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Original Research Article

Phytochemical investigation, isolation and estimation of total phenolic contents & total flavonoids contents of leaves and flowers extracts of Hippeastrum vittatum

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Abstract: ***Background:*** Adolescent depression is a growing global concern, often underdiagnosed and undertreated. Cognitive Behavioral Therapy (CBT) is a well-established psychological intervention; however, its digital adaptation—Digital Cognitive Behavioral Therapy (dCBT)—offers the potential for greater accessibility and engagement. This study aimed to evaluate the effectiveness of dCBT in reducing depressive symptoms among adolescents compared to standard care. ***Materials and Methods***: A randomized controlled trial was conducted involving 120 adolescents aged 13–18 years diagnosed with moderate to severe depression using the PHQ-9 scale. Participants were randomly allocated into two groups: the intervention group (n = 60) received an 8-week dCBT program via a mobile application, while the control group (n = 60) received standard school counseling. Depression severity was assessed at baseline and post-intervention using PHQ-9 and Beck Depression Inventory-II (BDI-II). Secondary outcomes included changes in anxiety (GAD-7) and quality of life (KIDSCREEN-27). Data were analyzed using paired and unpaired t-tests, with a significance level set at $p < 0.05$. ***Results:*** The intervention group showed a statistically significant reduction in PHQ-9 scores from a mean of 15.2 ± 2.3 at baseline to 7.8 ± 1.9 post-intervention ($p < 0.001$), whereas the control group showed a smaller reduction from 14.9 ± 2.6 to 12.4 ± 2.1 ($p = 0.04$). Similarly, BDI-II scores in the dCBT group dropped from 27.1 ± 4.8 to 13.5 ± 3.2. Improvement in anxiety and quality of life scores was also significantly greater in the intervention group compared to controls ($p < 0.01$). ***Conclusion:*** Digital CBT is a highly effective intervention for reducing depressive symptoms in adolescents, outperforming standard counseling in both primary and secondary outcomes. Its scalability and accessibility make it a promising tool for broader implementation in school and community mental health settings.

Keywords: Digital CBT, adolescent depression, randomized controlled trial, PHQ-9, mental health intervention, mobile therapy, school counselling.

INTRODUCTION

Human beings have always involved themselves in various activities to ensure their wellbeing and survival [1]. More than fifty species of Hippeastrum were brought to Europe during the mid-19th and early 20th centuries. Recent years have seen a great deal of research on amaryllis bulbs, roots, and flowers, with both local and international researchers isolating extracts from a range of chemical components [2].

The bulbs are tunicate, meaning they have mushy concentric inner scales or leaf bases and a protective, dry outer shell. Two to seven durable evergreen or deciduous leaves, measuring 30 to 90 cm in length and 2.5 to 5 cm in width, are produced by bulbs that are typically 5 to 12 cm in diameter. The leaves are sessile, hysteranthous, sub-petiolate, and infrequently persistent. Two free bracts form a bivalve spathe with free leaflets at its base, and the flowers are arranged in

umbelliform inflorescences [3]. There are two to fifteen huge, spectacular flowers, which are more or less hermaphrodite and zygomorphic, depending on the species. The native species are often red or purple, with each blossom measuring 13 to 20 cm (5-8) for diameter. Their shapes are declinate (curving downward and then upward at the tip) and funnelform (funnel shaped). Three outer sepals and three inner petals make up the perianth's six vibrantly colored tepals, which can have a similar or drastically distinct look. The segments of the perianth are either uneven or subequal. The tepals are joined at the base to form a small tube, which typically has a callose ridge at the neck or a simple scaly paraperigonium with fimbriae [4][5].

a. Flower **b. Leaves**

Fig 1. *Hippeastrum vittatum*

Taxonomy

Kingdom : Plantae
Order : Asparagales
Family : Amaryllidaceae
Genus : *Hippeastrum*
Species : *vittatum*

The ethanolic fresh flower extract from *Hippeastrum vittatum* revealed the unique alkaloids, tannins, glycosides, etc. [6].

- Ismine
- Lycorine
- Crinine
- Vittacarboline
- Haemanthamine
- Narciclasine
- Galanthamine
- Tazettine

The current study was based on the Phytochemical Investigation and estimation of total phenolic contents & total flavonoids contents of leaves and flowers extracts of *Hippeastrum vittatum.*

MATERIALS AND METHODS

Experimental requirements

Fresh leaves and flowers of *Hippaestrum vittatum*, methanol, ethanol, DPPH, distilled water, Wistar albino rats (either sex), rotatory evaporator and weighing machine.

Collection, authentication, and preparation of extract

The Fresh leaves and flowers of *Hippaestrum vittatum* were collected from UP East region and authenticated by a Scientist at BSI, Prayagraj. The leaves and flowers were washed making dust-free and dried at room temperature or shade. The dried leaves and flowers were rendered into coarse powders and then finally into fine ones. The powders of each were weighed and soaked in methanol and hydroalcoholic solvent (1:1), separately for fifteen days with gradual stirrings. The obtained slurry of mixture was kept for drying under partial vacuum using a rotary evaporator or water-bath [7].

Phytochemical screening

The plant extracts were screened for different phytoconstituents to check their presence [8][9].

Detection of Alkaloids

Extracts are dissolved individually in dilute HCl and filtered.

Mayer's Test: Filtrates are treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates are treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of

alkaloids.

Hager's Test: Filtrates are treated with Hagers Reagent. Formation of yellow ppt indicates the presence of alkaloids.

Detection of Glycosides

Fehling's test: With distilled water dilution, Fehling's solutions A and B are heated for one minute. There were 8 drops of plant extract added to this transparent blue solution. It is then combined with 1 ml of Fehling's solution and heated for 5 minutes in a water bath. Brick red precipitation is an indication of glycoside content.

Detection of Saponins

Foam test: About 2g of the plant extract was mixed with 10ml of distilled water and shaken vigorously for a stable persistent froth. Appearance of froth indicates the presence of saponins.

Detection of Tannins

Ferric chloride test: 0.5g of the dried powdered sample is boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% $FeCl_3$ is added and observed for brownish green-black or a blue-black coloration.

Lead acetate test: 2ml of plant extract is combined with 2ml of distilled water. 0.01g lead acetate is added to this combined solution and shaken well. Development of white turbidity and precipitate indicates the presence of tannins.

Detection of Flavonoids

NaOH test: A small amount of extract is treated with aqueous NaOH and HCl, and observed for the formation of yellow orange color.

H_2SO_4 test: A fraction of the extract is treated with Conc.H_2SO_4 and observed for the formation of orange color.

Detection of terpenoids

5 ml of the aqueous plant extract is combined with 2.0 ml of chloroform, which is then added, evaporated on the water path, and boiled with 3 ml of concentrated H2SO4. As terpenoids took shape, a grey colour emerged.

Detection of Steroids

2 ml of chloroform and concentrated H_2SO_4 are added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicates the presence of steroids.

Test for Reducing Sugars and Carbohydrates

Molisch test

To 2-3ml extract of individual solvents add few drops of α-naphthol solution in alcohol, shake and add concentrate H_2SO_4 from sides of test tube. Violet ring at the junction of two liquids.

Fehling's test

It is utilised to find decreasing sugars. Make a volume of 500ml by dissolving 34.66 grammes of copper sulphate in distilled water (solution A). 50 g of sodium hydroxide and 17.3 grammes of potassium sodium tartrate should be dissolved in distilled water to a volume of up to 50 millilitres (Solution B). Prior to usage, combine two solutions in an equal volume. Fehling's A and B solution in a 1 mL mixture should be boiled for one minute. Add the test solution in an equal amount. Heat in a pot of boiling water for 5-10 minutes. A first yellow and then a brick red hue was seen.

Determination of total phenolics content

Folin Ciocalteu reagent was used for analysis of total phenolics content [10]. Briefly, 0.5 ml of the extract was mixed with 0.5ml of Folin-Ciocalteu reagent. The solution was kept at 25°C for 5-8 min before adding 2 ml of sodium carbonate solution 7.5 % and adjusting the volume to 8 ml with water. After 2 h, the absorbance was measured at 725 nm. Gallic acid was used as standard for the calibration curve. Total phenolic content was expressed as mg gallic acid equivalents per gram of sample (mg/g).

Determination of total flavonoids content

The total flavonoid content was measured by a colorimetric assay [11]. One hundred micro liters of extract was added to 4 ml of distilled water. Then, 0.3 ml 5% sodium nitrite was added. After 5 min, 0.3ml of 10% aluminium chloride was added. In 6 min, 2 ml of 1 M sodium hydroxide was added to the mixture. Immediately, the mixture was diluted by the addition of 3.3 ml distilled water and mixed thoroughly. The absorbance was determined at 510 nm versus a blank. Catechin was used as standard for the calibration curve. Total flavonoids content of the extract was expressed as mg catechin equivalents per gram of sample (mg/g).

Isolation of bioactive compounds [12]

- **TLC analysis**

Both the herbal extracts (after dissolving in respective solvents) are placed to the precoated TLC plate in the form of dots using a fine capillary. The top of the plate had identification markings. Chromatography test is performed in rectangular glass vessels. A smooth sheet of filter paper was inserted in the TLC chamber and left in the developing solvent to prevent insufficient chamber saturation and the unwanted edge effect. Anisaldehyde-sulphuric acid is sprayed on the plate, and then it heated at 115 degrees Celsius for 5 minutes. The solvent system utilised was chloroform: ethyl acetate: acetone (7:1.5:1.5). Plates are developed, allowed to air dry, and then analysed for spot count, colour, and Rf values. Agents used for spraying anisaldehyde and sulfuric acid.

➢ HPTLC Analysis

The stationary phase is typically a thin layer of silica gel or other adsorbents. The choice of stationary phase depends on the nature of the sample components. The mobile phase can be a mixture of solvents, depending on the solubility of the analytes. Common mobile phase can be a mixture of polar and non-polar solvents, which allow for optimal separation of components.

Sample preparation

Prepare the sample by dissolving the analyte in a suitable solvent. Filtration might be necessary to remove any particular matter. The sample is applied to the TLC plate in small volumes using a micropipette or a syringe. Sample application should be uniform and concentrated in one area.

Chromatographic Separation

The plate is put in a developing chamber, where capillary action will cause the mobile phase to rise. Separation results from the sample's components moving at various speeds depending on how they interact with the stationary phase. After development, the plate is dried and observed under UV light or through staining techniques for colorimetric analysis. Densitometric scanning (measurement of intensity of bands) will also be performed to quantity the separated components.

RESULTS AND DISCUSSION

Percentage yield

The percentage yield was obtained as 63.47% and 58.26% in methanolic and hydroalcoholic leaves extract of *Hippaestrum vittatum* (Lily), respectively. However, the methanolic and hydroalcoholic flowers extract of *Hippaestrum vittatum* showed the percentage yield of 53.41% and 48.16%, respectively.

Phytochemical investigation of Lily extracts

Methanolic leaves extract of *H. vittatum* showed the alkaloids, tannins, saponins, terpenoids, flavonoids, phenol and starch in moderate. However, hydroalcoholic leaves extract showed presence of flavonoids. While, glycosides, steroids, sugars, and proteins were absent in both the fractions.

Table 1. Phytochemical investigation of *Hippaestrum vittatum* extracts

Phytochemical	Leaves extract	
	Methanolic	Hydroalcoholic
Alkaloids	++	+
Glycosides	_	_
Tannins	++	+
Saponins	++	+
Terpenoids	++	+
Steroids	_	_
Flavonoids	++	++
Phenols	++	+
Reducing sugars	_	_
Proteins	_	_
Carbohydrate	+	+
Starch	++	+

+++: Abundance, ++: Moderate; +: Presence -: Absent

Flowers extract (methanolic) showed the alkaloids, saponins, flavonoids in abundance. However, tannins, terpenoids, phenol, and starch was found in moderate quantity. Methanolic flower extract of *H. vittatum* reported the absence of glycosides, steroids, sugars and proteins.

Table 2. Phytochemical investigation of *Hippaestrum vittatum* extracts

Phytochemical	Flower extract	
	Methanolic	Hydroalcoholic
Alkaloids	+++	++
Glycosides	_	_
Tannins	++	+
Saponins	+++	++
Terpenoids	++	+
Steroids	_	_
Flavonoids	+++	++
Phenols	++	+
Reducing sugars	_	_
Proteins	_	_

Carbohydrate	+	+
Starch	++	+

+++: Abundance, ++: Moderate; +: Presence -: Absent

Isolation of bioactive compounds

- **TLC analysis of *H. vittatum* extract**

In methanolic fraction, the Rf value was observed as 0.81 in methanol: ethyl acetate (30:70) in leaves extract of *H. vittatum* (Lily). In hydroalcoholic fraction, Rf value was observed as 0.72 in the methanol: ethyl acetate (30:70). However, *H. vittatum* flowers extract (methanolic fraction), methanol: ethyl acetate (30:70) exhibited the Rf value of 0.83. *H. vittatum* flowers extract (hydroalcoholic fraction), methanol: ethyl acetate (30:70) exhibited the Rf value of 0.76.

Table 3. TLC analysis of *H. vittatum* extract

HPTLC

Herbal extract	Fractions	Solvent system	R_f Value
Leaves extract of *H. vittatum*	Methanolic	Methanol + ethyl acetate (30:70)	0.81
	Hydroalcoholic	Methanol + ethyl acetate (30:70)	0.72
Flowers extract of *H. vittatum*	Methanolic	Methanol + ethyl acetate (30:70)	0.83
	Hydroalcoholic	Methanol + ethyl acetate (30:70)	0.76

Analysis

In HPTLC analysis, leaves and flowers extracts of *H. vittatum* showed the expected compounds as flavonoids, saponins and terpenoids according the Rf values of plant constituents.

Table 4. HPTLC analysis of of *H. vittatum*

Herbal extract	Solvent system	R_f Value	Expected compounds
Leaves extract of *H. vittatum*	Methanol + ethyl acetate (30:70)	0.81	Flavonoids & Saponins
	Methanol + ethyl acetate (30:70)	0.72	
Flowers extract of *H. vittatum*	Methanol + ethyl acetate (30:70)	0.83	Flavonoids & Terpenoids
	Methanol + ethyl acetate (30:70)	0.76	

Determination of Total Phenolic Content (TPC)

The methanolic and hydroalcoholic leaves extract of *H. vittatum* demonstrated the total phenolic content as 64.29mg and 59.63mg, respectively.

Table 5. Determination of TPC in leaves and flowers extracts of *H. vittatum*

H. vittatum Extract	Total Phenolic Content (mg)	
	Methanolic	Hydroalcoholic
Leaves	64.29	59.63
Flowers	67.53	63.19

Moreover, the methanolic and hydroalcoholic flowers extract of *H. vittatum* demonstrated the total phenolic content as 67.53mg and 63.19mg, respectively. It found that flowers extract was more significant in having the total phenolic contents as compared to leaves.

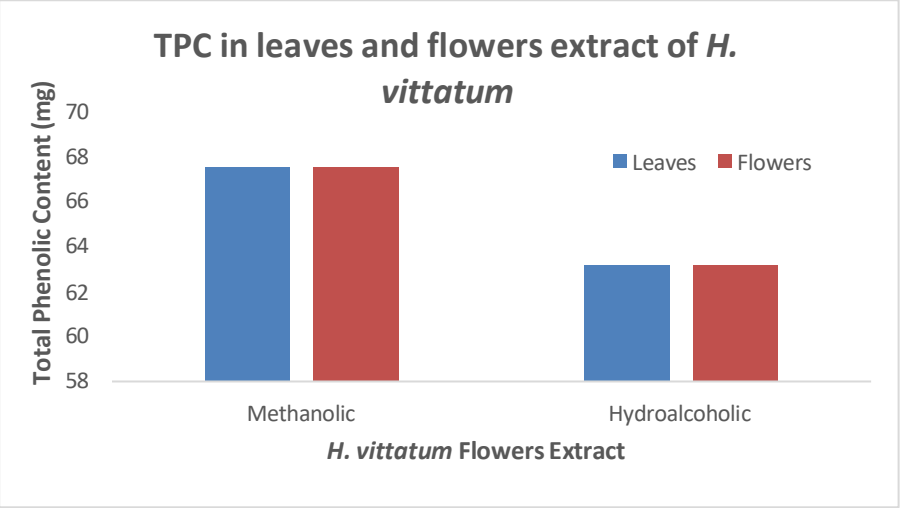

Fig 2. TPC in leaves and flowers extract of *H. vittatum*

Determination of Total Flavonoids Content (TFC)

In estimation of TFC, the methanolic and hydroalcoholic leaves extract of *H. vittatum* demonstrated the total flavonoids content as 44.28mg and 41.63mg, respectively.

Table 6. Determination of TFC in leaves and flowers extracts of *H. vittatum*

H. vittatum Extract	Total Flavonoids Content (mg)	
	Methanolic	Hydroalcoholic
Leaves	44.28	41.63
Flowers	56.72	52.14

Moreover, the methanolic and hydroalcoholic flowers extract of *H. vittatum* demonstrated the total flavonoids content as 56.72mg and 52.14mg, respectively. Similar to TPC, the total flavonoids content was found significant in amount in flowers extracts as compared to leaves.

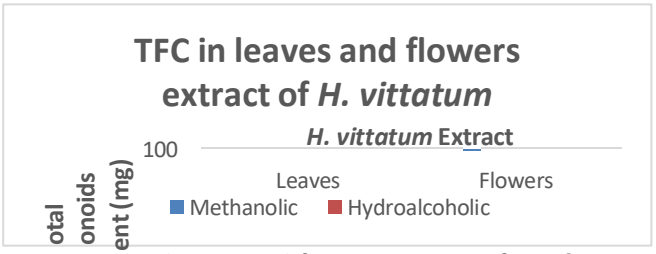

Fig 3. TFC in leaves and flowers extract of *H. vittatum*

In addition to the extensive array of Amaryllidaceae alkaloids identified in H. vittatum, the present findings reveal this species' ability to generate a variety of non-alkaloidal compounds that exhibit significant chemical similarity to those synthesized by other members of the subfamily Amaryllidoideae, thereby enhancing the potential chemotaxonomic significance of these metabolites. Furthermore, the findings of the present study may serve as a foundation for subsequent exploration of various extracts from H. vittatum plants, particularly regarding the antioxidant and anti-SARS-CoV-2 properties of their phytoconstituents, while considering potential structural modifications of these natural scaffolds aimed at developing targeted anti-COVID-19 therapeutics [13].

The quantification of total phenol utilized gallic acid, a polyphenolic compound, as the standard. It is ubiquitous in nearly all plants, therefore serving as a benchmark for measuring. These organic acids possess a pure and stable phenolic composition [14]. The quantification of total flavonoid levels utilized quercetin as a reference solution, as quercetin is a flavonoid classified within the flavonol group [15]. This study's findings on the phenolic and flavonoid content in the ethanol extract of tamarillo peel differ from prior research that employed alternative procedures and solvents. The extraction process, together with variations among farmers, growing settings, nutritional levels, and temperatures, might result in differing concentrations of flavonoids [16]. This research employed the DPPH method, which relies on the color change of DPPH resulting from the reaction between the DPPH free radical and an electron or hydrogen atom released by a compound present in the material, yielding a yellow 1,1-diphenyl-2-picrylhydrazyl

compound. The ethanol extract of tamarillo peel was assessed using the DPPH technique, with vitamin C serving as a reference. Vitamin C can be employed as a comparator in this investigation due to its high natural antioxidant properties [17]. This research shown that the ethanol extract of tamarillo peel exhibits strong antioxidant activity. This study parallels the research conducted by Hawa et al., which asserts that diverse kinds have strong antioxidant capacity [18]. The extraction of tamarillo fruit and skin utilizing various solvents, including 80% methanol and aqueous solutions, was assessed by the DPPH, FRAP, and ABTS test methods. Nallakurumban et al. demonstrated that tamarillo contains a substantial quantity of phenolics and flavonoids, which enhance the fruit's antioxidant potential [19]. Diep et al. revealed that tamarillos had a fairly high antioxidant capacity, which is strongly correlated with elevated levels of total phenolics. The presence of these bioactive compounds highlights tamarillo's potential for further use in culinary and medical fields [20].

CONCLUSION

It concluded that flowers extract of Hippaestrum vittatum (Lily) demonstrated moderate concentration of phytochemical constituents in contrast leaves extract of Lily. Moreover, methanolic fractions reported a higher level of total phenolic and total flavonoids contents than hydroalcoholic fractions which might be due to better solubility of phytocomponents. Hippaestrum vittatum is the rice source of saponins, tannins, alkaloids and flavonoids which could be isolated and used in the management of various ailments.

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