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In Vitro Antidiabetic and Anti- Inflammatory Analysis of Rhizome Extract of Hedychium Flavescens

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Abstract
The principle of this study was to assess the potential anti-inflammatory and anti-diabetic properties of *Hedychiumflavescens*. The anti-diabetic activity was determined using the α-amylase inhibitory assay with the DNSA method, while the anti-inflammatory activity was evaluated using a modified version of the method developed by Mizushima et al. In the examination of the antidiabetic effects, the acetone extract of *Hedychiumflavescens* rhizome demonstrated an IC50 value of 50.25 µg/ml, indicating higher activity compared to the standard Acarbose. However, the acetone rhizome extract exhibited relatively lower inhibition in terms of anti-inflammatory activity, with an IC50 value of 82.26 µg/ml compared to the standard.

Key-Words: *Hedychiumflavescens*, Antidiabetic, Anti-inflammatory, Zingiberaceae, Medicinal plant.

1. Introduction

The metabolic disease called diabetes mellitus is characterized by low or faulty insulin action, which raises blood sugar levels. It affects approximately 25% of the global population, particularly in industrialized and developing countries[1]. Diabetes is associated with some of the primary factors contributing to mortality on a global scale. In India, the number of diabetic patients has doubled in the past two decades, and it is projected that by 2025, around 69.9 million people will have diabetes. The economic burden of diabetes-related deaths in India is estimated to be around $210 billion annually, According to the World Health Organization, it is projected that this figure will increase to $335 billion in the next decade[2].

The treatment of diabetes is complex, and the available drugs have various adverse effects, including weight gain, disruption of intestinal function, acidosis of the intestines, liver damage, drug resistance, and toxicity[1]. Other side effects include hypoglycemia, gastrointestinal upset[3], increased risk of urinary tract infections[4], and higher susceptibility to upper respiratory tract infections[5]. The existing treatments for diabetes offer restricted advantages and are accompanied by exorbitant expenses and potential risks. The disease is not cured by them; instead, they suppress the symptoms, which can have long-term negative effects on overall health. Traditional treatments will remain the cornerstone of diabetes care, however, alternative and emerging therapies possess the capacity to supplement or potentially supplant current treatments, thereby facilitating optimal diabetes management[6].

A typical sign of many chronic illnesses is inflammation. It is an innate reaction to tissue damage brought on by hazardous substances, microbiological pathogens, or physical trauma. Its goal is to remove damaging stimuli and start the damaged tissue's healing process[7]. The inflammation is caused by the denaturation of proteins, and the denaturation can be inhibited by anti-inflammatory drugs. Nevertheless, these medications can lead to gastrointestinal ulcers, which can further result in complications such as ulcer bleeding, perforation, and obstruction. They might also be involved in major cardiovascular events such as acute renal failure, hypertension development, myocardial infarction, and the worsening of established heart failure[8].

Safer and more effective alternatives can be observed in medicinal plants that have traditionally been used to treat diabetes and inflammation. India is home to a plentiful number of medicinal plants. The beneficial effects

of these plants are primarily attributed to the presence of secondary metabolites, which are responsible for their health-promoting properties. Numerous therapeutic plants have been found to contain a range of phytocompounds, including flavonoids, polyphenols, tannins, terpenes, saponins, phytosteroids, and other compounds[9]. One particularly valuable family found widely in the Indian subcontinent is Zingiberaceae, also known as the ginger family, which consists of many important ornamental, spice and medicinal plants. Around 1600 species are known from this family, which consists of approximately 50 genera [10]. Among the rhizomatous perennials in this family, the genus *Hedychium* contains about 70–80 species that are native to Madagascar, Southeast Asia, and India[11]. *Hedychiumflavescens*, also known as yellow ginger flower, is a perennial flowering plant with thick flesy rhizomes and erect, leafy pseudostems reaching heights of 1-3m [12]. It is indigenous to Assam, Myanmar, East Himalaya, China South-Central, Nepal, Vietnam and other places; it has been naturalised in a number of other places, including Madagascar, Mauritius, India, Sri Lanka, West Himalaya, South Africa, and Hawaii[11].

Traditionally, *H. flavescens* has been used as a remedy for swellings[12], to treat fractures[13], for gastritis[14], as an anti-rheumatic[15], and for abdominal swellings, colic, and hemorrhoids[16]. Phytochemical analysis of *Hedychiumflavescens* rhizome revealed the presence of carbohydrates, protein, tannin, cardiac glycoside, starch, phenols, alkaloids, saponins, sugar, and terpenoids, while its flowers showed the presence of carbohydrates, ketose protein, phenols, saponins, and terpenoids[15]. In a GC-MS study, the essential oil extracted from its rhizome exhibited the presence of monoterpene ß-pinene[16]. Strong antibacterial and antifungal effects have been reported for *H. flavescens* leaves and rhizomes against a variety of bacteria. *Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis, Streptococcus mutans, Streptococcus pyogenes, and Staphylococcus aureus*are some of the bacteria that can be found in the samples.*Aspergilluslavus, Aspergillusustus, Aspergillustubigenesis, Aspergillusniger, Aspergillus fumigates, Candida albicans, Cryptococcus neoformans, and Sporothrixschenckii*aresome fungi studied[17].

This study focuses on the acetone extract of *H. flavescens* rhizome and its potential antidiabetic and anti-inflammatory activities. Significantly, this report represents the first documentation of the antidiabetic and anti-inflammatory properties exhibited by the extract derived from *H. flavescens*.

2. Methods And Meterials

2.1. Verification and Collection

From Moolayar stream in Palani hills, Dindigul district, Tamil Nadu, plant specimens were collected and verified at St. Joseph's college (Autonomous), Tiruchirappalli, Tamil Nadu, Rapinat Herbarium and Centre for molecular systematics. This voucher specimen (RHT 68885) can be used as a reference in the future.

2.2. Extraction

The rhizomes were cleansed with water, fragmented into smaller pieces and dried in
shade. The rhizomes were finely ground into powder and carefully stored in containers that were sealed tightly. A 72-hour shaker experiment was conducted at room temperature with powdered rhizomes soaked in acetone. Afterwards, the extracts were filtered through muslin cloth and concentrated using a rotary evaporator. The end product was a dark brown solid extract with a gummy consistency, which was then used for analysis of its antidiabetic and anti-inflammatory properties.

2.3. Assay for Anti-Diabetic Activity (Inhibition of α-Amylase):

The α-amylase inhibitory assay was conducted to study the antidiabetic activity of the rhizome extract from *Hedychiumflavescens*. Test tubes containing various concentrations (10, 50, 100, 250, and 500 µg/ml) of the rhizome extract and the standard drug Acarbose were mixed with α-amylase solution (1.0 mg/ml in phosphate buffer, pH 6.9). After incubating the mixtures for 30 minutes at 25°C, a 0.25% starch solution in a phosphate buffer with a pH level of 6.9 was added to initiate the reaction. The process continued for five minutes at 37°C. After adding the DNS reagent in 0.4 M NaOH, the reaction was stopped by adding the sodium potassium tartrate solution with 1% 3, 5-dinitrosalicylic acid. Following 10 minutes of heating in boiling water, the test tubes were cooled to room temperature. At 540 nm, absorbance (A) was measured after diluting the reaction mixture with distilled water. The blank incubation used buffer solution instead of the enzyme solution. All tests were performed three times. The α-amylase inhibitory activity was determined by calculating the percentage of inhibition using the following formula:

$$\% \, Inhibition = \frac{A1 - (A2 - A3)}{A1} \times 100$$

A1, A2, and A3 denote the absorbance values of 100% enzyme activity, the test sample containing the enzyme, and the test sample lacking the enzyme, respectively.

2.4. Anti-inflammatory Activity:

The aim of this study was to investigate the ability of the rhizome extract of *Hedychiumflavescens* to suppress protein denaturation, in order to assess its anti-inflammatory properties. A modified version of the technique described by Mizushima and Kobayashi and Sakat*etal*. [18, 19] was employed for this evaluation. In this method, 500 µl of 1% bovine serum albumin was mixed with varying concentrations of the standard Acetyl salicylic acid and the rhizome extracts (10, 50, 100, 250, and 500µg/ml). An incubation period of ten minutes at room temperature was followed by a 20-minute incubation period at 51°C . After the sample had reached the ambient temperature, the absorbance was quantified at a wavelength of 660 nanometers. The % inhibition for protein denaturation during the triplicate experiment was determined using the following formula:

$$\% \, Inhibition = \frac{A1 - A2}{A1} \times 100$$

where A1 denotes the control sample's absorbance and A2 the test sample's absorbance. In addition, a dosage response curve was created in order to ascertain the IC50 values.

2.5. Statistical analysis:

The mean ± standard deviation was used to express the acquired data. Students t-test was used to statistically analyse the data, and significance was determined when $p<0.05$. Furthermore, in both analyses, IC50 values were computed for the standard and sample.

3. Results

In this study, the researchers investigated the α-amylase inhibitory activity of the acetone extract obtained from the rhizomes of *Hedychiumflavescens*. The extract was tested at various concentrations ranging from 10 to 500 µg/ml to evaluate its inhibitory effects. For comparison, acarbose, a standard drug for α-amylase inhibition, was also included in the study. The results revealed that the extract displayed significant inhibitory activity, ranging from 29.07% to 82.43%, with an IC50 value of 50.25 µg/ml [Table 1]. These findings suggest that the inhibitory activity of the extract is comparable to that of the standard drug, which had an IC50 value of 55.64 µg/ml. Additionally, it was observed that the inhibitory activity of the rhizome extract was dependent on the dosage administered [Fig. 1].

In this study, another aspect was examined which involved assessing the anti-inflammatory properties of the rhizome extract against bovine serum albumin. The extract displayed a concentration-dependent inhibition of protein denaturation, with average inhibition percentages of 3.25%, 8.42%, 38.89%, 46.36%, and 61.73% for doses of 10, 50, 100, 250, and 500 µg/ml, respectively [Table 2]. The IC50 value for this inhibition was determined to be 82.26 µg/ml. Although the rhizome extract exhibited moderate activity in inhibiting albumin denaturation compared to the positive control Acetyl salicylic acid, which had an IC50 value of 99.13 µg/ml, the findings still suggest its potential as an anti-inflammatory agent[Fig. 2].

Tabel 1:Antidiabetic Activity Of Rhizome Extract Of Hedychium Flavescens

Sl.no.	Concentration (µg/ml)	Percentage of inhibition	
		Sample	Standard
1	10	29.07 ±0.35	34.96 ±0.23
2	50	52.71 ± 0.26	55.15 ±0.33
3	100	68.47 ±0.23	69.71 ±0.62

4	250	75.71 ±0.63	77.01 ±0.54
5	500	82.43 ±0.45	86.13 ±0.67
IC_{50} value		50.25	55.64

Table 2: Anti-Inflammatory Activity Of Rhizome Extract Of *Hedychium Flavescens*

Sl. no.	Concentration (µg/ml)	Percentage of inhibition	
		Sample	Standard
1	10	3.25 ±1.25	8.37 ±0.17
2	50	8.42 ±0.98	11.09 ±0.22
3	100	38.89 ± 0.47	41.26 ±0.46
4	250	46.36 ±1.71	62.67 ±0.21
5	500	61.73 ± 0.33	74.28 ±0.37
IC_{50} value		82.26	99.13

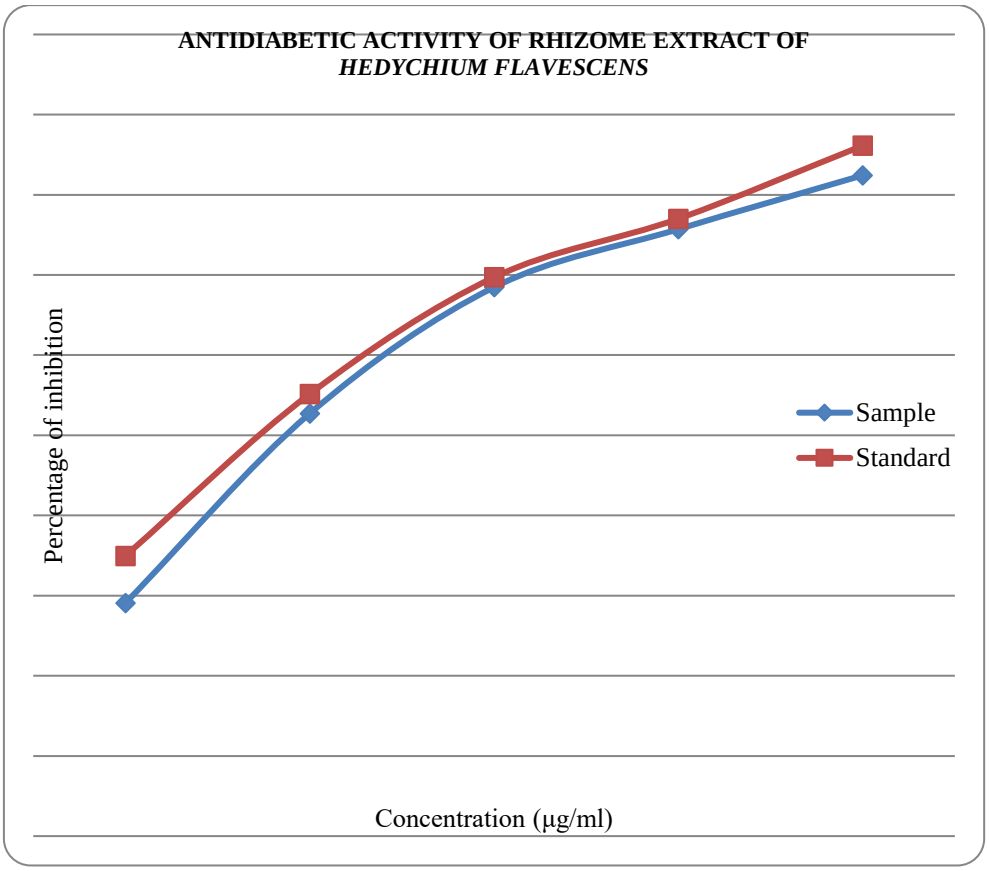

Fig. 1: Anti-inflammatory activity of rhizome extract of *Hedychiumflavesence*

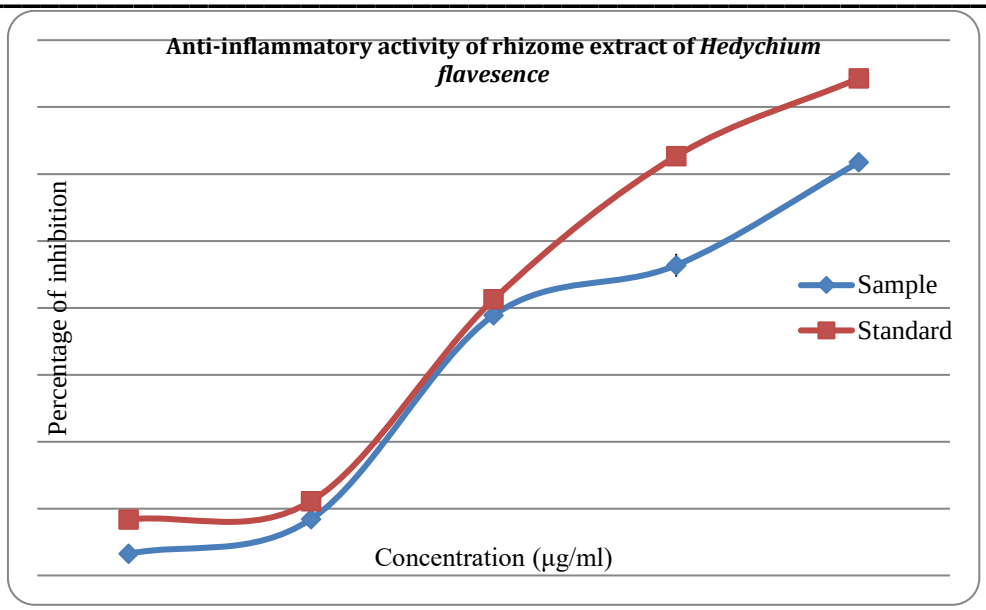

Fig. 2: Anti-inflammatory activity of rhizome extract of *Hedychiumflavesence*

4. Discussion

Diabetes mellitus is a severe and potentially life-threatening metabolic disorder that is becoming more prevalent worldwide. It is characterized by elevated levels of glucose in the blood. While there are numerous effective medications available for treating diabetes mellitus, they often come with significant side effects. However, there are several plant-based sources that show promise as antidiabetic drugs. The antidiabetic properties of certain phytoconstituents like glycosides, alkaloids, terpenoids, flavonoids, and carotenoids are responsible for these effects. These plant extracts have been observed to impact multiple mechanisms, including the functioning of pancreatic beta cells, inhibition of insulinase enzyme, improvement of insulin sensitivity, insulin-like activity, utilization of glucose in the peripheral tissues, synthesis of hepatic glycogen, inhibition of glucose absorption in the intestines, reduction of glycaemic index, and modulation of glutathione effects. In the current study, the inhibitory potential of α-amylase of one such medicinal plant of the Zingiberaceae family, *H. flavescens* was investigated.

One of the main causes of inflammation, which can result in a number of illnesses like neurological, cardiovascular, intestinal, dental, and renal issues, is protein denaturation. Furthermore, diseases like diabetes, ageing, obesity, multiple sclerosis, pancreatitis, and cancer have all been related to inflammation[22–32]. Non-steroidal anti-inflammatory medications (NSAIDs) are widely used to treat inflammation and reduce pain because they can prevent protein denaturation[33]. It is crucial to remember that these medications may have specific negative effect on the hepatic system, cardiovascular system, renal system and hematologic system in addition to the gastric mucosa[34]. Additional mild side effects could include urticaria and aspirin-exacerbated respiratory diseases as well as anaphylactoid responses affecting the skin and pulmonary systems[35, 36]. Throughout history, medicinal plants have been utilized as a natural remedy for inflammation, offering a safer and effective alternative to NSAIDs available in the market. In this particular study, the anti-inflammatory effect of the rhizome extract of *H. flavescens* was evaluated against the denaturation of bovine serum albumin *in vitro*, as there are ethical concerns associated with animal use.

5. Conclusion

The current examination of *H. flavescens* uncovered that the extract from its rhizome effectively hinders α-amylase, showcasing its antidiabetic activity. Additionally, the investigation demonstrated a concentration-dependent inhibition of protein denaturation (albumin) with an IC50 value of 82.26 µg/ml, suggesting that the rhizome extract displayed moderate anti-inflammatory characteristics. Further research is required to investigate approaches for enhancing the plant's *in vivo* potential for antidiabetic and anti-inflammatory effects.

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