In vitro antibacterial activity and phyto-chemistry of samples from the roots of *Viola pilosa*

Maria Khan Panni and Jehan Bakht
Institute of Biotechnology and Genetic Engineering, the University of Agriculture Peshawar, KPK, Pakistan

Abstract: Anti-microbial activity and phytochemical analysis of samples from the roots of *Viola pilosa* was studied against six strains of bacteria. Data indicated that the tested bacterial strain differed in its sensitivity to the root extracts of *Viola pilosa.* The results showed that *Xanthomonas campestris, Bacillus subtilis* and *S. aureus* were more susceptible to butanol extracted fraction. *S. aureus* was totally resistant to aqueous extracted fraction at all concentrations and *.Xanthomonas campestris* and *Bacillus subtilis* were least susceptible to the same fraction at 0.5 mg disc^{-1}. Similarly, ethyl acetate at 2 mg disc^{-1} concentrations was effective against *Pseudomonas aeruginosa* and *Escherichia coli. Klebsiella pneumoniae* was completely resistant to all the tested concentrations. Phytochemical analysis of the different solvent extracted samples suggested the presence or absence of different various metabolites including alkaloids, saponins, tannins, sterols, flavonoids, protein, carbohydrates and fats.

Keywords: Antibacterial activity, disc diffusion assay, *Viola pilosa,* phyto-chemistry.

INTRODUCTION

Medicinal plants are in use by human being since long time for the treatment of different health issues (Redzic, 2007). Interest in herbal medicine has increased due to unjudicious use of antibiotics and prevalence drug resistant microorganisms (Chopra *et al*., 1997). Plant contains different phytochemicals which are used for various pharmaceutical and nutritional purposes (Oktay *et al*., 2003; Wangensteen *et al*., 2004). Medicinal plants are rich in biologically active compounds that hold antimicrobial properties (Bakht *et al*., 2018; Bilal *et al.*, 2018; Ayaz *et al.*, 2017; 2018; Wajid *et al.*, 2017; Ullah *et al.*, 2015). Extracts of plants and phytochemicals are receiving extra significance as potential bases for preventing different infections during the modern era.

Viola pilosa known as "Banafsha or smooth leaf white violet belongs to the family Violaceae (Mabberley, 1987). There is 1 genus (*Viola*) and 17 species in Pakistan (Perveen and Qaiser, 2009). Flowering usually occurs in March-May. *Viola pilosa or Viola serpens* Wall is usually found in Swat and Siran valley of Pakistan. *Viola serpens* has been testified to treat various diseases. Astringent, demulcent, diuretic, diaphoretic, purgative, emollient, refrigerant and purgative activity have been reported from the leaves and flowers of *Viola pilosa* (Shinwari and Khan, 2000; Ahmed *et al*., 2006). Phytochemical screening revealed that *Viola serpens* is rich in flavanoids and glycosides (Adhikary *et al*., 2011).

MATERIALS AND METHODS

Collection and identification of plant material

The present research was conducted at IBGE, The University of Agriculture Peshawar, Pakistan. Plants *Viola pilosa* were collected from Northern areas (Swat) and identified at the Department of Botany, University of Peshawar.

Preparation of crude extracts from the roots

Plant materials (roots) were removed carefully and washed with distilled water. Plant material was dried in shade at room temperature for two weeks before grinding to fine powder by means of an electric grinder (Thomas Scientific USA). Crude extract and their fractionation were carried out as described by Madiha *et al*. (2018). Antimicrobial activity of different samples from *Viola pilosa* against different strains of bacteria (Table 1) and phytochemical analysis was determined by the methods described by Madiha *et al*. (2018).

STATISTICAL ANALYSIS

The experiment was repeated in triplicate and MSTAT computer software was used for the analysis of the data. Significant differences among means were separated by Least Significant Difference (LSD) test at $p<0.05$ (Steel *et al*., 1997).

RESULTS

Our results revealed that *K. pneumoniae* was highly resistant to samples isolated from *Viola pilosa* measuring no activity at any concentration. *Pseudomonas aeruginosa* revealed varying degree of growth inhibition at all the tested concentrations of different samples from the roots of *Viola pilosa* (fig. 1). Growth reduction of the tested microbe was dose dependent. Maximum growth inhibition of 75.41% was noted by ethyl acetate followed by butanol (74.04%) at 2 mg disc^{-1} concentration. Similarly, minimum activity (34.25% ZI) against

**Corresponding author:* e-mail: jehanbakht@yahoo.co.uk

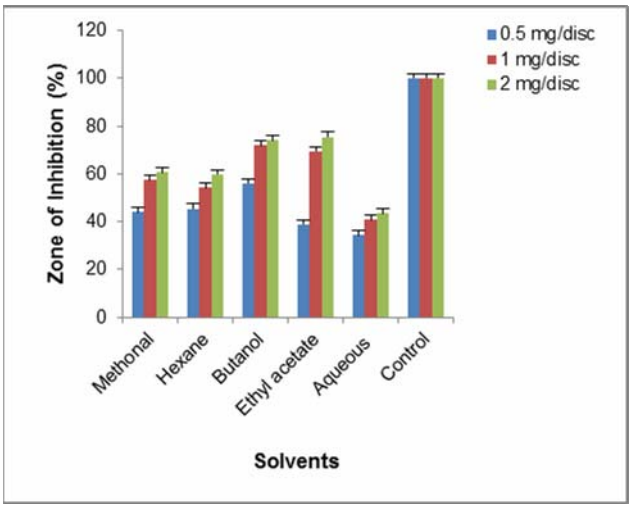

Fig. 1: Antibacterial activity of crude methanol, hexane, butanol, ethyl acetate and aqueous extracted samples from the roots of *Viola pilosa* against *P. aeruginosa* by disc diffusion assay (Bar shows LSD at p<0.05).

Pseudomonas aeruginosa was measured by aqueous samples at 1 mg disc^{-1}. Butanol fraction inhibited the activity of the tested bacterium by 41.19% followed by hexane (40.83%) at 2 mg disc^{-1}. Aqueous samples from the tested plant measured 0% ZI. The growth of *S. aureus* was also inhibited by hexane, crude methanolic extract and ethyl acetate to varying degree (fig. 2). Results in fig. 3 showed that highest growth inhibition of *B. subtilis* (52.60%) was noted by butanol at 2 mg disc^{-1} followed by 45.6% ZI of the same extract at 1 mg disc^{-1}. Lowest antimicrobial activity of 20.44% was revealed by aqueous fraction at 0.5 mg disc^{-1}. *E. coli* was also susceptible to different solvent samples of the tested plant at all concentration. Ethyl acetate fraction resulted in maximum growth inhibition (41.19% ZI) at 2 mg disc^{-1} followed by 39.41% ZI each of the same extract and crude methanolic sample at 1 mg disc^{-1}. Minimum activity of 25.06% was noted for the same microbe by aqueous at 1 mg disc^{-1} (fig. 4). *X. campestris* also showed susceptibility to samples from the tested plant (fig. 5). Butanol samples showed maximum activity (62% ZI) at 2 mg disc^{-1} followed by crude methanolic (55.9% ZI) at 1 mg disc^{-1}. Minimum activity against *X. campestris* was measured by aqueous samples at 0.5 mg disc^{-1} (27.58% ZI).

In the present study samples from the roots of *Viola pilosa* were also screened for alkaloids, tannins, fats and oils, proteins, carbohydrates, sterols, flavonoids and saponins (table 2). Phytochemical screening revealed traces of proteins, moderate quantity of alkaloids and saponins and high quantity of tannins, fats and oils, carbohydrates, sterols and flavonoids. The study revealed that n-hexane samples were found negative for alkaloids, proteins and tannins and was rich in fats, oils and flavonoids. The butanol extracted fraction presented good content of tannins, carbohydrates and saponins and moderate content of fats, oils, sterols, flavonoids and alkaloids. The results further suggested that ethyl acetate was found negative for alkaloids, fats and oils and had traces of proteins, carbohydrates and saponins. In the present study it was demonstrated that water extracted fraction exhibited high content of flavonoids, fats and oils and a good content of tannins, carbohydrates and sterols. The lowest level was found for proteins, alkaloids and saponins.

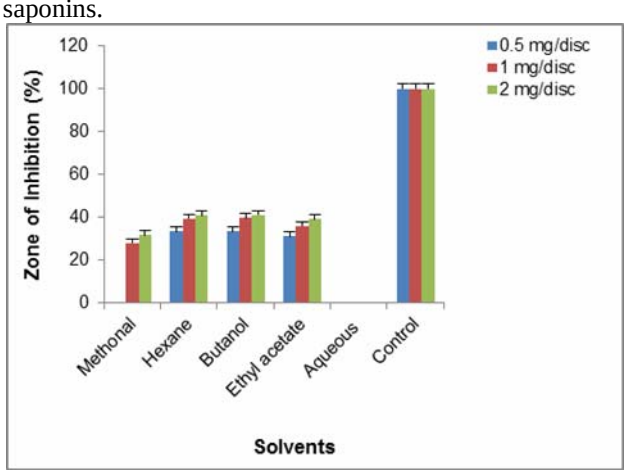

Fig. 2: Antibacterial activity of crude methanol, hexane, ethyl acetate, butanol and aqueous extracted samples from the roots of *Viola pilosa* against *S. aureus* by disc diffusion assay (Bar shows LSD at p<0.05).

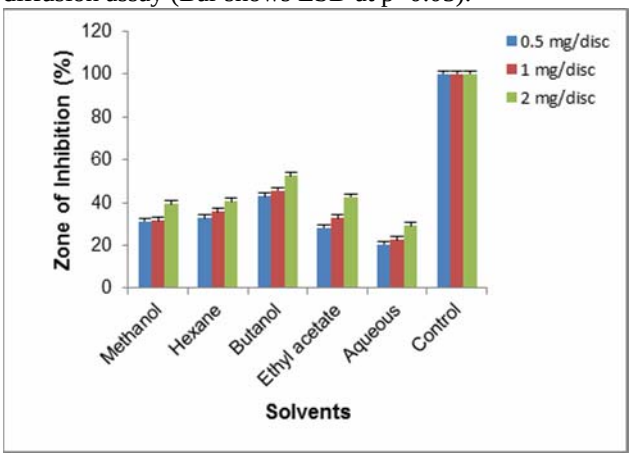

Fig. 3: Antibacterial activity of crude methanol, hexane, ethyl acetate, butanol and aqueous extracted samples from the roots of *Viola pilosa* against *B. subtilis* by disc diffusion assay (Bar shows LSD at p<0.05).

DISCUSSION

The current study investigates the antibacterial activity of six different bacterial strains and phytochemical screening of samples from the roots of *Viola pilosa*. Our results revealed that *K. pneumoniae* showed maximum resistance to different samples and revealed no activity at any concentration. Adhikary *et al.* (2011) concluded that crude methanolic extracted samples of *Viola pilosa* did not show antibacterial activity against *Klebsiella sp*. Ethyl

Table 1: Microbial strains tested during the present experiment

Microbial Species	Gram strain type	Details of the Microbial strains used
Klebsiella pneumoniae	Negative	Clinical isolate obtained from The Department of Microbiology Quaid-I-Azam University Islamabad, Pakistan
Pseudomonas aeruginosa	Negative	ATCC # 9721
Staphylococcus aureus	Positive	ATCC # 6538
Bacillus subtilis	Positive	Clinical isolate obtained from The Department of Microbiology, Quaid-I-Azam University Islamabad, Pakistan
Escherichia coli	Negative	ATCC # 25922
Xanthomonas campestris	Negative	ATCC # 33913

Table 2: Phytochemical profile of solvent extracted samples from shoots of *Viola pilosa.*

EXTRACT	Alkaloids	Proteins	Tannins	Carbohydrates	Sterols	Flavonoids	Saponins	Fats and Oils
CRUDE	++	+	+++	+++	+++	+++	++	+++
N-HEXANE	_	_	_	+	+	+++	+	+++
BUTANOL	++	+	+++	+++	++	++	+++	++
E. ACETATE	_	+	+++	+	++	++	+	_
AQUEOUS	+	+	++	++	++	+++	+	+++

+++: shows the presence in abundance
++ : shows presence in moderate quantity
+ : shows presence but in less amount
_ : shows complete absence of the compound

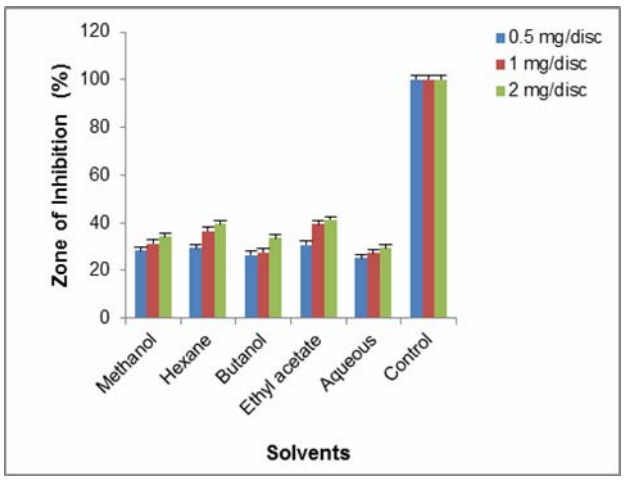

Fig. 4: Antibacterial activity of crude methanol, hexane, ethyl acetate, butanol and aqueous extracted samples from the roots of *Viola pilosa* against *E. coli* by disc diffusion assay (Bar shows LSD at p<0.05).

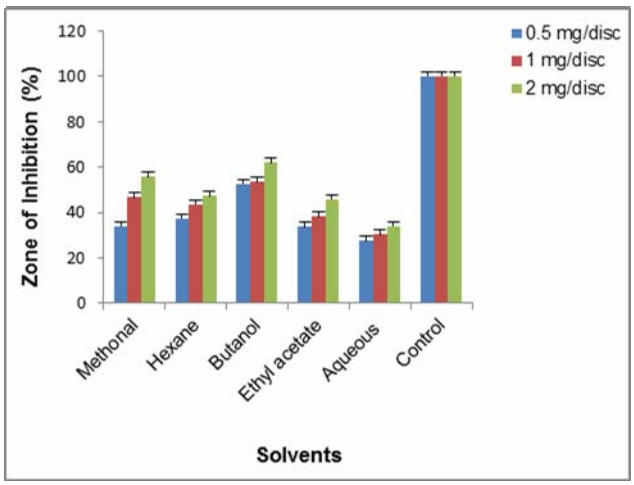

Fig. 5: Antibacterial activity of crude methanol, hexane, ethyl acetate, butanol and aqueous extracted samples from the roots of *Viola pilosa* against *X. campestris* by disc diffusion assay (Bar shows LSD at p<0.05).

acetate fraction reduce the growth of *P. aeruginosa* at 2 mg disc^{-1} followed by butanol, crude, n-hexane and aqueous samples.

These results agree with Banaszczak *et al*. (2005), Arora *et al*. (2007), Pranting *et al.* (2010) and Akhbari *et al.* (2012) who reported that the activity of *P. aeruginosa* was inhibited by crude methanolic samples from *Viola tricolor* and *Viola odorata.* Maximum activity against *S. aureus* was measured by butanol fraction followed by n-hexane and ethyl acetate extracted samples. Crude methanolic extracted samples measured minimum growth reduction in *S. aureus* at concentration of 0.5 mg disc^{-1}.

No activity was also recorded by aqueous samples against *S. aureus* at any concentrations (Arora *et al*., 2007; Vuuren *et al.*, 2008). All samples reduced the activity *B. subtilis* and n-butanol showed maximum activity at 2 mg disc^{-1} (Sahin *et al.,* 2003; Ahmed *et al.,* 2006; Vuuren *et al.,* 2008). *E. coli* was moderately susceptible to all the concentrations of the extracts under study. Ethyl acetate extracted fraction, when compared with other samples and controls was the most effective to reduce the activity of *E. coli.* These results corresponds Sahin *et al.* (2003) and Daoud *et al.* (2011). The growth of *X. campestris* was controlled by all samples in concentration dependent

manner. Maximum growth reduction was noted by butanol at maximum concentration and lowest by aqueous at 2 mg $disc^{-1}$ (Karaman *et al*., 2003; Mahesh and Satish, 2008).

Analysis of the extracts revealed that crude methanolic extract exhibited good phytochemicals profile showing the presence of alkaloids, tannins, carbohydrates, sterols, fats and oils, flavonoids, saponins and proteins followed by butanol, aqueous, n-hexane and ethyl acetate. The results of our study showed the absence of alkaloids in hexane and ethyl acetate samples, moderate concentration of alkaloids in crude methanolic and butanol extracted samples while low concentration in water extracted samples. Proteins and tannins were detected in all the methanolic extracts except hexane. Similarly, fats and oils were found in all the root extracts except ethyl acetate extracted samples. The results further confirmed the presence of carbohydrates, saponins, flavonoids and sterols in the crude methanolic root extracted samples of *Viola pilosa.* These findings are similar to Parekh and Chanda (2006), Vukics *et al.* (2008 a,b), Muhammad and Saeed (2012) and Barkatullah *et al.* (2012).

CONCLUSION

It can be concluded that samples from the roots of *Viola pilosa* revealed varying degree of activity against *X. campestris, B. subtilis* and *S. aureus. Klebsiella pneumoniae* was completely resistant to all concentrations under study. Phytochemical screening indicated the presence or absence alkaloids, saponins, tannins, sterols, flavonoids, protein, carbohydrates and fats.

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