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Research Paper

Evaluation of Antioxidant potential of 50% Hydroethanolic Leaf Extract of Hydrocotyle verticillata Thunb

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ABSTRACT

Medicinal plants are a vital source of bioactive compounds, widely used in traditional medicine and increasingly integrated into modern healthcare. Phytochemicals, particularly secondary metabolites such as alkaloids, flavonoids, tannins and phenols, exhibit significant therapeutic potential due to their antioxidant properties. Free radicals, including reactive oxygen and nitrogen species, contribute to oxidative stress and degenerative diseases, while plant-derived phenolic compounds can neutralize these radicals. Hydrocotyle verticillata Thunb., commonly known as water pennywort, has long been employed in indigenous medicine. The present study evaluates the antioxidant activity of 50% hydroethanolic leaf extract of H. verticillata, highlighting its potential as a natural therapeutic agent..

INTRODUCTION

Medicinal plants have long played a pivotal role in indigenous healthcare systems across India and other parts of the world. Traditional knowledge, when integrated with modern medical practices, offers a promising approach to addressing the health needs of wider populations [1]. Plants are rich in secondary metabolites, which have been extensively utilized for treating diverse diseases, particularly in developing countries where herbal remedies remain the primary source of medication [2].

Phytochemicals, natural bioactive compounds found in various plant parts, act synergistically with nutrients and fibres to protect against chronic diseases such as cancer, cardiovascular disorders, and hypertension [3]. These compounds are broadly classified into primary constituents, including sugars, amino acids, and proteins, and secondary constituents such as alkaloids, terpenoids, flavonoids, tannins, and phenolic

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compounds, which are largely responsible for the therapeutic properties of plants [4].

Oxidative stress, caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS), is a major contributor to degenerative diseases [5]. Natural antioxidants, particularly phenolic compounds, play a crucial role in neutralizing free radicals through redox mechanisms, thereby preventing cellular damage [6,7]. Ethnopharmacological research has proven effective in identifying novel plant-derived anti-infective agents [8], and increasing attention has been directed toward natural antioxidants due to their efficacy and minimal side effects [9].

Hydrocotyle verticillata Thunb., commonly known as water pennywort, belonging to the family Apiaceae, is widely distributed in freshwater habitats across the Americas and the West Indies. Traditionally, its juice, poultice, and decoction have been used to treat fevers, wounds, boils, abscesses, coughs, hepatitis, influenza, and sore throats [10]. Based on its established ethnopharmacological importance, this study evaluated the antioxidant potential of the 50% hydroethanolic leaf extract of *Hydrocotyle verticillata*, emphasizing its potential as a source of natural antioxidants.

MATERIALS AND METHODS

Plant collection and authentication

The leaves of *Hydrocotyle verticillata* Thunb were collected from local areas in Hosur. The plant was authenticated by Dr. Soosairaj, Associate Professor, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil Nadu. A reference specimen has been deposited in the Department of Botany, St. Joseph's College, Tiruchirappalli, under the accession number 8315.

Preparation of the extracts for phytochemical analysis

Fresh leaves of *Hydrocotyle verticillata* Thunb were carefully rinsed under running tap water to eliminate adhering soil and debris. The cleaned leaves were subjected to shade dry at ambient temperature for approximately a week. The dried leaves were then pulverized into a coarse powder, which were used for extract preparation. The coarse powder was extracted using different solvents *viz*, aqueous, ethanol, 50% hydro ethanol, methanol and acetone. The obtained crude extracts were concentrated, filtered using Whatman No. 1 filter paper, and then kept at 4 °C for the qualitative analysis of phytochemicals.

Qualitative phytochemical analysis

Phytochemical evaluation was carried out on five extracts of *Hydrocotyle verticillata* to identify bioactive secondary metabolites, including flavonoids, alkaloids, sterols, tannins, triterpenoids, saponins, cardiac glycosides, and phenols. The screening was performed by using protocols in earlier studies [11,12,13].

Preparation of 50 % hydroethanolic extract

Following qualitative screening, quantitative evaluation was carried out using a 50% hydroethanolic extract. Approximately 200 g of *Hydrocotyle verticillata Thunb* leaves were immersed in 400 ml of 50% hydroethanol and subjected to cold maceration for three days (72 hours), with intermittent stirring to facilitate extraction. The mixture was then passed through fine muslin cloth, and the filtrate obtained was concentrated on a water bath until complete dryness. This procedure yielded dark brown crystalline residues, which were stored in airtight containers. The dried extract was subsequently reconstituted and employed for further study.

Quantitative Phytochemical Analysis

Estimation of Flavonoid

Total Flavonoid present in the 50% ethanolic extract of *Hydrocotyle verticillata* were estimated by the aluminium chloride colorimetric method. Total flavonoid was calculated based on the standard calibration curve and expressed as weight of quercetin equivalent mg/g of extract [14,15].

Estimation of Tannin

Tannin content in the sample was quantified using the Folin–Ciocalteu method. The absorbance was recorded at 700 nm, and concentrations were determined from a standard calibration curve prepared with tannic acid. Results were expressed as milligrams of tannic acid equivalents per gram of dried extract [16].

Estimation of Total Phenolics

The phenolic constituents in the 50% ethanolic extract of *Hydrocotyle verticillata* were estimated by using the Folin–Ciocalteu reagent using previous studies. Based on the standard calibration curve, the total phenolic content was calculated and expressed as catechol equivalents (mg/g of extract) [17].

Estimation of total alkaloid content

The total alkaloid content in the 50% ethanolic extract of *Hydrocotyle verticillata* was determined using a UV-spectrophotometric method as described by Manjunath et al., 2012 [18]. The values were expressed as milligrams of alkaloid equivalents per gram of dried extract.

Free radical scavenging assay

DPPH radical scavenging assay

The stable DPPH (2,2-Diphenyl-1-picryl hydrazyl) radical was used for the determination of free radical scavenging activity of hydroethanolic extracts of *Hydrocotyle verticillata* leaves [19]. Briefly, 1 ml of DPPH solution in methanol was added to 0.5ml of plant extracts in different concentrations. After 15 minutes, the absorbance was recorded at 517nm, using ascorbic acid as a positive control [20].

ABTS Radical Scavenging Assay

The antioxidant activity of the plant extracts against ABTS was evaluated by the method described by Re et al. (1999) [21]. The extracts (20–100 µg/ml) were mixed with 1 ml of ABTS solution (7 mM) and incubated for 6 minutes. The absorbance was recorded at 734 nm. Ascorbic acid served as the positive standard. The inhibitory concentration (IC_{50}) was determined as the extract concentration required to inhibit 50% of ABTS radicals, based on linear regression analysis.

Statistical analysis

Data are represented as mean values with their standard error of the mean (SEM).

RESULTS AND DISCUSSION

Qualitative analysis of phytochemicals

The results of phytochemical analysis of leaves of *Hydrocotyle verticillata Thunb* are shown in the Table 1. The qualitative phytochemical analysis revealed the presence of secondary metabolites such as phenols, flavonoids, tannins, glycosides, alkaloids, triterpenoids and plant nutrients such as carbohydrates and proteins in the various extractive solvents. Presence of phytochemicals was indicated by the positive (+) sign and absence by the negative (-) sign. The 50% ethanolic leaf extract of *Hydrocotyle verticillata Thunb* showed the presence of higher levels of phytochemicals when compared to other solvent extracts.

This may be due to the higher solubility of each plant constituents in the respective solvents. Polyphenolic phytochemicals are believed to reduce the risk of several major diseases including neurodegenerative disorders [22]. Thus, presence of polyphenols, flavonoids, alkaloids and tannin compounds may be indicative of medicinal value of *Hydrocotyle verticillata.*

Table 1: Qualitative analysis of phytochemicals in different extracts of *Hydrocotyle verticillata Thunb* leaves

Plant Constituents	Extractive solvents				
	Aqueous	Ethanol	50% ethanol	Acetone	Methanol
Carbohydrate	+	+	+	-	+
Proteins	+	-	+	+	+
Flavonoids	+	+	+	+	+
Phenolics	-	+	+	+	-
Steroids	-	-	-	-	-
Tannins	-	+	+	+	-
Glycosides	+	+	+	+	+
Alkaloids	+	+	+	-	-
Thiols	-	-	-	-	-
Tri terpenoid	+	+	+	-	-

+ve and –ve symbols indicate the presence and absence respectively of plant constituents with respect to extractive solvents in increasing order of polarity. Experiments are carried out in multiples of three sets for each test.

Quantitative analysis of phytochemicals in Hydroethanolic leaf extract of *Hydrocotyle verticillata Thunb*

Flavonoids

Hydroethanolic leaf extract of *Hydrocotyle verticillata Thunb* was found to contain 19.45 mg/g of flavonoids. Flavonoids are group of naturally occurring compounds widely distributed as secondary metabolite in plants. Hence the medicinal plants containing flavonoid compounds are repeatedly screened for antioxidant activity [23].

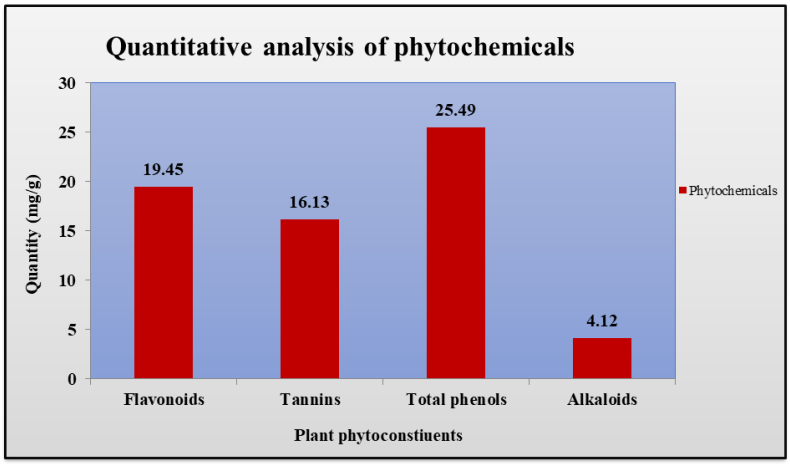


Figure 1: Quantitative analysis of phytochemicals

Tannins

The level of tannin was found to be 16.13 mg/g in 50% ethanolic leaf extract of *Hydrocotyle verticillata Thunb*. Tannins and tannin-like substances are wide spread in all the medicinal plants. These are the polyphenolic compounds divided into two main groups- hydrolysable and condensable. Hydrolysable tannins contain a polyhydric alcohol usually and condensed tannins are mostly flavanols and are probably polymers of flavan-3-ol (catechin) [24].

Total phenols

The level of total phenol was found to be 25.49 mg/g in the 50 % ethanolic leaf extract of *Hydrocotyle verticillata Thunb*. Phenolic constituents are very important in plants because of their scavenging ability due to their hydroxyl

groups [25]. Phenolic compounds are powerful chain breaking antioxidants [26].

Alkaloids

The level of total alkaloid was found to be 4.12 mg/g in the 50 % ethanolic leaf extract of *Hydrocotyle verticillata Thunb*. According to previous studies, alkaloids have a wide range of pharmacological activities including antimalarial, anticancer, antibacterial and antihyperglycemic activity. Alkaloids have equally been exploited for their importance in traditional pharmaceutical usage [27,28].

FREE RADICAL SCAVENGING ASSAY

DPPH radical scavenging assay

The percentage inhibition increases when the DPPH radicals were scavenged by antioxidants in the plant extract. The DPPH radical scavenging activity of hydroethanolic extract of *Hydrocotyle verticillata Thunb* is shown in the figure with ascorbic acid as the standard. The scavenging activity of 50% ethanolic extract of *Hydrocotyle verticillata* was found to be in a dose-dependent manner.

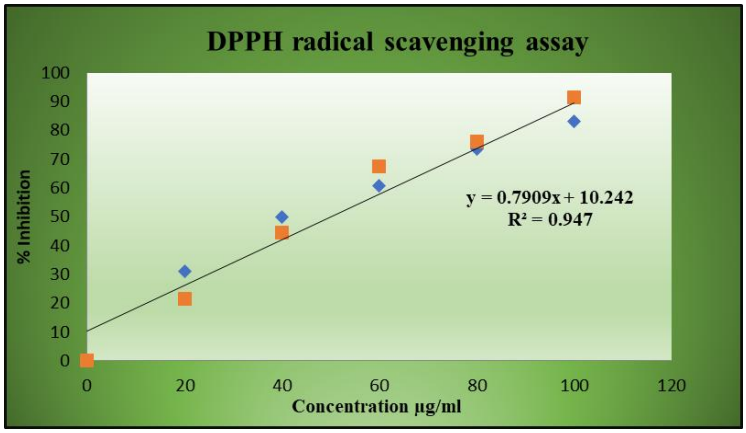


Figure 2: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

ABTS Radical Scavenging Assay

The percentage inhibition increased progressively as the ABTS radicals were neutralized by antioxidants present in the plant extract. The ABTS radical scavenging activity of the hydroethanolic extract of *Hydrocotyle verticillata* Thunb is depicted in the figure, with ascorbic acid serving as the reference standard.

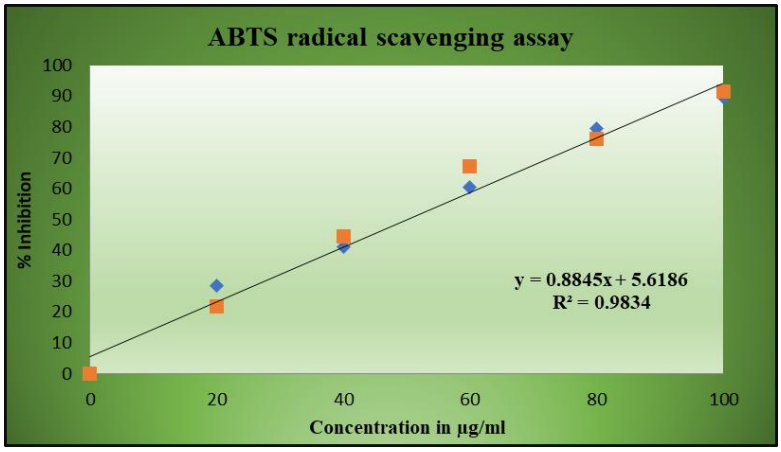


Figure 3: ABTS 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

The scavenging potential of the 50% ethanolic extract of *Hydrocotyle verticillata* was observed to be concentration-dependent, showing enhanced activity with increasing extract levels.

CONCLUSION

The present study revealed that the leaves of *Hydrocotyle verticillata* Thunb are a rich source of bioactive phytochemicals, including flavonoids, phenols, tannins, glycosides, alkaloids, triterpenoids, carbohydrates, and proteins. Quantitative estimation confirmed appreciable levels of flavonoids, tannins, phenols, and alkaloids in the hydroethanolic leaf extract, supporting its phytochemical richness. The extract exhibited strong free radical scavenging activity in both DPPH and ABTS assays, with inhibition increasing in a concentration-dependent manner when compared to the standard antioxidants. These findings highlight the medicinal properties of *H. verticillata*, particularly its polyphenolic constituents, which may contribute to its antioxidant efficacy and possible therapeutic applications against oxidative stress-related disorders. Further studies are required to isolate the active constituents and confirm the mechanisms of action.

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