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Extraction, Phytochemical Screening and Antioxidant Potential of Hydroalcoholic Extract of *Viola odorata Linn*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Bioactive chemicals found in medicinal plants are used to treat a variety of human ailments and serve a vital part in healing. Alkaloids, flavonoids, phenol, saponin, steroids, and tannins are examples of secondary components. Anticancer, antibacterial, antidiabetic, anti-diuretic, and anti-inflammation properties are all found in medicinal plants. The growing interest in secondary metabolites' significant biological action has highlighted the need of assessing their presence in therapeutic plants. *Viola odorata* (Viola odorata, Violaceae) is a Viola species native to Europe and Asia that has recently been introduced to North America and Australasia. In India, the plant is known as Banafsa, Banafsha, or Banaksa, and it's often used to treat sore throats and tonsillitis. It was known to be used to treat cancer-related pain. It has traditionally been used to treat anxiety, sleeplessness, and high blood pressure. Alkaloids, glycosides, saponins, methyl slicylate, mucilage, and vitamin C are all found in it. Antioxidant, analgesic, antihypertensive, and diuretic properties have been documented for the plant. The goal of this research is to identify phytochemicals, assess phenolic and flavonoid content, and measure the antioxidant potential of *V. odorata* flowers. The well-known test methodology was used to determine qualitative analysis of various phytochemical elements as well as quantitative analysis of total phenol and flavonoids. The antioxidant activity of a hydroalcoholic extract of *V. odorata* flowers was tested in vitro using the 1,1-diphenyl, 2-picryl hydrazyl (DPPH) assay and the hydrogen peroxide radical scavenging

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technique. Phenols, flavonoids, tannins, saponins, alkaloids, and other phytochemicals were discovered by phytochemical investigation. The total phenolic and flavonoids content of the hydroalcoholic extract of V. odorata flower was 0.521 and 0.637 mg/100 mg, respectively. In the investigated models, the extract showed dose-dependent free radical scavenging properties. For the DPPH technique, the IC_{50} value of *V. odorata* floral extract was 109.78g/ml, which was close to that of ascorbic acid (IC_{50}=27.82g/ml). The IC_{50} value for hydrogen peroxide was discovered to be 85.85g/ml, which compares well to ascorbic acid (IC_{50}=16.48g/ml). The present study describes the phytochemical profile and antioxidant activity of *V. odorata* flower which will further used for medicinal applications.

Keywords: Viola odorata; qualitative; quantitative phytochemical; antioxidant activity.

1. INTRODUCTION

Exogenous substances and endogenous metabolic activities in the human body produce free radicals, or highly reactive oxygen species. “These are capable of oxidising biomolecules such as nucleic acids, proteins, lipids, and DNA, and can cause neurological problems, cancer, emphysema, cirrhosis, atherosclerosis, arthritis, and other degenerative illnesses” [1,2]. “Antioxidants are chemicals that stop free radicals from attacking and thereby lessen the risk of various diseases” [3]. “Almost all organisms are protected from free radical damage to some extent by antioxidant substances such as ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids, and glutathione, as well as enzymes such as superoxide dismutase and catalase. According to Prior and Cao [4], antioxidant supplements or dietary antioxidants protect against free radical damage”. “Natural antioxidants are currently receiving a lot of interest as a way to protect the human body, particularly brain regions, from oxidative damage produced by free radicals. Several medicinal plants have proven such efficacy using classic psychoneuropharmacology approaches in the last two decades” [5]. With this in mind, the current study was carried out to assess the antioxidant activity of *V. odorata*, which has been traditionally used for a variety of purposes. Banafshah is the common name for Viola odorata (Violaceae). It may be found at high altitudes in the Himalayas, Europe, and North America. It is a trailing plant with a height of less than 6 inches. The subterranean stem of the plant is thick and scaly, containing rooted runners. “It has heart-shaped leaves with scalloped or slightly serrated edges that are dark green, smooth, or occasionally downy beneath and create a rosette at the plant's base. The flowers range in hue from deep purple or blue to pinkish or even whitish-yellow” [6,7]. The odorous principle, blue colouring substance, and sugar, a glucoside, are all found in the flowers of *V. odorata*. Viola-quercetin may be found everywhere over the plant. This plant has also yielded salicylic acid (natural aspirin) [8]. The roots, leaves, flowers, and seeds of *V. odorata* contain the alkaloid violine. Ionine, saponins, glycosides, methyl salicylate, mucilage, vitamins A and C, and alkaloids are all found in V. odorata essential oil [9]. 4.0 percent anthocyanins, 1.1 percent flavonoids, 18.0 percent mucilage, and 8.5 percent ash are found in *V. odorata* flowers [10]. “In bronchitis, cancer, cough, fever, urinary infections, rheumatism, sneezing, and kidney and liver problems, the entire aerial section of *V. odorata*, including stem, flowers, and leaves, is utilised" [11,12]. The most common form of the medication is a syrup produced from flower petals, which 1 to 2 drachms can be administered to newborns for coughs and chest tightness. In cases of fever, a cooling infusion (2 drachms of the flower in a pint of warm water) is administered in doses of 1–2 oz [6]. The cytotoxicity and antipyretic efficacy of *V. odorata* have been studied [13,14]. The present study was focused to evaluate the phytochemical analysis and antioxidant activity of flowers of *V. odorata*.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh *V. odorata* flowers were harvested from Vindhya Herbals Bhopal's Minor Forest Produce Processing & Research Centre. The plant material (flower portion) chosen for the research was properly cleaned under running tap water and then rinsed in distilled water before being allowed to dry at room temperature for a period of time. The plant material was then shade dried for 3 to 4 weeks without being contaminated. An electric grinder was used to ground dried plant material. Color, odour, taste, and texture of powdered plant material were evaluated. For

phytochemical and biological experiments, dried plant material was placed in an airtight container and preserved.

2.2 Chemical Reagents

Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) provided all of the chemicals utilised in this work (Mumbai, India). The chemicals and solvents utilised in this experiment were all of analytical grade.

2.3 Defatting of Plant Material

The 53 gramme shade dried powder of flowers of *V. odorata* was defatted using the maceration process and extracted with petroleum ether. The extraction process was maintained until the material had been defatted.

2.3.1 Successive extraction with different solvents by maceration method

Water, hydroalcoholic, ethyl acetate, and chloroform were used to extract plant material in four different polarity solvents. The maceration process was used to remove powdered plant components. To get the dry concentrated extract, the resulting content was filtered using Whatman filter paper no. 1 and stored for solvent evaporation. The extractive yield was calculated by weighing the dried crude concentrated extract, which was then transferred to glass vials (6 2 cm) and kept in a refrigerator (4°C) until utilised for analysis [15].

2.3.2 Screening for phytochemicals

Standard phytochemical screening protocols were used to determine the presence of bioactive compounds [16,17]. The tests were identified by visual inspection of colour change or precipitate formation after the addition of particular reagents to the solution.

2.3.3 Estimation of total phenolic content

The modified Folin-Ciocalteu technique [18] was used to determine the total phenolic content of the extract. In methanol, 10 mg Gallic acid was dissolved in 10 ml methanol, and different aliquots of 5-25g/ml were produced. We dissolved 10 mg of dried extracts in 10 ml methanol and filtered it. For the phenol determination, two millilitres (1 mg/ml) of this solution were utilised. 1 ml Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) sodium carbonate were combined with 2 ml of each extract or standard. The mixture was vortexed for 15 seconds before being let to sit for 15 minutes to develop colour. A spectrophotometer was used to measure the absorbance at 765 nm.

2.3.4 Estimation of total flavonoids content

The total flavonoids content was determined using the aluminium chloride technique [19]. In 10 ml methanol, 10 mg quercetin was dissolved, and different aliquots of 5- 25g/ml were made in methanol. We dissolved 10 mg of dried extracts in 10 ml methanol and filtered it. The flavonoid concentration was determined using three millilitres (1 mg/ml) of this solution. 1 ml of a 2 percent $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to remain at room temperature for 15 minutes before measuring absorbance at 420 nm.

2.4 Antioxidant Activity

2.4.1 DPPH radical scavenging assay

The spectrophotometer was used to test DPPH scavenging activity with minor modifications [20]. The stock solution (6 mg in 100 mL methanol) was produced to give an initial absorbance of 1.5 mL in 1.5 mL methanol. After 15 minutes, there was a decrease in absorbance in the presence of sample extract at various concentrations (10-100 g/ml). 1.5 mL of DPPH solution was added to 3 mL of methanol, and the absorbance was measured at 517 nm for the control reading. A series of volumetric flasks were filled with 1.5 ml of DPPH and 1.5 ml of the test material at various concentrations. For each concentration, the absorbance at zero time was measured. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

2.4.2 Hydrogen peroxide

The hydrogen peroxide (H_2O_2) scavenging activity of the plant extract was evaluated using the Ruch et al, technique [21]. 0.6 ml of 4 mM H2O2 solution made in phosphate buffer (0.1 M pH 7.4) was combined with 4 ml of ethanolic extract (4 ml) prepared in distilled water at varied concentrations and incubated for 10 minutes.

The solution's absorbance was measured at 230 nm. The chemical ascorbic acid was utilised as a positive control. Using the following equation, the percentage of inhibition was estimated by comparing the absorbance values of the control and test samples.

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

Where Acontrol = absorbance of the blank control (containing all reagents except the extract solution) Asample = absorbance of the test sample.

Table 1. Results of percentage yield of flowers extracts

Sr. No	Solvents	% Yield (W/W)
1	Petroleum ether	1.52
2	Chloroform	4.35
3	Ethyl acetate	3.78
4	Hydroalcoholic	9.33
5	Distilled water	5.44

3. RESULTS AND DISCUSSION

To achieve the real yield of extraction, the crude extracts produced after each consecutive maceration extraction step were concentrated on a water bath by fully evaporating the solvents. Table 1 shows the yield of extracts produced from plant flowers employing solvents such as chloroform, ethyl acetate, hydroalcoholic, and water. Table 2 shows the findings of a qualitative phytochemical study of the crude powder of *V. odorata* flower. Alkaloids, diterpenes, glycosides, flavonoids, saponins, phenols, proteins, and carbohydrate were found in hydroalcoholic and aqueous extracts of *V. odorata*. Using the equation derived from the calibration curve, the total phenolic content (TPC) of the dry extract sample was expressed as mg/100mg of gallic acid equivalent: $y = 0.019x + 0.016$, $R^2 = 0.999$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $y = 0.032x + 0.002$, $R^2 = 0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance. TPC of *V. odorata* hydroalcoholic extract revealed concentration values of 0.521, which were followed by TFC of 0.637. Figs. 1&2 and Table 3 the hydrogen donating nature of extracts was evaluated using the DPPH radical scavenging assay [22]. The inhibitory concentration 50 percent (IC_{50}) of *V. odorata* hydroalcoholic floral extract was determined to be 109.78g/ml when compared to ascorbic acid (27.82g/ml) in DPPH radical scavenging activity. Table 4 and Fig. 3 show dose-dependent action with regard to concentration. Numerous oxidase enzymes and activated phagocytes produce hydrogen peroxide in vivo, and it is known to have a role in the death of several bacterial and fungal species [23]. “There is growing evidence that hydrogen peroxide can operate as a messenger molecule in the creation and activation of numerous inflammatory mediators, either directly or indirectly via its reduction product, Oho” [24]. “The loss of H2O2 may be determined when a scavenger is treated with H_2O_2 using a peroxidase test method. The effectiveness of *V. odorata* hydroalcoholic floral extract and ascorbic acid to scavenge hydrogen peroxide at varied concentrations is shown in Table 5 and Fig. 4. At all concentrations examined, extracts were capable of scavenging

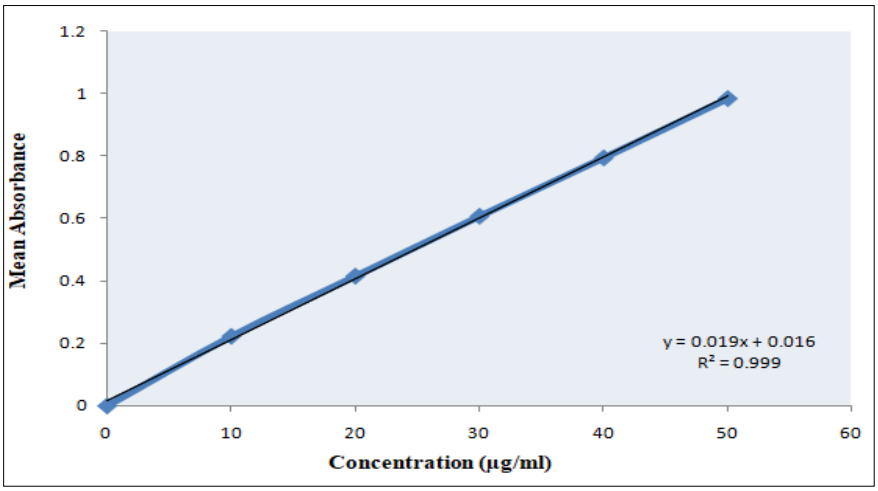

Fig. 1. Graph of calibration curve of gallic acid

Table 2. Result of phytochemical screening of extracts of *V. odorata*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Hydroalcoholic extract	Aqueous Extract
1.	**Alkaloids**				
	Hager's Test:	-Ve	-Ve	+Ve	-Ve
2.	**Glycosides**				
	Legal's Test:	-Ve	-Ve	+Ve	-Ve
3.	**Flavonoids**				
	Lead acetate Test:	-Ve	+Ve	+Ve	-Ve
	Alkaline test:	-Ve	-Ve	+Ve	+Ve
4.	**Diterpenes**				
	Copper acetate Test:	-Ve	-Ve	+Ve	+Ve
5.	**Phenol**				
	Ferric Chloride Test:	-Ve	-Ve	+Ve	+Ve
6.	**Proteins**				
	Xanthoproteic Test:	+Ve	-Ve	+Ve	-Ve
7.	**Carbohydrate**				
	Fehling's Test:	+Ve	-Ve	+Ve	+Ve
8.	**Saponins**				
	Froth Test:	-Ve	-Ve	+Ve	+Ve
9.	**Tannins**				
	Gelatin test:	-Ve	-Ve	-Ve	-Ve

Table 3. Results of total phenol and flavonoids content

S. No.	Extracts	Total phenol content	Total flavonoids content
		mg/100mg	
1	Ethyl acetate	0.253	0.365
2	Hydroalcoholic	0.521	0.637
3	Aqueous	0.425	0.569

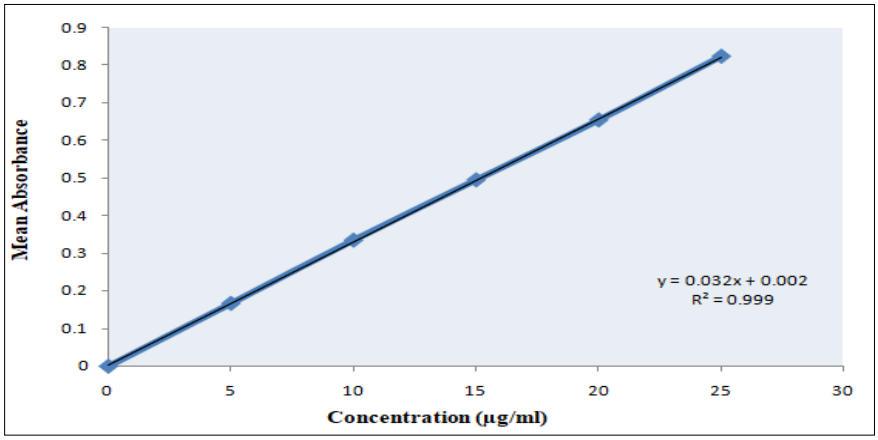

Fig. 2. Graph of calibration curve of Quercetin

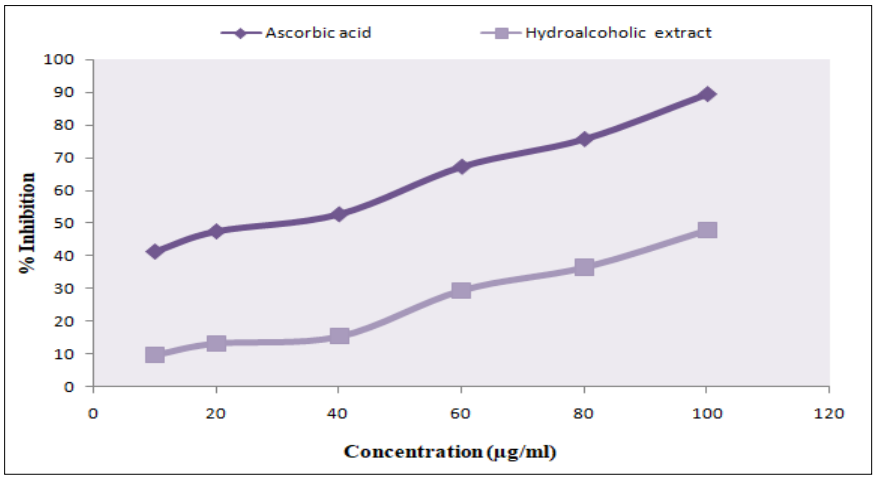

Fig. 3. % Inhibition of ascorbic acid and extract of *V. odorata*

Table 4. % Inhibition of ascorbic acid and extract of *V. odorata* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	41.52	9.8
2	20	47.70	13.3
3	40	52.92	15.4
4	60	67.43	29.52
5	80	75.89	36.51
6	100	89.63	47.85
IC 50		**27.82**	**109.78**

Table 5. % Inhibition of ascorbic acid and extract of *V. odorata* using H_2O_2 method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	20	46.58	15.85
2	40	67.52	23.52
3	60	76.52	30.54
4	80	83.52	48.21
5	100	91.85	59.52
IC 50		**16.48**	**85.85**

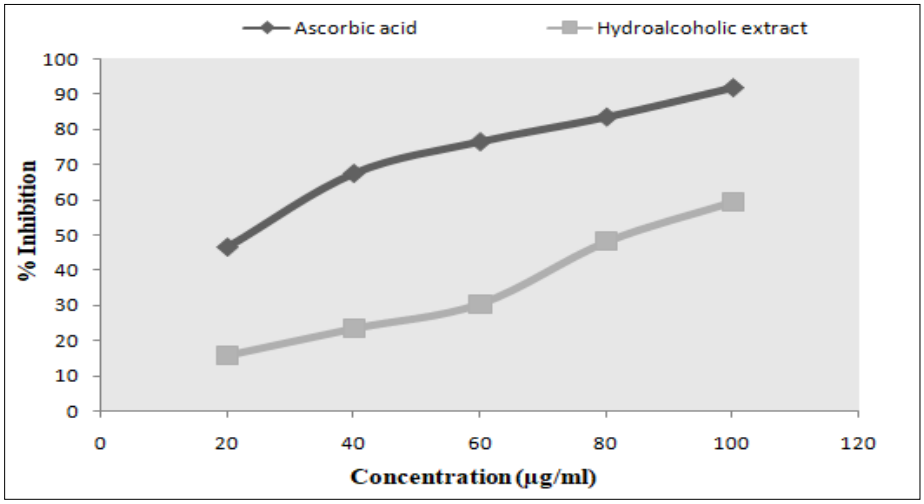

Fig. 4. % Inhibition of ascorbic acid and extract of *V. odorata*

hydrogen peroxide in an amount-dependent manner. At normal ambient or cellular concentrations and temperatures, hydrogen peroxide is a very weak oxidant, and most organic compounds (save for few sulfur-containing molecules) are almost immune to it. In the presence of decreased transition metal ions, however, the Fenton reaction converts hydrogen peroxide to the far more aggressive oxidant, hydroxyl radical, in the cells. Other transition metals, such as copper (I), cobalt (II), and nickel (II), have also been shown to participate in the process" [23]. As a result, the removal is critical for antioxidant defence in cell and food systems.

4. CONCLUSION

The findings of this study show that the extract may efficiently scavenge several reactive oxygen species/free radicals in vitro. This might be because it can produce a large number of stable oxidised products after oxidation or radical scavenging. The extracts' wide spectrum of action shows that antioxidant activity is mediated by many pathways. The extract's various antioxidant activities exhibited in this study clearly reveals the plant's potential application value. However, before V. odorata may be used as an antioxidant component in animal feeds or human health foods, its in vivo safety must be carefully explored in experimental rodent models. The above results showed that *V. odorata* hydroalcoholic flower extract could exhibit antioxidant properties. Further studies, on the use of above plants for their antioxidant role in various systems may provide potential natural antioxidants.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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