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## **Qualitative Analysis of *In Vivo* and *In Vitro* Propagated *Viola odorata* L.**

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### **ABSTRACT**

The present study was conducted to evaluate the qualitative phytochemical composition of ethanolic extracts obtained from *invivo* and *in vitro* propagated *Viola odorata* L. Phytochemical screening was carried out using standard qualitative tests for alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates, terpenoids, and glycosides. The results revealed the presence of most phytochemical constituents in both extracts, indicating comparable phytochemical profiles between *in vivo* and *in vitro* propagated plants. Only Wagner's test for alkaloids showed negative results. The findings support the medicinal importance of *Viola odorata* L. and suggest that *in vitro* propagated plants can serve as a reliable alternative source of bioactive compounds for pharmaceutical and medicinal applications.

**Keywords:** *Viola odorata* L., phytochemical screening, medicinal plants, *in vitro*, *in vivo* propagation, secondary metabolites, ethanol extract

## INTRODUCTION

Phytochemical screening is used to extract different plant chemicals to evaluate their biological activity and medicinal potential. Plants possess medicinal value because they contain specific chemical components that produce clear physiological effects on biological systems (Aslam F. *et al.* 2009). Medicinal plants contain various phytochemicals such as alkaloids, carbohydrates, terpenoids, steroids, flavonoids, and tannins, which exert specific physiological effects on the human body and are valuable in the treatment and cure of human disease (Krishnaiah D. *et al.* 2007). Living organisms synthesize these compounds through both primary and secondary metabolic processes. Secondary metabolites are highly diverse in terms of their chemical nature and taxonomy, and their exact functions often remain unclear. These compounds are widely applied in human medicine, veterinary practice, agriculture, scientific research, and numerous other fields (Vasu, K. *et al.* 2009). Plant products have been used in phytomedicine since ancient times. These medicinal substances can be obtained from various plant parts such as bark, leaves, flowers, roots, fruits, and seeds (Criagg, G.M. & David, J.N., 2001).

## MATERIAL AND METHODS

### PREPARATION OF PLANT EXTRACT

The collected plant material of *Viola odorata* L. from natural habitat was washed with tap water to remove soil and dust particles. Washed plant material was first dried in shade after that it was dried in the oven. It was crushed into an electric grinder and weighted. In other hand for *in vitro* propagated plants, firstly removed from culture vessels and then media removed from roots by rinsing with distilled water then dried in the oven and made fine powder by using electric grinder. The powdered plant samples of *in vitro* and *in vivo* propagated *Viola odorata* L (vegetative part). (15g/250 ml) were extracted successively with ethanol using soxhlet apparatus at 55-85°C for 20-24 hr. The method used with slight modification (Eglorashi and Van Staden, 2004). The solvents of the respective extracts were reduced by Rotary evaporator and stored at 4°C for further use.

### PREPARATION OF TESTING REAGENTS

**Mayer's reagent-** 5 gm KI was dissolved in 10ml D/W and 5gm HgCl<sub>2</sub> was in 60 ml D/W. Both were mixed with this solution and the final volume 100ml was made by adding D/W.

**Wagner's reagent-** 2 gm of iodine 6gm of KI was dissolved in 100 ml distilled water.

**Molisch's reagent-** naphthol was added in 95% ethanol.

**Ferric chloride solution-** 2 gm ferric chloride dissolves in 50 ml distilled water.

**Dragendorff reagent-** 2 ml filtrate of plant drug extract, 2 ml of reagent was mixed.

#### TEST FOR ALKALOIDS:

**Mayer's reagent:** Two drops of Mayer's reagent were added along the sides of the test tube into a small amount of plant extract. The presence of alkaloids is depicted by white creamy precipitate.

**Wagner's test:** A few drops of Wagner's reagent were added to a few amounts of plant extract and a reddish-brown precipitate indicated by the presence of alkaloids (Banu and Cathrine, 2015).

**Dragendorff reagent:** To 2 ml filtrate of plant drug extract, 2 ml of reagent was mixed. Formation of reddish brown precipitate indicated the presence of alkaloids.

#### TEST FOR FLAVONOID

**Lead acetate test:** Extract was treated with few drops of lead acetate solution. Yellow colour solution indicated the presence of flavonoids (Tiwari *et al.*, 2011).

**Alkaline reagent test:** A small amount of the extract mixed with few drops of sodium hydroxide. The intense yellow colour solution becomes colourless in addition to dilute acid proved the presence of flavonoids (Saxena *et al.*, 2013).

#### TEST FOR PHENOL

**Ferric chloride test:** 2ml extract was added followed by ferric chloride solution in the test tube. The solution results black colour indicating the presence of phenol.

**Lead acetate test:** 2ml extract was added followed by lead acetate solution in the test tube. The solution results yellow colour indicates the presence of phenol (Santhi and Seggottuvel, 2006).

#### TEST FOR TANNINS

**Ferric chloride test:** 1 ml of extract was added with 10 ml of deionised water and then treated with 3 drops of ferric chloride. A greenish-brown precipitate indicated the presence of tannins.

#### TEST FOR SAPONINS

**Foam test:** A 5 ml aliquot of the extract was diluted with 20ml of deionised water, shaken vigorously and observed. Persistent foaming indicated the presence of saponins (Harbourne, 1998).

#### TEST FOR CARBOHYDRATES

**Molisch's reagent test:** A 3 ml of the aqueous extract was added to 2 ml of Molisch's reagent and the resulting mixture was shaken; 2 ml of concentrated sulfuric acid was poured carefully down the side of the test tube. Formation of a red or dull violet color at the inter-phase of the two layers was indicative of positive test.

### TEST FOR TERPENOIDS

**Salkowski test:** 5ml of plant extract was mixed with 2 ml of chloroform. Then 3ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids (Edioga *et al.*, 2005).

### TEST FOR GLYCOSIDES

**Kellar – Kiliani test:** 2 ml of filtrate was added with 1ml of glacial acetic acid. Then 1ml of ferric chloride was added with 1ml concentrated sulfuric acid. Green-blue coloration of solution indicated the presence of glycoside (Parekh and Chanda, 2007).

### RESULT

Phytochemicals are important plant metabolites classified into primary and secondary metabolites. Secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, glycosides, and saponins are synthesized through primary metabolic pathways and are mainly found in leaves, roots, and stems (Jones and Kossel, 1953; Krishnaiah *et al.*, 2007). These compounds exhibit diverse biological and pharmacological activities, including anesthetic, antioxidant, anti-inflammatory, antimicrobial, antiviral, and therapeutic effects (Hérouart *et al.*, 1988; Hoffmann, 2003; Mahato and Sen, 1997).

*Viola odorata* L. contains several phytochemical constituents, including carbohydrates, saponins, terpenoids, phenols, flavonoids, tannins, coumarins, and proteins (Aslam *et al.*, 2019). In the present study, ethanol extracts of *in vitro* and *in vivo* propagated *Viola odorata* L. were prepared using the Soxhlet extraction method. Preliminary phytochemical screening was carried out to detect major phytochemical groups such as alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates, terpenoids, and glycosides using standard qualitative tests.

The alkaloids were tested by using Mayer's reagent, Wagner's reagent and Dragendorff reagent. The flavonoids were tested by Lead acetate test and Alkaloid reagent test. The phenolic compounds were tested by using Ferric chloride and Lead acetate test. Tannins were detected by Ferric chloride test. Saponins were tested by using foam formation. Carbohydrates tested by Molisch's reagent. Terpenoids were tested by Salkowski test. Glycosides were tested by Kellar – Kiliani test.

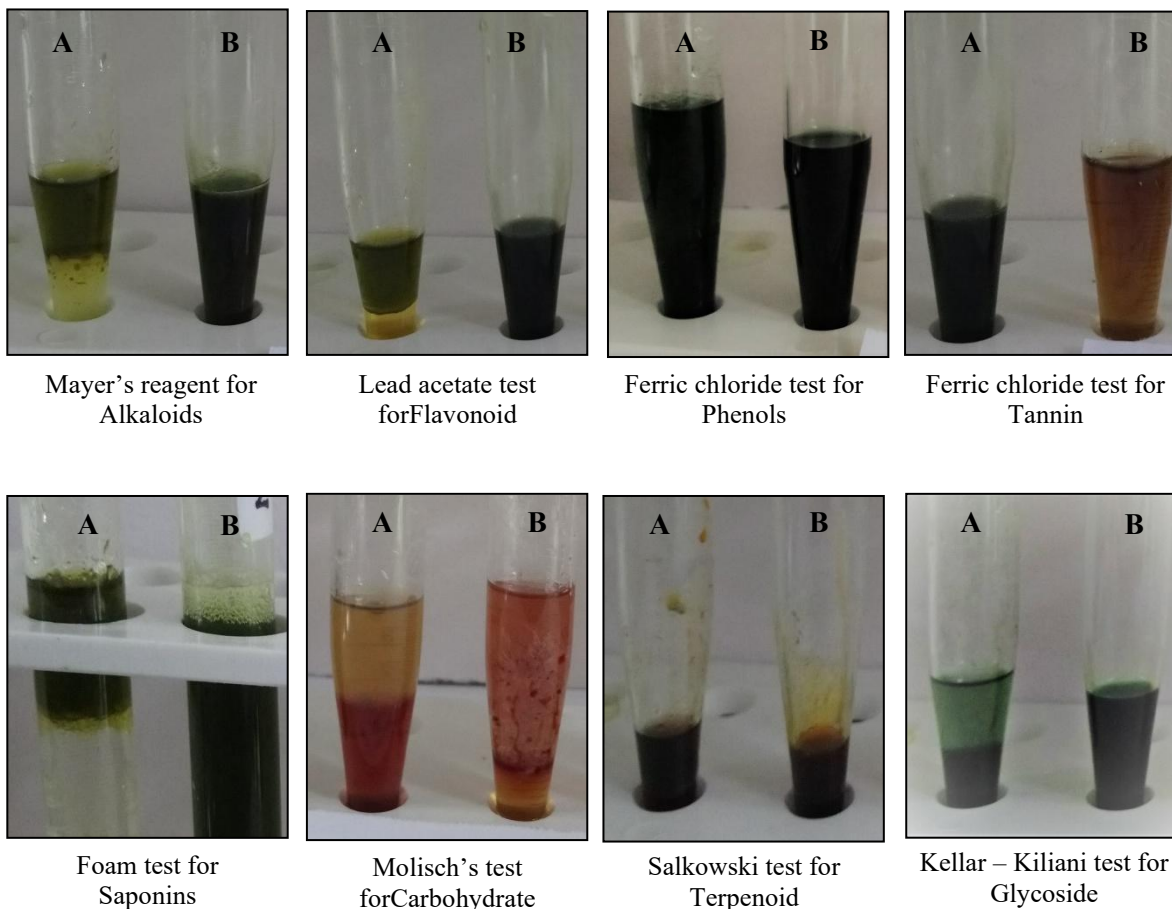
The ethanol extract of *in vitro* and *in vivo* propagated *Viola odorata* L. was tested for presence of phytochemicals. Results are shown in table no.1 and in Figure 1. The tested all phytochemicals like alkaloid, flavonoid, phenol, tannin, saponins, carbohydrate, terpenoids and glycosides were present in ethanol extract of *in vitro* as well as *in vivo* propagated *Viola odorata* L. only alkaloid test by Wagner's reagent showed negative results.

Table No. 1. Qualitative Phytochemical test of ethanol extract of *Viola odorata* L.

Sr. no	Phytochemicals	Test	Ethanol extract of <i>in vitro</i> propagate dplants.	Ethanol extract of <i>in vivo</i> propagated plants.
1	Alkaloids	Mayer's reagent	+	+
		Wagner's reagent	-	-
		Dragendorff reagent	+	+
2	Flavonoids	Lead acetate test	+	+
		Alkaloid reagent test	+	+
3	Phenols	Ferric chloride test	+	+
		Lead acetate test	+	+
4	Tannins	Ferric chloride test	+	+
5	Saponins	Foam formation test	+	+
6	Carbohydrates	Molisch's reagent	+	+
7	Terpenoid	Salkowski test	+	+
8	Glycoside	Keller - Kiliani	+	+

("+" present, "-" Absent)

Figure 1. Qualitative analysis of ethanol extract of *Viola odorata* L.



A- Extract of *in vitro* propagated *Viola odorata* L.,

B- Extract of *in vivo* propagated *Viola odorata* L.

## DISCUSSION

The qualitative analysis of phytochemicals was carried out using simple screening tests. The ethanolic extracts of both *in vivo* and *in vitro* propagated *Viola odorata* L. were found to contain various phytochemicals, including alkaloids, flavonoids, phenolic compounds, saponins, glycosides, and carbohydrates. Similar findings have been reported in the aqueous leaf extract of *Viola odorata* L. (Siddiqi *et al.*, 2012) as well as in its flower extract (Sharma *et al.*, 2019).

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