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Research Article

Antimicrobial evaluation of crude extracts of bark and stem of virginia creeper (*Parthenocissus quinquefolia* (L.) Planch)

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ABSTRACT

The ***In vitro*** antimicrobial potential of bark and stem of virginia creeper (*Parthenocissus quinquefolia* (L.) Planch) was measured in order to authenticate its ethnopharmacological significance. Bark and stem parts were extracted by static maceration technique in non-polar and polar solvents, such as n-hexane, chloroform, ethanol and double distilled water. Agar well diffusion technique was used to verify the antimicrobial activity of these extracts against two Gram-negative, Gram-positive and fungal strains. Minimum inhibitory concentration (MIC) was measured through modified broth dilution method. However, ethanolic and aqueous extracts of stem resulted in highest inhibition zone (52.5±1.1 mm) against Pseudomonas aeruginosa and augmented antifungal activity (46±0.48 mm) against *Fusarium solani*, respectively. Whereas n-hexane extract of bark exhibited least antibacterial activity in the form of inhibition zone of 15±0.22mm against S. aureus. Similarly, minimum activity (16.5±0.21 mm) was observed in ethanolic extract of bark, against *F. solani*. The most resistant MIC value was shown by ethanolic bark extract, i.e. 0.5 mg/mL against P. aeruginosa. Thus, virginia creeper can be recommended as a remedy to combat efficaciously with the resilient contagious strains.

Keywords: Antimicrobial activity; *E. coli*; inhibition zone; minimum inhibitory concentration; virginia creeper.

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INTRODUCTION

Plants are the significant source of bioactive compounds utilized in herbal drugs. They contain secondary metabolites which strengthen the immune system to combat infectious diseases. The World Health Organization (WHO) has assessed that 80% of the population of developing countries uses plants as a source of traditional medicines for their primary health care needs (Izzo, 2004). Researchers have been interested in isolation of bioactive compounds from plants for the preparation of novel medicines (Essawi and Srour, 2000). WHO has catalogued about 2100 therapeutic plants present all over the world (Priya et al., 2002).

Despite of development in allopathic drugs, bacteria and fungi are still the causative agents of contagious diseases in human beings. Due to scarcity of medicines in developing countries, their impact is predominantly very huge. By the evolution of novel bacterial strains having multi-resistance ability, actions should be taken to diminish the effects of these bacteria by understanding their genetic mechanism and introduction of new synthetic or natural drugs (Field and Lettinga, 1992). According to one estimate, about 20,000 to 35,000 species of plants are utilized as medicines,

pharmaceuticals, cosmetics and neutraceuticals by many ethnic groups over the whole world. The studies on physiochemical, pharmaceutical, antibacterial and antifungal activities of plant extracts have shown a unique way to explore new sources of medicines (Frey and Meyers, 2010). In 1990, near about $15.5 billion was consumed on phyto-pharmaceuticals derivation and this industry is more enhanced in next years. At present, the leaves, shoot, roots, flowers, bark and fruit of plants are the basis of approximately 42% of 25 uppermost marketed prescriptions globally marketed (Ramya et al., 2008).

Jayachitra *et al*. (2013) assessed the phytochemical constituents and antimicrobial potential of methanolic extract of *Cissus setosa* Roxb. using agar well diffusion. The plant exhibited strong antibacterial activity against gram positive bacterium, *Micrococcus luteus* and antifungal activity against *Candida parapsilopsis*. Ajaib *et al*. (2013) also investigated the antimicrobial activity of *Iris aitchisenii* against two Gram-positive bacteria (*Streptococcus faecalis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The demand for more and more herbal drugs is continuously increasing which necessitates the screening of medicinal plants with promising biological activity. Thus, the present study was conducted to investigate antimicrobial efficiency of Virginia creeper (family Vitaceae) for its therapeutic uses.

MATERIALS AND METHODS

Collection and Preparation of Samples

The plant material, *Parthenocissus quinquefolia* (L.) Planch was collected from Lahore. Bark and stem were separated, dried at room temperature and powdered. About 300 g of each plant part was extracted by soaking in 600 mL for 8 days in each non-polar and polar solvent, such as n-hexane, chloroform, ethanol and double distilled water. Dried extracts were incorporated for evaluation of antimicrobial activity.

Microorganisms

Four bacterial strains including Gram-positive, (*Staphylococcus aureus* & *Bacillus subtilis*); Gram-negative bacteria, (*Escherichia coli* & *Pseudomonas aeruginosa*) and two fungal strains (*Aspergillus niger* & *Fusarium solani*) were selected as test microorganisms. Bacterial strains were obtained from Pathology Department, Mayo Hospital Lahore whereas; fungal strains were obtained from Institute of Industrial Biotechnology and Microbiology, GC University, Lahore.

Preparation of Medium

Nutrient Agar medium and Potato Dextrose Agar (PDA) medium were employed for culturing bacteria and fungi respectively. Nutrient Agar medium was prepared by following the standard composition provided by American Public Health Association (APHA, 1917) and Association of Official Analytical Chemists (AOAC, 1995). Similarly, PDA was made by following the recipe of Johansen (1940).

Zone of Inhibition

The inhibition zone was estimated by using Agar-well diffusion technique after Jorgensen et al. (2007). According to this technique, dilute inoculums of tested bacteria and fungi were spread in the Petri plates containing desired media, standard hole was made in the centre of all Petri plates using corn borer no.6 and 1 mL of crude extract was filled in the hole in the laminar air flow. Each plate was labelled, covered with cling film and placed in incubator at 35±2°C and 25±2°C for 24 and 48 hours for investigation of antibacterial and antifungal activity, respectively. After incubation, diameter of inhibited area by plant extracts was measured.

Minimum Inhibitory Concentration (MIC)

The ethanolic extracts of *P. quinquefolia* were employed for the evaluation of minimum inhibitory concentration (MIC). The MIC assessment was taken by following modified broth dilution assay of Murry et al. (1999). Optical densities were measured at 595 nm through UV-spectrophotometer.

Statistical Analysis

All parameters were executed in three replicates. The results obtained were statistically analyzed by applying Analysis of Variance (ANOVA) and Duncan's Multiple Range Test to find out the significant value of analysis at $P < 0.05$ and least significant difference (LSD), after Steel et al. (1997).

RESULTS AND DISCUSION

Antimicrobial potential of crude extracts of bark and stem of *P. quinquefolia* (L.) Planch was analyzed by measuring the zone of inhibition and Minimum Inhibitory Concentration (MIC). The standard discs were used to determine the

susceptibility of bacterial and fungal strains. The results revealed that *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *A. niger* and *F. solani* had intermediate response against standard discs as compared to the plant extracts (Table1).

Table 1. Zone of inhibition (mm) indicated by standard antibiotic and antifungal discs against test microbes.

Microbial strains	Standard discs	Concentration of standard discs (µg)	Zone of Inhibition (mm)
E. coli	Amikacin	20	18.7±1.04
P. aeruginosa	Cephalaxin	20	18±0.76
S. aureus	Erythromycin	20	15±0.98
B. subtilis	Amikacin	20	17±0.58
A. niger	Griseofulvin	20	22±1.52
	Terbinafine	20	20.5±2.01
F. solani	Griseofulvin	20	21±2.54
	Terbinafine	20	19±2.32

Mean values followed by ± standard error.

The solvents, in which extracts of different parts of *P. quinquefolia* were prepared, proceeded as negative control against test microorganisms. No activity was reported by any solvent used. The maximum zone of inhibition was measured by using ethanolic extract of stem, i.e. 52.5±1.10 mm against *P. aeruginosa* while the minimum activity was exhibited by the n-hexane extract of bark, i.e. 15±0.2 mm against *S. aureus*. The ethanol bark extract executed maximum zone of inhibition, i.e. 51± 0.9 mm against *P. aeruginosa*. The same trend was observed when *B. subtilis* was evaluated against the plant extracts under investigation, with maximum activity reported by ethanol extract of bark, i.e. 47±1.4 mm. Chloroform and ethanol extracts of stem exhibited 41±0.4 mm and 40.05±0.8 mm inhibitions zones against B. subtilis, respectively (Table 2).

Table 2. Zone of inhibition produced by extracts of different parts of *Parthenocissus quinquefolia* against various microbial strains.

Plant parts	Solvents	Zone of Inhibition (mm)					
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*Aspergillus niger*	*Fusarium solani*
Bark	n-Hexane	17±0.12f	22±0.40e	15±0.22e	18.5±0.6f	21±0.31ef	24±0.22e
	Chloroform	29±0.30d	26±0.53d	36±0.73ab	29±0.2d	34±0.73b	27±0.43d
	Ethanol	38±0.52b	51±0.91ab	35.5±1.04b	47±1.4a	18±0.23f	16.5±0.21g
	Water	20±0.41e	18±0.25f	21.5±0.36d	28.5±0.4de	27±0.56de	21±0.35f
Stem	n-Hexane	18.5±0.15ef	21±0.20e	31±0.89c	26.5±0.37e	40±1.82a	34±0.56b
	Chloroform	32±0.74c	50±0.71b	38±0.72a	41±0.44b	35±0.81b	31±0.51c
	Ethanol	37±0.99b	52.5±1.10a	35.5±0.4b	40.1±0.80b	39±0.96a	30±0.73c
	Water	51±1.41a	37±0.47c	30±0.57c	36±1.02bc	34±0.55bc	46±0.48a
LSD		2.228	2.289	2.164	2.163	2.8210	2.770

Mean followed by various letters in same column is significantly different at a level of $P < 0.05$ according to Duncan's new multiple range test while ± indicates standard error and LSD means least significant difference. The antimycotic potential of *P. quinquefolia* was evaluated against *A. niger* and *F. solani* (Table 2). The best results were depicted by stem extract of n-hexane, i.e. 40±1.82 mm and lowest zone of inhibition was reported by ethanol bark extract, i.e. 18±0.23mm against *A. niger*. Likewise, the maximum and minimum activity against *F. solani* was documented by aqueous stem and ethanol bark extract, i.e. 46±0.48 mm and 16.5±0.21 mm, respectively. The modified broth dilution assay was carried out according to Murray *et al*. (1999) to further assess the minimal consistency of the plant extracts at which the growth of the bacteria and fungi could be inhibited. The results revealed that MIC value for ethanolic bark extract was quantified as 0.5 mg mL-1 against E. coli (Fig. 1). However, ethanol bark and stem extracts had minimum absorbance against *P. aeruginosa* at MIC 0.5 mg mL-1 and 0.6 mg mL-1 respectively (Figure 2).

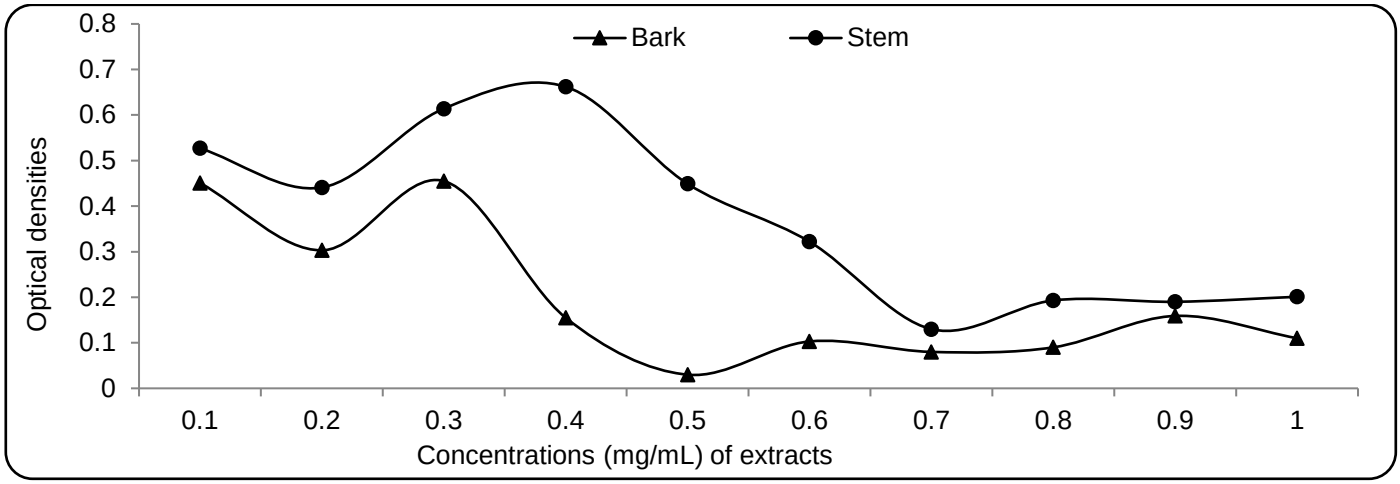

Figure 1. MIC values (mg/mL) exhibiting the antibacterial potential of the extracts of *P. quinquefolia* against *E. coli.*

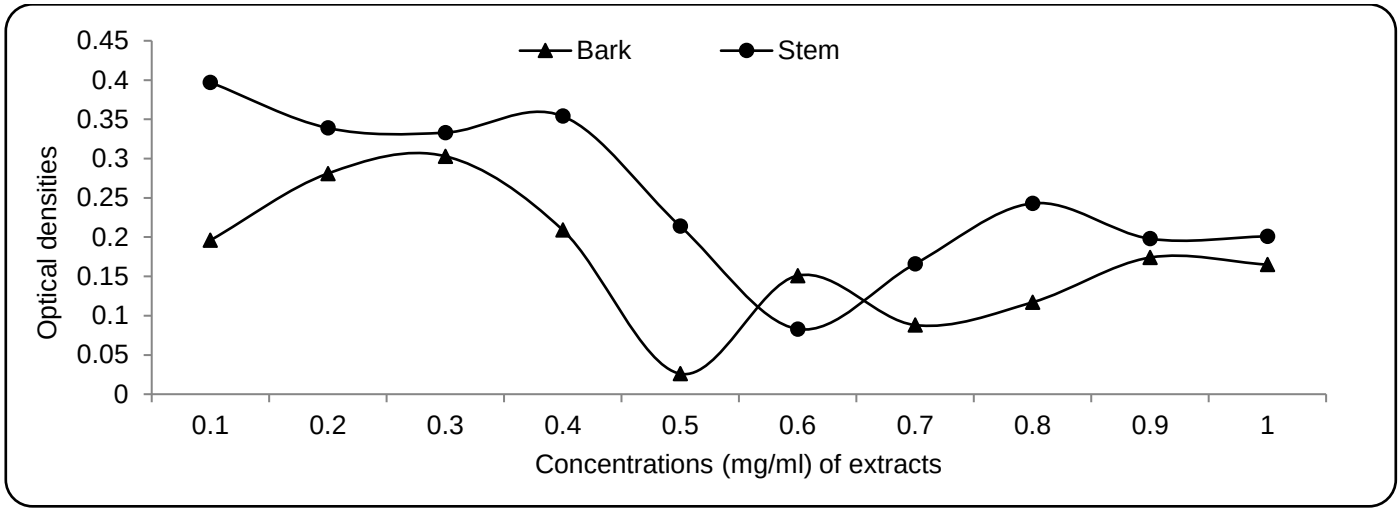

Figure 2. Graphical representation of the MIC values (mg/mL) of *P. quinquefolia* against *P.aeruginosa.*

Likewise, MIC values of bark and stem extracts (ethanol) against S. aureus and B. subtilis were given in figure 3 and 4, respectively.

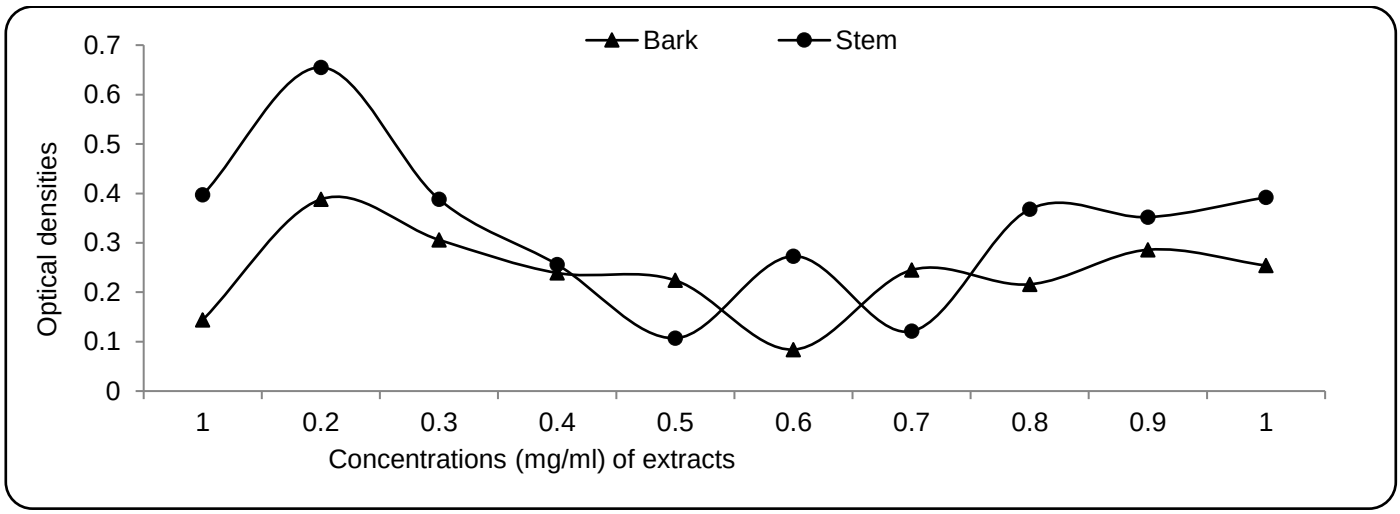

Figure 3. Graphical representation of the MIC values (mg/mL) exhibiting the antibacterial potential of the extracts of *P. quinquefolia* (L.) Planch against *S. aureus*.

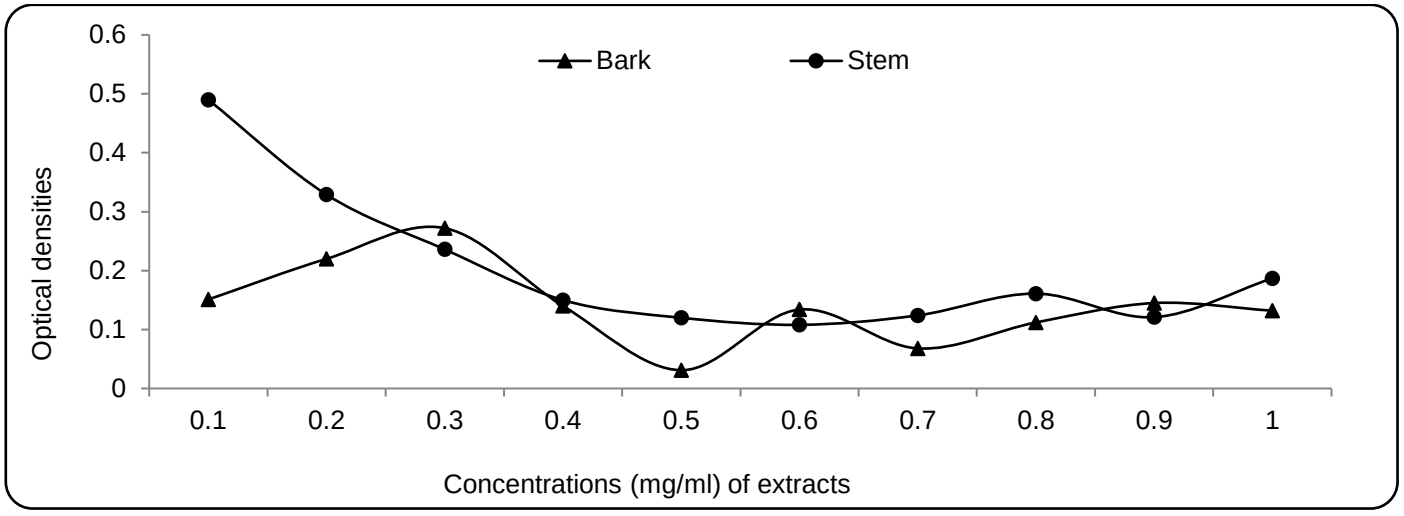

Figure 4. MIC values (mg/mL) exhibiting the antibacterial potential of the extracts of *P. quinquefolia* against *B. subtilis*.

MIC results against fungal strains revealed that minimum absorbance (0.009±0.2nm) was obtained by ethanolic bark extract at 0.9 mg mL^{-1} concentration while ethanol stem extract executed MIC 0.5 mg mL^{-1} against *A. niger* (Figure5). In the same manner, bark and stem extracts had 0.8 mg mL^{-1} and 0.5 mg mL^{-1} MIC values against *F. solani*, respectively (Figure 6).

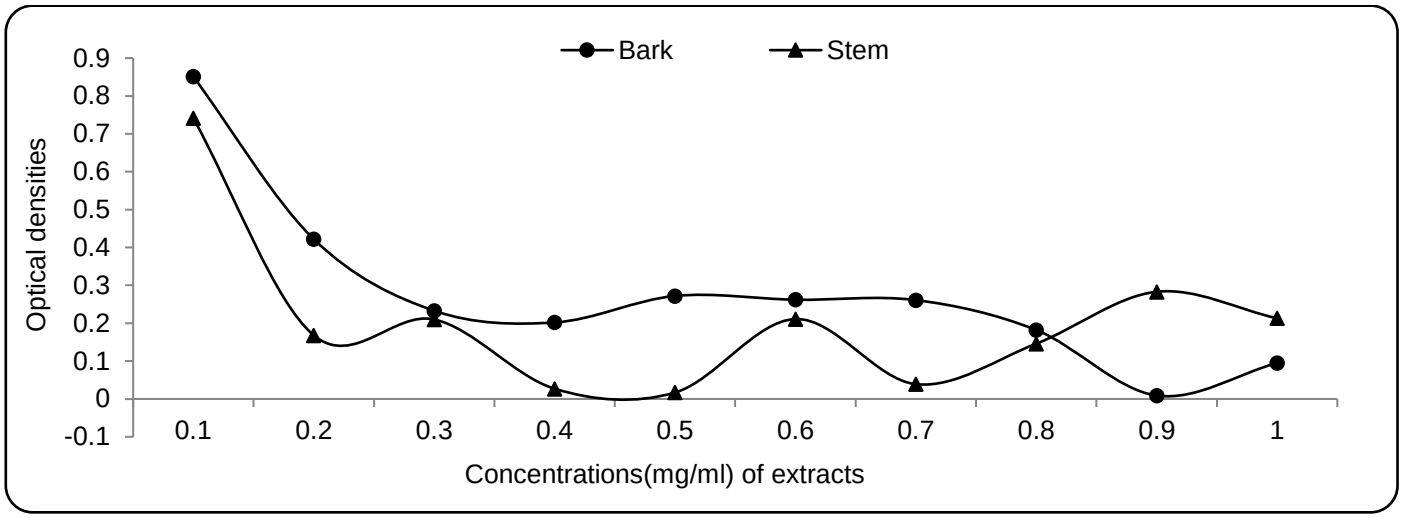

Figure 5. MIC values (mg/mL) exhibiting the antifungal potential of the extracts of *P. quinquefolia* against *A. niger*.

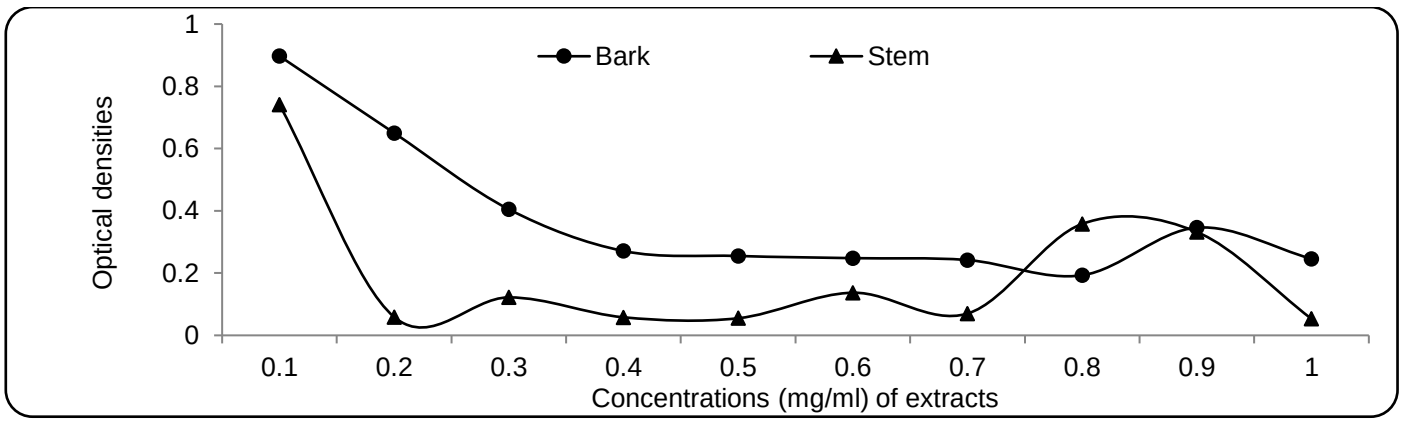

Figure 6. Graphical representation of the MIC values (mg/mL) exhibiting the antifungal potential of the bark and stem extracts of *P. quinquefolia* against *F. solani*.

Ethanolic stem extract of *P. quinquefolia* showed the highest inhibition zone (52.5±1.1 mm) against *P. aeruginosa*. The least inhibition zone was reported by n-hexane extract of bark (15±0.22 mm) against S. aureus. Similar results were also reported by Ajaib et al. (2013) while estimating antimicrobial activity of *Iris aitchisonii* (Bakar) Boiss. They examined maximum antibacterial activity (45±1.73 mm) against *P. aeruginosa*. Summarizing the results of antibacterial activity, it was observed that ethanol and chloroform extracts of bark and stem of *P. quinquefolia* exhibited significant activity against all the test bacterial strains. Whereas, n-hexane extracts of all the parts of Virginia creeper showed minimum antibacterial activity against all the bacterial strains. The standard discs, i.e. Amikacin, Cephalaxin and Erythromycin showed less inhibition as compared to plant extracts. The highest and lowest antimicrobial activity was documented by aqueous stem and ethanol bark extract in the form of inhibition zone, i.e. 46±0.48 mm and 16.5±0.21 mm, respectively against *F. solani*. These results are also supported by the findings of Hosamani et al. (2012) while estimating the antimicrobial potential of leaf extracts of *Psoralea corylifolia* L. In the same trend, most resistant MIC value was shown by ethanolic bark extract, i.e. 0.5mg/ml against P. aeruginosa and ethanolic fruits extract, i.e. 0.4mg/ml against *F. solani* Similar results were also documented by Tangarife-Castano *et al*. (2012) while assessing the antifungal activity of Verbenaceae and Labiatae families. Concluding all the results, different concentrations of bark and stem of *P*. *quinquefolia* can be used in formulation of antibiotics and antimycotics as medication for human ailments caused by under studied bacterial and fungal strains.

CONCLUSION

From present study, it was concluded that ethanol and chloroform extracts of bark, stem, leaves and fruits of *P*. *quinquefolia* had significant activity against all the test microbial strains. The antimicrobial potency of bark and stem of *P. quinquefolia* was more effective than standard antimicrobial drugs. So, this plant can be used as an alternative of synthetic standard medicine against respiratory diseases, diarrhea as well as other diseases caused by under tested microbes.

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