Antimicrobial, Antioxidant and bioactive compounds from four Folk medicinal plants of Solan, Himachal Pradesh, India

Sharma Isha, Thakur Monika, Rangra Avinash and Tripathi Astha*
Faculty of Applied Sciences and Biotechnology, Shoolini University, Bajhol, Solan, H.P, INDIA
*asthatripathi4u@gmail.com

Abstract

In the present research, antimicrobial, antioxidant, bioactive compounds screening was done for four folk medicinal plants (Viola odorata flower, Tinospora cordifolia stem, Bacopa monnieri leaves and Mentha piperita leaves). Methanol extract was prepared and antimicrobial properties examined against gram positive and gram negative bacterial strains (Staphylococcus aureus and Pseudomonas aeruginosa) and one fungal strain (Candida albicans). Antimicrobial assay significantly showed maximum antimicrobial activity of Viola odorata against the bacterial strain Pseudomonas aeruginosa whereas Tinospora cordifolia stem extract had good antifungal activity. Tinospora cordifolia and Viola odorata were selected for antioxidant and bioactive screening.

It was exhibited that the both plant extract had antioxidant activity in dose-dependent manner. Fourier-transform infrared spectroscopy (FT-IR) and bioactive compounds screening showed the presence of flavanoids, phenols, alkaloids and saponins compounds in both plant extract. This study concluded that two selected folk medicinal plants Viola odorata flower and Tinospora cordifolia stem had good antibacterial and antifungal activity. Both plant extract also had antioxidant properties and can be used for alternative herbal treatment of diseases. Methanol extract of both plants had the presence of various phytochemicals that can be used as pharmaceutical products.

Keywords: *Viola odorata*, *Tinospora cordifolia*, *Bacopa monnieri*, *Mentha piperita*, antimicrobial activity, antioxidant activity, FT-IR, bioactive compounds.

Introduction

In recent year, antibiotic resistance property is the major problem faced by the health sectors overall the world. The pathogenic infections are difficult to cure as rapid development of microorganism resistance mechanisms.[11] Researchers are finding alternative natural source for the treatment of infections. In India different regions have local folk medicinal plants to cure various infections. 50 % of the rural population of India depends upon the folk medicinal plants for the cure of different bacterial, fungal and viral diseases.[20] Ayurdeva has also mentioned many medicinal plants for the pathogenic diseases.

Himachal Pradesh is well known for its forest areas and it contains many medicinal plants having medicinal value. Number of drug formulations are prepared from traditional medicinal plants with no side effects.[4] Medicinal plants also contain antioxidant property for the reduction of harmful free radicals. Plants play important role in the treatment of atherosclerosis and cardiovascular diseases by reducing lipids per oxidation.[7] Medicinal plants are reported to contain bioactive compounds which provide them medicinal properties. These medicinal plants and their derivatives have been used in the formulation of more than 75 % of current medicines.

Different parts of the plants have been used for the treatment of various health problems. Many pharmacological active bioactive compounds are isolated from medicinal plants. Alkaloids, flavanoids, saponins, tannins and terpenoids are few such secondary bioactive compounds produced during the metabolic processes of plants.[14]

The selection of *Mentha piperita, Viola odorata, Bacopa Monnieri, Tinospora cordifolia* was based on its medicinal use by the folk people of Solan rural areas. Therefore, the aim of this study was to compare the antimicrobial activities of methanol extract of *M. piperita, B. Monnieri, T. cordifolia* and *V. odorata* plant parts against respiratory pathogens and then to analyze the folk plants with maximum antimicrobial potential for antioxidant and bioactive compounds profile.

Material and Methods

Collection of the samples: Naturally grown *M. piperita*, *B. monnieri*, *T. cordifolia*, *V. odorata* plants were collected from the rural area of Solan, Himachal Pradesh, India. The leaves, stem and flowers parts were taken on the basis of traditional medicinal uses. The collected parts were thoroughly washed with water to remove soil. After washing, the parts were air dried for the removal of moisture. The dried plant material was powdered using a blender.

Extraction of plant material: Methanol extracts were prepared by weighing 10 g dry powder of *V. odorata* flower, *M. piperita* leaves, *B. monnieri* leaves and *T. cordifolia* stem. 100 ml of methanol was added in each medicinal plant powder. Then the mixture was kept at room temperature for 2 days under shaking and dark conditions. After two days the plant extracts were centrifuged for 15 minutes at 7000 rpm. Supernatant was collected in the china dish and placed over

water bath for evaporation at temperature 45°C. The dried residue after complete evaporation was collected in tight container and stored at 4°C. Final concentration 100 mg/ml of each plant extract was prepared by dissolving in 10% DMSO solvent.[2]

Preparation of inoculums: Test strains *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans were* used in antimicrobial assay. The inoculums suspension was prepared by culturing the microbes in the nutrient broth for overnight at 37°C for bacterial culture and sabrose dextrose broth for fungal culture at 25°C.

Antimicrobial assay: Agar well diffusion method was used to check the antimicrobial potential of methanol extract of *V. odorata, B. monneria, T. cordifolia* and M. *piperita*. Fresh inoculums suspension of test strains was prepared for each assay. Muller Hinton agar media plates were prepared for the well diffusion. 4 mm diameter wells were prepared by using sterile cork borer. Lawn culture of microbial suspension was prepared by using sterile cotton swabs.100µl of *V. odorata*, *B. monnieri*, *T. cordifolia* and *M. piperita* extracts were inoculated in separate wells in aseptic conditions. Then the plates were kept for 2 hours in room temperature.

The plates were incubated at 37ºC for 24 hours in case of bacteria and 25ºC for 72 hours in case of *C. albicans*. Positive control and negative control were also introduced in the plates. Ciprofloxacin and fluconazole (10 mg/ml) were used as positive control for both bacteria and *C. albicans* respectively. 10% DMSO was used as negative control for all test strains.[12]

Antioxidation activity by DPPH (1, 1-diphenyl;-2-picrylhydrazyl) Radical Scavenging assay: This assay was used to determine the ability of an antioxidant agent to donate hydrogen radical to DPPH radical. DPPH radicals have deep purple color and stable free form. When an electron is added to the free radical of DPPH in the presence of an antioxidant agent, the purple color changes.

The free radical of DPPH gets converted into hydrazine DPPH-H form. The conversion of DPPH free radical could be detected by the decrease in the absorption at 517 nm. The antioxidant potential was determined by the rapid decease in the absorption.

For the determination of antioxidant activity of extracts on the stable free radical of DPPH different concentration of extracts were prepared. 0.1 ml of each concentration was added to 3 ml of 0.004% methanol solution of DPPH.

Then this mixture was kept in dark room without disturbance for 30 minutes. After that the absorbance of the reaction mixture was taken at 517 nm. Percentage of inhibition was calculated by using the following formula:

$$I\ \% = [(A_{Blank} - A_{sample})/A_{Blank}] \times 100$$

where A_{blank} = absorbance of the control reaction (containing all reagents except the test sample) and A_{sample} = absorbance of sample/standard.

IC_{50} value was calculated from the graph plotted I% versus concentration curve. The free radical scavenging activity was measured for different concentrations of sample and compared with standard ascorbic acid.[17]

FTIR spectroscopy: The bioactive compounds screening was further analyzed by the analysis of functional groups present in the methanol extract. Fourier transform infrared spectroscopy was used to determine the functional groups. The small amount of dry methanol extracts (5mg) of medicinal plant with high antimicrobial potential was used for this analysis. The dry extract was dispersed in the dry potassium bromide. The mixture was mixed using mortar and then pressed by applying 6 bars pressure for 2 min for making a thin disc of mixture. Then this thin disc was placed under the ionic resonance. The IR spectrum was obtained by using Perkin Ekmer 2000 infrared spectrometer. Scanning of sample was done 16 times to increase the signal to noise ration.

Phyto-chemical screening: For the detection of alkaloids compounds, Mayer's test was done. Saponins were analyzed by frothing test. Alkaline reagent test was performed for the flavonoids compounds detection. Carbohydrates (reducing sugars) presence was observed by Benedict test. Phenolic compound was detected by lead acetate test. A bulky white precipitate indicated the presence of phenolic compounds.[9]

Statistical Analysis: All the experiment analyses were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's HSD test using SAV v.9.1.3 program. Differences at $p^{*}<0.05$ were considered to be significant.

Results and Discussion

The antimicrobial assay: To determine the antimicrobial potential of selected folk medicinal plants, methanol extracts of each plant parts having medicinal value were prepared. The antibacterial activity was observed against gram positive and gram negative bacteria and one fungal strain causing the infections of respiratory tract. *S. aureus* (Gram positive cocci), *P. aeruginosa* (Gram negative cocci) and *C. albicans* (fungus) strains procured from IMTECH Chandigarh were used as test strains in the agar well diffusion method. The zone to inhibition was measured to determine the maximum antimicrobial activity of folk medicinal plants.

Methanol extracts of *V. odorata* flower significantly showed maximum zone of inhibition against *P. aeruginosa* and *S. aureus* (16mm and 8.5mm respectively) as shown in table 1 and fig. 1. The methanol extracts of *T. cordifolia* stem had shown antifungal activity (19.75mm zone of inhibition)

against *C. albicans* with significant difference (Table 1, Fig. 3). Maximum antimicrobial activity was shown by *V. odorata* flower in case of both bacterial strains and *T. cordifolia* stem in case of fungal strain. The antioxidant potential and phytochemical analysis were further done in both methanol extract of *V. odorata* flower and *T. cordifolia* stem.

DPPH Free Radical Scavenging Assay: DPPH was used in the form of free radical to know the antioxidant potential of the compounds present in the plants methanol extracts of *T. cordifolia* and *V. odorata*. The plants *T. cordifolia* and *V. odorata* exhibited an antioxidant activity in dose- dependent manner (Fig: 4 and 5 respectively). The solvent extract of *T. cordifolia* stem at different doses exhibited non-significant ($p<0.05$) antioxidant activity as compared to *V. odorata* flower.

The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower is the IC_{50} value, the higher is the antioxidant activity of samples.[21] The IC_{50} value of methanol extract of *V. odorata* was 3.8 µg/ml and for *T. cordifolia* it was 3.4 µg/ml as shown in table 2.

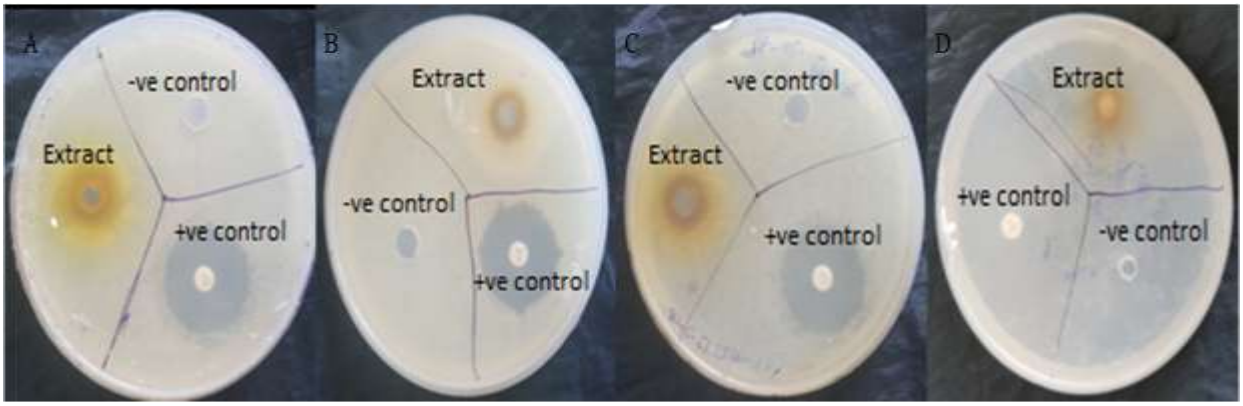

Fig. 1: Antimicrobial assay of methanol extract of plant (A) *V. odorata* flower, (B) *B. monnieri* leaves, (C) *T. cordifolia* stem and (D) *M. piperita* leaves against bacteria *P. aeruginosa*

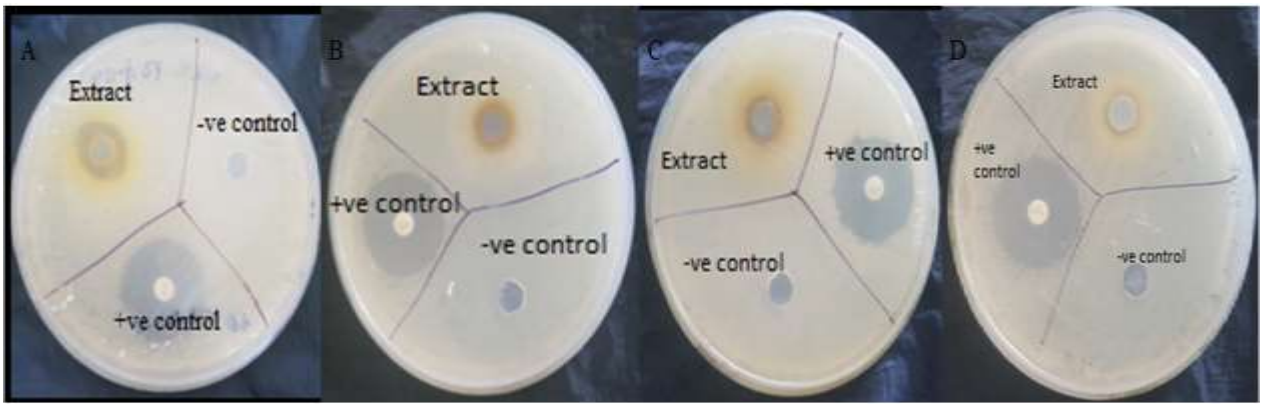

Fig. 2: Antimicrobial assay of methanol extract of plant (A) *V. odorata* flower, (B) *B. monnieri* leaves, (C) *T. cordifolia* stem and (D) *M. piperita* leaves leaves against bacteria *S. aureus*

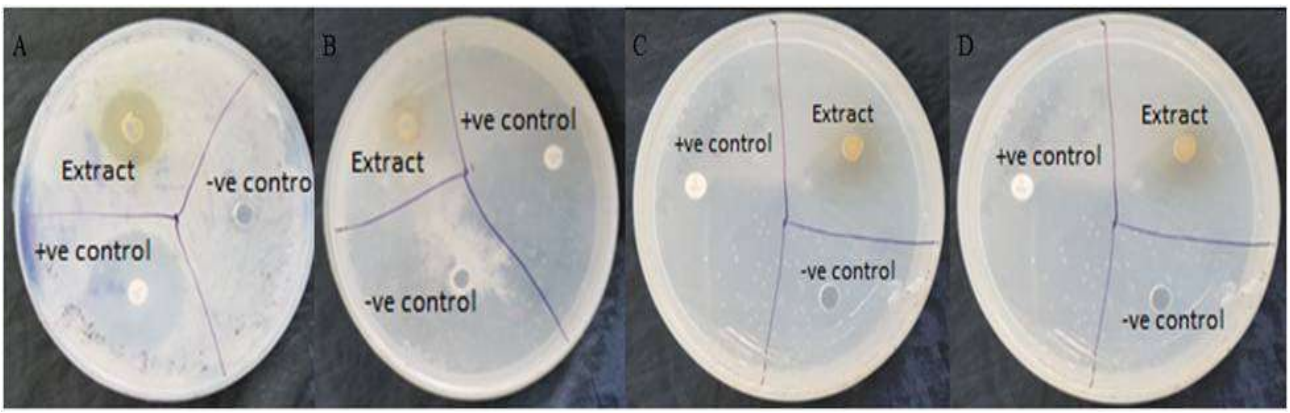

Fig. 3: Antimicrobial assay of methanol extract of plant (A) *V. odorata* flower, (B) *T. cordifolia* stem, (C) *B. monnieri* leaves and (D) *M. piperita* leaves against Fungus *C. albicans*

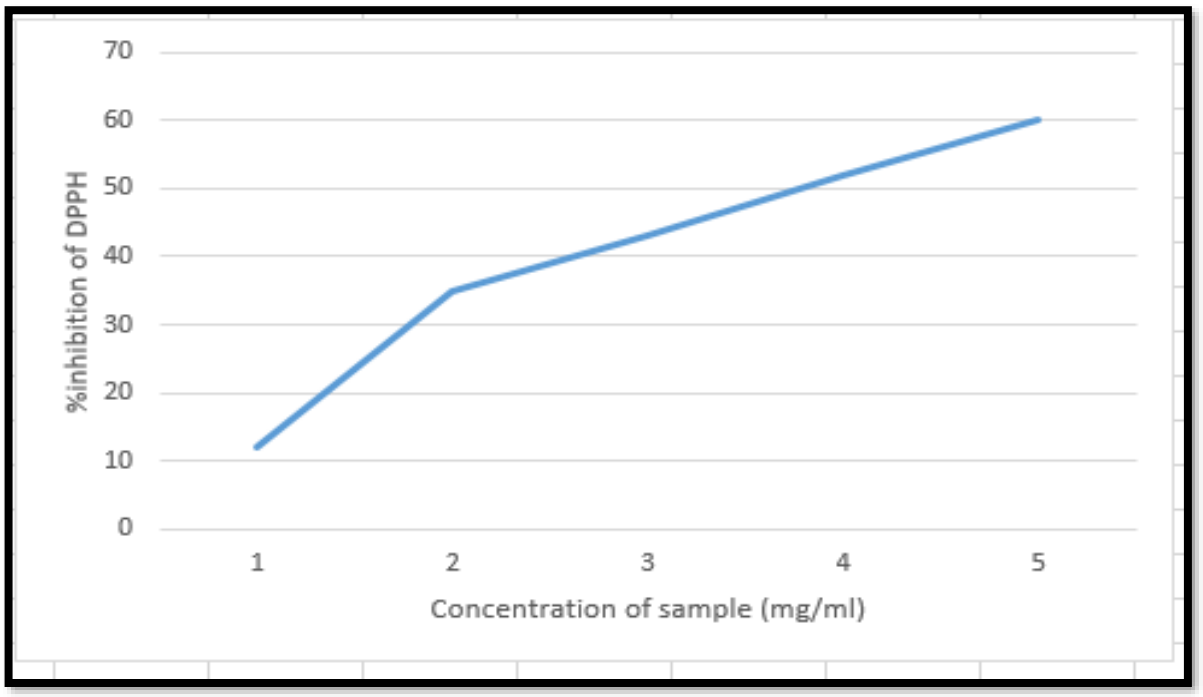

Fig. 4: Graphical representation of antioxidant activity of *V. odorata* flower

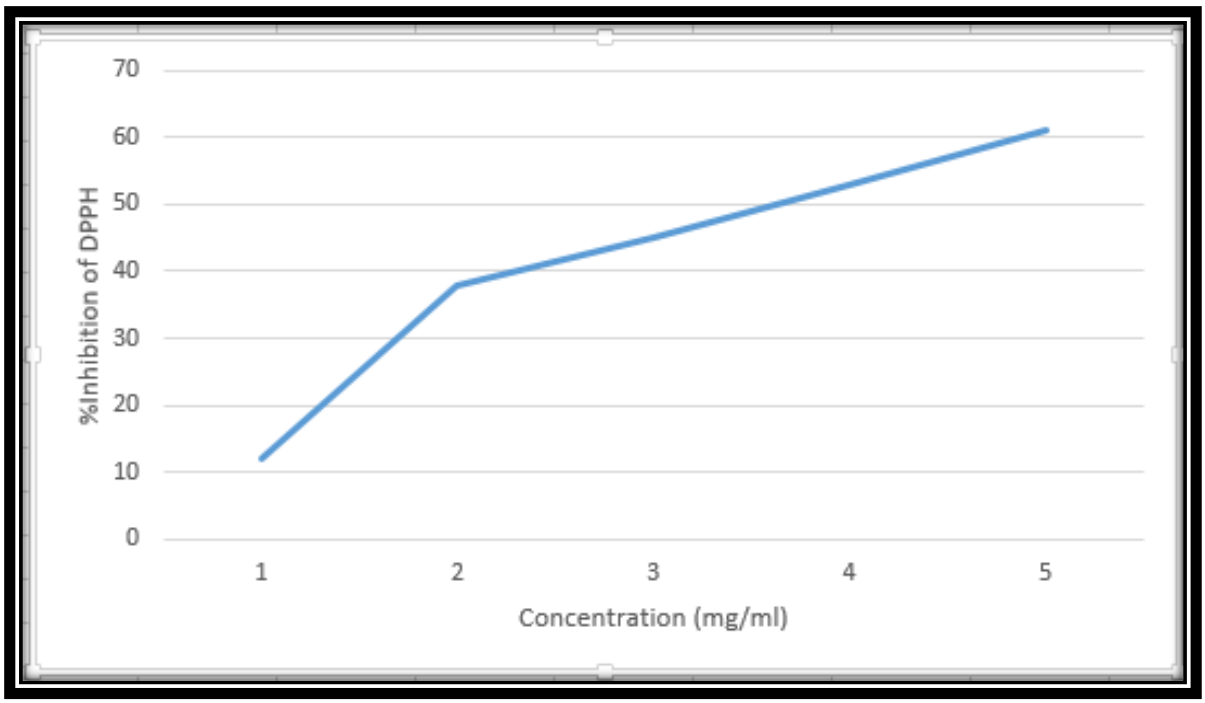

Fig. 5: Graphical representation of antioxidant activity of *T. cordifolia* stem

Table 1
The antibacterial and antifungal extents of plants were seen in form of zone of inhibition (Mean ± SD).

Bacterial isolates	Methanol extract (100mg/ml)			
	Zone of Inhibition (mm)			
	***V.odorata* flower**	***T. cordifolia* stem**	***B. monnieri* leaves**	***M. piperita* leaves**
S. aureus	8.5±1.41 [a]	2.5±0.70[b]	3.5±0.80 [b]	2.25±0.70 [b]
P. aeroginosa	16±0.70 [a]	14.5±0.40[a]	2.5±1.4 [b]	0.00
C. albicans	18.5±1.41[ab]	19.75±1.25[a]	15.5±1.23[c]	16±0.35[bc]

Note: In each row different letters means significant difference (p*<0.05)

FTIR spectroscopy of *V. odorata* and *T. cordifolia* plant extract: The methanol extracts of *V. odorata* and *T. cordifolia* were examined using FTIR spectroscopy for the presence of functional groups present. The FTIR spectrum of *V. odorata* and *T. cordifolia* had been shown in fig. 6 and fig. 7 respectively and explained in table 3 and 4.

Bioactive Compound screening of *V. odorata* and *T. cordifolia* plant extract: Several tests were performed to test the presence of bioactive compounds present in the methanol extract of *V. odorata* and *T. cordifolia*. Summary of the entire test performed and there results is shown in table 5. The color change and the precipitate formed determine whether the test is positive or negative. The colored compounds and precipitate formed are shown in fig. 8. Bioactive compound screening of both plant extracts showed the presence of bioactive components like alkaloids, saponins, flavonoids, carbohydrates and phenolic compounds.

Discussion

Previous studies had reported the antimicrobial potential of *V. odorata* against pathogens of respiratory infections. In this study, *V. odorata* showed zone of inhibition against *P. aeruginosa*, *S. aureus*, *Streptococcus pneumonia* and *Streptococcus pyogenes*. Methanol extract has been observed to have more antimicrobial potential than aqueous and acetone solvents.[22] Similar results have also been reported in case of methanol leaf extact of *M. piperita* against various pathogens such as *Escherichia coli, Acinetobacter, S. aureus* and *C. albicans.*[19] A study done to compare the antimicrobial activity of methanol and ethyl acetate leaf extracts of *T. cordifolia* against *E.coli* showed that both plant extract have antibacterial potential.[13]

Different polar extracts of the leaf callus of *B. monnieri* were also reported to have antibacterial activity against gram positive and gram negative bacteria. The polar solvents had shown antibacterial activity but the aqueous extract exhibited no antimicrobial activity.[15] Another study observed the antifungal activity of methanol leaf extact of *Bacopa monnieri*.[1]

The medicinal plants are well known for their antioxidant properties. In various cosmetic products, medicinal plant extracts are used as a potential antioxidant agent.[6]

Table 2
Antioxidant activities (DPPH radical scavenging activity) of methanol extract of *V. odorata* flower and *T. cordifolia* stem.

Samples	Concentration of methanol extract used (µg/ml) (Scavenging activity of free DPPH radicals) (%)				
	10	**20**	**30**	**40**	**50**
Viola odorata	12±0.03 [a]	35±0.02 [b]	43±0.03 [b]	52±0.48 [b]	60±0.32 [b]
Tinospora cordifolia	12±0.05 [a]	38±0.14 [a]	45±0.04 [a]	53±0.02 [b]	61±0.08 [b]
Ascorbic acid	10.56±0.12 [c]	32.71±0.06 [b]	48.46±0.02 [a]	66.33±0.01 [a]	83.57±0.01 [a]

Different letters of each column mean significant difference at p*<0.05. Values are Mean ± SD (n=3)

Table 3
FT-IR analysis of methanol extract of *V. odorata* flower

S.N.	Wave no. (cm^{-1}) (cm^{-1}) test sample	Wave no. (cm^{-1}) Reference	Function group	Bioactive compounds Identified
1.	3365	3570- 3200	O-H Stretch, Hydroxy group, H-bonded	Polyhydroxy compound
2.	3324	3570-3200	O-H Stretch, Hydroxy group, H- Bonded	Polyhydroxy compound
3.	2211	2300- 1990	Multiple bonding	Nitrile compound
4.	2145	2300- 1990	Multiple bonding	Nitrile compound
5.	2117	2300- 1990	Multiple bonding	Nitrile compound
6.	2041	2300- 1990	Multiple bonding	Nitrile compound
7.	2030	2300- 1990	Multiple bonding	Nitrile compound
8.	1641	1650- 1600	C=O Stretch	Ketone compound
9.	1410	1410- 1310	O-H Bend, Alcoholic group	Phenol or Tertiary compound
10.	1322	1340- 1250	CN Stretch	Aromatic primary amine
11.	1011	1100- 1000	PO3 Stretch	Phosphate ion
12.	953	995- 850	P-O-C	Aromatic phosphate
13.	691	700-600	C- Br Stretch	Aliphatic bromo
14.	668	700-600	C- Br Stretch	Aliphatic bromo

Table 4
FT-IR analysis of methanol extract of *T. cordifolia*

S.N.	Wave no. (cm^{-1}) test sample	Wave no. (cm^{-1}) Reference	Function group	Bioactive compounds Identified
1.	3520	3570- 3200	O-H Stretch, Hydroxy group, H- Bonded	Polyhydroxy compound
2.	3373	3570- 3200	O-H Stretch, Hydroxy group, H- Bonded	Polyhydroxy compound
3.	2924	2865- 2845	Symmetric stretching of –CH (CH_2)	Lipids, proteins
4.	2190	2300- 1990	Multiple bonding	Nitrile compound
5.	2177	2300- 1990	Multiple bonding	Nitrile compound
6.	2151	2300- 1990	Multiple bonding	Nitrile compound
7.	2127	2300- 1990	Multiple bonding	Nitrile compound
8.	2097	2300- 1990	Multiple bonding	Nitrile compound
9.	2037	2300- 1990	Multiple bonding	Nitrile compound
10.	2021	2300- 1990	Multiple bonding	Nitrile compound
11.	2008	2300- 1990	Multiple bonding	Nitrile compound
12.	1655	1100- 1000	PO3 Stretch	Phosphate ion
13.	1439	1510- 1450	C=C-C, Aromatic	Aromatic compound
14.	1410	1410- 1310	O-H Bend, Alcoholic Group	Phenol or TertiaryCompound
15.	1320	1340- 1250	CN Stretch	Aromatic primary amine
16.	1013	1100- 1000	PO3 Stretch	Phosphate ion
17.	953	995- 850	P-O-C	Aromatic phosphate
18.	905	995- 850	P-O-C	Aromatic phosphate
19.	706	700- 600	C- Br Stretch	Aliphatic bromo compounds
20.	666	700- 600	C- Br Stretch	Aliphatic bromo

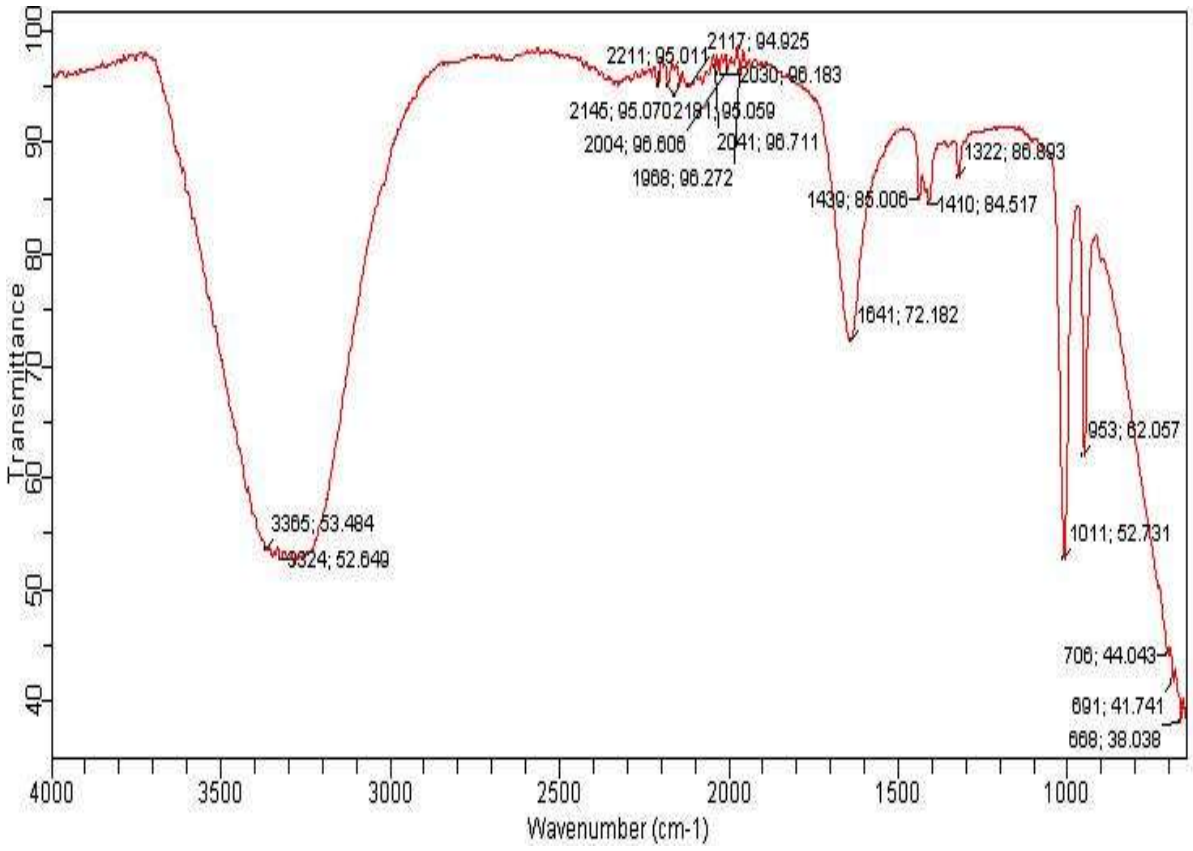

Fig. 6: FT-IR of methanol extract of *V. odorata*

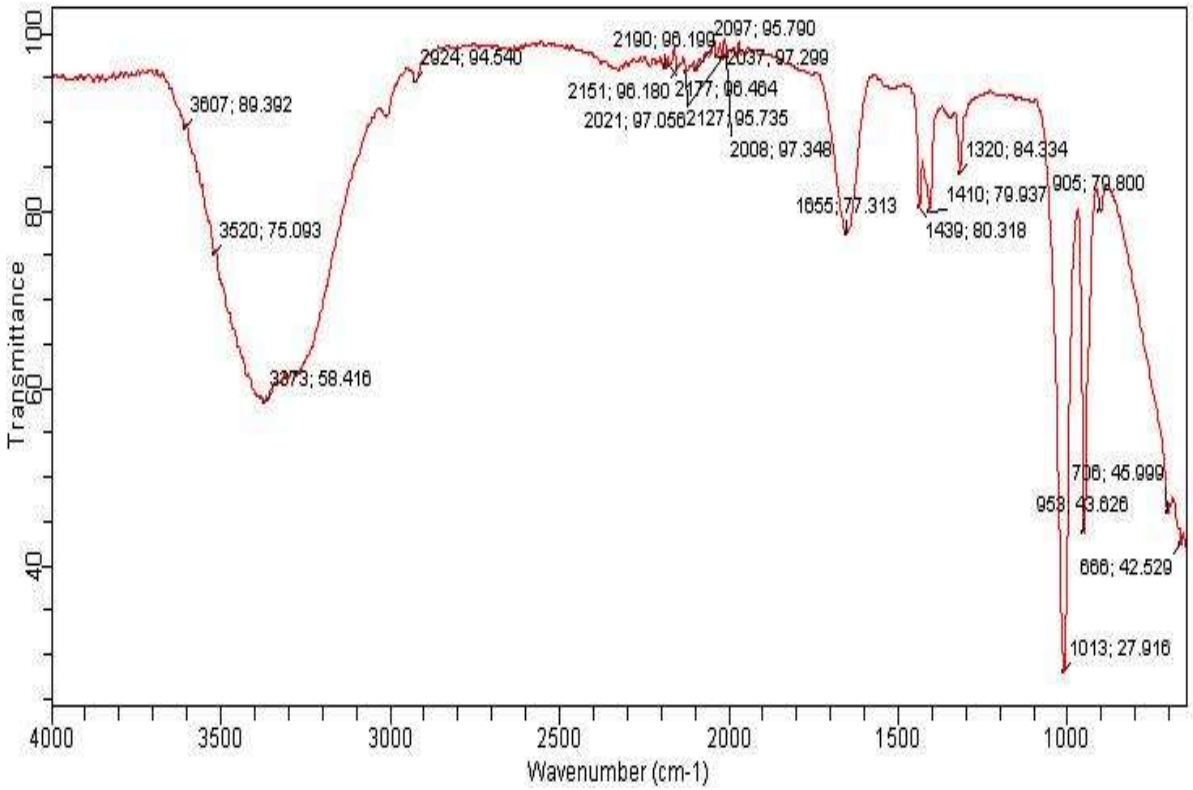

Fig. 7: FT-IR of methanol extract of *T. cordifolia*

Table 5
Bioactive compounds analysis of *V. odorata* flower and *T. cordifolia* stem

Phytochemical test	Compound detected	*V. odorata* flower	*T. cordifolia* stem
Mayer's test	Alkaloids	+++	+++
Lead Acetate test	Flavonoids	+++	++
Froth's test	Saponins	++	++
Ferric Chloride test	Phenolic Compounds	+++	++
Benedict's Test	Carbohydrates	+++	+++

The notations +++, ++, + and refer to appreciable intensity of compound (Positive within 5 minutes), moderate amounts (positive after 5 min but within 10 min), trace amounts (positive) after 10 min but within 15 min) and completely absent respectively.

V. odorata flowers are previously reported to have antioxidant properties.[23] The leaf and stem parts of *T. cordifolia* were also analyzed for its antioxidant potential. In this study it was observed that the stem solvent extract had high antioxidant activity.[10]

The medicinal values are provided to the medicinal plants by the production of various secondary metabolites. The bioactive compounds are the compounds synthesised by the plant for protection against bacterial and fungal infections.[16] A comparative study done on the different medicinal plants revealed the presence of various bioactive compounds like saponins, tannins, flavonoids and steroids. These bioactive compounds provide the antimicrobial potential to medicinal plants and use of these plants in the development of new drugs.[5] The photochemical screening of *V. odorata* had shown the presence of alkaloids, saponins and flavonoids.[8] *T. cordifoila* stem had also been reported to contain phenolic and alkaloids compounds. The present study had shown the similar results as per previously reported findings.

Conclusion

The present study concluded that antimicrobial activity of *V. odorata* flower methanol extract significantly showed maximum zone of inhibition against *P. aeruginosa* and *S. aureus*. The antifungal activity was best shown by the *T. cordifolia* stem extract. The antioxidant potential was analyzed for the plants which showed maximum antimicrobial and antifungal activity. The antioxidant assay concluded that the plants *T. cordifolia* and *V. odorata* exhibited an antioxidant activity in dose- dependent manner with no significant difference.

The methanol extract of *T. cordifolia* stem at different doses exhibited no significant ($p^{*}<0.05$) difference in the antioxidant activity as compared to *V. odorata.* In case of FT-IR spectroscopy analysis, it was observed that *V. odorata* plant extract had maximum bioactive compounds or functional group present as compared to *T. cordifolia.* Bioactive compounds analysis of both plant extracts showed the presence of bioactive components like alkaloids,

saponins, flavonoids, carbohydrates and phenolic compounds in varying amount. This study indicates that the selected folk medicinal plants have medicinal value and can be further used for drug discovery.

Acknowledgement
Department of Biotechnology and Applied Sciences, Shoolini University, Solan, H.P.

References
1. Alam K. et al, Antimicrobial activity of leaf callus of *Bacopa monnieri*, *Literature of Pharmaceutical*, **3**, 287-91 **(2011)**

2. Al-Asmari A.R., Siddiqui Y.M., Athar M.T., Al-Buraidi A., Al-Eid A.S. and Horaib G.B., Antimicrobial activity of aqueous and organic extracts of a Saudi medicinal plant: *Rumex nervosus*, *J Pharm Bioaplli Sci*., **7(4)**, 300-3 **(2015)**

3. Bains A. and Tripathi A., Antibacterial and antioxidant properties of wild mushrooms collected from Himachal Pradesh, *Inter J Biol Pharm and Allei Sci*., **4(10),** 6161-70 **(2015)**

4. Balunas M.J. and Kinghorn A.D., Drug discovery from medicinal plants, *Life Sciences*, **18**, 431-441 **(2005)**

5. Bhasin P., Bansal D., Punia A. and Sehrawat A.R., Medicinal plant used in folklore remedies in India, *J Pharmaceutical Research*, **5(3)**, 1643-45 **(2012)**

6. Bhatt I.D., Rawat S. and Rawal R.S., Antioxidants in medicinal plants. In: Biotechnology for medicinal plants, Chandra S., Lata H. and Varma A., eds., Springer, Berlin, Heidelberg, 295 **(2013)**

7. Cragg G.M. and Newman D.J., Biodiversity: A continuing source of novel drug leads, *Pure App Chem*., **77**, 7-24 **(2005)**

8. Feyzabadi Z., Ghorbani F., Vazani Y. and Zarshenas M.M., A critical review on phytochemistry, pharmacology of *Viola ordorata* L. and related multipotential products in tradition Persian medicine, *Phytotherapy Research*, **31(11)**, 1669-75 **(2017)**

9. Gul R., Jan S.U., Faridullah S., Sherani S. and Jahan N., Preliminary phytochemical screening, quantitative analysis of alkaloids andantioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan, *The Scientific World Journal*, DOI: 10.1155/2017/5873648 **(2017)**

10. Ilaiyaraja N. and Farhath K., Antioxidant potential of *Tinospora cordifolia* extracts and their protective effect on oxidation of biomolecules, *Pharmacognosy J*., **3(20)**, 56-62 **(2011)**

11. Joshi S., Singh R. and Singh S.P., Antibiotic resistance profile of *Escherichia coli* isolates from colibacillosis in and around Pantnagar, India, *Veterinary World*, **5(7)**, 405-408 **(2012)**

12. Kaminidevi S., Thangavelu T., Udayabhanu J. and Senthil T.M., Antimicrobial activity of methanolic extracts of indigenous traditional Indian folk Medicinal Plant, *Gnaphalium polycaulon*, *Inter J Green Pharm*, **9**, 39-44 **(2015)**

13. Kumar D.V., Geethanjali B., Avinash K.O., Kumar J.R., Chandrashekrappa G.K. and Kanthesh M., Basalingappa. *Tinospora cordifolia*: Antimicrobial property of the leaves of amruthaballi, *J Bacteriol and Mycol Open Access*, **3(11)**, 957-59 **(2017)**

14. Kumar R.S., Venkateshwar C., Samuel G. and Rao S., Phytochemical Screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at Adilabad District andhra Pradesh, India, *Inter J of Engineering Sci Invention*, **2**, 65-70 **(2013)**

15. Mkk F., Sp D. and Irfan M., Antibacterial and antifungal activity of various extracts of *Bacopa monnieri*. arXiv preprint arXiv:1909.01856 **(2019)**

16. Olowosulu A.K. and Ibrahim Y.E.K., Studies on the phytochemical screening of aqueous extracts of medicinal plants used in folk medicine in Nigeria, *West Africa Journal of Biologi Sci*, **3**, 21-26 **(2006)**

17. Paixao N., Perestrelo R., Marques J.C. and Câmara J.S., Relationship between antioxidant capacity and total phenolic content of red, rose and white wines, *Food Chem.*, **105**, 204–14 **(2007)**

18. Pradhan D., Ojha V. and Pandey A.K., Phytochemical analysis of *Tinospora cordifolia* (WILLD.) MIERS EX HOOK. F. and THOMS stem of varied thickness, *Inter J Pharmaceutical Sci Research*, **4(8)**, 3051-56 **(2013)**

19. Pramila D.M. et al, Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (*Mentha piperita*: Laminaceae), *J Medicinal Plant Research*, **6(2)**, 331-35 **(2012)**

20. Verma Rachna, Tapwal Ashwani and Puri Sunil, Phytochemical Profiling and GCMS Study of *Adhatoda vasica* Nees. an Ethnomedicinal Plant of North Western Himalaya, *Biological Forum – An Inter Jour*, **8(2)**, 268-273 **(2016)**

21. Saxena M. and Saxena J., Phytochemical screening of *Acorus calamus* and *Lantana camara*, *Inter Resear J Pharm.*, **3(5)**, 323-26 **(2012)**

22. Shanker G.S., Bithel N., Kumar S., Painuly D. and Singh J., A new derivative of ionone from aerial parts of *Viola odorata* Linn and its antibacterial role against respiratory pathogens, *Clinical Phytosci.*, **2(1)**, 4 **(2017)**

23. Stojkovic D., Glamoclija J., Ciric A. and Sokovic M., Free radical scavenging activity of *Viola ordorata* water extracts, *Herbs Spices and Medicinal Plants*, **17(3)**, 285-90 **(2011)**.

(Received 23rd December 2019, accepted 11th March 2020)