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Research Article

Comparative analysis of *in vitro* antibacterial activity of extracts of *Viola patrinii* on pathogenic microorganisms

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ABSTRACT

The antibacterial activities of hexane (H), petroleum ether (PE), acetone (AC), choloroform (C), ethanolic (E) and water (W) extracts of whole plant (1mg/ml) of *Viola patrinii* were determined against wide variety of pathogenic bacteria. The extracts were tested against various bacteria's like *Escherichia coli* (EC)*, Staphylococcus aureus* (SA)*, Staphylococcus pyogens* (SP), *Bacillus subtilis* (BS), *Klebsiella pneumoniae* (KP*) and lactococcus* (LC) by well diffusion method. Minimum inhibitory concentration (MIC) and Minimum lethal concentration (MLC) values of each extract were determined. It is concluded that ethanolic extract of whole plant of *Viola partinii* exhibited significant antibacterial activity. These findings established the potential of the plant *Viola partinii* as an effective antibacterial agent. However, further studies are needed to evaluate active compounds and probable medicinal benefits in chemotherapy among humans.

Keywords: *Viola partinii*; antimicrobial activity; MIC; MLC; pathogenic microorganisms; extracts

INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer et al., 1999). It has been estimated that 14 - 28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethnomedicinal use of the plants. Researcher and scientists throughout the world are trying to explore new medicine from plants to help the humanity (Bachheti et al., 2011). Phytochemicals which can be derived from leaves, stems, barks and flowers of plants are applied as natural anti pathogenic (Yousuf et al., 2011).

In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient time (Bhattacharjee, 1998). India is rich in medicinal plant diversity. All known types of agro-climatic, ecologic and edaphic conditions are met within India. India is rich in all three levels of biodiversity, as species diversity, genetic diversity and habitat diversity (Zafar et al., 1999). India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Martins et al., 2001). Natural products are known to play an important role in both drug discovery and chemical biology. In fact, many of the current drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from natural motifs (Cheesbrough, 2000). Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms (Gordon and David, 2001). Consequently, it is believed that the whole plant has more effective healing properties than its isolated constituents. Any part of the plant may contain active components (Nair and Chanda, 2004). In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju, et al., 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin, et al., 1985). Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments (Tanaka et al., 2002).

Viola is a genus of flowering plants in the violet family Violaceae, with around 400–500 species distributed around the world. Over 30 species are found wild in the Indian gardens. *Viola Patrinii* is a glabrous or pu-

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Table 1: Antibacterial activity of various solvent extracts of *Viola partinii*

Micro-organism	Zone of Inhibition(mm)						
	A	C	PE	E	W	AC	H
Escherichia coli	27	17	11	22	15	14	13
Staphylococcus aureus	23	15	NA	18	NA	NA	12
Staphylococcus pyogens	21	NA	NA	14	NA	NA	NA
Bacillus subtilis	25	NA	NA	15	NA	NA	NA
Klebsiella pneumoniae	22	NA	NA	11	NA	NA	10
Lactococcus	27	12	13	20	11	NA	11

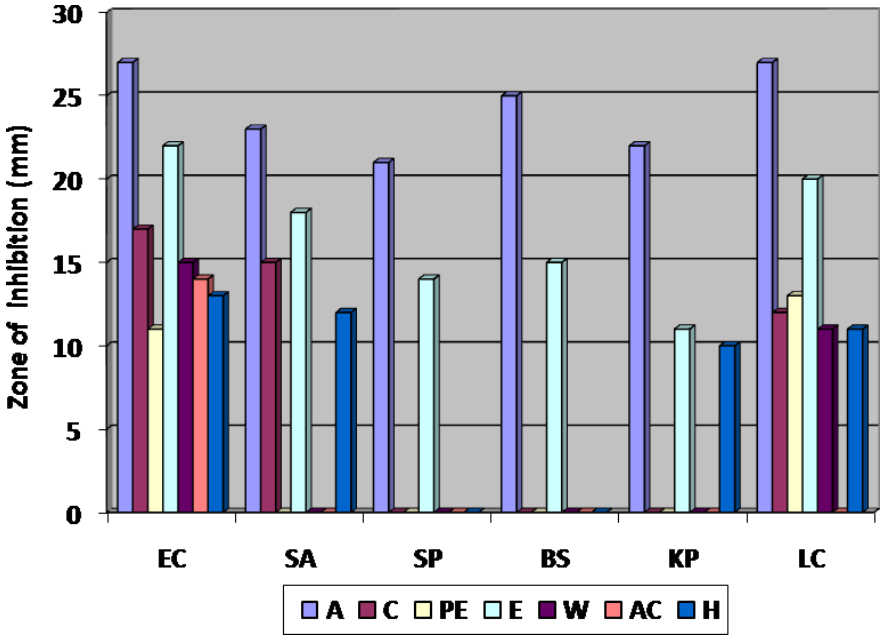

Figure 1: Zone of inhibition of various solvent extracts of *Viola patrinii*

bescent herb, with a woody rootstock, distributed in the temperate Himalayas from Kashmir to Bhutan, extending to the hills of Arunachal Pradesh, Meghalaya and Manipur in the East, southwards to the hills of the eastern and western ghats, at altitudes of 900-2400m. Leaves triangular-ovate, crenate or serrate, very variable; flowers lilac, often scented capsules straight 6-12 mm. long (wealth of India, 1976). *Viola Patrinii* is used in the for a variety of therapeutic applications including the purification of blood and the treatment of bruises and ulcers (Kirtikar & Basu, 1995) in the Chinese system of medicine it is recommended for use against cancer disorders. The dried flowers are used as a purgative and for cough and cold. It is also used in Unani recipes, such as Joshanda and Rogan Banafshah (CSIR, 1976). Viola spp. are also used for ornamental purposes. The roots of Viola spp. contain saponins, glucosides, methyl salicylate, and an emetic principle called violin (Chopra et al., 1958)

MATERIALS AND METHODS

Collection and Identification of Plant

The plants of *Viola partinii* were collected from the hilly areas of Kashmir (J&K) The plant species was identified by Dr. Sumer chand, Systematic Botany Division, Forest Research India (FRI), Dehradun, Uttarakhand, India.

Preparation of Solvent extracts

The method (Alade and Irobi, 1993) was adopted for preparation of plant extracts with little modifications. Briefly 20 g portions of the powdered plant material were soaked separately in 100 ml of each hexane (H), petroleum ether (PE), acetone (AC), choloroform (C), ethanolic (E) and water (W) for 72 h. Each mixture was stirred after every 24 h using a sterile glass rod. At the end of extraction, each extract was passed through Whatman filter paper no. 1 (Whatman, England). The filtrate obtained were concentrated in vacuum rotary evaporator at 30°C.

Test organisms used

The test organism's *Escherichia coli (EC), Staphylococcus aureus (SA), Staphylococcus pyogens (SP), Bacillus subtilis (BS), Klebsiella pneumoniae (KP) and lactococcus (LC)* were the bacterial strains obtained from Institute of Microbial Technology (IMTECH) Chandigarh, India. These were obtained from pure lab cultures of Dept. of Biotechnology, Graphic Era University, Dehradun, India.

Determination of antibacterial

Table 2: Antibacterial activity of various solvent extracts of *Viola partinii*

Micro-organism	Minimum Inhibitory Concentration (MIC) (mg/ml)						Minimum Lethal Concentration (MLC) mg/ml					
	C	PE	E	W	AC	H	C	PE	E	W	AC	H
Escherichia coli	0.8	NA	0.8	0.8	0.8	0.7	0.9	NA	0.9	0.9	0.9	0.9
Staphylococcus aureus	0.7	NA	0.6	NA	NA	0.6	0.9	NA	0.8	NA	NA	0.8
Staphylococcus pyogens	NA	NA	0.8	NA	NA	NA	NA	NA	0.9	NA	NA	NA
Bacillus subtilis	N A	NA	0.8	NA	NA	NA	NA	NA	0.9	NA	NA	NA
Klebseilla pneumonia	NA	NA	NA	NA	NA	0.8	NA	NA	NA	NA	NA	0.9
Lactococcus	NA	NA	0.8	0.7	NA	0.8	NA	NA	0.9	0.9	NA	0.9

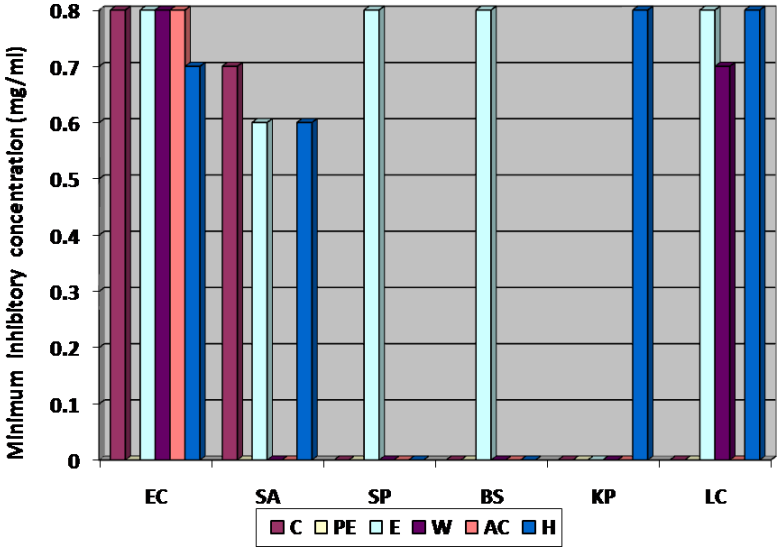

Figure 2: Minimum inhibitory concentration of various solvent extracts of *Viola partinii*

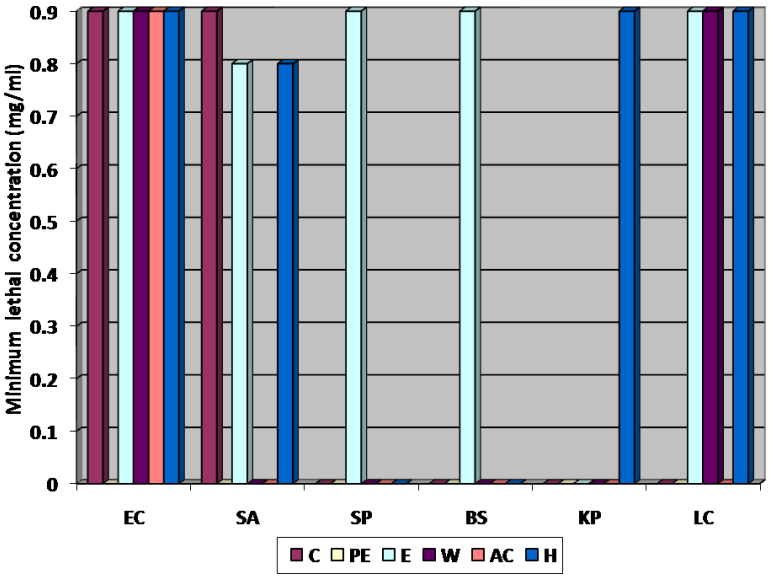

Figure 3: Minimum lethal concentration of various solvent extracts of *Viola partinii*

The agar well diffusion method (Perez and Anesini, 1993) was modified. Nutrient agar medium (NAM) was used for bacterial cultures. The culture medium was inoculated with the microorganism separately suspended in Nutrient broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts (1mg/ml) and solvent blanks (hexane (H), petroleum ether (PE), acetone (AC), choloroform (C), ethanolic (E) and water (W) as the case may be). Standard antibiotic (Amoxicillin (A), concentration 1mg/ml) was simultaneously used as positive control. The bacterial plates were then incubated at 37°C for 18 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition observed. The extracts that showed antimicrobial activity were subjected to minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) assay by two fold dilution method. The minimum dilution of the plant extract

that kills the bacterial growth was taken as MLC (Minimum lethal count) while as MIC was interpreted as the lowest concentration of the sample, which showed clear fluid without development of turbidity.

RESULTS AND DISCUSSION

The antibacterial activities of the ethanolic, Hexane and choloroform extracts of *Viola partinii* showing significant variations as shown in Table 1. Among the three extracts tested, ethanolic extract had greater antibacterial potential. The largest zones of inhibition were observed for ethanolic extract against *E. coli (22mm)* and *lactococcus* (20mm). Antimicrobial potency of the leaf extract of *Viola partinii* against the tested bacteria were expressed in MIC as presented in Table 2 respectively. The MIC values against these bacteria strains ranged from 0. 6 to 0. 8 mg/ml while as MLC values ranged from 0. 8 to 0. 9 mg/ml.

This may indicate that the *Viola partinii* extracts have broad inhibitory activities to pathogenic microorganisms and promising to act as potential antibacterial from natural plant sources. The experiments were performed in triplicates. The results are indicated in Table 1 and Table 2.

CONCLUSION

The extracts of the plant used showed prominent antibacterial activity against *Escherichia coli, lactococcus* and *Staphylococcus aureus* while as less activity against *Klebsiella pneumoniae, staphylococcus pyogens, Bacillus subtilis* which are severe pathogens. Thus the use of these plants in the treatment of pathogenic diseases associated with the infection of these pathogens is validated, scientifically supported by the results obtained in this work.

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