

Screening Of Extracts Of *Valeriana hardwickii* For Their Antibacterial Activity

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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 *Valeriana hardwickii* 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 and choloform extract of whole plant of *Valeriana hardwickii* exhibited significant antibacterial activity. These findings established the potential of the plant *Valeriana hardwickii* as an effective antibacterial agent. However, further studies are needed to evaluate active compounds and probable medicinal benefits in chemotherapy among humans.

Key words: *Valeriana hardwickii*, antimicrobial activity, MIC, MLC, pathogenic microorganisms, extracts.

Introduction:

For many centuries, it is a known fact that humankind depends on plants as an indirect source of energy, and shelter. It has been found that near about 80% of all established natural products originate from plants [1]. Scientific communities throughout the world are trying to explore new medicine from plants and its various parts [2-4]

These natural products have a significant use in the finding and production of new pharmaceuticals which are then clinically useful. They can be used as primary materials to produce some drugs of synthetic origin or they can be used to make products, which then assist in making fully synthetic drugs [5]. Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants. They also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes [6-7]. In the recent past, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects [8-9]. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as in tannin [10].

Valeriana hardwickii is a pubescent herb, up to 1.5m in height, found in the temperate Himalayas from Kashmir to Bhutan at altitudes of 1,200-3,600m, and in the Khasi and Jaintia hills between 1,500 and 1,800m. Rootstocks descending, fibrous; radical leaves few, long-petioled, ovate, dropping off before fruiting; cauline leaves pinnate or deeply pinnatifid with lanceolate leaflets or segments; flowers white, often unisexual, incymose clusters, forming axillary, compound corymbs or panicles; fruit ovate-oblong compressed, 2-3 mm long brown spreading calycinal hairs [11]. The root is bitter, carminative, diuretic, expectorant, nervine and stimulant [12-13]. It is used as a nerve tonic and in the treatment of conditions such as epilepsy and hysteria. It is also used in the treatment of rheumatism and low blood pressure [13].

Materials And Methods:

Collection and Identification of Plant:

The plants of *Valeriana hardwickii* 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 [14] 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), chloroform(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 vacuo using rotary evaporator at 30°C.

Test organisms used:

The test organism's *Escherichia coli*(EC), *Staphylococcus aureus*(SA), *Staphylococcus pyogenes*(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:

The agar well diffusion method [15] 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), chloroform(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, chloroform and acetone extracts of *Valeriana hardwickii* showing significant variations as shown in Table 1. Among the all extracts tested, ethanolic and chloroform extract had greater antibacterial potential. The largest zones of inhibition were observed for ethanolic extract against *E.coli* (20mm) and chloroform extract also against *E.coli* (19 mm).

Antimicrobial potency of the leaf extract of *Valeriana hardwickii* 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 0.7 to 0.9 mg/ml.

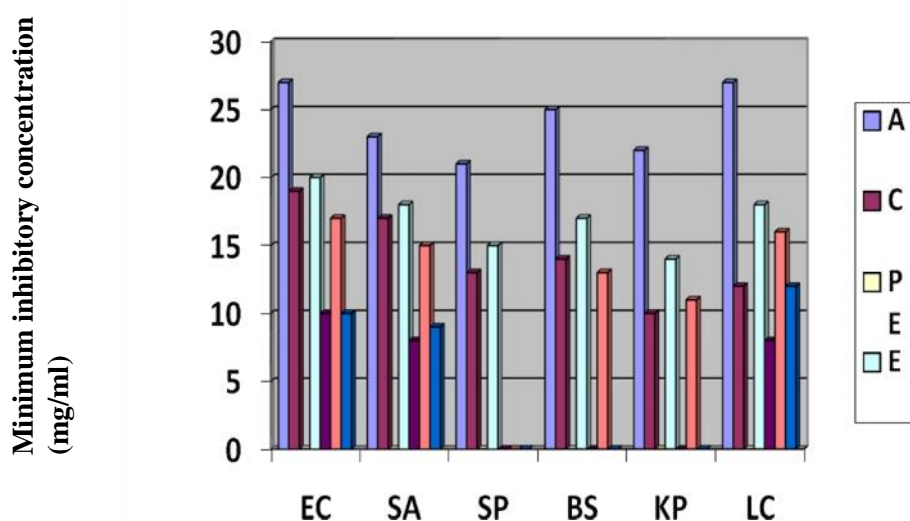
This may indicate that the *Valeriana hardwickii* extracts have broad inhibitory activities to pathogenic microorganisms and promising to act as potential antibacterial from natural plant sources. The experiments were performed in triplicates. The results are indicated in Table 1 and Table 2.

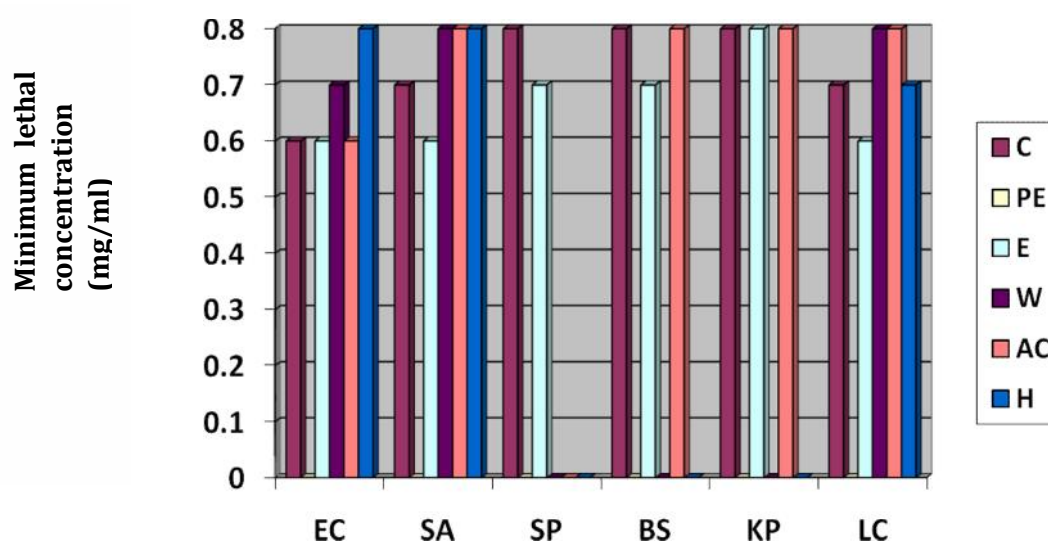
Table 1: Antibacterial activity of various solvent extracts of *Valeriana hardwickii*.

Micro-organism	Zone of Inhibition(mm)						
	A	C	PE	E	W	AC	H
<i>Escherichia coli</i>	27	19	NA	20	10	17	10
<i>Staphylococcus aureus</i>	23	17	NA	18	08	15	09
<i>Staphylococcus pyogens</i>	21	13	NA	15	NA	NA	NA
<i>Bacillus subtilis</i>	25	14	NA	17	NA	13	NA
<i>Klebsiella pneumoniae</i>	22	10	NA	14	NA	11	NA
<i>Lactococcus</i>	27	12	NA	18	08	16	12

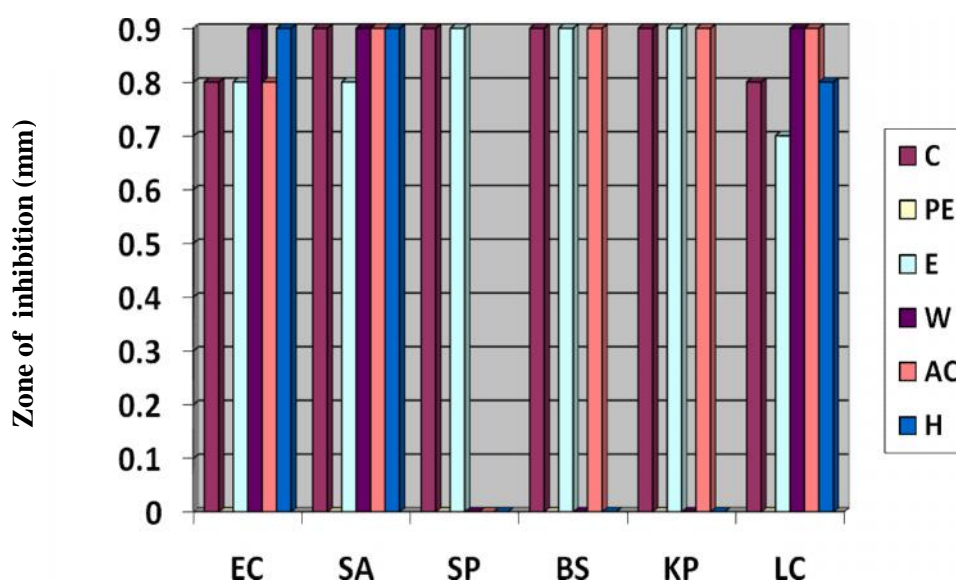
Table 2: Antibacterial activity of various solvent extracts of *Valeriana hardwickii*

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
<i>Escherichia coli</i>	0.6	NA	0.6	0.7	0.6	0.8	0.8	NA	0.8	0.9	0.8	0.9
<i>Staphylococcus aureus</i>	0.7	NA	0.6	0.8	0.8	0.8	0.9	NA	0.8	0.9	0.9	0.9
<i>Staphylococcus pyogens</i>	0.8	NA	0.7	NA	NA	NA	0.9	NA	0.9	NA	NA	NA
<i>Bacillus subtilis</i>	0.8	NA	0.7	NA	0.8	NA	0.9	NA	0.9	NA	0.9	NA
<i>Klebsiella pneumonia</i>	0.8	NA	0.8	NA	0.8	NA	0.9	NA	0.9	NA	0.9	NA
<i>Lactococcus</i>	0.7	NA	0.6	0.8	0.8	0.7	0.8	NA	0.7	0.9	0.9	0.8

**Graph 1: Zone of inhibition of various solvent extracts of *Valeriana hardwickii***



Graph 2: Minimum inhibitory concentration of various solvent extracts of *Valeriana hardwickii*



Graph 3: Minimum lethal concentration of various solvent extracts of *Valeriana hardwickii*

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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