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Determination of Invitro Antibacterial and Antifungal Potency Of Essential Oil Obtained By Hydrodistillation of Valeriana hardwickii

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Abstract

Multi-drug resistance in disease causing micro-organisms is the medical emergency going throughout the world. The medicinal plants contains secondary metabolites that possesses antimicrobial potency. Essential oil obtained from medicinal plants could be used as the substitute of synthetic drugs. In this regard, the aim of this study was to find the antimicrobial properties of Essential oil obtained from Valeriana hardwickii. The essential oil of Valeriana hardwickii showed lowest inhibition zone about 7.82 mm against K. rhizophila at the concentration of 500 µg/ml and showed highest zone of inhibition about 12.34 mm against E.coli at the concentration of 1000 µg/ml for bacterial strains. The essential oil of Valeriana hardwickii showed lowest inhibition zone about 7.12 mm against C.albicans at the concentration of 125 µg/ml and showed highest zone of inhibition about 11.67 mm against C.albicans at the concentration of 1000 µg/ml for fungal strains. The essential oil of Valeriana hardwickii almost showed same antimicrobial activity against bacterial strains in comparison to the fungal strains. The order of antibacterial activity for these strains were E.coli>P.aeruginosa>S.aureus>S.typhi>K.rhizophila>M.luteus>S.epidermis. This work lead to the findings that described the antimicrobial potential of Valeriana hardwickii essential oil and usefulness of essential oil against pathogenic bacteria and fungi. However, further studies are needed to evaluate active compounds and probable medicinal benefits in chemotherapy among humans

Keywords: Valeriana hardwickii, Essential oil, antibiotic resistance, antimicrobials, Zone of inhibition

1. Introduction

Valeriana hardwickii is a plant belongs to the family, *Valerianaceae*. It is found in the temperate regions of Himalayas, from Bhutan to Kashmir, Khasi and Jaintia hills at high altitudes of 1200-3600 m, 15,00 and 1,800 m respectively. Roots are bitter, acts as carminative, diuretic, expectorant, nervine and stimulant, also used as a nerve tonic. Epilepsy, hysteria, rheumatism and low blood pressure are also treated by its roots [1, 2]. *Valeriana hardwickii* showed potent antimicrobial activities and can can promising candidate to act as antibacterial from natural wealth [3]. The different plant extracts showed potent antioxidant activities as per total phenol content (TPC), 2,2-diphynyl-1-picrylhydrazyl (DPPH) and superoxide anion method [4]. In one study it has been reported that the antimicrobial and antioxidant properties of plant essential oils is contributed by these monoterpenes and sequiterpenes [5, 6]. The main focus of this study is to explore pharmacological components of essential oil obtained from whole plant of *Valeriana hardwickii* so that it can be exposed and evaluated against oxidants, pathogens like bacteria and fungi.

2. Materials and Methods

Collection and Preparation of Plant Materials

Fresh plants of *Valeriana hardwickii* are collected from Chakrata forest, which is located at high altitude of Chakrata, Uttarakhand, India. The plant samples were cleaned from soil and debris first by running tap water and than by sterilized water. After cleaning the plant sample was air dried and than converted to fine powdered form. The powedered sample was kept in air tight amber coloured bottles for further use.

Extraction Of Essential Oil

The technique of hydrodistillation was used almost for four hours to extract oil from whole plant of *Valeriana hardwickii* in a Clevenger type apparatus. After hydrodisatillation moisture was removed from oil sample by the help of anhydrous sodium sulphate.

Percentage Yield

Detemination of amount of extracted oil was done and percentage yield of extracted oil was calculated that was obtained from samples of *Valeriana hardwickii*. The percentage yield of oil is calculated by:-

$$\text{Percentage Yield of oil} = \frac{\text{Weight of Oil}}{\text{Weight of Aerial Plant parts}} \times 100$$

Test Microorganisms used

Gram positive bacterial species: *Staphylococcus aureus (MTCC-3160), Kocuria rhizophila (1541), Staphylococcus epidermis (MTCC 3615), Micrococcus luteus (MTCC 1541),* **Gram negative bacterial species:** *E.coli (MTCC-614, Pseudomonas aeruginosa* (MTCC-424)*, Salmonella typhi (NCTC 786) and* **Fungal species :***Candida albicans (MTCC-227), Aspergillus niger (MTCC 1344),* were used for the screening of antimicrobial properties of essential oil of *Valeriana hardwickii*.

Determination of diameter of zone of inhibition by well diffusion method

The essential oils obtained from plants are also known for their therapeutic or pharmacological properties [7]. Invitro antibacterial and antifungal screening was done using agar disc diffusion method [8]. The inoculum of gram positive bacteria like *Kocuria rhizophila* (MTCC 1541), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615) , *Micrococcus luteus* (MTCC 1541) and gram negative bacteria like *Salmonella typhi* (NCTC 786), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia Coli* (MTCC 614)were assayed after the incubation of 24 hours at 37 ^{0}C on nutrient agar broth while as the inoculums of fungi *Aspergillusniger*(MTCC 1344), *Candida albicans* (MTCC 227) were assayed after incubation of 5 days at 25 ^{0}C on Saboured dextrose broth. The nutrient broth or media was autoclaved and 500 µl of inoculums was mixed. The inoculum was screened to facilitate 10^5 CFU/ml of both bacteria and fungi [9]. The essential oil was further serially diluted in 5% of DMSO (dimethylsulfoxide) to the different concentrations like 1, 0.50, 0.250 and 0.125 mg/ml. These different concentrations of essential oil were applied on filter paper discs of 6 mm diameter and then soaked on particular inoculated medium present in petridish plates. Ciprofloxacin and fluconazole are the standard drugs used as a positive control for bacteria and fungi, respectively. Sample producing inhibition zone were screened also for MIC in millimetres to check antimicrobial or antifungal activities of essential oil by the help of dilution broth method [8]. Thus, dilution broth method was used to calculate the MIC of varying samples of essential oils. The sample of oil was diluted with 5% DMSO at the levels of 10^{-2} to 10^{-5}. Both negative and positive controls were obtained in similar medium. The results of MIC were obtained due to the level of turbidity produced by microbial and fungal growth.

3. Results and Discussion

Antibacterial and antifungal activities of Valeriana hardwickii essential oil

The essential oil of *Valeriana hardwickii* showed lowest inhibition zone about 7.82 mm against *K. rhizophila* at the concentration of 500 mg/ml and showed highest zone of inhibition about 12.34 mm against *E.coli* at the concentration of 1000 mg/ml for bacterial strains. The essential oil of *Valeriana hardwickii* showed lowest inhibition zone about 7.12 mm against *C.albicans* at the concentration of 125 mg/ml and showed highest zone of inhibition about 10.13 mm against *A.niger* at the concentration of 1000 mg/ml for fungal strains. The essential oil *Valeriana hardwickii* almost same antimicrobial activity against bacterial strains in comparison to the fungal strains. The order of antimibacterial activity for these strains were *E.coli>P.aeruginosa> S.aureus>K.rhizophila.* When inhibition zones were observed, it was analyzed that Plant essential oil showed

antimicrobial activity against Gram positive, Gram negative and Fungal strains. 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 essential oil *Valeriana hardwickii* almost same antimicrobial activity against bacterial strains in comparison to the fungal strains. Thus the results clearly proves the essential oil of *Valeriana hardwickii* can be efficient in the field of pharmaceutical industry as it will help in the treatment of diseases, caused by bacteria, fungus or oxidation, due to its antimicrobial chemometric profile.

The experiments were performed in triplicates. The results of antibacterial activities of essential oil are summarized in **Table 1** and **Figure 1**, while as the results of antifungal activities are depicted by **Table 1** and **Figure 2**.

4. Conflict of Interest

The authors hereby declare that there is no conflict of interest as per the publication of this paper.

5. Acknowledgments

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Figure and table legends:

Figure 1: Antibacterial activity of *Valeriana hardwickii* Essential Oil

**SA,Staphylococcus aureus., KR, Kocuria rhizophila., SE, Staphylococcus epidermis., ML, Micrococcus luteus., EC, E.coli, PA, Pseudomonas aeruginosa*., ST, *Salmonella typhi.*

*X-axis, Concentration of essential oil, Y-axis, Zone of inhibition

Figure 2: Antifungal activity of *Valeriana hardwickii* Essential Oil

*CA, *Candida albicans.*, AN, *Aspergillus niger*, *X-axis, Concentration of essential oil, Y-axis, Zone of inhibition

Table 1: Antibacterial And Antifungal activity of *Valeriana hardwickii* Essential Oil

*****Postive control for antibacterial activity is Ciprofloxacin, Fluconazole is positive control for antifungal activity.

Figure 1:

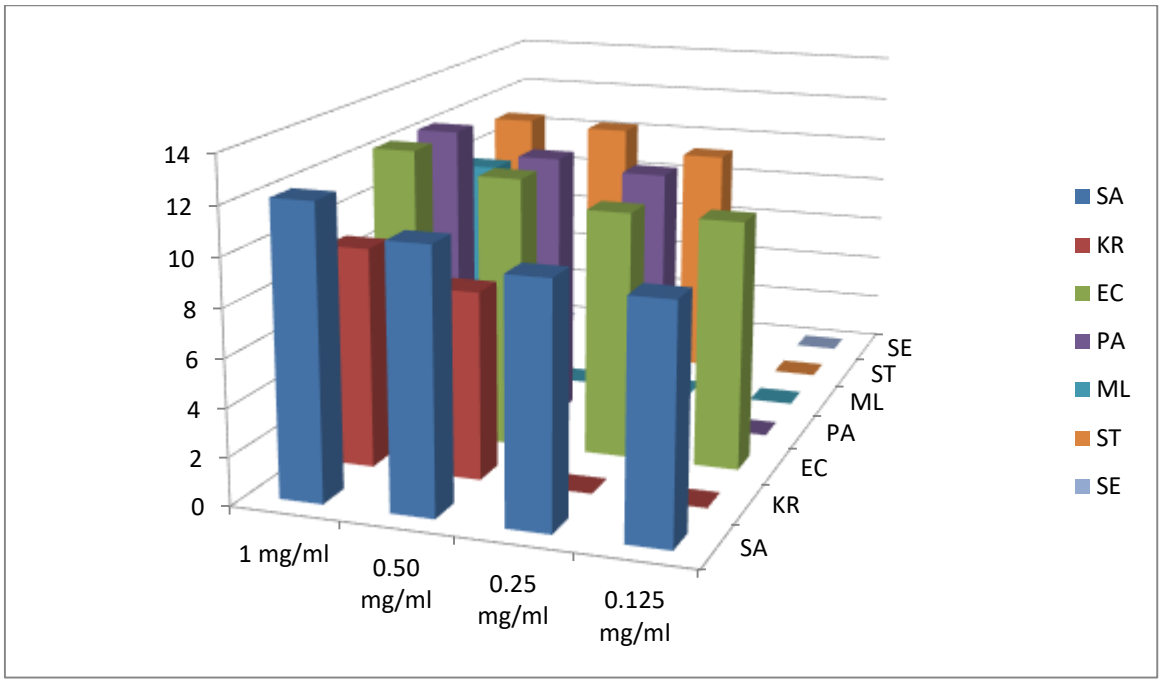

Figure: 2

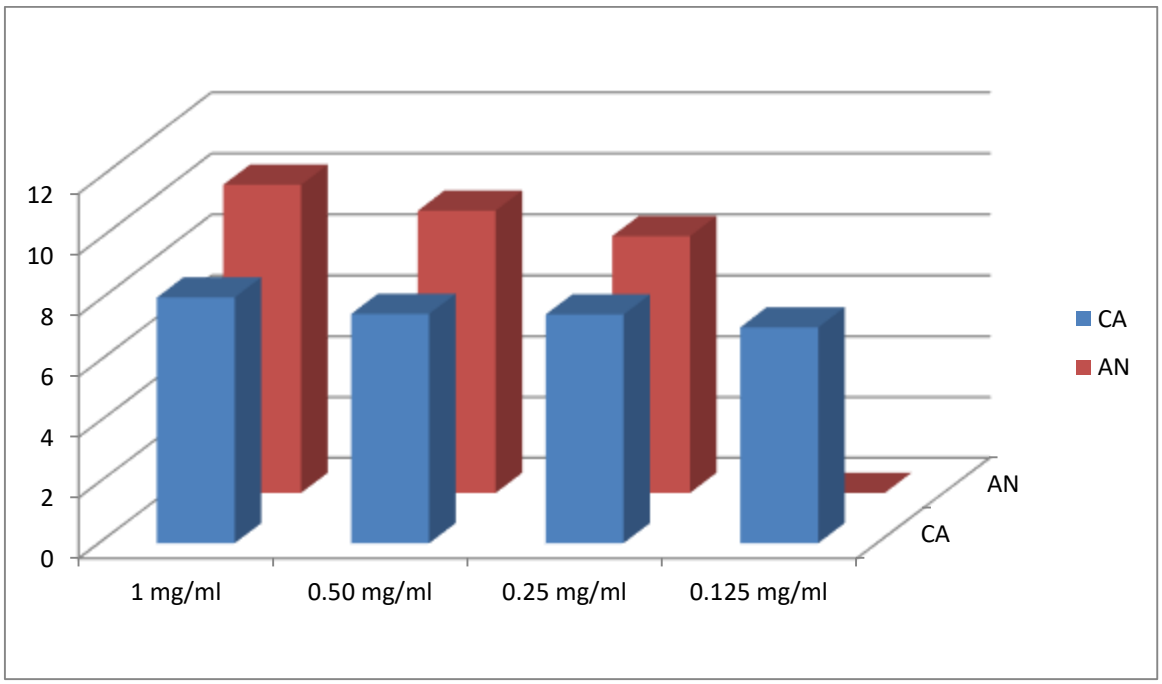

Table 1*:* Antibacterial And Antifungal activity of *Valeriana hardwickii* Essential Oil

S. No.	Gram Positive Bacteria	Concentration of Standard and sample (mg/ml)	Zone of Inhibition (mm)	Disc size (mm)
1.	*Staphylococcus aureus* **(MTCC 3160)**	10 (Ciprofloxacin)	37.86	6
		1000 (Sample)	12.12	6
		500 (Sample)	10.83	6
		250 (Sample)	9.95	6
		125(Sample)	9.57	6
2.	*Kocuria rhizophila* **(MTCC 1541)**	10 (Ciprofloxacin)	27.24	6
		1000 (Sample)	9.20	6
		500 (Sample)	7.82	6
		250 (Sample)	0	6
		125(Sample)	0	6
3.	*Staphlococus epidermis* **(MTCC 3615)**	10 (Ciprofloxacin)	38.07	6
		1000 (Sample)	0	6
		500 (Sample)	0	6
		250 (Sample)	0	6
		125(Sample)	0	6
4.	*Micrococcus luteus* **(MTCC 1541)**	10 (Ciprofloxacin)	35.55	6
		1000 (Sample)	9.87	6
		500 (Sample)	0	6
		250 (Sample)	0	6
		125(Sample)	0	6
Gram Negative Bacteria				
1.	*Escherchia Coli* **(MTCC 614)**	10 (Ciprofloxacin)	27.63	6
		1000 (Sample)	12.34	6
		500 (Sample)	11.47	6
		250 (Sample)	10.40	6
		125(Sample)	10.39	6
2.	*Pseudomonas aeruginosa* **(MTCC 424)**	10 (Ciprofloxacin)	42.25	6
		1000 (Sample)	12.33	6

		500 (Sample)	11.44	6
		250 (Sample)	11.05	6
		125(Sample)	0	6
3.	*Salmonella typhi* **(NCTC 786)**	10 (Ciprofloxacin)	41.75	6
		1000 (Sample)	11.28	6
		500 (Sample)	11.09	6
		250 (Sample)	10.12	6
		125(Sample)	0	6
Fungal Strains				
1.	*Candida albicans* **(MTCC 227)**	10 (Fluconazole)	28.03	6
		1000 (Sample)	8.10	6
		500 (Sample)	7.56	6
		250 (Sample)	7.54	6
		125(Sample)	7.12	6
2.	*Aspergilus niger* **(MTCC 1344)**	10 (Fluconazole)	17.56	6
		1000 (Sample)	10.13	6
		500 (Sample)	9.28	6
		250 (Sample)	8.45	6
		125(Sample)	0	6