

ANTIMICROBIAL ACTIVITY OF SWERTIA CILIATA, ACORUS CALAMUS AND VIOLA SERPENS

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ABSTRACT

Natural products have been studied aiming to understand their biological properties. Thus, this study aimed to investigate the antimicrobial activity of three essential oils (EOs) used in aromatherapy procedures, a natural therapy with great emphasis currently used against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Salmonella enterica* strains. Three medicinally important plants *Swertia ciliata*, *Acorus calamus* and *Viola serpens* were evaluated for their antimicrobial activities. The pure oil of *Swertia ciliata* exhibit maximum activity against *Bacillus subtilis* with strong inhibition zone of 21.0 ± 0.26 mm diameter and MIC 200

$\mu\text{L}/\text{mL}$. The pure oil of *Acorus calamus* exhibit maximum activity against *Klebsiella pneumonia* with strong inhibition zone of 21.4 ± 0.12 mm diameter and MIC 200 $\mu\text{L}/\text{mL}$, and The pure oil of *Viola serpens* exhibit maximum activity against *Bacillus subtilis* with strong inhibition zone of 20.0 ± 0.16 mm diameter and MIC 200 $\mu\text{L}/\text{mL}$.

KEYWORDS: Antimicrobial, aromatherapy, EOs.

INTRODUCTION

Herbs and their essential oils have been used since the beginning of human history for flavored foods and beverages, they have been empirically used to disguise unpleasant odors, attract other individuals and control health problems, contributing to the welfare humans and animals, thus demonstrating the cultural and economic importance use of these products (Franz, 2010).

The essential oils are typically liquid, clear and unusually colored, complex and the present compounds are volatile, characterized by a strong odor and synthesized by aromatic plants during secondary metabolites, which act to protect the plant against microorganisms and insects. Their synthesis takes place various parts of plants such as buds, flowers, leaves, stems, branches, seeds, berries, roots, wood or bark, and are stored in secretory cells, cavities, channels, epidermal cells or trichomes (**Bakkali et al., 2008**).

Temporal and spatial variations in the total content of secondary metabolites products from plants occur at different levels and despite the existence of a genetic control, the expression may undergo changes resulting from biochemical, physiological, ecological and evolutionary interactions that represent an important interface between chemistry and the environment surrounding the plants.

Essential oils have several biological properties, such as larvicidal action (**Rajkumar and Jebanesan, 2010**) antioxidant (**Wannes et al., 2010**) analgesic and anti-inflammatory (**Mendes et al., 2010**) fungicide (**Carmo et al., 2010**) and antitumor activity (**Silva et al., 2008**). The *in vitro* antimicrobial activity of essential oils has been researched extensively against a variety of microorganisms (**Lopez et al., 2005**).

Nevertheless, the emergence of multidrug resistant bacteria poses a challenge to treating infections, so the need to find new substances with antimicrobial properties for use in the fight against these microorganisms is evident (**Hemaiswarya et al., 2008**). Historically, most antibiotics come from a small set of functional molecular structures whose lives were extended by generations of synthetic reorganizations and arrangements (**Fischbach and Walsh 2009**). Moreover, the food, pharmaceutical and cosmetic industries have shown great interest in the antimicrobial properties of essential oils, as the use of natural additives has received importance as a trend in the replacement of synthetic preservatives (**Okoh et al., 2010**).

The essential oils extracted from plants are indispensable materials in the pharmaceutical, food, cosmetics sectors because of the increasing concern with harmful synthetic additives (**Sacchetti et al, 2005**). A great majority of the essential oils are used as fragrance in perfumes and aromas in food industry.

The essential oils have a number of biological activities, including antibacterial, antifungal and antioxidant properties (**Fatouma et al., 2011 and Jihua et al., 2011**). With the growing interest in the use of essential oils in both food and pharmaceutical industries, a systematic examination of the plant extracts has become increasingly important (**Biljana et al., 2011 and Termentzi et al., 2001**).

The role of natural products as medicine in treatment of ailments has always been very important all over the world. Sources of natural products are both terrestrial and aquatic that includes plants, microorganisms, vertebrates and invertebrates (**Newman et al., 2000**). Plants produce a variety of toxic compounds that can act as drugs against pathogenic microorganisms.

There is a long history related to the plants used in treatment of human diseases. For example licorice (*Glycyrrhiza glabra*), myrrh (*Commiphora* species) and poppy capsule latex (*Papaver somniferum*), have written historic record to be used in 2600 B.C. and these plants are still used in treatments either as a part of drug or as herbal preparations in traditional medicine (**Newman et al., 2000**). Traditional use of plants as therapeutic tool especially those with ethno pharmacological uses serve as basis for their use in modern medicines. According to a recent analysis 80% of 122 plant-derived drugs are related to their original traditional uses (**Fabricant & Farnsworth, 2001**).

About 50% of all deaths occur due to infectious diseases in tropical areas of world (**Iwu et al., 1999**). As far as causes of deaths are concerned, recent surveys proves it to be the second major cause of death worldwide and third major reason in developed countries (**Nathan, 2004**). Although antibiotics are major means of treating such infectious diseases but they are not effective in one major limitation of efficacy of antibiotics is development of resistance in microbes and this resistance is spreading all over the world (**Livemore, 2003 and Walsh & Amyes, 2004**). As a result cases of therapy failure much use of antibiotics (**Solomon et al., 2003 and Alder, 2005**). Although more than 99% known organic compounds are synthetic but still natural products are the basis of more than a third of all drugs sales (**Newmann et al., 2003**).

Keeping in view importance of natural sources especially plants, this study was conducted to evaluate antibacterial potential of three important medicinal plants *Swertia ciliata*, *Acorus*

calamus and *Viola serpens*. For antibacterial analysis, agar well diffusion method was adopted while antifungal assay was performed through tube dilution method.

Microorganisms Used for the Test

1. *Bacillus subtilis* (MTCC 441)
2. *Staphylococcus aureus* (MTCC3103)
3. *Klebsiella pneumonia* (MTCC3384)
4. *Salmonella enterica* (MTCC3224)

Bacillus subtilis

Bacillus subtilis is a ubiquitous naturally occurring saprophytic bacterium that is commonly recovered from soil, water, air and decomposing plant material. Under most conditions however it is not biologically active and is present in the spore form. Different strains of *B. subtilis* can be used as biological control agents under different situations. There are two general categories of *B. subtilis* strains, those that are applied to the foliage of a plant and those applied to the soil or transplant mix when seeding.

Staphylococcus aureus

The gram-positive bacteria, such as *Staphylococcus aureus* is a very common bacterium, living on many healthy individuals without causing any problem. When it enters in the body, it is responsible for several infections. This bacteria is responsible for post operative wound infection, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning.

Klebsiella pneumonia

Klebsiella is a type of Gram-negative bacteria that can cause different types of healthcare-associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. Increasingly *Klebsiella* bacteria are normally found in the human intestines (where they do not cause disease). They are also found in human stool (feces). In healthcare settings *Klebsiella* infections commonly occur among sick patients who are receiving treatment for other conditions. Patients whose care requires devices like ventilators (breathing machines) or intravenous (vein) catheters, and patients who are taking long courses of certain antibiotics are most at risk for *Klebsiella* infections. Healthy people usually do not get *Klebsiella* infections.

Salmonella enteric

Salmonella enterica is a gram-negative, rod-shaped, flagellated bacterium that is of interest due to its ability to cause infectious disease in humans and animals. As their name suggests Salmonella enterica are involved in causing diseases of the intestines (enteric means pertaining to the intestine).

The three main serovars of Salmonella enterica are Typhimurium, Enteritidis, and Typhi. Salmonella enterica serovar Typhi also called Salmonella typhi. This bacterium is the causative agent of typhoid fever. Salmonella enterica serovar Typhimurium also called Salmonella Typhimurium. The most common cause of food poisoning by Salmonella species was due to *S. Typhimurium*. Salmonella enterica serovar Enteritidis also called Salmonella enteritidis.

Experimental

Microorganisms were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India, as Microbial Type Culture Collection (MTCC). All microbial strains were cultured and maintained in Microbiology Laboratory, Department of Biotechnology, Bhimtal Campus, Kumaun University, Nainital, India.

Antimicrobial Activity

Screening essential oil for antimicrobial activity was done by the well diffusion method which is normally used as preliminary check for antimicrobial efficiency of essential oil.

Culture Media

The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

Composition Nutrient broth

Beef extract	1.0 g
Yeast extract	2.0 g
Peptones	5.0 g
NaCl	5.0 g
Agar	15.0 g
Distilled Water	1.0 L
pH	7.2-7.4

Based on the screening essential oil was identified to have potent antibacterial activity and their Minimum Inhibitory Concentrations (MIC) were determined by Agar Well Diffusion

method. The organisms were cultured in nutrient broth (bacterial strains) and malt yeast broth (fungal strains) and the tests were carried out on Mueller Hinton agar and Potato Dextrose agar plates respectively.

The inoculums of the microbial strains were prepared from 24 h broth cultures, the cultures were adjusted to 10^6 CFU/ml with sterile water. The different concentration range from 5 μ l/ml to 1000 μ l/ml of essential oil was prepared by dissolving them in DMSO. Muller Hinton agar and Potato Dextrose agar was poured into petriplates. After solidification, 100 μ L of test strains were spread on the media plates separately. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. After the inoculation, a well was made in the plates with sterile borer (3 mm).

The oil sample (30 μ L/well) of different concentrations (5 μ l/ml to 100/ml μ l) were introduced into the well and plates were incubated at 37°C for 24 hrs. Microbial growth was determined by measuring the zone of inhibition. Kanamycin (50 μ g/ml) for bacterial strain and Nystatin (30 μ g/ml) for fungal strain was used as positive control. DMSO was used as negative control. Experiments were carried out in triplicate. Inhibition of bacterial growth in the plates containing test oil was analyzed by its comparison with growth in blank control plates. The MICs were determined as the lowest concentration of oil inhibiting visible growth of each organism on the agar plate.

RESULTS AND DISCUSSION

The essential oil was screened for antimicrobial activity against four standard strains of bacteria representing two gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and two gram-negative bacteria *Klebsiella pneumoniae*, *Salmonella enterica* bacterial stains.

The tests were repeated in triplicate and the average values along with standard deviation (\pm SD) were taken to show the inhibitory activity of the pure essential oils against the selected set of bacteria as shown in (**Table-1**). The antibacterial activity was compared with the standards Gentamycin and Kanamycin (antibiotics).

Essential oil of *Swertia ciliata*

The neat essential oil of *Swertia ciliata* was screened for antimicrobial activity against four standard strains of bacteria. The results of in vitro test (**Table 1**) and **Fig.1**, showed that the

oil had moderate to good activity against the tested pathogens on the basis of zone of inhibition and MIC values.

All the pathogens were inhibited by the whole tested plants oils. The pure oil exhibit maximum activity against *Bacillus subtilis* with strong inhibition zone of 21.0 ± 0.26 mm diameter and MIC 200 $\mu\text{L}/\text{mL}$. The essential oil showed moderate activity against *Staphylococcus aureus* 19.3 ± 0.15 mm; MIC 200 $\mu\text{L}/\text{m}$, *Klebsiella pneumonia* 22.1 ± 0.12 mm; MIC 200 $\mu\text{L}/\text{m}$ respectively. while low activity against *Salmonella enteric*.

To the best of our knowledge, this is the first report on the antimicrobial activity of the essential oil of *Swertia ciliata* collected from Munsiyari, District-Pithoragarh, India.

Table: 1 Antimicrobial activity of the essential oils of *Swertia ciliata* aerial part

S.No	Microorganism	MTCC Code	MIC $\mu\text{L}/\text{m}$	<i>Swertia ciliata</i> ZI (mm)	Standard Gentamycin ZI (mm)	Standard kanamycin ZI (mm)
1	<i>Staphylococcus aureus</i>	3103	200	19.3 ± 0.15	27.7 ± 0.06	25.0 ± 0.10
2	<i>Bacillus subtilis</i>	441	200	21.0 ± 0.26	21.3 ± 0.15	21.0 ± 0.10
3	<i>Klebsiella pneumonia</i>	3384	200	22.1 ± 0.12	25.3 ± 0.12	22.3 ± 0.15
4	<i>Salmonella enteric</i>	3224	200	10.3 ± 0.21	20.3 ± 0.12	21.3 ± 0.06

*MTCC- Microbial Type Culture Collection

*MIC- Minimum inhibitory concentration

*ZI- Zone of inhibition

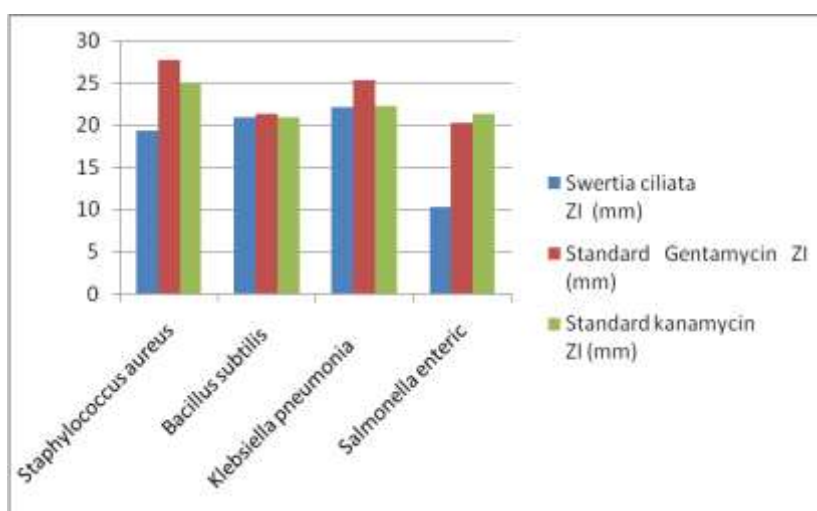


Fig.1 Comparative antimicrobial activity of *Swertia ciliata* with respect to Gentamycin and Kanamycin

Essential oil of *Acorus calamus*

The neat essential oil of *Acorus calamus* was screened for antimicrobial activity against four standard strains of bacteria. The results of in vitro test (**Table 2**) and **Fig.2**, showed that the oil had moderate to good activity against the tested pathogens on the basis of zone of inhibition and MIC values. All the pathogens were inhibited by the whole tested plants oils. The pure oil exhibit maximum activity against *Klebsiella pneumonia* with strong inhibition zone of 21.4 ± 0.12 mm diameter and MIC $200 \mu\text{L}/\text{mL}$. The essential oil showed moderate activity against *Staphylococcus aureus* 20.3 ± 0.15 mm; MIC $200 \mu\text{L}/\text{m}$, *Salmonella enteric* 17.8 ± 0.19 mm; MIC $200 \mu\text{L}/\text{m}$ respectively. while low activity against *Bacillus subtilis*.

Table: 2 Antimicrobial activity of the essential oils of *Acorus calamus* aerial part

S.No	Microorganism	MTCC Code	MIC $\mu\text{L}/\text{m}$	<i>Acorus calamus</i> ZI (mm)	Standard Gentamycin ZI (mm)	Standard kanamycin ZI (mm)
1.	<i>Staphylococcus aureus</i>	3103	200	20.3 ± 0.15	27.7 ± 0.06	25.0 ± 0.10
2.	<i>Bacillus subtilis</i>	441	200	15.1 ± 0.19	21.3 ± 0.15	21.0 ± 0.10
3.	<i>Klebsiella pneumonia</i>	3384	200	21.4 ± 0.12	25.3 ± 0.12	22.3 ± 0.15
4.	<i>Salmonella enteric</i>	3224	200	17.8 ± 0.19	20.3 ± 0.12	21.3 ± 0.06

*MTCC- Microbial Type Culture Collection

*MIC- Minimum inhibitory concentration

*ZI- Zone of inhibition

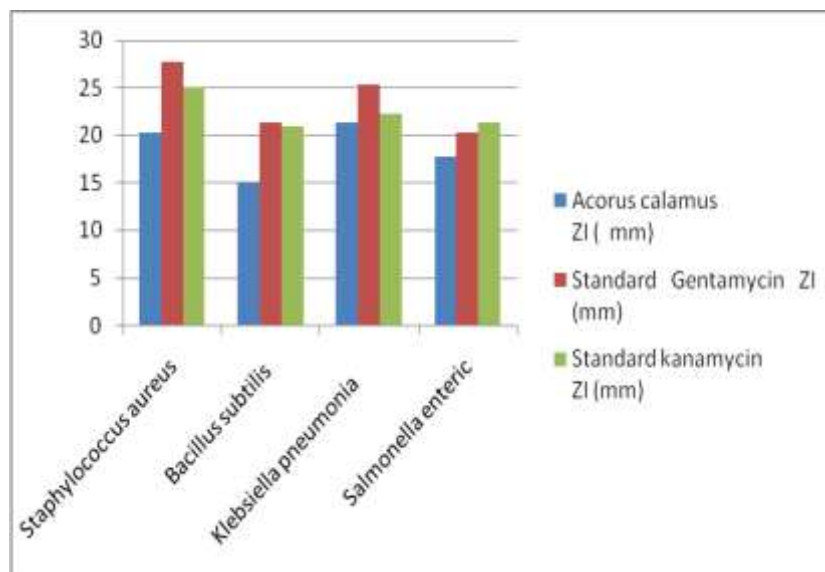


Fig. 2 Comparative antimicrobial activity of *Acorus calamus* with respect to Gentamycin and Kanamycin

Essential oil of *Viola Serpens*

The essential oil of *Viola serpens* was screened for antimicrobial activity against four standard strains of bacteria. The results of in vitro test (**Table 3**) and **Fig.3**, showed that the oil had moderate to good activity against the tested pathogens on the basis of zone of inhibition and MIC values. All the pathogens were inhibited by the whole tested plants oils. The pure oil exhibit maximum activity against *Bacillus subtilis* with strong inhibition zone of 20.0 ± 0.16 mm diameter and MIC 200 $\mu\text{L}/\text{mL}$. The essential oil showed moderate activity against *Staphylococcus aureus* 20.5 ± 0.12 mm; MIC 200 $\mu\text{L}/\text{m}$, *Klebsiella pneumonia* 16.2 ± 0.12 mm; MIC 200 $\mu\text{L}/\text{m}$ respectively. while low activity against *Salmonella enteric*.

To the best of our knowledge, this is the first report on the antimicrobial activity of the essential oil of *Viola serpens* collected from (Shama Kapkot), District-Bageshwar, India.

Table-3 Antimicrobial activity of the essential oils of *Viola serpens* aerial part

S.No	Microorganism	MTCC Code	MIC $\mu\text{L}/\text{m}$	<i>Viola serpens</i> ZI (mm)	Standard Gentamycin ZI (mm)	Standard kanamycin ZI (mm)
1.	<i>Staphylococcus aureus</i>	3103	200	20.5 ± 0.12	27.7 ± 0.06	25.0 ± 0.10
2.	<i>Bacillus subtilis</i>	441	200	20.0 ± 0.16	21.3 ± 0.15	21.0 ± 0.10
3.	<i>Klebsiella pneumonia</i>	3384	200	16.2 ± 0.12	25.3 ± 0.12	22.3 ± 0.15
4.	<i>Salmonella enteric</i>	3224	200	14.0 ± 0.13	20.3 ± 0.12	21.3 ± 0.06

*MTCC- Microbial Type Culture Collection

*MIC- Minimum inhibitory concentration

*ZI- Zone of inhibition

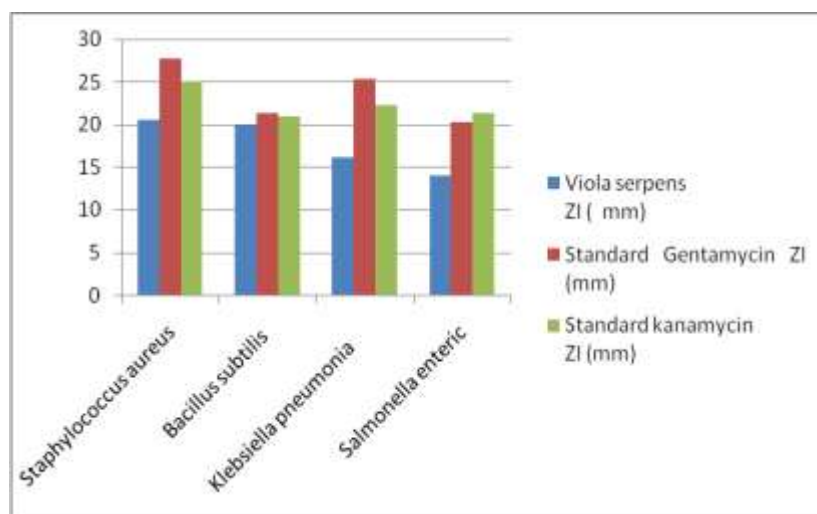


Fig: 3 Comparative antimicrobial activity of *Viola serpens* with respect to Gentamycin and Kanamycin

CONCLUSION

In summary, this is the first report on the antimicrobial activity of the essential oil of *Viola serpens* collected from (Shama Kapkot), District-Bageshwar, India. According to the results of antimicrobial activity, *Swertia ciliata* exhibit maximum activity against *Bacillus subtilis* with strong inhibition zone, *Acorus calamus* exhibit maximum activity against *Klebsiella pneumonia* with strong inhibition zone and *Viola serpens* exhibit maximum activity against *Bacillus subtilis* with strong inhibition zone.

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