract of *C. roseus*. Flavonoids have been investigated in various studies as antioxidant, anti-inflammatory, and antitumor agents (Compean & Ynalvez, 2014). Terpenoids along with other phytochemicals displayed antibacterial potential (Abdulhamid *et al.*, 2014). The active role of glycosides (Tagousop *et al.*, 2018), Anthaquinine (Malmir *et al.*, 2017), Tannins (Abdulhamid *et al.*, 2014), Saponins (Oboh, 2010), and alkaloids (Mariita *et al.*, 2011) in antimicrobial activities justify the application of methanolic extract of *C. roseus* as an antibacterial drug. These metabolites along with the other phytochemicals contribute to the antimicrobial potential.

DPPH free radical Scavenging method relies on hydrogen-donating activity which represents an important mechanism of antioxidant study of phenolic compositions. In the present study, the antioxidant activity of methanol leaf extract of *Madagascar periwinkle* at selected concentrations (250ppm, 500ppm, and 1000ppm) was studied by DPPH free radical assay against the standard ascorbic acid and elaborated that methanolic extracts of *C. roseus* has the ability to donate a proton and could serve as a free radical inhibitor. The antioxidant potential of standard Ascorbic acid was found greater than the methanolic leaf extracts. These findings are supported by the findings presented by (Mir *et al.*, 2018; Tiong *et al.*, 2013). More recently, Alaraidh, (2020) explored the protective role of salicylic acid, indoleacetic acid, and tryptophan on the antioxidant activities and gene expression in *C. roseus*.

In vitro antibacterial potential of methanolic leaf extracts of *C. roseus* was evaluated against three different tested bacterial strains (*Staphylococcus aureus*, *Micrococcus luteus*, and *E. coli*) by disc diffusion method. In our study, the methanolic leaf extract of *C. roseus* showed excellent antibacterial potential against *S. aureus*, *M. luteus* and *E. coli* *In vitro*. *Madagascar periwinkle* serves as a potential source for alkaloids (Ramya, 2008) and many other important secondary metabolites which support the antibacterial potential of *C. roseus*. Since the qualitative tests of methanol extracts of *C. roseus* showed the presence of flavonoids, terpenoids, glycosides, anthraquinone, tannins, proteins, carbohydrates, saponins, and alkaloids in the test samples, it was reported that the presence of these metabolites supports the antimicrobial efficacy of *C. roseus* thus it highlights the application of *C. roseus* in treating the diseases (Fa *et al.*, 2019). In a similar research study, leaf extracts of *C. roseus* prepared in organic solvents showed strong antibacterial potential against various bacterial strains (Ramya, 2008).

Our research findings related to the antimicrobial potential of *C. roseus* are in line with the other researches (Patil & Ghosh, 2010; Kumari & Gupta, 2013; Safhi *et al.*, 2013). Antitumor potential of methanolic extract of *C. roseus* was studied against *A. tumefaciens* in potato disc bioassay *In vitro*. Methanolic leaf extracts of *C. roseus* showed antitumor potential and no tumor cells were observed in the potato discs treated with the methanolic extracts of *C. roseus*. These findings are supported by similar research in some previous studies (Sayeed *et al.*, 2014; Costa-Lotufo *et al.*, 2005). Our study findings implicate that if active molecules are identified from these plant extracts, plant tissue culture techniques can be employed for the production of secondary metabolites and there is a dire need to explore the antibacterial and antitumor potential of more native medicinal plants.

Conclusion

Methanolic based leaf extract of *C. roseus* contains various important metabolites including flavonoids, terpenoids, glycosides, anthraquinone, tannins, proteins, carbohydrates, saponins, and alkaloids which play a significant role in supporting antimicrobial activity. This study suggests that leaf extract of *C. roseus* possess strong antibacterial and antitumor potential thus can be used to substitute the application of synthetic chemicals for control of bacterial infections and other ailments.

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