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Investigating the Phytochemical Analysis and Antioxidant Activity of *Hedychium flavescens*

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ABSTRACT

For ages, medicinal plants have served as a valuable reservoir of healing substances, with their efficacy often attributed to the existence of phytocompounds and antioxidants. The purpose of this research paper is to investigate the chemical composition and antioxidant capabilities of extracts derived from both the leaves and rhizomes of Hedychium flavescens, a traditional medicinal plant found in the Western Ghats and Eastern Himalayan range in India. Preliminary phytochemical screening of rhizome extracts of the plant showed better results than the leaf extracts. On comparing the extracts, acetone and methanol extracts of plant parts- rhizome and leaf showed notable outcome. Antioxidant potential was analysed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical scavenging and Hydrogen peroxide scavenging methods. Antioxidant tests suggest promising results especially shown by the acetone extract of the rhizome, indicating the potential of Hedychium flavescens as a natural reservoir of antioxidants.

Keywords: Phytochemical analysis, Antioxidant activity, *Hedychium flavescens*, Zingiberaceae, Medicinal plants

1. INTRODUCTION

For millennia, medicinal plants have been pivotal in human healthcare, forming the foundation of traditional medical practices globally. Indigenous communities across the ages have developed intricate knowledge of local flora for therapeutic purposes. Msonthi (1984) suggests these traditions, often transmitted orally, encompass the utilization of particular plants for diverse ailments and rituals aimed at fostering both spiritual and physical wellness. Phytochemical analysis and evaluation of antioxidant activity are essential aspects of researching medicinal plants, offering valuable understanding into their therapeutic potential and biological effects. Phytochemical analysis involves the process of recognizing and measuring bioactive compounds found in medicinal plants. A study done by Harborne (1973) shows that these compounds, which include alkaloids, flavonoids, phenolic acids, terpenoids, and saponins, among others, are known to possess diverse pharmacological properties. Cragg and Newman (2005) have observed that by comprehending the phytochemical makeup of medicinal plants, researchers can link particular chemical components with observed biological effects, thus directing the choice of plant extracts or fractions for further investigation. Moreover, Molyneux (2004) have put forward that phytochemical analysis contributes to the standardization and quality assurance of herbal products, guaranteeing uniformity and effectiveness in therapeutic preparations.

Antioxidants are substances that counteract detrimental free radicals and oxidative stress, safeguarding cells against harm and damage. An imbalance exists between the body's ability to use antioxidants to combat free radicals and the amount of them that are produced. This imbalance leads to oxidative stress. This disproportion can cause damage to cells and play a role in the emergence of several illnesses. (Pham-Huy et al., 2008) show in their study that antioxidants help in decreasing oxidative stress and prevent onset of illnesses. Prior and Cao (1999) state that many medicinal plants are rich in antioxidants, which are pivotal in contributing to their potential health benefits. According to Halliwell (1994), assessing the antioxidant potency of plant extracts or isolated compounds provides valuable insights into their capacity to counteract free radicals and mitigate oxidative harm. Benzie and Strain (1996) report that *in vitro* antioxidant tests, like FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and Hydrogen peroxide scavenging assays provide quantitative measures of antioxidant capacity, allowing scientists to compare plant extracts based on their

antioxidative properties. Aune (2019) in his study has revealed that antioxidants help lower oxidative stress, which is associated with chronic conditions like cancer and heart disease and (Cho et al., 2004) in their research show that antioxidants reduce the likelihood of age-related macular degeneration, a significant contributor to vision loss among the elderly. (Yang, 2023; Zhao, 2022; Steinmetz, 1993) have found that consumption of dietary antioxidants was connected to reduced likelihood of certain cancers, like lung cancer and colorectal cancer. (Knekt et al., 2002) claims that antioxidants such as flavonoids present in vegetables and fruits might decrease the chances of heart disease by enhancing endothelial function, reducing inflammation and averting the oxidation of low-density lipoprotein (LDL) cholesterol. (Mizrahi, 2009 and Joseph, 2004) suggest that antioxidants, including vitamins C and E, flavonoids, and polyphenols, may provide neuroprotective advantages, potentially aiding in the prevention of neurodegenerative disorders. These studies highlight the importance of antioxidants in promoting health and preventing a range of diseases. Incorporating antioxidant-rich foods into our diet and embracing a balanced lifestyle can improve general well-being and decrease the chances of chronic diseases.

Hedychium flavescens, commonly referred to as yellow ginger or cream ginger, is a plant of the Zingiberaceae family, native to Asia and found in China, India, Nepal, and Bhutan. *Hedychium flavescens* has traditionally been used in Asian cultures for its medicinal properties. Singh and Sharma (2018) point out that its rhizomes and leaves have been used to ease stomach discomfort, aid digestion, and alleviate inflammation and pain. Although not as extensively utilized in cooking as its cousin ginger (Zingiber officinale), certain cultures integrate the rhizomes of *Hedychium flavescens* into their cuisine for both flavor enhancement and medicinal benefits. Studies exploring the chemical composition and taste characteristics of these rhizomes could offer valuable insights into their culinary utility. The aromatic flowers of *Hedychium flavescens* are notable for their fragrant qualities. (Canton, 2023 and Gauvin-Bialecki, 2023) have shown the potential use of the plant's flowers in the production of perfumes and essential oils due to their pleasant aroma. (Anandi, 2023a, 2024b) has shown significant findings regarding the antibacterial, antifungal, antidiabetic and anti-inflammatory properties while (Mani, 2023 and Suksathan, 2018) demonstrated the antioxidant characteristics of *Hedychium flavescens*. These studies highlight the existence of phenols and other bioactive elements in the plant, contributing to these activities.

The objective of this study was to examine the phytoconstituents through phytochemical screening of the various extracts of *Hedychium flavescens* leaves and rhizome and to determine the antioxidant potential of the extracts through DPPH and Hydrogen peroxide scavenging methods.

2. MATERIALS AND METHODS

2.1 Authentication of plant

The plant specimens were collected from the Moolayar stream, at the Palani hills of Dindigul district, Tamil Nadu and was authenticated by Dr. S. John Britto S.J. at the Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli. The voucher specimen (RHT 68885) has been stored.

2.2 Extraction

The collected plant parts were washed and allowed to air dry. The dried plant parts were powdered using a mechanical grinder and stored in airtight containers for future use. The leaf and rhizome powders were then extracted using solvents, such as methanol (ME), aqueous (AQ), acetone (AC) and chloroform (CH). For 3 days they were kept in a shaker at 37˚C and then filtered through Whatman No. 1 filter paper. They were then concentrated using a rotary evaporator. The concentrated extracts, which had the appearance of sticky, black solids, were diluted in dimethyl sulfoxide (DMSO) to get a 50 µg/µl concentration in order to be used for preliminary phytochemical analysis and antioxidant testing.

2.3 Preliminary Phytochemical Tests

Qualitative phytochemical screening was done to check the presence of flavonoids, phenols, alkaloids, fixed oils, proteins, carbohydrates, quinones, terpenoids, sterols, glycosides, amino acids, saponins, coumarins, and phlobatannins in the methanol, aqueous, acetone and chloroform extracts of leaves and rhizomes of *H. flavescens*. The extract's qualitative phytochemical analysis was carried as per the standard procedures outlined by (Brain, 1975 and Evans, 2009).

Test for alkaloids

Mayer's test: A little amount of Mayer's reagent was added to two millilitres of the extract. Alkaloids' presence was indicated by a cream-colored precipitate.

Hager's test: Few drops of Hager's reagent was added to two millilitres of the extract. Alkaloids' presence was confirmed when a yellow precipitate started to form.

Wagner's test: Two millilitres of the extract and a small amount of Wagner's reagent were combined. Alkaloids' presence was indicated by reddish-brown precipitate.

Test for amino acids

2 millilitres of the extract was added to 1 millilitre of recently made 0.25% ninhydrin solution and briefly heated to boiling. A blue tint appeared, signifying that amino acids were present.

Test for carbohydrates

Molisch's test: 1 millilitre of extract was added to a mixture of 5 millilitres of water, 1 millilitre of concentrated sulphuric acid (H_2SO_4) and 2 drops of ethanolic α-naphthol (20%). Carbohydrates were present because a violet ring appeared at the intersection.

Test for coumarins

Three millilitres of a 10% sodium hydroxide (NaOH) solution were used to treat two millilitres of extract. Emergence of a yellow hue indicated existence of coumarin compounds.

Test for fixed oils

One millilitre each of 1% CuSO4 solution and 10% NaOH solution were mixed with two millilitres of extract. Emergence of blue hue suggested the existence of fixed oils.

Test for flavonoids

1 millilitre of extract was added to 1 millilitre of 10% lead acetate solution. Flavonoids' presence was revealed by the production of yellow precipitate.

Alkaline test: A little amount of NaOH solution was added to two millilitres of extract. Flavonoids' presence was indicated by the emergence of yellow colour that vanished when diluted hydrochloric acid (HCl) was added.

Test for glycosides

Molisch's test: In a test tube, 1 millilitre of extract and few drops of Molisch's reagent were mixed. Then, 2 millilitres of concentrated H_2SO_4 were carefully added, tilting the test tube. Glycosides were indicated by the creation of a violet ring at the intersection of the two liquids.

Glycoside test: One millilitre of extract and one millilitre of water were added in a test tube and well shaken. The mixture was then supplemented with aqueous NaOH. Glycosides' presence was revealed by yellow colour formation.

Keller-Kiliani test: 2 millilitres of the extract was added to 1 millilitre of glacial acetic acid, 1 millilitre of concentrated H_2SO_4, and few drops of 5% ferric chloride ($FeCl_3$). Glycosides' presence was suggested by the formation of a brown ring at the juncture.

Test for phenols

Lead acetate test: To 2 millilitres of extract, 10% lead acetate solution was added. Phenols were confirmed by the appearance of a large, white precipitate.

Potassium dichromate test: A 5% potassium dichromate solution was combined with two millilitres of the extract. There were phenols present because a brown precipitate formed.

Ferric chloride test: A little amount of a 5% ferric chloride was added to 2 millilitres of extract. Phenols' presence were confirmed by bluish-black colour.

Test for phlobatannins

Hydrochloric acid (HCl) test: Two millilitres of the extract were combined with diluted HCl. Phlobatannins were present because a crimson precipitate appeared.

Test for proteins

Concentrated sulfuric acid (H_2SO_4) test: A little amount of concentrated H_2SO_4 was mixed with two millilitres of the extract. Proteins were present because a white precipitate formed.

Xanthoprotetic test: A little amount of concentrated nitric acid (HNO_3) was combined with 2 millilitres of extract. Proteins were present because a yellow tint started to appear.

Biuret test: Two millilitres of the extract was added to two millilitres each of solutions containing one percent copper sulphate ($CuSO_4$) and five percent sodium hydroxide (NaOH). Proteins and free amino acids were suggested by the development of a violet or purple colour.

Test for quinones

1 millilitre of the extract was mixed with one millilitre of alcoholic KOH. Quinones were present because a reddish-blue colour started to appear.

Test for saponins

5 ml of distilled water were mixed with 1 ml of the extract, then the mixture was slowly heated. After fifteen minutes of shaking the mixture, the appearance of a continuous froth suggested the existence of saponins.

Test for sterols

To perform Salkowski's test, two millilitres of extract was combined with 2 ml of concentrated H_2SO_4 and two millilitres of chloroform. Sterols' presence was confirmed when the H_2SO_4 layer fluoresced a greenish yellow colour and chloroform layer formed a red colour.

Liebermann-Burchard test: Two millilitres were dissolved in two millilitres of acetic anhydride. Few drops of concentrated sulphuric acid was gradually added to this. Sterols were indicated by a variety of colour changes.

Test for terpenoids

Liebermann-Burchard test: One millilitre of the extract underwent treatment with acetic anhydride, chloroform, and a little amount of sulphuric acid. Dark green color indicated terpenoids' presence.

Salkowski's test: One millilitre of the extract was mixed with two millilitres of chloroform and a little amount of concentrated sulphuric acid. Development of reddish-brown color indicated terpenoids' presence.

2.4 Antioxidant tests

2.4.1 DPPH Radical scavenging assay

DPPH assay is widely used in antioxidant research involving natural products, largely due to its simplicity and sensitivity. This method operates on the principle that antioxidants act as hydrogen donors, effectively scavenging radicals. It quantifies compounds that are radical scavengers. The decrease of DPPH in the examined samples is closely correlated with the antioxidant efficacy.

A methanol solution containing 100 µM of 2,2-diphenyl-1-picrylhydrazyl was produced, and 100 µl of this solution was mixed with 300 µl of acetone extract of rhizome (AC) at several concentrations (10, 20, 30, 40 and 50 µg/ml). The combinations were mixed well, then they were left at room temperature for thirty minutes. UV-VIS spectrophotometer was used to measure the absorbance at 517 nm, with ascorbic acid as the standard. Lower absorbance values indicate higher free radical scavenging activity in the reaction mixture. Following formula was used to determine the capacity to scavenge the DPPH radical:

$$\%\ inhibition) = \left[\frac{absorbance\ (control) - absorbance\ (reaction\ mixture)}{absorbance\ (control)}\right] X\ 100$$

2.4.2 Hydrogen peroxide scavenging method

By oxidising crucial thiol (-SH) groups, hydrogen peroxide functions as a moderate oxidising agent and can directly disable certain enzymes. Because of its rapid permeability across cell membranes, it can enter cells and combine with ferrous and possibly cupric ions to produce OH radicals. Its harmful effects could be caused by this method. Consequently, it is critical that cells control the build-up of hydrogen peroxide.

The approach described by (Ruch et al., 1983) was used to test how well plant extracts can remove hydrogen peroxide (H_2O_2). A 0.043 M H_2O_2 was made using 1 M phosphate buffer (pH 7.4). Various rhizome acetone extract concentrations (AC) (10, 20, 30, 40 and 50 µg/ml) were mixed with the above solution and kept for ten minutes. At 230 nm, absorbance of H_2O_2 was measured, comparing it to a blank solution that had phosphate buffer but no H_2O_2, with ascorbic acid as the standard. The percentage inhibition was calculated using:

$$\%\ inhibition = \left[\frac{absorbance\ (control) - absorbance\ (reaction\ mixture)}{absorbance\ (control)}\right] X\ 100$$

2.5 Statistics

All measurements were done in triplicate ($n = 3$). Standard deviation (± SD) and IC50 values were calculated with Microsoft Excel.

3. RESULTS AND DISCUSSION

The preliminary phytochemical screening of extracts of *Hedychium flavescens* leaves and rhizomes revealed the presence of flavonoids, phenols, alkaloids, proteins, amino acids, coumarins, glycosides, carbohydrates, sterols, quinones, terpenoids, saponins, fixed oils and phlobatannins as shown in Table 1. The results were displayed as high (+++), moderate (++), low (+) and nil (-) according to their intensity of the response towards reaction chemicals and nature of expected outcome. The qualitative estimation revealed that the rhizome extracts showed notable presence of phytochemical constituents compared to leaf extracts. Acetone extract of the rhizome had better results when compared to other extracts. Aqueous extract of rhizome and chloroform extract of leaf showed moderate amounts of phytochemical

constituents. All the four extracts of both leaf and rhizome revealed presence of terpenoids, glycosides, saponins, carbohydrates, proteins and fixed oils.

Tables 2, 3, 4, and 5 display the findings on *Hedyhium flavescens*' antioxidant activity. Ascorbic acid, the positive control in the DPPH experiment, demonstrated 85.4±0.81% radical scavenging activity at a 50 µg/ml dosage. The acetone extracts of both rhizome and leaves showed the highest scavenging activity (79.06±0.74% and 81.48±0.69%, respectively) at 50 µg/ml, while the aqueous extracts from the same sources showed the lowest radical scavenging activity (70.64±0.33% and 71.15±1.27%, respectively) (Tables 2, 3 and Figures 1,2). Ascorbic acid was shown to have an IC50 value of 12.78 µg/ml, whilst acetone extracts from leaves and rhizome demonstrated IC50 values in the DPPH experiment of 23.47 and 16.64 µg/ml.

Ascorbic acid, the positive control in the hydrogen peroxide scavenging assay, demonstrated 76.2±1.08% radical scavenging activity at 50 µg/ml. At 50 µg/ml, acetone extracts of both plant parts displayed the highest scavenging activity, at 72.27±0.71% and 74.98±0.61%, respectively. In contrast, the aqueous extracts from the same materials and the rhizome showed the lowest radical scavenging activity, at 63.13±0.66% and 68.82±1.33%, respectively (Tables 4, 5 and Figures 3, 4). Ascorbic acid was discovered to have an IC50 value of 18.17 µg/ml, whilst acetone extracts from leaves and rhizome demonstrated IC50 values in the DPPH experiment of 24.74 and 20.21 µg/ml.

Table 1: Preliminary phytochemical screening of *Hedychium flavescens* leaf and rhizome extracts

Sl. No.	Phytochemical constituents	LEAF				RHIZOME			
		CH	AC	ME	AQ	CH	AC	ME	AQ
1.	Alkaloids	-	+++	++	-	+++	+++	+++	-
2.	Amino acids	-	++	-	-	+	+++	-	-
3.	Carbohydrates	+	++	+++	++	+++	+++	++	++
4.	Coumarins	-	+++	+	-	+++	+++	+++	+
5.	Fixed oils	++	++	+++	++	+++	++	+++	++
6.	Flavonoids	-	++	++	-	+++	+++	+++	+

7.	Glycosides	+	++	+++	++	+++	+++	++	++
8.	Phenols	-	-	++	-	-	+++	++	-
9.	Phlobatannins	-	-	-	-	++	-	+++	+++
10.	Proteins	+	+	+++	+	+++	+	+++	+
11.	Quinones	+	-	-	-	++	+	-	+
12.	Saponins	+++	+++	+++	+++	-	+++	+++	+++
13.	Sterols	-	++	+	-	-	+++	-	+++
14.	Terpenoids	+	+++	++	+	++	+++	+	+++

+ + +: High; + +: Moderate; +: Trace; - : Absence

Table 2: DPPH assay of leaf extracts of *Hedychium flavescens*

Sl. No.	**Concentration (µg/ml)**	**Percentage of inhibition (%)**				
		CH	**AC**	**ME**	**AQ**	**Standard (Ascorbic acid)**
1	10	30.76±1.73	33.17±1.03	30.39±1.52	21.74±0.24	46.5±1.32
2	20	38.39±1.02	45±1.23	44.32±0.17	33.75±1.54	57.8±0.6
3	30	58.23±1.75	60.76±1.85	56.02±1.02	49.80±1.38	66.7±0.7
4	40	61.02±0.95	70.24±1.30	64.75±1.67	52.45±1.53	77.8±0.6
5	50	73.34±0.78	79.06±0.74	77.76±0.17	70.64±0.33	85.4±0.81
IC50 value		27.82	23.47	25.96	33.71	12.78

Table 3: DPPH assay of rhizome extracts of *Hedychium flavescens*

Sl. No.	**Concentration (µg/ml)**	**Percentage of inhibition (%)**				
		CH	**AC**	**ME**	**AQ**	**Standard (Ascorbic acid)**
1	10	42.56±0.79	44.21±0.81	41.67±0.74	37.06±1.51	46.5±1.32
2	20	48.04±0.35	52.83±0.84	50.65±0.76	43.03±1.13	57.8±0.6
3	30	60.13±0.56	61.65±1.12	58.11±1.32	50.43±1.57	66.7±0.7
4	40	69.23±0.69	73.21±0.98	70.63±0.92	63.07±0.39	77.8±0.6

5	50	78.65±0.09	81.48±0.69	77.11±0.62	71.15±1.27	85.4±0.81
IC50 value		19.58	16.64	19.40	26.66	12.78

Table 4: Hydrogen peroxide scavenging assay of leaf extracts of *Hedychium flavescens*

Sl. No.	Concentration (µg/ml)	Percentage of inhibition (%)				
		CH	AC	ME	AQ	Standard (Ascorbic acid)
1	10	34.82±1.44	36.63±1.02	33.02±0.61	31.89±0.43	41.9±1.96
2	20	43.13±1.42	47.31±0.09	43.89±1.03	38.93±1.45	52.6±1.81
3	30	52.74±0.47	52.83±0.77	51.10±1.11	48.02±0.48	60.3±1.08
4	40	63.94±0.43	64.13±0.95	60.56±0.70	59.23±0.21	69.7±1.34
5	50	69.59±0.65	72.27±0.71	68.53±0.81	63.13±0.66	76.2±1.08
IC50 value		26.85	24.74	28.38	32.13	18.17

Table 5: Hydrogen peroxide scavenging assay of rhizome extracts of *Hedychium flavescens*

Sl. No.	Concentration (µg/ml)	Percentage of inhibition (%)				
		CH	AC	ME	AQ	Standard (Ascorbic acid)
1	10	38.02±0.95	39.84±1.61	32.08±1.51	33.63±1.52	41.9±1.96
2	20	45.56±0.26	50.22±0.79	49.29±0.78	36.70±0.47	52.6±1.81
3	30	57.77±1.26	60.27±1.29	53.77±1.54	51.87±0.98	60.3±1.08
4	40	65.47±1.61	67.58±0.91	65.34±1.14	58.94±0.90	69.7±1.34
5	50	70.16±1.59	74.98±0.61	72.12±0.50	68.82±1.33	76.2±1.08
IC50 value		23.59	20.21	25.30	30.01	18.17

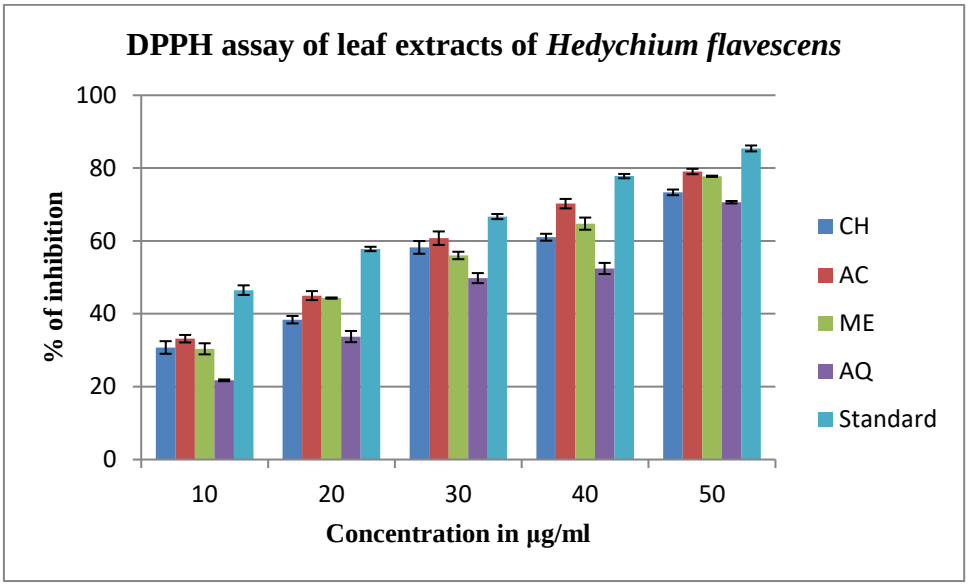

Figure 1: DPPH assay of leaf extracts of *Hedychium flavescens*

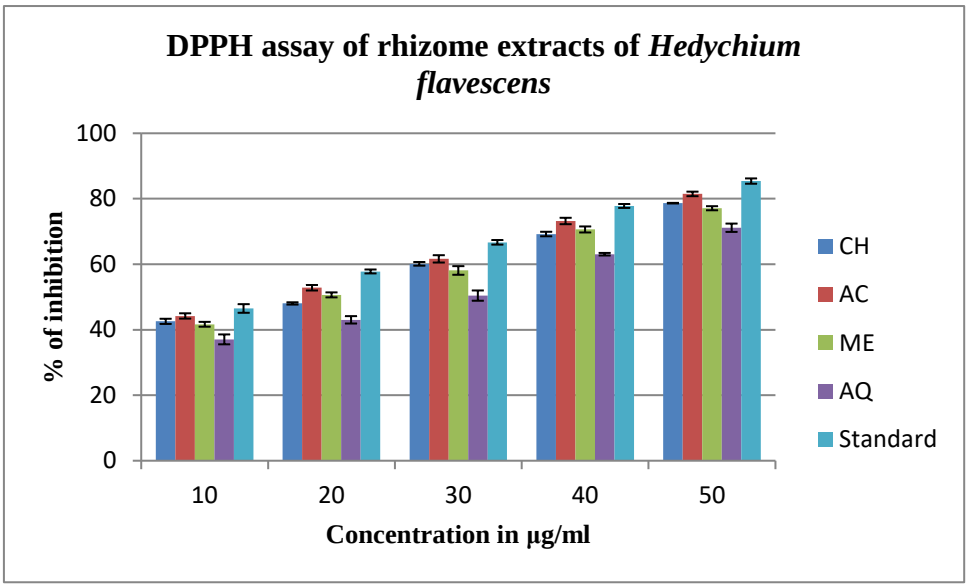

Figure 2: DPPH assay of rhizome extracts of *Hedychium flavescens*

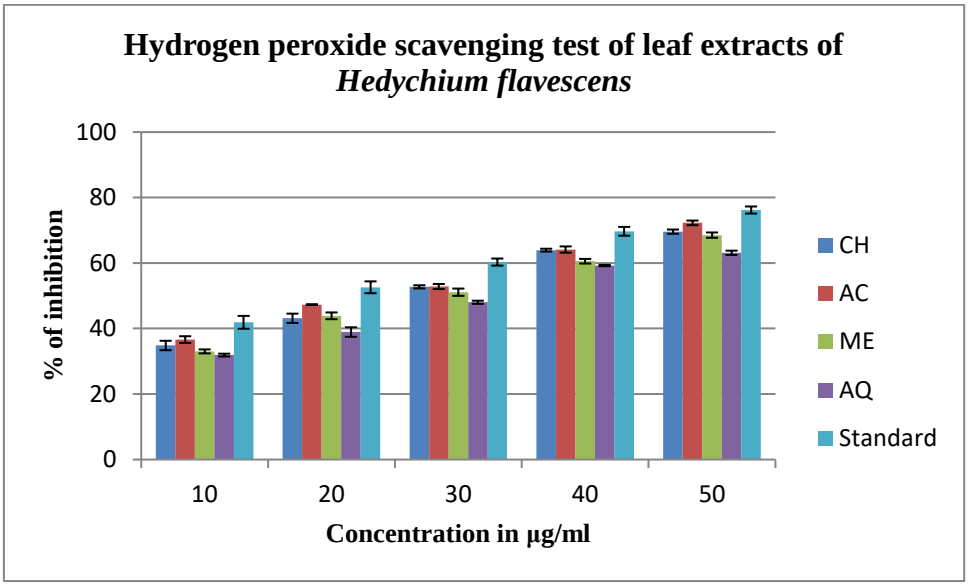

Figure 3: Hydrogen peroxide scavenging test of leaf extracts of *Hedychium flavescens*

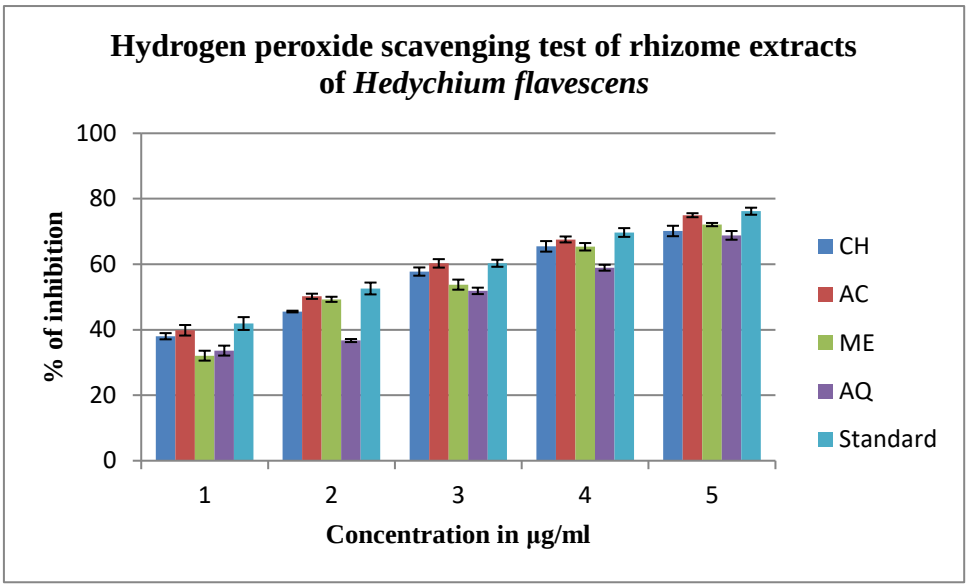

Figure 4: Hydrogen peroxide scavenging test of rhizome extracts of *Hedychium flavescens*

4. CONCLUSION

This study involves phytochemical screening, antioxidant assays and exploring potential uses in pharmaceuticals. Alkaloids are known to possess antiviral, antibacterial, anti-inflammatory and anticancer properties. Flavonoids are well-known for having anti-inflammatory and antioxidant characteristics. They have been linked to several health advantages, such as

cancer prevention and heart protection. Due to their antioxidant qualities, phenolic compounds can aid in preventing oxidative cell damage. When combined with water, saponins can create foamy formations and behave as detergents. Their capability to decrease cholesterol and stimulate the immune system has been the subject of research. Terpenoids possess wide range of biological actions, including as anti-inflammatory, antimicrobial, and anti-tumorous effects. Quinones, aromatic compounds containing two carbonyl groups bonded to a benzene ring, have been studied for their potential antioxidant and anticancer activities. Coumarins have been shown to have anti-inflammatory, anticoagulant, and anticancer effects. The extracts of *Hedychium flavescens* contain phytoconstituents such as flavonoids, phenols, alkaloids, fixed oils, proteins, carbohydrates, quinones, terpenoids, sterols, glycosides, amino acids, saponins, coumarins, and phlobatannins that indicate their potential as a source of novel medicines. The extracts' high concentrations of quinone, phenolic, alkaloid, flavonoid, and terpenoids may be due to their pharmacological action in scavenging free radicals.

Additionally, testing for antioxidant activity using DPPH and hydrogen peroxide scavenging tests demonstrated the crude extracts' strong ability to scavenge free radicals. Compared to standard, acetone extracts of the plant parts showed significant antioxidant activity followed by methanol extracts. The current findings will undoubtedly aid in determining the potential of *Hedychium flavescens* for medicinal and nutraceutical purposes. Hence, additional research is needed to isolate and identify the plant's active compounds, as well as to understand the mechanism behind its biological and antioxidant effects.

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