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Comparative Analysis of Antimicrobial, Antioxidant and Phytochemicals from the Folk Medicinal Plants of Dharampur, Solan

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Abstract

Objective: The main objective of the present research work was to examine the antimicrobial, antioxidant, qualitative phytochemicals screening and FT-IR analysis of some folk medicinal plants (*Viola odorata* flower, *Tinospora cordifolia* stem, *Bacopa monnieri* leaves and *Mentha piperita* leaves).

Method: Aqueous extract was prepared of selected plant material and antimicrobial properties examined against the pathogenic bacterial strains (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and fungal strain (*Candida albicans*) by well diffusion assay. DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay was used to check the antioxidant potential of *Tinospora cordifolia* and *Viola odorata*. FT-IR (Fourier Transform Infrared) spectroscopy was done to analyze the functional groups present in the medicinal plants (*Viola odorata* and *Tinospora cordifolia*). Phytochemical screening was done of both plant extract to observe the presence of bioactive components.

Result: In the antimicrobial assay, best effect was observed in case of *Viola odorata* against the bacterial strain (*Pseudomonas aeruginosa*) whereas all the plant aqueous extract had good antifungal activity against the fungal strain (*Candida albicans*). The plants *Tinospora cordifolia* and *Viola odorata* exhibited an antioxidant activity in dose-dependent manner. FT-IR and phytochemicals screening showed the presence of flavanoids, phenols, alkaloids, and saponins compounds in both plant aqueous extract.

Conclusion: This study concluded that all the selected folk medicinal plants can be used as alternative herbal treatment of diseases. *Viola odorata* and *Tinospora cordifolia* had antioxidant properties. Aqueous 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; Phytochemicals; Medicinal plant

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Introduction

Medicinal plants have been used as natural medicines. This kind of activities had been in existence since pre-historic times. In India, most of the population depends on the conventional type of medicine [1]. Indian folk-medicine and ethnobotany had includes 2532 plant species and many of which have medicinal properties [2]. Due to rise in side effects of unfavorable drug reactions, the interests of government and academics in conventional medicines are growing rapidly. Plants have formed the basis of worldly traditional medicine systems that have been in existence for thousands of years and continue to provide new improvements for humanity. In today's world natural products and their derivatives represent more than 50% of all the drugs which are useful for clinical purposes [3]. Medicinal plants are used as source for great economic value all over the world. Natural products are important sources for biologically active drugs. There has been an increasing interest in the study of medicinal plants as natural products in different parts of the world. In India for thousands of years, it is believed to be existence of Ayurvedic form of medicine [4]. Medicinal plants have natural antioxidant properties which are further used for the treatment of diseases throughout the world. Due to beneficial effects of antioxidants, we need to explore natural anti-oxidants to treat various diseases. Medicinal plants which contain antioxidant activity are considered to be very useful for treatment of atherosclerosis and cardiovascular diseases by reducing lipids per oxidation [5]. Nowadays, most of the researchers are dependent on the medicinal plants

for the discovery of new drugs having fewer side effects [6]. Since ancient times, medicinal plants have received more attention because of their health benefits, such as anti-infectious properties [7]. These medicinal plants can be considered as a valuable source of ingredients which can be used in drug development.

The selection of *Mentha piperita*, *Viola odorata*, *Bacopa Monnieri*, *Tinospora cordifolia* was based on its medicinal use. Therefore, the aim of this study was to analyze antimicrobial and phytochemical activities of aqueous extract of *M. piperita*, *B. Monnieri*, *T. cordifolia* and *V. odorata* against respiratory pathogens that usually cause upper and lower respiratory tract infections.

Materials and Methods

Collection of the samples

Indigenously grown *Mentha piperita*, *Bacopa monnieri*, *Tinospora cordifolia*, *Viola odorata* plants were collected from local village Dharampur, in Solan, Himachal Pradesh, India. The identification of plant material was confirmed by a botanist in the Department of Basic Sciences, Shoolini University, India. The plant material was thoroughly washed with clean water to remove soil and other dirt. Then the plant material were separated, air dried for complete drying. The dried plant material was powdered using a blender.

Extraction of plant material

Plant extracts were prepared by immersing 10g of *Mentha piperita* leaves, *Bacopa monnieri* leaves, *Tinospora cordifolia* stem and *Viola odorata* flower powdered plant material in 100 ml solvent i.e. water and kept it in the dark room for 2 days. After that each plant extracts were centrifuged at 7000 rpm for 15 minutes. The supernatant were poured in the china dish and then placed over the water bath at (50°C to 60°C) for 24 h. The dried residues were in air tight containers and kept at (4°C to 5°C) for further use. Extracts were dissolved in 10% Dimethyl Sulfoxide (DMSO) to a final concentration of 100 mg/ml [8].

Preparation of inoculums

Human oral pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* used as test organisms. Cultures of bacteria were grown for 12 h in nutrient broth at 37°C. Culture of *Candida albicans* was initially grown in potato dextrose broth at 25°C for 24 h.

Antibacterial activity

The antimicrobial activity of aqueous extract of *V. odorata*, *B. monneria*, *T. cordifolia* and *M. piperita* was determined by agar well diffusion method. The test strains were first cultured in nutrient broth for 24 h before use. 100 µl of the standardized cell suspension were spread on a Mueller Hinton agar. Sterile 4 mm diameter of Cork borer was used to bored wells into the Mueller Hinton agar. Approximately 100 µl of V. odorata, B. monnieri, T. cordifolia and M. piperita aqueous extracts were introduced into the wells separately, allowed to stand at room temperature for about 2 h and then incubated at 37°C for 24 h in case of bacteria and 25°C for 72 h in case of *Candida albicans*. The plates were observed for zones of inhibition after incubation time and compared with Ciprofloxacin and Fluconazole at a concentration of 100 mg/ml [9].

Antioxidation activity by DPPH radical scavenging assay

The DPPH assay is based on the capability of an antioxidant to donate hydrogen radical or an electron to DPPH radical, which is stable free radical with deep violet color. When an odd electron becomes paired in the presence of free radical scavenger of antioxidant agent, DPPH radicals get reduced to corresponding hydrazine, DPPH-H form [10]. Due to quenching of its free radicals by antioxidants present in the medium the deep purple color disappears, i.e., by electron donation or by providing hydrogen atom *via.,* free radical attack on DPPH molecule and convert them to colorless bleached product known as 2,2 diphenyl-1-hydrazine. This results in decrease in the absorption at 517 nm, hence, more rapidly the decrease in absorbance more potent the antioxidant activity of the extract.

The antioxidant activity of the extracts on the stable radical 1,1-Diphenyl;-2-Picrylhydrazyl (DPPH) was determined. The 0.1 ml of mushroom ethanol extract, at various concentrations was added to 3 ml of a 0.004% methanol solution of DPPH and was allowed to stand for 30 min for the reaction to occur. The absorbance of the resulting solution was measured at 517 nm from this values the corresponding percentage of inhibitions were calculated by using the following equation:

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

Where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample) and A_{sample} is the absorbance of sample/standard. To get the effective concentration IC_{50}, (defined as the concentration of antioxidant required to reducing the concentration of initial DPPH° 50%) for each extract, concentration providing 50% inhibition (IC_{50}) 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) [11].

FTIR spectroscopy

Fourier transform infrared spectroscopy was used to identify the characteristic functional groups in the extract. A small quantity (5 mg) of the extract was dispersed in dry potassium bromide. The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min to form a K-Br thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Perkin Ekmer 2000 infrared spectrometer. The sample was scanned from 4000 cm for 16 times to increase the signal to noise ratio.

Phytochemical screening

Mayer's test: Mayer's reagents prepared by mixture of mercuric chloride (1.36 g) and potassium iodide (5 g) in 100 ml water. It gives cream colored precipitate.

Saponins test: Dissolve the extract in pyridine (2 ml) and add sodium netropruside solution (2 ml) and make alkaline with NaOH solution.

Flavonoids test: In this test extract was mixed with few drops of NaOH solution.

Carbohydrates test (Benedict test): Complex mixture of sodium carbonate, sodium citrate, copper sulphate. It is used to detect the pressure of reducing sugars.

Phenolic compound: Treat the extract with 3 ml of 10% lead acetate solution. A bulky white precipitate indicated the presence of phenolic compounds [12].

All the experiment analyses were carried out in triplicates. The

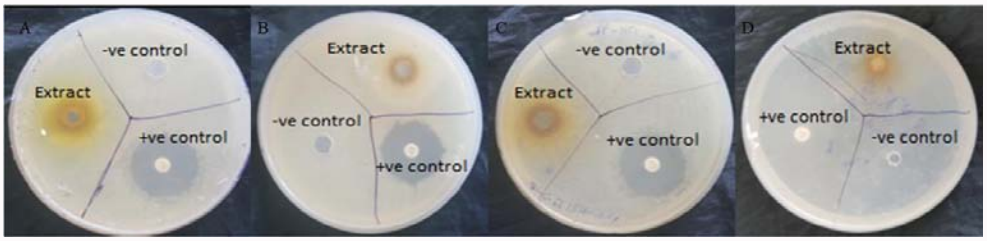

Figure 1: Antimicrobial assay of aqueous extract of plant (A) *Viola odorata* flower, (B) *Tinospora cordifolia* stem, (C) *Bacopa monnieri* leaves and (D) *Mentha piperita* leaves against bacteria *Pseudomonas aeruginosa*.

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

Bacterial isolates	Aqueous extract (100 mg/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
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 ($p^*<0.05$).

Table 2: Antioxidant activities (DPPH radical scavenging activity) of aqueous extract of *Viola odorata* flower and *Tinospora cordifolia* stem.

Samples	Concentration of aqueous 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).

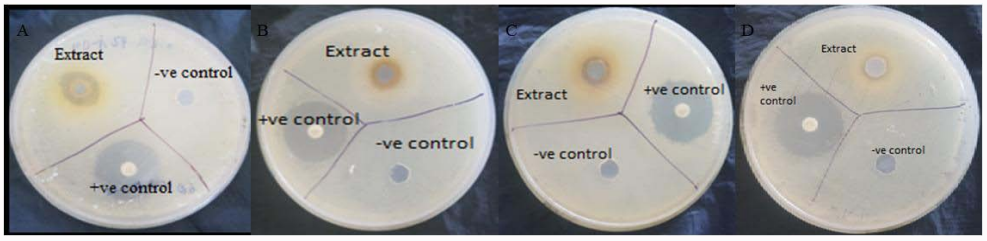

Figure 2: Antimicrobial assay of aqueous extract of plant (A) *Viola odorata* flower, (B) *Tinospora cordifolia* stem, (C) *Bacopa monnieri* leaves and (D) *Mentha piperita* leaves against bacteria *Staphylococcus aureus*.

results are expressed as mean values and Standard Deviation (SD). The results were analyzed using one-way Analysis of Variance (ANNOVA) 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 activity

To determine the antimicrobial properties of medicinal plants, aqueous extracts of these medicinal plants prepared and observed the activity of these plants against pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*) and fungus (*Candida albicans*) strains by well diffusion method.

The results of the antimicrobial activity of aqueous extracts of *Viola odorata* flower showed maximum zone of inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (16 mm and 8.5 mm, respectively) as shown in (Table 1) and (Figure 1). The aqueous extracts of *Tinospora cordifolia* stem, *Viola odorata* flower, *Mentha piperita* and *B. monnieri* leaves had shown antifungal activity against *Candida albicans* with no significant difference (Table 1) and (Figure 3).

Antioxidation activity by DPPH Free Radical Scavenging Assay: DPPH (1,1-diphenyl-2-picrylhydrazyl) was used in the form of free radical to know the antioxidant potential of the compounds present in the plants extracts (*Tinospora cordifolia* and *Viola odorata*). The plants *Tinospora cordifolia* and *Viola odorata* exhibited an antioxidant activity in dose- dependent manner (Figure 4 and 5) respectively. The aqueous extract of *Tinospora cordifolia* stem at different doses exhibited non significant ($p<0.05$) antioxidant activity as compared to *Viola odorata*.

The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower the IC_{50} value, the higher the antioxidant activity of samples (Li et al. 2009). The IC_{50} value of aqueous extract of *Viola odorata* was 3.8 µg/ml and for *Tinospora cordifolia* it was 3.4 µg/ml as shown in (Table 2).

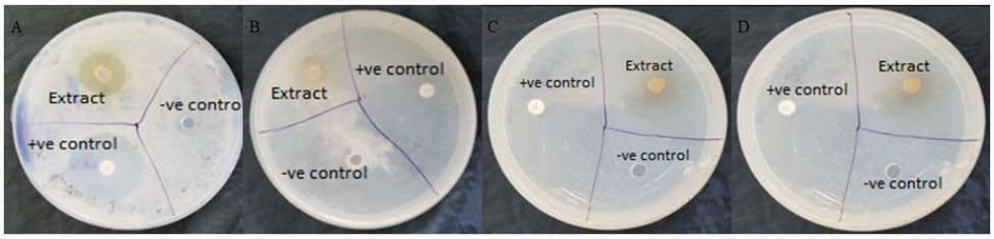

Figure 3: Antimicrobial assay of aqueous extract of plant (A) *Viola odorata* flower, (B) *Tinospora cordifolia* stem, (C) *Bacopa monnieri* leaves and (D) *Mentha piperita* leaves against Fungus *Candida albicans*.

Table 3: FT-IR analysis of aqueous extract of *Viola odorata* flower.

Sr. no.	Wave no. (cm^{-1}) (cm^{-1}) test sample	Wave no. (cm^{-1}) Reference	Function group	Phytocompounds 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 aqueous extract of *Tinospora cordifolia*.

Sr. no.	Wave no. (cm^{-1}) test sample	Wave no. (cm^{-1}) Reference	Function group	Phytocompounds Identified
1	3520	3570- 3200	O-H Stretch, Hydroxy	Polyhydroxy compound
2	3373	3570- 3200	O-H Stretch, Hydroxy group, H- Bonded	Polyhydroxy compound
3	2924	2865- 2845	Symmetric stretching of –CH (CH2)	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 Tertiary Compound
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

Table 5: Phytochemicals analysis of *Viola odorata* flower and *Tinospora cordifolia* stem.

Phytochemical test	Compound detected	*Viola odorata* flower	*Tinospora 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.

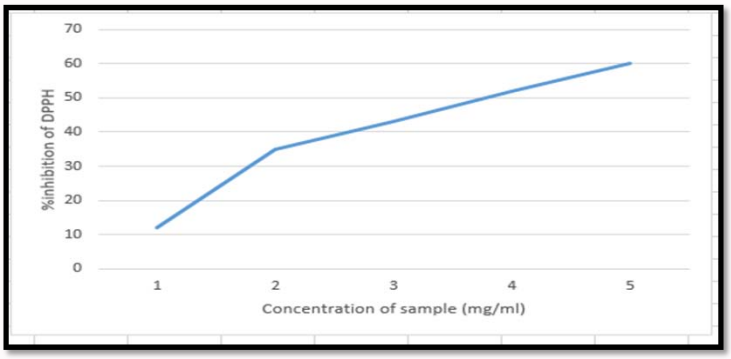

Figure 4: Graphical representation of antioxidant activity of *Viola odorata* flower.

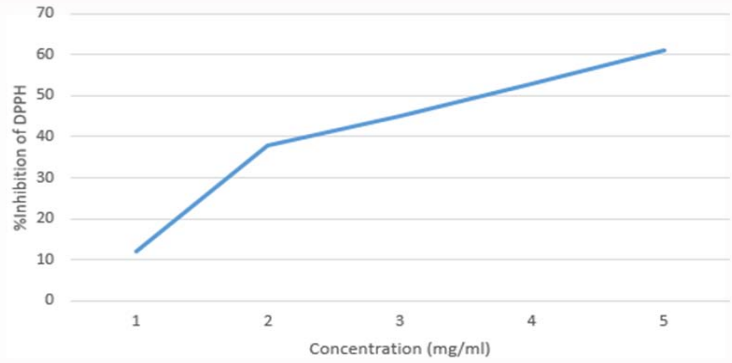

Figure 5: Graphical representation of antioxidant activity of *Tinospora cordifolia* leaves.

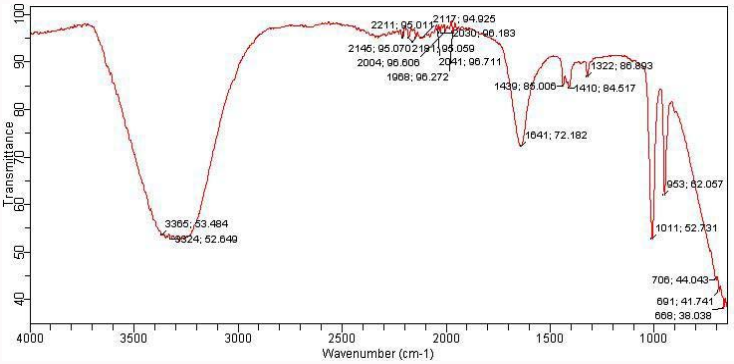

Figure 6: FT-IR of Aqueous extract of *Viola odorata* flower.

FTIR spectroscopy

The aqueous extracts of *Viola odorata* and *Tinospora cordifolia* was examined using FTIR spectroscopy for the presence of functional groups present. The FTIR spectrum for aqueous of *Viola odorata* and *Tinospora cordifolia* had shown in (Figures 6 and 7), respectively and explained below in (Tables 3 and 4).

Phytochemicals analysis of plant extract

Several tests were performed to test the presence of phytochemicals in the aqueous extract of *Viola odorata* and *Tinospora cordifolia*. Summary of the entire test performed and there results are shown in (Table 5). The color change and the precipitate formed determine whether the test is positive or negative. The colored compounds and

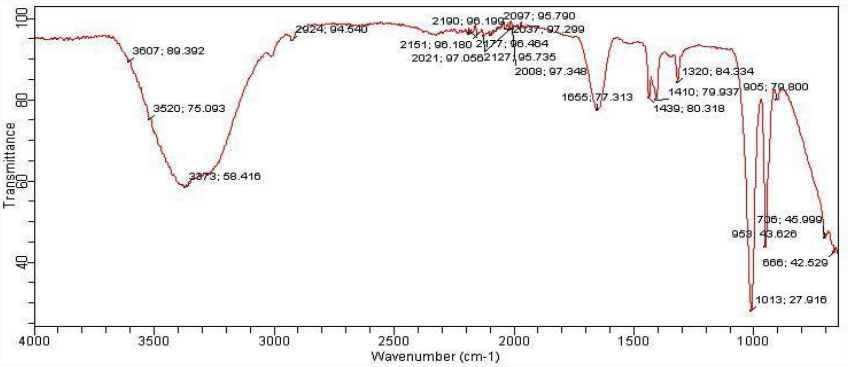

Figure 7: FT-IR of aqueous extract of *Tinospora cordifolia*.

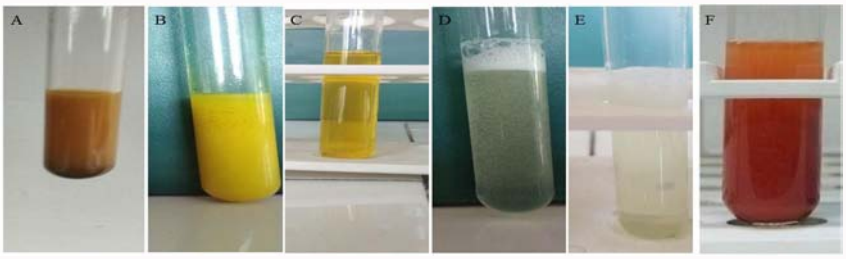

Figure 8: Phytochemical assay of aqueous extract of plant *Viola odorata* flower (A) Sample, (B) Mayer's Test for Alkaloids (C) Alkaline test for Flavonoids (D) Saponins test for Saponins (E) Lead acetate's test for Phenolic compounds (F) Benedict's test for Carbohydrates.

precipitate formed are shown in (Figure 8). Phytochemical properties of plant extracts showed the presence of bioactive components like alkaloids, saponins, flavonoids, carbohydrates and phenolic compounds in varying amount.

Discussion

A comparative study was done on *Viola odorata* against selected respiratory tract pathogens i.e. Haemophilus influenza, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Streptococcus pyogenes* [13]. Methanol extracts exhibited a higher degree of antibacterial activity as compared to aqueous, acetone and petroleum ether extracts. A similar study was done on antimicrobial activity of the methanolic leaf extract of locally available *Mentha piperita*, which showed activity against clinical isolates of *Escherichia coli*, *Acinetobacter, Staphylococcus aureus* and two fungi such as *Candida albicans*, *Candida glabrata* [14]. A comparative was also done on anti-microbial character of shade dried and powdered *T. cordifolia* against *Escherichia coli*. It is seen that the ethanolic and methanolic extracts of the leaf showed good zone of inhibition [15].

Another study was done on anti-microbial activity of the methanolic extract of the leaf callus of *Bacopa monnieri* at the concentrations 0.25, 0.05 and 0.03 mg/disc was investigated for its antimicrobial activity by modified Kirby-Bauer diffusion method. The finding of this study revealed that the extract of the leaf callus of *Bacopa monnieri* possessed a dose dependent antimicrobial activity against all the tested bacterial and fungal species indicated by the zones of inhibition of the microbial growth [16].

Antioxidant properties of medicinal plants are well known and have emerged as potential antioxidant agent. Medicinal plants contain various compounds having the scavenging properties of free radicals [17]. Previously antioxidant analysis of *Viola odorata* flower collected from Serbia showed free radical scavenging activity [18]. A similar study was conducted on different solvent extracts of *T. cordifolia* leaf and stem. This study concluded that the methanol and ethyl acetate extract of this plant stem had most potent antioxidant properties [19].

The phytochemicals present in plants are responsible for preventing disease and promoting health. Phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of Low Density Lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol normalizing blood pressure and clotting [20]. A comparative study was done on the phytochemical screening of various plant extracts revealed the presence of tannins, steroids, flavonoids, cardiac glycosides and saponins. These results suggested that solvent extracts of plants had the presence of many phytochemicals and can also be used for developing novel antimicrobial biorationals of plant origin [21]. Flavonoid, saponin and alkaloids had been reported in *V. odorata* [22]. The phytochemical screening of *T. cordifoila* stem revealed the presence of phenols, flavanoids, alkaloids and saponins. This study concluded that the stem of this plant can used in the pharmaceutical industries [23].

Conclusion

From the above study we have concluded that antimicrobial activity of aqueous extracts of *Viola odorata* flower showed maximum activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The aqueous extracts of all the selected folk medicinal plants had shown good antifungal activity against *Candida albicans* with no significant difference. It was used in the form of free radical to know the antioxidant potential of the compounds present in the plants extracts (*Tinospora cordifolia* and *Viola odorata*) concluded that the plants *Tinospora cordifolia* and *Viola odorata* exhibited an antioxidant activity in dose-dependent manner with no significant difference. The aqueous extract of *Tinospora cordifolia* stem at different doses exhibited no significant ($p^{*}<0.05$) difference in the

antioxidant activity as compared to *Viola odorata*. In case of FT-IR spectroscopy analyses, it showed that in *Viola odorata* had maximum phytocompounds or functional group present as compared to *Tinospora cordifolia*. Phytochemical properties of plant extract (*Viola odorata*) showed the presence of bioactive components like alkaloids, saponins, flavonoids, carbohydrates and phenolic compounds in varying amount.

This study indicates that these folk medicinal plants have nutraceutical potential in them and can be further use for drug discovery.

Acknowledgment

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