Original Article

Phytochemical Screening And Antimicrobial Potential Of Viola Odorata Flower Hydroalcoholic Extract

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Abstract

Infectious (or communicable) diseases are not only a concern in the past, but they are also an issue now in both developing and rich countries. Pathogenic germs such as fungus, bacteria, parasites, and viruses cause it. Pathogenic microorganisms have been fought with therapeutic herbs. Herbal medications are commonly used in healthcare due to their inexpensive cost and abundance of antibacterial qualities. Herbal remedies have been utilised in most countries since ancient times, but in Asia, various medicinal plants are extensively employed as a therapy for infectious diseases in rural and backward areas. The goal of this study was to determine the antibacterial properties and phytochemical content of Viola odorata flower hydroalcoholic extract. The well-known test technique in the literature was used to determine the qualitative analysis of several phytochemical elements. The standard ofloxacin, ciprofloxacin (10-30g/ml), was used to test in vitro antimicrobial activity against *Streptococcus mutans, Bacillus subtilis, Staphylococcus aureus, Salmonella bongori, Klebsiella pneumoniae, and Escherichia coli* using the well diffusion assay method. The antibacterial activity was measured in millimetres by measuring the diameter of the inhibitory zone (mm). Hydroalcoholic extract of flower had antibacterial efficacy against all microbes in a concentration-dependent manner, but it was less than standard medication. The presence of these phytochemicals is thought to be responsible for the plant's antibacterial properties. Phytochemicals responsible for these effects can be identified and manipulated for pharmaceutical purposes in future investigations.

Keywords: Infectious diseases, *Viola odorata*, Antimicrobial activity, Hydroalcoholic extract.

INTRODUCTION

Due to the widespread idea that green medicine is safe, easy to obtain, and has fewer side effects, herbal medicines have become more popular in the treatment of all ailments. Many plants are less expensive and more accessible to the majority of people, especially in developing countries, than orthodox treatment, and they have a lower risk of side effects. These factors may explain for their widespread popularity and use [1]. Some researches have documented the therapeutic benefits of certain plants [2-4]. The main source of innovative medications and healthcare items is medicinal plants [5]. The extraction and characterisation of various phyto chemicals from these green factories has resulted in the development of a number of medicines with high activity profiles [6]. Indeed, market and public demand have grown to such an extent that many therapeutic plants are now at risk of extinction or genetic diversity loss [7]. The continuing appearance or persistence of drug-resistant organisms, as well as pathogenic organisms' increasing evolutionary adaptations to routinely used antimicrobials, have lowered the efficacy of currently used antimicrobials. Furthermore, antibiotics are linked to side effects, necessitating the hunt for alternative medications from unconventional sources such as plants [8]. Plants are still a key source of commercially consumed medications. Many synthetic medications are derived from natural plant components as well. In recent years, there has been an upsurge in the use of natural goods, and active plant extracts are regularly evaluated for new medication discoveries [9]. Because there is little information on the antimicrobial activity of the Viola odorata Linn flower, the current study looks at the antimicrobial activity of hydroalcoholic extracts from the flower extract against bacterial and fungal species. These extracts are also subjected to preliminary phytochemical analyses in order to identify bioactive chemicals with antibacterial activity.

MATERIALS AND METHODS

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. Mumbai, India), SD Fine- Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade. Quercetin, atropine and gallic acid were kindly provided by Scan Research Laboratories, Bhopal (India). The test

organisms *Streptococcus mutans*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella bongori*, *Klebsiella pneumonia* and *Escherichia coli* were obtained from the stocks of Scan Research Laboratories, Bhopal (India).

Collection of plant

Fresh flowers of *Viola odorata* Linn were collected from Minor Forest Produce Processing & Research Centre, Vindhya Herbals Bhopal. The flowers were washed thoroughly with normal tap water followed by sterile distill water. Then flowers were dried under shaded condition at room temperature. Flowers were crushed to powder using grinding machine. Powder was stored at 4ºC in tight air container bottle.

Extraction by maceration Method

53 gram of powdered flowers of *Viola odorata* Linn were exhaustively extracted with different solvent (Petroleum ether, Chloroform, Ethyl acetate, Hydroalcoholic and Distilled water) by maceration method. The extract was evaporated above their boiling points. Finally, measured the percentage yield of the dried extracts. The recovered extracts were then reduced in a rotary evaporator and finally stored in airtight containers at 4◦C for further use.

Phytochemical screening

The qualitative chemical experiments were carried out with some modifications for different extracts according to the methods mentioned in parkhe et al., [10].

Antimicrobial activity

Sterilization of Instruments and glassware's: At first, all instruments which were used in laboratory were made sterile, all glassware's like Erlenmeyer flask, graduated cylinders, stirring rods, beakers, test tubes, petri dishes, inoculating loops, that were used in the assay were placed in an autoclave at 121°C under 15 psi pressure for 25 min by using autoclave and followed aseptic technique method.

Preparation of nutrient agar media (NAM): Nutrient agar media was prepared for growing of bacteria inside the laboratory. The standard size (100mm× 15mm) petri dishes as required for whole experiment. For preparation of NAM, 28 gram NAM powder was mixed with 1000 ml of distilled water and stirred to obtain homogenized mixture. After which, NAM mixture was placed in Autoclave under 15 psi pressure, at 121°C for 25 min for sterilization of media. After that poured the culture media into petri dishes at ratio of 20 ml/dish and was left half covered on the table to let the agar cool down and solidify at room temperature.

Antimicrobial activity: Antimicrobial activity of hydroalcoholic extract obtained from of *Viola odorata* Linn was determined, using the agar well diffusion assay method as described by Holder and Boyce [11]. The aqueous extract used to suitably dilute upto the concentrations of 100, 50 and 25 milligram per ml. The plates were done in triplicates and were incubated at 37°C. The antimicrobial activity was taken on the basis of diameter of zone of inhibition, which was measured after 24 hours of incubation and the mean of three readings is presented. The aqueous extract was dissolved in distilled water.

Statistical analysis: Each experiment has three replicates and three determinations were conducted. Means of variable and standard deviation were recorded.

RESULTS AND DISCUSSION

The crude extracts so obtained after each of the successive maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the flower extract of *Viola odorata* using hydroalcoholic as solvent isdepicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder flower extract of *Viola odorata* are shown in Table 2. The antimicrobial activity of hydroalcoholic flower extract of *Viola odorata* showed bioactivity by inhibiting growth of microbial species selected for the test as shown in table 4 and 5. The zone of inhibition shown by the extracts was comparable to the standard drug. It is effective against *Streptococcus mutans*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella bongori*, *Klebsiella pneumonia* and *Escherichia coli* in concentration dependent manner.

Table 1: Extractive values of *Viola odorata* Linn

1.	Hydroalcoholic	9.33	Green

Table 2: Result of Phytochemical screening of *Viola odorata* Linn

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Hager's Test:	 +Ve
2.	**Glycosides** Legal's Test:	+Ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 +Ve +Ve
4.	**Diterpenes** Copper acetate Test:	 +Ve
5.	**Phenol**	

	Ferric Chloride Test:	+Ve
6.	**Proteins** Xanthoproteic Test:	+Ve
7.	**Carbohydrate** Fehling's Test:	+Ve
8.	**Saponins** Froth Test:	+Ve
9.	**Tannins** Gelatin test:	-Ve

+Ve = Positive, -Ve= Negative

Table 3: Antimicrobial activity of standard drug against selected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition (mm)		
			10 µg/ml	20 µg/ml	30 µg/ml
1	Ofloxacin	*Streptococcus mutans*	12±0.15	15±0.13	17±0.19
2.	Ciprofloxacin	*Bacillus subtilis*	12±0.5	17±0.74	20±0.15
		Staphylococcus aureus	25±0.47	29±0.47	34±0.47
		Salmonella bongori	17±0.15	23±0.86	25±0.5
		Klebsiella pneumoniae	23±0.47	26±0.47	28±0.47
		Escherichia coli	22±0.47	26±0.47	30±0.47

*Average of three determination, Mean ± SD

Table 4: Antimicrobial activity of hydroalcoholic extract of *Viola odorata* Linn against selected microbes

S. No.	Name of microbes	Zone of inhibition (mm)		
		Hydroalcoholic extract		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Streptococcus mutans*	9±0.47	10±0.47	12±0
2.	*Bacillus subtilis*	7±0	9±0.47	10±0.47
3.	*Staphylococcus aureus*	10±0.47	11±0.47	13±0.47
4.	*Salmonella bongori*	9±0.47	11±0.47	13±0.47
5.	*Klebsiella pneumoniae*	11±0.47	12±0	15±0.47
6.	*Escherichia coli*	10±0.47	11±0.47	13±0.47

*Average of three determination, Mean ± SD

CONCLUSION

Extracts of *Viola odorata* Linn in this study demonstrated a broad-spectrum of antimicrobial activity against selected microbial species. The antimicrobial activity of the plant extract, possibly due to the identified phytoconstituents, further confirms its use as a health remedy in folklore medicine. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial infections. Identification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view of formulating novel chemotherapeutic agents should be the future direction for investigation

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