ISSN No:-2456-2165

Pharmacological Activities of Essential Oil Extracted from *Viola patrinii* against Some Gram Positive and Gram Negative Bacteria as Well Fungal Strains

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Abstract:- The rapid alarming drug resistance against pathogenic microorganisms is a medical catastrophe for the world. The possible deferring force is to concise the use of antimicrobials synthesised at industrial level and investigate for natural medicinal plant derived compounds having properties of fighting microorganisms. In this regard, the aim of this study was to find the antimicrobial properties of Essential oil obtained from *Viola patrinii*. The essential oil of *Viola patrinii* showed lowest inhibition zone about 6.62 mm against *S.aureus* at the concentration of 250 µg/ml and showed highest zone of inhibition about 16.37 mm against *E.coli* at the concentration of 1000 µg/ml for bacterial strains. The essential oil of *Viola patrinii* showed lowest inhibition zone about 8.02 mm against *A.niger* at the concentration of 250 µg/ml and showed highest zone of inhibition about 15.23 mm against *C.albicans* at the concentration of 1000 µg/ml for fungal strains. The essential oil *Viola patriii* showed high antimicrobial activity for both fungal strains and bacterial strains. The order of antimibacterial activity for these strains were *E.coli*>*M.luteus*>*P.aeruginosa*> *S.aureus*>*S.typhi*>*K.rhizophila*>*S.epidermis.* This investigation lead to the findings that described the antimicrobial potential of this plant essential oil and usefulness of oil against antibiotic resistance. However, further studies are needed to evaluate active compounds and probable medicinal benefits in chemotherapy among humans.

Keywords:- *Viola Patrinii, Violaceae, Essential Oil, Antibiotic Resistance, Antimicrobials, Inhibition Zone.*

I. INTRODUCTION

The mixture of terpenoids makes essential oil that is obtained from aromatic and medicinal plants. The phytotype composition present in the essential oil is specific for a specific plant species [1]. Plants and plant derived compounds have played essential roles for the synthesis of new pharmaceutical products and many known drugs. From that time higher plants have become main source for the discovery of many drugs and among the discovered drugs a handful of 100 drugs with well known structure are commonly used [2]. The great number of chemicals have been derived from plants as medicine by scientific evaluation, and these plants are used traditionally for the treatment of bacterial infection and hence exploiting the power of plant derived compounds for the discovery of anti-microbials with a great therapeutic potential [3].

Medicinal plants are to be screened from recent years to develop noble compounds for treating the Multi-drug resistant (MDR) bacterial diseases. Some natural and synthetic compounds are also used for the purpose which includes screening for bioactive compounds or plant derived extracts against MDR efflux pump inhibition, α-lactamase inhibitors, synergistic approaches such as antibiotic–phytocompound interaction, and targeting the virulence and pathogenicity of bacteria and use of quorum-sensing inhibitors [4].

ISSN No:-2456-2165

The herb namely as *V. patrinii* (China violet) is having short and thin stem, as the thickening of its stem is inhibited by a secondary woody growth. This plant contains leaves which are glabrous and triangular in shape and are narrow, elongated, and superficially chordate. The flowers of this plant are having lilac colour. This plant is found in the temperate regions of Himalaya, they are also found at high altitudes (900-2400 m) of Manipur, Meghalaya and Arunachal Pradesh. The plant is expanded from the east south wards to the hills of the Eastern and Western Ghats [5]. *Viola patrinii* is a plant belongs to the family *Violaceae*, it is a medicinal herb also known as China violet, it is a therapeutic agent of traditional medicine used for the treatment of bruises, ulcers and purification of blood. In the Chinese system, it is also recommended for treating cancer and disorders related to it [6]. For cough and cold, its dried flowers are used and these dried flowers are also used as purgative. Different recipes are also made from it like Joshanda and Rogan Banafshah [5].

All these findings observed that *Viola patrinii* is having potent antibacterial activity. *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *lactococcus* were tested against extracts from *Viola patrinii* by well diffusion method, each extract were determined by their Minimum inhibitory concentration (MIC) and Minimum lethal concentration(MLC) values. Ethanolic extract is having significant antimicrobial activity, when extracted from whole plant i.e from *Viola patrinii* [7]. Gelatinase (A and B) and collagenase are inhibited by this plant [8]. Oxidative stress and inflammation in the brain is the reason for the diseases caused in central nervous system. In murine hippocampal HT22 cells and BV2 microglia, ethanolic extracts of *Viola patrinii* showed anti-inflamatory and antioxidative activities [9]. In vitro grown callus of *Viola patrinii* showed more antioxidant activity in comparison to wild plant, when this activity was determined by DPPH (α, α-diphenyl-β-picrylhydrazyl) method [10]. This plant can be used for treating complications like skin disorders, upper respiratory tract infections and abdominal pain. Different activities like anti-inflammatory, purgative properties and diuretic properties were shown by this plant. **[**11-13]**.** Therefore, the objective of our study is to find the antimicrobial activities of essential oil from whole plant of *Viola patrinii* using Agar well diffusion method.

II. MATERIALS AND METHODS

Collection and preparation of plant materials

Fresh plants of *Viola patrinii* are collected from Rishiganga Valley, District Chamoli located at high altitude, Uttarakhand, India. The plants were authenticated at Forest Research India (FRI), India.

Extraction of essential oil

Clevenger type apparatus was used to extract essential oil from *Viola patrinii*. The extraction of oil is based on hydro-distillation that was carried for four hours. After this procedure, anhydrous sodium sulphate was used to remove moisture from extracted plant oil. The amount of extracted oil was determined by following formula:-

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

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 for the screening of antimicrobial properties of essential oil of Viola patrinii.

Determination of diameter of zone of inhibition by well diffusion method.

By using clinical isolates disc /well diffusion method [Marmonier] and MIC by microdilution were the two methods used for the determination of antimicrobial activity of plant essential oils. Nutrient agar/broth (pH 7.2) and Saboured dextrose agar/broth were used for bacterial culture and fungal culture respectively. The media was autoclaved and 500µl of inoculum was mixed with it. The bacteria inoculated in the media was checked to provide 10^5 CFU/ml. The media having inoculum was then poured in sterilized petridishes, paper discs containing plant essential oil (size 6mm) were placed on the smooth and dry surface of the poured media in the petriplates. 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. Wells were filled with sample dilution (0.1µl) when well diffusion method was performed. These petriplates were left in Laminar air flow for half an hour for the diffusion of plant essential oil in the media. Then these petriplates were transferred to B.O.D incubator and were incubated at 37±1ºC for 24 hrs for bacterial growth and at 22-25ºC for 5 days for fungal growth. After proper incubation period zones of inhibition were measured with the help of zone reader, these samples were run along with positive and negative controls. Ciprofloxacin and Fluconazole (1mg/ml) were used as antibacterial and antifungal positive controls respectively. These inhibition zones of plant essential oil showed bactericidal and fungicidal activity. The terms bactericidal and fungicidal means that the plant essential oils kills the bacteria and fungi respectively by showing the clear zone around the discs. The negative controls were used and showed no inhibitory zones around the discs used for bacterial and fungal strains.

III. RESULTS AND DISCUSSION

When inhibition zones were observed, it was analyzed that Plant essential oil showed antimicrobial activity against Gram positive, Gram negative and Fungal strains. The essential oil of *Viola patrinii* showed lowest inhibition zone about 6.62 mm against *S.aureus* at the concentration of 0.250 mg/ml and showed highest zone of inhibition about 16.37 mm against *E.coli* at the concentration of 1 mg/ml for bacterial strains. The essential oil of *Viola patrinii* showed lowest inhibition zone about 8.02 mm against *A.niger* at the concentration of 0.250 mg/ml and showed highest zone of inhibition about 15.23 mm against *C.albicans* at the concentration of 1 mg/ml for fungal strains. The essential oil *Viola patriii* showed high antimicrobial activity for both fungal strains and bacterial strains. The order of antimibacterial activity for these strains were *E.coli>M.luteus>P.aeruginosa> S.aureus>S.typhi>K.rhizophila>S.epidermis.* The essential oil *Viola patriii* showed high antimicrobial activity against fungal strains in comparison to the bacterial strains. This may indicate that the *Viola partinii* essential oil has broad inhibitory activities to pathogenic microorganisms and promising to act as potential antibacterial as well as antifungal from natural plant sources. 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.

Conflict of Interest

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

Acknowledgments

The authors are very thankful to the Department of Biological Sciences, Maharishi University of Information technology, Lucknow (UP), India and JNU, Delhi, India for their support and coordination to carry this research work.

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ISSN No:-2456-2165

FIGURE AND TABLE LEGENDS:

Figure 1: Antibacterial activity of *Viola patrinii* 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 *Viola patrinii* 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 *Viola patrinii* Essential Oil
*****Postive control for antibacterial activity is Ciprofloxacin, Fluconazole is positive control for antifungal activity.

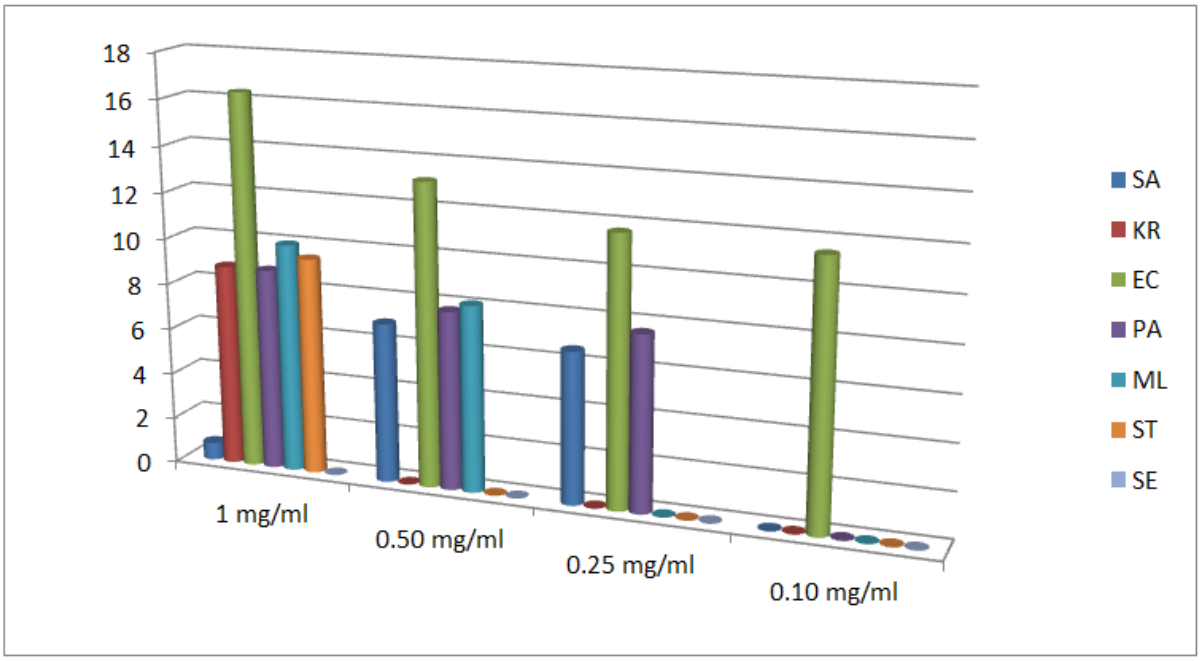

Figure 1:

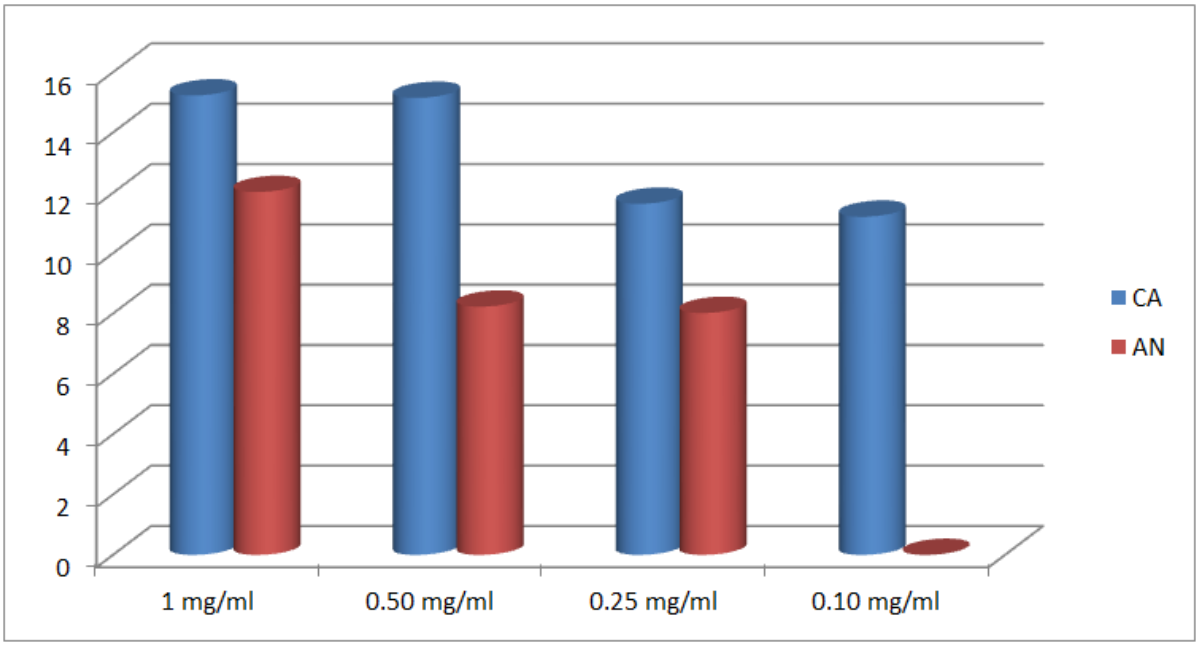

Figure: 2

ISSN No:-2456-2165

Table 1*:*

S. No.	Gram Positive Bacteria	Concentration (µg/ml)	Zone of Inhibition (mm)	Disc size (mm)
1.	*Staphylococcus aureus* **(MTCC 3160)**	10 (Ciprofloxacin)	26.25	6
		Negative Control	0	6
		1000 (Sample)	7.52	6
		500 (Sample)	6.96	6
		250 (Sample)	6.62	6
		125(Sample)	0	6
2.	*Kocuria rhizophila* **(MTCC 1541)**	10 (Ciprofloxacin)	28.43	6
		Negative Control	0	6
		1000 (Sample)	8.80	6
		500 (Sample)	0	6
		250 (Sample)	0	6
		125(Sample)	0	6
3.	*Staphlococus epidermis* **(MTCC 3615)**	10 (Ciprofloxacin)	38.76	6
		Negative Control	0	6
		1000 (Sample)	0	6
		500 (Sample)	0	6
		250 (Sample)	0	6
		125(Sample)	0	6
4.	*Micrococcus luteus* **(MTCC 1541)**	10 (Ciprofloxacin)	35.34	6
		Negative Control	0	6
		1000 (Sample)	9.98	6
		500 (Sample)	8.12	6
		250 (Sample)	0	6
		125(Sample)	0	6
Gram Negative Bacteria				
1.	*Escherchia Coli* **(MTCC 614)**	10 (Ciprofloxacin)	30.32	6
		Negative Control	0	6
		1000 (Sample)	16.37	6
		500 (Sample)	13.17	6
		250 (Sample)	11.7	6
		125(Sample)	11.5	6
2.	*Pseudomonas aeruginosa* **(MTCC 424)**	10 (Ciprofloxacin)	43.36	6
		Negative Control	0	6
		1000 (Sample)	8.79	6
		500 (Sample)	7.77	6
		250 (Sample)	7.64	6
		125(Sample)	0	6
3.	*Salmonella typhi* **(NCTC 786)**	10 (Ciprofloxacin)	43.02	6
		Negative Control	0	6
		1000 (Sample)	9.45	6
		500 (Sample)	0	6
		250 (Sample)	0	6
		125(Sample)	0	6
Fungal Strains				
1.	*Candida albicans* **(MTCC 227)**	10 (Fluconazole)	25.52	6
		Negative Control	0	6
		1000 (Sample)	15.23	6
		500 (Sample)	15.15	6

ISSN No:-2456-2165

		250 (Sample)	11.64	6
		125(Sample)	11.21	6
2.	*Aspergilus niger* **(MTCC 1344)**	10 (Fluconazole)	17.72	6
		Negative Control	0	6
		1000 (Sample)	12.03	6
		500 (Sample)	8.23	6
		250 (Sample)	8.02	6
		125(Sample)	0	6