INTRODUCTION
Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. The plants are source of medicines since ancient times. According to World Health Organization, 80% of the populations in the world depend on traditional medical practitioners for their medicinal needs. Numerous surveys on antimicrobial medicinal plants had been made in United States and in many countries throughout the world. Such study had demonstrated the wide occurrences of active compounds in higher plants. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. More than 64 plants have been found to possess significant antibacterial properties, and more than 24 plants have been found to possess antidiabetic properties. Viola odorata is a species of the genus Viola native to Europe and Asia, but has also been introduced to North America and Australia. It is commonly known as Sweet Violet, English Violet, Common Violet, or Garden Violet. The herb is known as Banasfa or Banaksa in India, where it is commonly used as remedy to cure sore throat and tonsillitis. The flowers and leaves of Viola odorata are made into syrup used in alternative medicine mainly for respiratory ailments associated with congestion, coughing, and sore throat. Large doses of the root contain an alkaloid called violine which is emetic (causing vomiting). Viola odorata provides best antimicrobial activity against Proteus vulgaris and Escherchia coli. Tea made from the entire plant is used to treat digestive disorders and new research has detected the presence of a glycoside of salsicid acid (natural aspirin) which substantiates its use for centuries as a medicinal remedy for headache, body pains and as a sedative. HPLC-DAD-ESI-MS analyses supported by extensive preparative chromatographic investigations and 2D NMR analyses revealed the predominance of complex flavon glycosides and permitted the complete characterization of the content of Viola odorata preparations. The plants constituents are being studied and show these uses to be valid. Eugene, Ferules-acid, Kaempferol, Quercetin, Scopoletin, also show promise in the treatment of many kinds of cancer, arthritis, AIDS and gum diseases. Cyclotide cyclovio- lacin O2 (CyO2) is the most active cyclotide from Viola odorata efficiently inhibited the growth of S. Enterica serovar Typhimurium LT2 and Escherchia coli in RDAs and MIC assays. In time-kill assays, cyO2 also had bactericidal activity against the Gram-negative species Pseudomonae and Pseudomonas aeruginosa. Cycloviolacin O1, a cyclotide from Viola odorata has antitumor effects and causes cell death by membrane permeabilization. The cycloptides from viola odorata has robust cytotoxicity effects that may be promising chemosensitizing agents against drug resistant breast cancer.

Key words: Viola species, Tinospora cordifolia, phytochemicals, herbal antibiotics

ABSTRACT
Study was undertaken to investigate the qualitative phytochemical screening and antimicrobial properties of different extracts of two Indian medicinal plants namely Viola species and Tinospora cordifolia, which were traditionally used in Indian folkloric medicine for the treatment of various bacterial infections. Plants were extracted using different solvents such as pure methanol, 50% methanol, water, acetone and hexane. Extracts of Viola species and Tinospora cordifolia plants were investigated for in vitro antimicrobial activity against pathogens, Escherichia coli, Staphylococcus aureus, Proteus Vulgaris, Klebsiella pneumonia, Pseudomonas aeruginosa and Enterobacter aerogenes by disc diffusion method. The presence of carbohydrates, proteins, steroids, phenolic compounds, tannins, alkaloids and saponins were indicated by the qualitative tests conducted. Plants of Viola species and Tinospora cordifolia extracted in acetone(1700µg,1100µg) and methanol (32000µg,4600µg) showed different diameters of zone of inhibitions ranging between 4 to 25 mm and exhibited the highest zone of inhibition against Staphylococcus aureus and Pseudomonas aeruginosa as compared to other tested organisms. The zone of inhibitions found from both the plant extracts were comparatively low to the antibiotic discs used against these microorganisms. The results provide justification for the use of these plants in folk medicine to treat infectious diseases but at higher concentration. However, high phytochemicals containing strains of these plants need to be screened to potentiate efficient herbal antibiotics.


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Viola species and Tinospora cordifolia as Herbal Antibiotics
Jha alok1, Kaur Prabhjot1, Khan M A1, Siddiqui S1, Sharma Neeta Raj1, 2
1 Lovely School of Bio-Sciences, Lovely Professional University, Phagwara-144402, India
2 Krishna Institute of Engineering and Technology, Ghaziabad, UP, India

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Fig.1 Tinospora cordifolia

Fig.2 Viola odorata
Sri Lanka. The plant is a climbing shrub found throughout India, typically growing in deciduous and dry forests. It is one of the most valuable traditional Indian medicinal herbs and has been used in ayurvedic preparations for the treatment of various ailments throughout the centuries. *Tinospora cordifolia* is reported to possess antispasmodic, anti-inflammatory, anti-alergic, antipyretic, antileptotic and anti-diabetic properties. It is generally prescribed in general debility, diabetes, fever, jaundice, skin diseases, rheumatism, urinary diseases, dyspepsia, goit, gonorrhoea and leucorrhoea. The root and stem of *Tinospora cordifolia* are prescribed in combination with other drugs as an anti-dote to snake bite and scorpion sting. A decoction of the stem is used for washing sore eyes and syphilitic sores. The stem is registered in the Thailand Pharmacopoeia, and commonly used in hospital to treat diabetes. The extracts of *Tinospora* also provide antifungal activity and nematicidal activity. The methanolic stem extract of *Tinospora cordifolia* possesses antifertility activity, which might be exploited to prevent unwanted pregnancy and control the ever increasing population explosion. The leaves are beaten with honey and applied to ulcers. Stem, root, whole plant is used in the treatment of wound, anthrax, pneumonia, asthma, and cough. *Tinospora cordifolia* can be used as effective protecting agents against oxidative stress and in treating diseases caused by the test organism present. The aim of present study was to evaluate the potential of *Viola species* and *Tinospora cordifolia* against various pathogens keeping in view to extend the scope of development of herbal antibiotics with minimum side effects.

**MATERIALS AND METHODS**

The *Viola* species was procured from Punjab and stem of *Tinospora Cordifolia* was collected from Indian Institute of Integrative Medicine (IIIM), Jammu, J & K (INDIA) in the month of February. The plants were shade dried at room temperature for 15-20 days. The dried plant material was made into coarse powder and passed through sieve and then used for crude extraction. Fine powder (5gm) of each was extracted in 100ml of methanol at 50-55°C for 24 hrs in shaker. The extract was filtered through Whatman filter paper No.1 and then concentrated at low temperature (40-50°C) in water bath. Further, the dried residue was preserved in airtight containers and kept at 4-5°C until further use. Likewise other extracts were prepared using different solvents such as 50% methanol, aqueous, acetone and hexane. The concentrated extracts were further dissolved in 500µl of sterile distilled water to prepare stock solutions. The extracts were then subjected to preliminary qualitative analysis of phytochemicals namely carbohydrate, alkaloid, terpenoid, protein, tannin and phenolics, steriod and saponins and found positive for all the constituents. Pure methanol, 50% methanol, water, acetone and hexane extracts were selected to test the antibacterial activity against six different microorganisms using disc diffusion method. The extraction value in methanol for *Viola* species was 33% followed by 23%, 39%, 2% & 1% in 50% aqueous methanol, aqueous, acetone and hexane respectively.

The extraction value in aqueous for *Tinospora* was 10% followed by 5%, 3%, 1% and 1% in methanol, aqueous methanol, acetone and hexane respectively. **Table 1.** *Pseudomonas aeruginosa* was inhibited maximally (12.5mm) followed by *Staphylococcus aureus* (8.5mm), *Proteus vulgaris* (8.5mm), *Escherichia coli* (6.5mm), *Enterobacter aerogenes* (6.5mm) and *Klebsiella pneumoniae* (4.5mm) by pure methanol extract of *Viola* species. **Table 2.** Zone of inhibition found in 50% methanol extract was maximum against *Proteus vulgaris* (9mm) followed by *Staphylococcus aureus* (8mm), *Klebsiella pneumoniae* (7mm), *Enterobacter aerogenes* (7mm) and *Escherichia coli* (6mm). The sensitivity of acetone extract of *Viola* species was detected high against *Staphylococcus aureus* (25mm) followed by *Pseudomonas aeruginosa* (6.5mm), *Escherichia coli* (4.5mm) and *Klebsiella pneumoniae* (2mm). Zone of inhibition was beyond detection limit in aqueous and hexane extract of *Viola* species against all microorganisms **Table 2.**

*Staphylococcus aureus* was inhibited maximally (14mm) followed by *Escherichia coli* (9mm), *Klebsiella pneumoniae* (7mm), *Enterobacter aerogenes* (6mm) in pure methanol extract of *Tinospora cordifolia*. Zone of inhibition found in 50% methanol extract against *Staphylococcus aureus* was (4.5mm) followed by *Escherichia coli* (3.5mm), *Proteus vulgaris* (2mm) and *Pseudomonas aeruginosa* (1mm). The sensitivity of acetone extract of *Tinospora cordifolia* was detected high against *Proteus vulgaris* (12mm) followed by *Staphylococcus aureus* (9mm), *Escherichia coli* (2mm). There was no zone of inhibition found in water and hexane extract of *Tinospora cordifolia* against all microorganisms tested **Table 3.** Streptomycin disc diffusion tests gave maximum zone of inhibitions that were used as control against these microorganisms.

**DISCUSSION**

In present study, aqueous extract of *Viola sp.* and *Tinospora Cordifolia* were not found effective even at 38 mg. and 10 mg. concentration of crude extracts respectively. In contrary, antibacterial activity of aqueous

extract of Viola odorata on Staphylococcus aureus and Pseudomonas aeruginosa has been reported with reference to temperature effect which showed that cold treatment has more effect than warm treatment\(^a\). With plant growth, antibacterial activity decreases in leaf and root, but in flowering stage it increases in flower organ (reference). Ethanolic and aqueous extracts of Tinospora cordifolia showed the toxicity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis. The ethanolic extract of plant has also been reported for better antibacterial activity than aqueous extract (Reference). This study is correlated with our study. According to earlier report (reference), agar well diffusion method allows better diffusion of the extracts into the medium thus enhancing contact with organisms. Paper discs may act as a barrier between the extract and the organisms thus preventing total diffusion of active components absorbed by the disc into the medium and may be responsible for the observed differences. Furthermore, extraction and isolation of herbal active principles and their potential as herbal antibiotics, is recommended. The variation in reports might be attributed to various environmental conditions and allelopathy.the selection of strains of Viola sp and Tinospora sp. containing high concentration of active components is in progress.

CONCLUSION

In conclusion, study depicts that Viola sp. and Tinospora cordifolia are having antimicrobial potential but at high concentration of extracts as compared to other antibiotics in use. However, the positive front of herbal antibiotics is that there is no side effect. Hence, such herbs in the form of decoction may be used in regular course to prevent infection. Further study is recommended for clinical evaluation, of efficacy of crude extract and individual purified nutraceuticals, to develop herbal antibiotics.

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