# PHYTO CHEMICAL ANALYSIS, ANTI-OXIDANT ASSAY AND INVITRO ANTI-INFLAMMATORY ACTIVITY AND α-AMYLASE ENZYME INHIBITION ACTIVITY OF *HEDYCHIUM CORONARIUM* J. KOENIG.

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**Abstract**

**BackGround:** *Hedychium coronarium* is a small, terrestrial, perennial rhizomatous herb, slightly aromatic plant commonly called as "Ginger Lily, Garland Flower, White Ginger". The plant rich in Phytochemicals and Antioxidant contents used in healing inflammations in human and to treat fever, common cold etc.

**Object:** The current study deals with the phytochemical composition and the anti-oxidant activities of the rhizomes of *Hedychium coronarium*. This study aims to evaluate the phytochemical and antioxidant properties of methonolic, hexane and ethyl acetate extract of rhizome of *Hedychium coronarium.* The plant extract shows various phytochemicals such as alkaloids, steroids, terpenoids, phenols, flavonoids, glycosides and saponins. To evaluate the anti-inflammatory activity and α-Amylase inhibition activity of rhizome extracts of *Hedychium coronarium* J. Koenig.

**Methods:** To elucidate antioxidant potential, DPPH˙ radical, superoxide radical, phoshomolybdenum reduction and $Fe^{3+}$ reducing power assays were done for methanol, hexane and ethyl acetate extracts of rhizome of H. *coronarium.* The present study aimed to evaluate the anti-inflammatory activity of alcoholic rhizome extract of *H. coronarium* by invitro HRBC membrane stabilization method and antidiabetic potential of methanolic rhizome extracts of *H. coronarium* by determining the ability to inhibit the enzyme α-Amylase.

**Results:** Alkaloids, Flavonoids, phenols and steroids were present in the rhizome extracts by the preliminary in vitro screening of rhizome extracts of *H. coronarium.* They contain excellent health beneficial antioxidants, non-toxic and in healing the inflammation and to treat diabetes mellitus. The maximum DPPH˙ radical scavenging activity was 91.13±0.36% at 120 µg/mL concentrations in ethyl acetate rhizome extract and the $IC_{50}$ was identified as 65.84 µg/mL. The maximum phosphomolybdenum reduction was 90.70±1.09% at 100 µg/mL concentrations in methanol extract and the $RC_{50}$ was 55.12 µg/mL. The invitro method showed significant anti- inflammatory property of different extract fraction tested. The n-hexane extract showed significant activity 99.31% to 15.12% of inhibition of RBC hemolysis produced by the extract shows maximum inhibition. When compared to the standard drug, aspirin shows 96.77% to 72.86% of inhibition of RBC hemolysis and other fractions were tested. The protection percentage of extract against inflammation at 0.68% to 84.87% for n-hexane rhizome extract. When compared to the standard drug, aspirin at the same concentration range shows 3.22% to 27.13% and other fractions were tested. The α-Amylase enzyme inhibition assay, the extract at concentration of 120µg/ml showed maximum α-Amylase inhibitory activity 63.25% compared to the standard drug, Acarbose 35.16 %. The methanolic extract had highest inhibitory activity against α-Amylase with 63.25 ± 0.67% inhibition at a concentration of 120µg/ml and $IC_{50}$ of 94.87 µg/ml.

**Conclusion:** This work has reported on the potential of the rhizome extracts of H. *coronarium* as anti-inflammatory and antidiabetic treatments. Hence this study concludes rhizome extract of *H. coronarium* to explore its bioefficiency and this plant have significant activity membrane protection. The plant extract may contain bioactive phytoconstituents which are effective to reduce the rate of digestion and absorption of carbohydrates and thereby contribute for effective management of diabetes. Future studies will provide an insight for the mode of action/mechanisms of the particular component.

**Keywords:** DPPH, Superoxide radical, Phosphomolybdenum reduction assay, Antioxidant, anti-inflammatory, α-Amylase inhibition, Standard drug, Aspirin, Acarbose.

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## 1. Introduction

Medicinal plants play an important role in the lives of rural people particularly in remote area of developing countries. Nearly 80% people living in developing countries still depend on plant-based traditional medicine for their primary health care [1,2]. Plants have been used for health and medicinal purposes for several thousands of years since ancient times for the treatment of many diseases and illnesses. Despite the advances in modern medicine, dependence on plant-based natural products has flourished due to its time tested, safety and efficacy. Bioactive compounds which are present in plants possess numerous health-related effects including antimicrobial, anti-inflammatory, antioxidant, anticancer properties etc. Therefore, plants account for a significant percentage of the pharmaceutical market and their utilization have been increasing rapidly in recent years [3,4]. Based on potent biological actions of natural product, chemists have been trying hard to isolate and identify bioactive leads from plant sources [5]. *Hedychium coronarium* J. Koenig is a small, terrestrial, perennial rhizomatous herb, slightly aromatic plant commonly called as "Ginger Lily, Garland Flower, White Ginger" indigenous to Native of Myanmar, Northeast India and Southern China, is cultivated as ornamental throughout tropics [6]. As a source material for paper preparation it is massively cultivated in Brazil [7]. *Hedychium coronarium* belonging to the family Zingeberaceae, commonly the leaves, stem, rhizome of *H. coronarium* used in ethnomedicine for fungal, bacterial and other microbial infections. *Hedychium coronarium* possesses a range of medicinal applications and its different plant parts are also used in traditional as well as modern medicine [8]. The therapeutic properties of this plant are mentioned in Ayurveda, Charka Samhita and Sushruta Samhita [9]. Medicinal potential of *Hedychium coronarium* increased its demand and uncovered plant for unsustainable harvesting. *Hedychium coronarium* contains a variety of biologically important substances which plays a crucial role in medicine, fragrances, flavor in food. Its utilization in different area in paper making, food for animals and also in other culinary items. The family Zingeberaceae consists of several important medicinal plants with wide range of pharmacological, biological activities and interesting phytochemical constituents. Certain parts of this plant traditionally used in the treatment of inflammation. The present study aimed to authenticate the traditional anti inflammatory activity of this species by invitro anti inflammatory screening [10]. Plants contain an array of natural compounds which have effects on the healing process and the potential to inhibit or reduce the inflammatory process [11]. A study conducted by Johnson [12] emphasised the importance of research in addressing the lack of alternative drugs, the promotion and development of new drugs as well as the management of therapeutic processes like inflammation and related metabolic conditions.

The medicinal plant used in the past days for curing many diseases and ailments in mankind which is known as Hedychium *coronaium*. It is a herbal plant grown in home garden easily available in hilly regions in pasttimes. This plant grown very well in the countries like Iraq, India etc. It is well known for its medicinal and therapeutic value in curing various inflamations in the organs of human body. Anti-pyertic effect in the plant helps in reliving common cold, fever and body ache. The Indian traditional healthcare system, Ayurveda provides relatively organized database and more exhaustive description of botanical materials, many of which have been used as templates for novel drug development [13]. These medicinal herbs constitute indispensable components of the traditional medicine practiced worldwide due to the low cost, easy access and ancestral experience [14]. Phytochemicals are biologically active, naturally occurring chemical compounds in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients [15]. About 80% of the world's population depends on traditional phyto medicines in numerous treatments and disorders [16]. Antioxidants are substances that either directly or indirectly protect cells against the adverse effects of xenobiotics, drugs, carcinogens and toxic radical reactions [17]. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. In a biological system, they protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants terminate the chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. They are believed to play a major role in preventing the development of chronic diseases like cancer, heart disease, stroke, AD, RA and cataracts [18].

Inflammation can be defined as a localised protective, non-specific immune response of vascular tissues to harmful stimuli such as damaged cells or irritants and pathogens [19]. Inflammation is a reaction of living tissues towards

injury and it comprises systemic and local responses [20]. The process of inflammation is a huge burden to human health care and involves many diseases [21]. Although there are multiple drugs available, many of the drugs that are anti-inflammatory agents come with extensive side-effects and have limited clinical use [22]. The safer and effective drugs that have anti-inflammatory properties are essential for the future use.

Inflammation is a part of immune response that depends both on the physical actions of white blood cells and the chemicals that they produce including antibodies, cytokines, etc. Lysosomal enzymes that are released during inflammation produce a variety of disorders by damaging biomolecules and causing lipid peroxidation of membranes, finally resulting in certain pathological conditions including heart attacks, rheumatoid arthritis and septic shocks [23]. Therefore, stabilization of the lysosomal membrane is important, in order to inhibit the release of lysosomal constituents from the activated neutrophil including enzymes and proteases, thus limiting the inflammation. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are taken externally to inhibit the release of lysosomal enzymes or stabilize the lysosomal membranes [24,25]. Human Red Blood Cell (HRBC) or erythrocyte membrane is similar to the lysosomal membrane and its stabilization implies that the extract may also stabilize lysosomal membranes. Hence in this assay stabilization of the HRBC by hypotonicity-induced membrane lysis is taken as an in vitro measure of anti-inflammatory activity of the plant extract [25].

A significant proportion of the population worldwide uses traditional medicinal practices including the use of herbal medicines amongst their armamentarium of therapeutic and prophylactic interventions for communicable and noncommunicable diseases including diabetes mellitus. The medicinal value of plants is attributed to their constituent biologically active phytochemicals [26]. Type 2 diabetes mellitus poses major public health and socioeconomic challenges. It is predicted that 23.9 million people in sub-Saharan Africa will be diagnosed with diabetes mellitus by 2030 [27]. The long-term effects of diabetes mellitus include damage and dysfunction of various organs. Diabetes mellitus also results in high concentrations of free radicals and a decline in the affected individual's natural antioxidant defence mechanisms ultimately causing oxidative stress and leading to damage of cellular organelles and increased lipid peroxidation [28]. Therapy with antioxidant supplements can reduce the amount of free radicals, thereby preventing or decreasing cellular damage brought on by oxidative stress. The management of type 2 diabetes mellitus includes dietary changes, exercise and pharmacologic interventions. There are currently several different classes of pharmacologic agents used to manage type II diabetes mellitus [29]. These pharmacological agents make use of different mechanisms including decreasing the amount of glucose released from the liver, increasing endogenous insulin secretion and increasing the sensitivity of cells to insulin [30] inmitigating the effects of type 2 diabetes mellitus.

## 2. Materials and Methods

*H. coronarium* plant used in this study were collected from the medicinal herb garden at the University of the Kerala. The plant was identified and authentication done by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC) West Tambaram, Chennai. The Reference number of Hedychium coronarium is PARK/2021/4546. Herbaria of voucher specimens were prepared and the specimens were lodged in the Queen Mary's College and Plant Anatomy Research Centre [31]. The Rhizomes dried in the shade, after they were ground to a powder. About 100 g of the grounded powder was extracted with 750ml of absolute methanol respectively, over a period of 48 h. The extracts were filtered through filter paper (Whatman®, No 4). The methanolic filtrate was concentrated under vacuum at 50 °C using a rotary evaporator. Thereafter, the concentrates were dried in an oven at 40°C. Then it can be used for future.

## 3. Procedure

### 3.1 Qualitative phytochemical analysis

**A. Alkaloids**

**Hager's test:** To 1ml of rhizome extract, saturated aqueous solution of picric acid was added and shaken well. yellow precipitate appears

**B. Terpenoids**

**Salkowski test**: To the1 ml of rhizome extract, chloroform was added and mixed well. Then, few drops of Conc.H2SO4 were added along the sides of the test tube. Red ring appears.

**Steroids**

**Libermann -Burchard's test**: To the 1 ml of rhizome extract, 1 mL of acetic anhydride was added and shaken well. To this, few drops of Conc.H2SO4 were added along the sides of the test tube. Dark violet colour appears

**C. Phenols:** To the 1 ml of rhizome extract Add few drops of Ferric chloride solution to the test tube. Add dilute Ammonium and shake well. Slight permanent turbidity occurs

**D. Flavonoids**

**Alkaline Reagent test**: To the 1 ml of rhizome extract, few drops of 2% NaOH solution was added and shaken well. Yellow colour appears

**E. Tannins**

**Lead acetate test**: To the 1 ml of rhizome extract, few drops of 5% Pb (CH3COO)2 solution was added and shaken well. White colour appears

**Glycosides**

**Legal's test:** To the extract, few drops of pyridine and few drops of alkaline sodium nitroprusside solution was added and shaken well. Blood red color appears.

**Saponins**

**Foam test:** To the extract, 3 mL of distilled water was added and shaken vigorously. Foam appears

### 3.2 In vitro antioxidant activity

#### 3.2.1 DPPH˙ radical scavenging activity

Antioxidant activity of rhizome extract of *H. coronarium* was calculated on the basis of stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) radical scavenging activity [32]. The reference standard used here was Ascorbic acid. The decrease in absorbance was measured at 517 nm. The same procedure can be repeated for the hexane and ethyl acetate rhizome extract. The percentage of inhibition was calculated as:

$$\% \text{ of DPPH radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

#### 3.2.2 Superoxide radical scavenging assay

Superoxide radical scavenging activity assay depend on the capacity of the rhizome extract to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system [33]. The standard reference used here was Ascorbic acid. The same procedure can be repeated for the hexane and ethyl acetate rhizome extract. The percentage of inhibition was calculated as:

$$\% \text{ of superoxide radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

#### 3.2.3 Phosphomolybdenum reduction assay

The antioxidant capacity of rhizome extract of *H.coronarium* was done by the method of [34]. The absorbance of the coloured complex was measured at 695 nm. The standard reference used here was the Ascorbic acid. The same procedure can be repeated for the hexane and ethyl acetate rhizome extract. The percentage of reduction was calculated as:

$$\% \text{ of Mo6+ reduction} = \text{Sample} - \frac{\text{Control}}{\text{Sample}} \times 100$$

#### 3.2.4 Ferric ($Fe^{3+}$) reducing power assay:

Reducing power of the rhizome extract of *H.coronarium* was determined by the modified(simply) potassium ferricyanide method [35]. The standard reference used here was the Ascorbic acid. The same procedure can be repeated for the hexane and ethyl acetate rhizome extract. The percentage of reduction was calculated as:

$$\% \text{ of } Fe^{3+} \text{ reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \times 100$$

### 3.3 Invitro anti-inflammatory assays

#### 3.3.1 Human Red Blood Cell (HRBC) Membrane Stabilization Assay

HRBC method was used for the estimation of anti-inflammatory activity in vitro [36] with slight modifications. To prepare suspension, a volume of 5 ml of blood sample was collected through a vein puncture from myself without taking any NSAIDs for 2 weeks prior to the experiment. It was centrifuged at 3000 rpm for 5 minutes and the supernatant was discarded. The carefully collected pellet was washed thrice with normal saline which is equal in volume to the pellet. It was then centrifuged until the yellowish colour of the

supernatant turned colourless with slight modifications. In order to prepare the reaction mixture 1 ml of the plant samples of each concentration were mixed with 200 µl of red blood cell suspension. The reaction mixtures were then incubated at 54 °C for 30 minutes. The tubes with reaction mixtures were cooled. The absorbance of the supernatant after centrifugation was measured at 560 nm using distilled water as the blank. The control was prepared using only red blood cell suspension. The same procedure was carried out with the concentration series of aspirin as the positive control instead of 1 ml of the plant sample [37]. The percentage of hemolysis and protection were calculated using Equations 1 and 2 [37].

Percentage hemolysis = [(optical density of test solution) ÷ (optical density of control)] × 100. (1)

Percentage Protection = 100- [(optical density of test solution) ÷ (optical density of control) × 100]. (2)

### 3.4 Invitro α-Amylase inhibition assay

The α-Amylase inhibiting activity of the solvent fraction was measured employing the starch-iodine method [38] with slight modifications and using of acarbose (conc. 60 µg/ml) as standard. The extract composed of concentration range (20-120 µg/ml) of rhizome extract of *H.coronarium* and 10 µl of 1% (w/v) solution of α- Amylase enzyme prepared in 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) and incubated at 37°C for 10 min. Followed by addition of soluble starch (1%, w/v) added and incubated at 37°C for 60 min. The enzymatic reaction stopped by adding 100 µl of 1 M HCl, then 200 µl of iodine reagent (5 mM I2 and 5 mM KI) added. The inference colour change noted, then absorbance read at 540 nm. The control without plant extract representing 100% enzyme activity. The inhibitors present in the extract, the starch added to the assay mixture not degraded and showed a dark-blue colour complex, whereas no colour complex is observed in the absence of the inhibitor, indicating that starch is completely hydrolyzed by α-Amylase [38]. % Inhibition of enzyme activity was calculated by

% Inhibition of enzyme activity = (A-B)/(A)$x$100 (3)

Where, A = absorbance of the plant sample and B= absorbance of control (no extract).

### Data Analysis

All tests were carried out in triplicate and the data shown in the table and the graphs are the average together with Standard Deviation (SD).

## 4. Results

The compounds which are biologically active from the natural plant materials have always been of great interest to botanist working on disease prevention. The in-depth research on the chemical and pharmacological nature of products of plant origin are very essential and this may eventually lead to the discovery of medicine that can be used in the treatment of several diseases including genetical and non-genetical diseases. plants having medicinal properties are resources of new drugs. The research needs to find out the scientific evidence for claims of plants. On the basis of the scientific approach the present study was to investigate the phytochemical constituent and antioxidant activities in *Hedychium coronarium* rhizome.In the Philippines, *Hedychium* is represented by only two known species, namely *H. coronarium* Koenig and *H. philippinense K.Schum*. The former is widely cultivated in the Philippines and other countries for ornamental purposes and therapeutic value, while the latter is endangered (DAO 2017-11). *H. coronarium*, locally known as camia, had been studied and became extremely popular. It had been the focus of majority of studies on inflorescence and flower development [39], variations in tissue development, phytochemical constituents [40], pollen biology, and medicinal value [41]. On the other hand, no new studies on *H. coronarium and H. philippinense* had been conducted in the last few decades on pharmaceutical basis. This paper discussed on the results came out from the qualitative and quantitate analysis which helps in the further identification and discovery of new components. Furthermore, investigations are achieved only through the analysis and various laboratory tests conducted on this plant. Here we undergone few laboratories test on quantitative and qualitative analysis on the *H. coronarium* rhizome and results are shown.

**Table :1.** Phytochemical content of the medicinal plant *H. coronarium*.

| Phytochemical tests | Methanol rhizome extract | Hexane rhizome extract |
|---|---|---|
| Alkaloids | ++ | - |
| Terpenoids | - | + |
| Steroids | + | + |
| Phenolic compounds | + | + |
| Flavonoids | + | - |
| Tannins | + | - |
| Glycosides | + | - |
| Saponins | + | - |

Expansions: (+ = Present) and (- = Absent)

The phytochemical analysis of rhizome extract of *H.coronarium* are done for the presence of alkaloids, terpenoids, steroids phenolic compounds, flavonoids, tannins, glycosides and saponins. (Table:1) The results shows that the methanolic extract of *H.coronarium* shows high amount of alkaloids, steroids, phenols, flavonoids, tannins, glycosides and saponins. But the hexane extract shows lesser amount of terpenoids, steroids and phenols.

### 4.1 DPPH˙ radical scavenging activity

The capability of methanol, hexane and ethyl acetate extract of *H.coronarium* to scavenge DPPH. (1,1-diphenyl-2-picrylhydrazyl) radical was evaluated by reducing the purple-colored stable DPPH (1,1-diphenyl-2- picrylhydrazyl) radical to the yellow colored 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increases with increasing concentration of the extract [42]. DPPH radical scavenging activity was carried out with the three different solvents namely methanol, hexane and ethyl acetate of rhizome and the results shows that DPPH˙ radical scavenging activity in methanol extract 84.17±0.37% at 120 µg/mL, in hexane extract 68.29±1.09% at120 µg/mL and in ethyl acetate extract 91.13±0.36% at 120 µg/mL (Table :2 and Fig:1). The maximum DPPH˙ radical scavenging activity was 91.13±0.36% at 120 µg/mL concentrations in ethyl acetate rhizome extract. The $IC_{50}$ of the three extracts are (71.28, 87.80 and 65.84) µg/mL concentrations respectively and the maximum $IC_{50}$ was reported in 65.84 µg/mL concentrations in the same extract. It was compared with the standard ascorbic acid ($IC_{50}$ = 142.68 µg/mL concentration).

**Table: 2.** Antioxidant activity by DPPH˙ radical scavenging assay on the extract of rhizome of *H. coronarium*

| DPPH* Radical Scavenging Activity | | | |
|---|---|---|---|
| Concentration (µg/mL) | Rhizome extract of Hedychium coronarium % of inhibition | | |
| | Methanol | Hexane | Ethyl Acetate |
| 20 | 14.16±7.60 | 22.41±1.91 | 46.30±3.94 |
| 40 | 49.73±1.09 | 25.36±1.69 | 57.02±4.17 |
| 60 | 61.75±1.27 | 40.52±2.19 | 66.32±1.45 |
| 80 | 78.09±2.72 | 47.75±0.47 | 76.3±4.71 |
| 100 | 80.67±1.84 | 61.94±3.44 | 88.62±2.07 |
| 120 | 84.17±0.34 | 68.29±1.90 | 91.13±0.36 |

**Fig 1:** DPPH˙ radical scavenging activity of the rhizome extract *H. coronarium*

### 4.2 Superoxide radical scavenging activity

Superoxide anion is also very harmful to cells and their effects can be magnified because it produces other kinds of free radicals and oxidizing agents. [43]. Flavonoids are effective antioxidants because they scavenge superoxide anions. Superoxide anions derived from dissolved oxygen from the riboflavin-light system and the evolving superoxide anions will reduce NBT. In this method, superoxide anion reduces the yellow dye ($NBT^{2+}$) to blue formazan, which was measured at 590 nm in UV-Vis spectrophotometer. Antioxidants have the ability to inhibit the blue NBT formation and the decreasing absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Superoxide radical scavenging activity was carried out with the 3 solvents namely as methanol, hexane and ethyl acetate and the results

shows that methanol rhizome extract 52.93±3.34% at 120 µg/mL, hexane rhizome extract 35.03±8.20% at 120 µg/mL and ethyl acetate rhizome extract 70.03±4.21% at 120 µg /mL (Table:3 and Fig:2). The maximum superoxide radical scavenging activity was 67.99±4.21% at 120 µg/mL concentrations in ethyl acetate rhizome extract and the $IC_{50}$ of the three extracts are (113.35 ,171.28 & 88.24) µg/mL concentrations respectively. The maximum $IC_{50}$ was reported in 88.24 µg/mL concentrations. It was compared with the standard of ascorbic acid ($IC_{50}$ = 144.47 µg/mL concentration).

**Table 3:** Superoxide radical scavenging activity of the rhizome extract *H. coronarium.*

| **Superoxide Radical Scavenging Activity** | | | |
|---|---|---|---|
| **Concentration** | **Rhizome extract of Hedychium coronarium** | | |
| **(µg/mL)** | **% of inhibition** | | |
| | **Methanol** | **Hexane** | **Ethyl Acetate** |
| 20 | 17.59±9.44 | 5.89±0.35 | 25.72±15.58 |
| 40 | 31.98±1.92 | 9.93±3.82 | 55.04±6.59 |
| 60 | 34.46±1.76 | 13.87±4.24 | 59.76±7.02 |
| 80 | 37.04±2.72 | 23.58±1.39 | 61.51±4.58 |
| 100 | 45.42±1.30 | 23.47±5.24 | 61.51±4.58 |
| 120 | 52.93±3.34 | 35.03±8.20 | 67.99±4.21 |

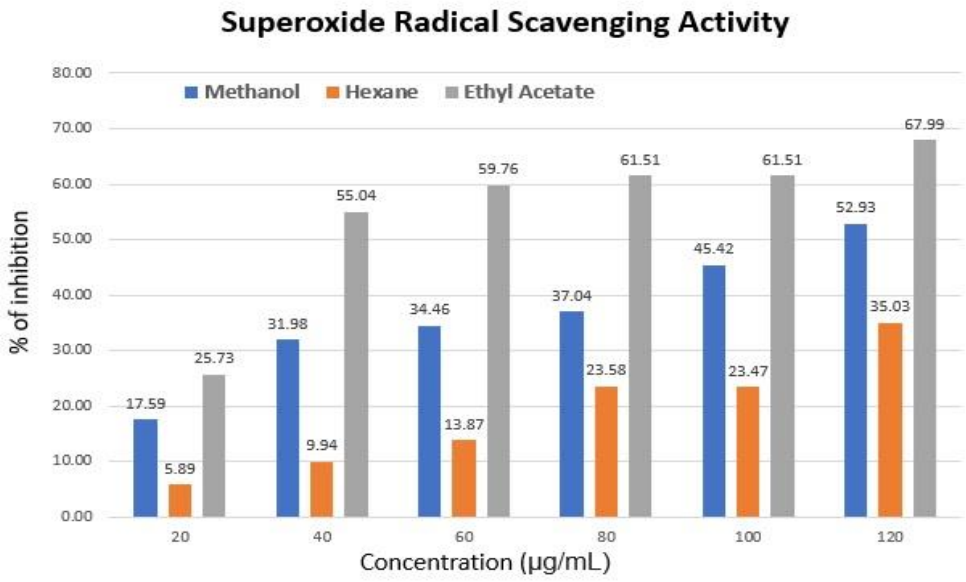



**Fig 2:** Superoxide radical scavenging activity of the rhizome extract *H. coronarium*.

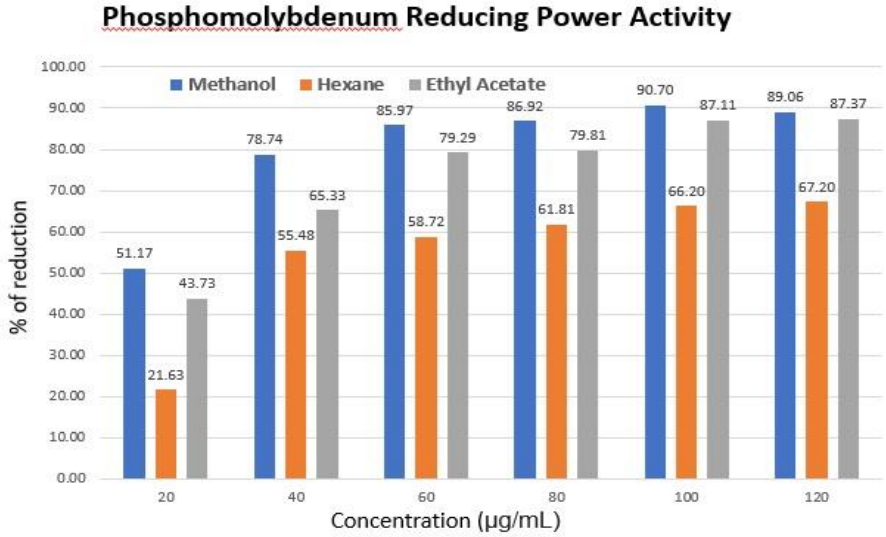
**4.3 Phosphomolybdenum reduction activity**

Antioxidant activity of rhizome extract of *H.coronarium* was measured by phosphomolybdenum reduction assay method which is based on the reduction of Mo (VI) to Mo(V) and the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm [44]. Phosphomolybdenum reduction assay was carried out with the 3 solvents such as methanol, hexane and ethyl acetate. It shows that methanol rhizome extract 90.70±1.09% at 100 µg/mL, hexane rhizome extract 67.20±1.24% at 120 µg/mL and ethyl acetate rhizome extract 87.37±1.87% at 120 µg/mL. The maximum phosphomolybdenum reduction was 90.70±1.09% at 100 µg/mL concentrations in methanol extract and the $RC_{50}$ of the three extracts are (55.12,89.28 and 68.67) µg/mL concentrations (Table:4 and Fig:3). The maximum $RC_{50}$ was reported in 55.12 µg/mL concentrations. It was compared with the standard ascorbic acid ($RC_{50}$ = 11.39µg/mL concentration).

**Table :4** Phosphomolybdenum reducing power activity of the rhizome extract *H. coronarium.*

| **Phosphomolybdenum Reducing Power Activity** | | | |
|---|---|---|---|
| **Concentration** | **Rhizome extract of Hedychium coronarium** | | |
| **(µg/mL)** | **% of reduction** | | |
| | **Methanol** | **Hexane** | **Ethyl Acetate** |
| 20 | 51.17±7.03 | 21.63±4.25 | 43.73±9.40 |
| 40 | 78.74±2.94 | 55.48±5.75 | 65.33±5.78 |
| 60 | 85.97±1.98 | 58.72±4.53 | 79.29±3.12 |
| 80 | 86.92±1.85 | 61.80±1.78 | 79.81±3.26 |
| 100 | 90.70±1.09 | 66.2±2.13 | 87.11±2.08 |
| 120 | 89.06±1.22 | 67.2±1.24 | 87.36±1.87 |

**Fig 3:** Phosphomolybdenum reducing power activity of the rhizome extract *H. coronarium.*




**4.4 Ferric ($Fe^{3+}$) reducing power assay**

The reducing power of $Fe^{3+}$ to $Fe^{2+}$ by the rhizome extract of *H.coronarium* was measured and the reduction ability increases with increasing concentration of the extract due to the formation of ferro-ferric complex [45]. Ferric ion reducing power activity determines the electron donating ability of an antioxidant. The flavonoids and phenolic compounds present in the rhizome extract have the ability to donate electrons, which reflects strong antioxidant activity The maximum $Fe^{3+}$ reduction assay was taken out with the solvents namely methanol, hexane and ethyl acetate and the results shows that methanol rhizome extract72.04±0.97% at 120 µg/mL, hexane rhizome extract77.52±0.95% at 120 µg/mL and ethyl acetate rhizome extract 59.39±0.26% at 120 µg/mL (Table:5and Fig:4). The maximum $Fe^{3+}$ reduction was 77.52±0.95 % at 120 µg/mL concentrations in the Hexane extract and the $RC_{50}$ of the three extracts are (83.28,77.39 and 101.02) µg/mL concentrations. The maximum $RC_{50}$ was reported in 77.39 µg/mL concentrations in the same extract. It was compared with the standard ascorbic acid (RC50= 15.14 µg/mL concentration.

**Table :**5 $Fe^{3+}$reducing power activity of the rhizome extract *H. coronarium.*

| **$Fe^{3+}$ reducing Power Activity** | | | |
|---|---|---|---|
| **Concentration** | **Rhizome extract of Hedychium coronarium** | | |
| **(µg/mL)** | **% of reduction** | | |
| | **Methanol** | **Hexane** | **Ethyl Acetate** |
| 20 | 36.14±14.37 | 6.48±3.61 | 28.10±3.59 |
| 40 | 51.86±2.41 | 15.32±4.04 | 39.81±0.93 |
| 60 | 64.05±0.90 | 47.25±1.79 | 42.87±0.26 |
| 80 | 65.46±1.98 | 56.06±1.27 | 50.36±0.70 |
| 100 | 69.69±0.80 | 67.92±1.18 | 58.48±0.47 |
| 120 | 72.04±0.97 | 77.52±0.95 | 59.38±0.26 |

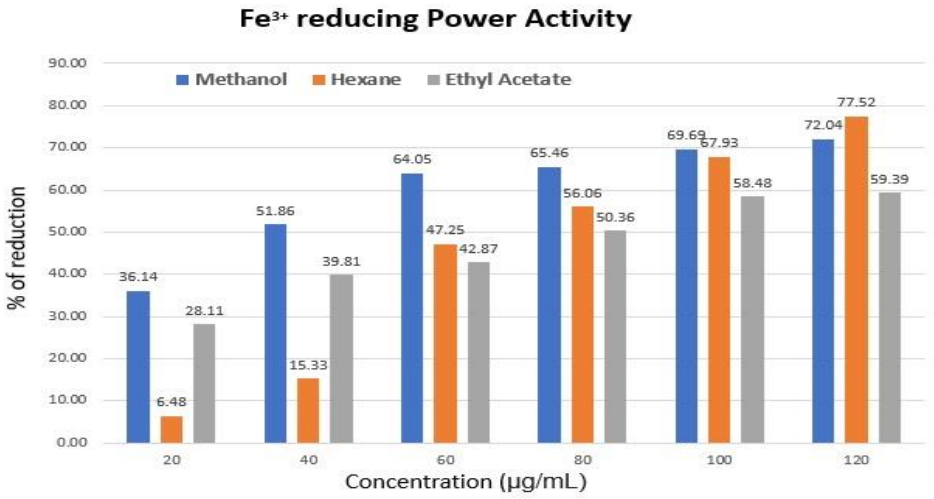



**Fig 4:** $Fe^{3+}$reducing power activity of the rhizome extract H. coronarium

The preliminary phytochemical screening was carried in broad sense to know its chemical content and it reveals the presence of considerable amount of Alkaloid, Flavonoid, Terpenoids, Steroid, Phenolic substances, Tannins and some amount of Saponins were identified from the phytochemical screening.

### 4.5 Anti-Inflammatory Activity

#### 4.5.1 Human Red Blood Cell (HRBC) Membrane Stabilization Activity

The inflammation process is a highly complex pathophysiological process which occurs when various processes of a variety of molecules and mediators that are signalling, occur in a series of catalytic enzymatic reactions [46]. Many plant extracts are known to exert their inhibitory enzyme effects via a range of diverse action mechanisms and target sites [47]. Stabilization of the HRBCs membrane was studied to further evaluate the anti-inflammatory action of rhizome extract of *H. coronarium*. The percentage of membrane stabilization for the plant was tested at different concentrations ranging from 20 µg/ml to 120 µg/ml at 560 nm and aspirin was used as the positive control. The percentages of hemolysis and protection percentage against inflammation was calculated for the plant extract are given in Table 6.

The results indicate that a concentration dependent increase in the HRBC membrane stabilization was observed at 20-120 µg/ml. At this concentration range *H. coronarium* methanolic rhizome extract produced 81.13% to 49.61% inhibition of RBC hemolysis and Ethyl acetate rhizome extract produced 71.13 % to 60.36 % inhibition of RBC hemolysis as compared to 99.31% to 15.12% of inhibition of RBC hemolysis produced by the n-hexane rhizome extract shows maximum inhibition. The n-hexane fraction of rhizome extract shows higher % of inhibition than the standard drug, aspirin shows 96.77% to 72.86% of inhibition of RBC hemolysis (Table 6 and Figure 5). The percentage of protection increased with increasing concentrations of the samples. The plant showed an increase in protection percentage of 18.86% to 50.38% in methanolic rhizome extract and protection percentage of 28.86% to 39.63% in Ethyl acetate rhizome extract and whereas the protection percentage of 0.68% to 84.87% for n-hexane rhizome extract shows maximum protection of hemolysis compared to other fractions. The standard drug, aspirin at the same concentration range shows 3.22% to 27.13% (Table 6 ang Figure 6). With the increasing concentrations of rhizome extract, membrane hemolysis decreases and membrane stabilization increases, thereby the protection is increased, which implies that the anti-inflammatory activity of the plant is concentration dependent.

As shown in (Figure 5), a concentration-response relationship was observed during the membrane stabilizing assay and a comparative activity was presented by the standard drug Aspirin. In this test Methanol, n-hexane and Ethyl acetate fractions revealed prominent inhibition of RBC hemolysis in the solution. Among these three fractions, n-hexane rhizome extract at the concentration of 120µg/ml showed highest level of membrane stabilizing activity (99.31% inhibition of hemolysis), then followed by (81.13% and 71.13 % inhibition) exhibited by Methanolic and Ethyl acetate rhizome extract fractions at the concentration of 120µg/ml, respectively. The standard drug, aspirin at the same concentration range shows 96.77% to 72.86% (Figure 5).

Membrane stabilization assay of erythrocytes is a very popular tool to investigate the anti-inflammatory potential of the plant extracts. The results (Figure 5) suggest that the rhizome extract have excellent anti-inflammatory potential via membrane stabilization mechanism.

As shown in (Figure 6), a concentration-response relationship was observed during the membrane stabilizing assay and a comparative activity was presented by the standard drug Aspirin. In this test Methanol, Ethyl acetate and n-hexane fractions revealed prominent protection percentage of 18.86% to 50.38% and protection percentage of 28.86% to 39.63% and whereas the protection percentage of 0.68% to 84.87% shows maximum protection compared to other fractions. The standard drug, aspirin at the same concentration range shows 3.22% to 27.13% (Figure 6). The results (Figure 6) suggest that the rhizome extract have excellent anti-inflammatory potential via membrane stabilization mechanism. Hence this study concludes n-hexane extract of *H. coronarium* to explore its bio efficiency and *H. coronarium* have significant activity (membrane protection) tabulated in (Table 6). The anti-inflammatory activity may be due to alkaloid or steroid present in the alcoholic extract of *H. coronarium*

**Table 6:** Percentage of hemolysis and Percentage of protection for the rhizomeextract of *Hedychium coronarium*..

| Solvent Fractions | Average percentage of hemolysis ± SD (%) | Average percentage of protection ± SD (%) |
|---|---|---|
| Methanol | 81.13±0.89 | 18.86±0.89 |
| | 71.95±5.81 | 27.95±6.01 |
| | 61.51±8.95 | 38.48±8.95 |
| | 59.47±8.88 | 40.52±8.88 |
| | 54.69±4.39 | 45.3±4.39 |
| | 49.61±7.23 | 50.38±7.23 |
| n- hexane | 99.31±0.80 | 0.68±0.80 |
| | 74.98±1.50 | 25.0±11.50 |
| | 65.18±0.80 | 34.81±0.80 |
| | 58.18±2.12 | 41.78±2.14 |
| | 49.08±3.46 | 50.91±3.46 |
| | 15.12±4.61 | 84.87±4.61 |
| Ethyl acetate | 71.13±2.01 | 28.86±2.01 |
| | 68.72±1.51 | 31.27±1.51 |
| | 66.43±2.69 | 33.56±2.69 |
| | 63.54±2.56 | 36.45±2.56 |
| | 61.34±3.75 | 38.65±3.75 |
| | 60.36±4.35 | 39.63±4.35 |

**Fig 5:** Percentage of hemolysis showing by the concentration series of rhizome extract of *Hedychium coronarium.*

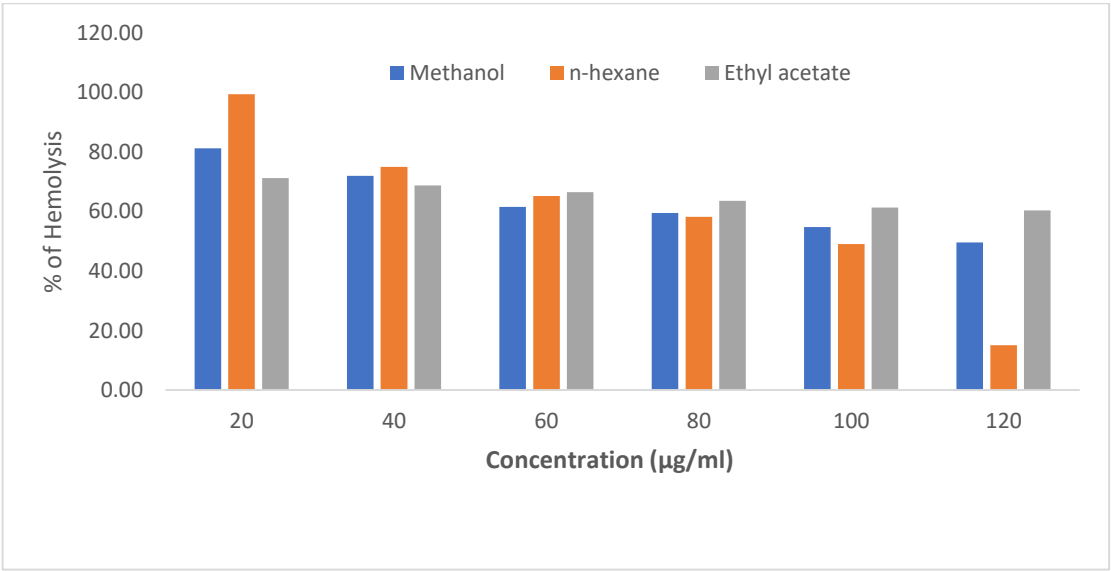
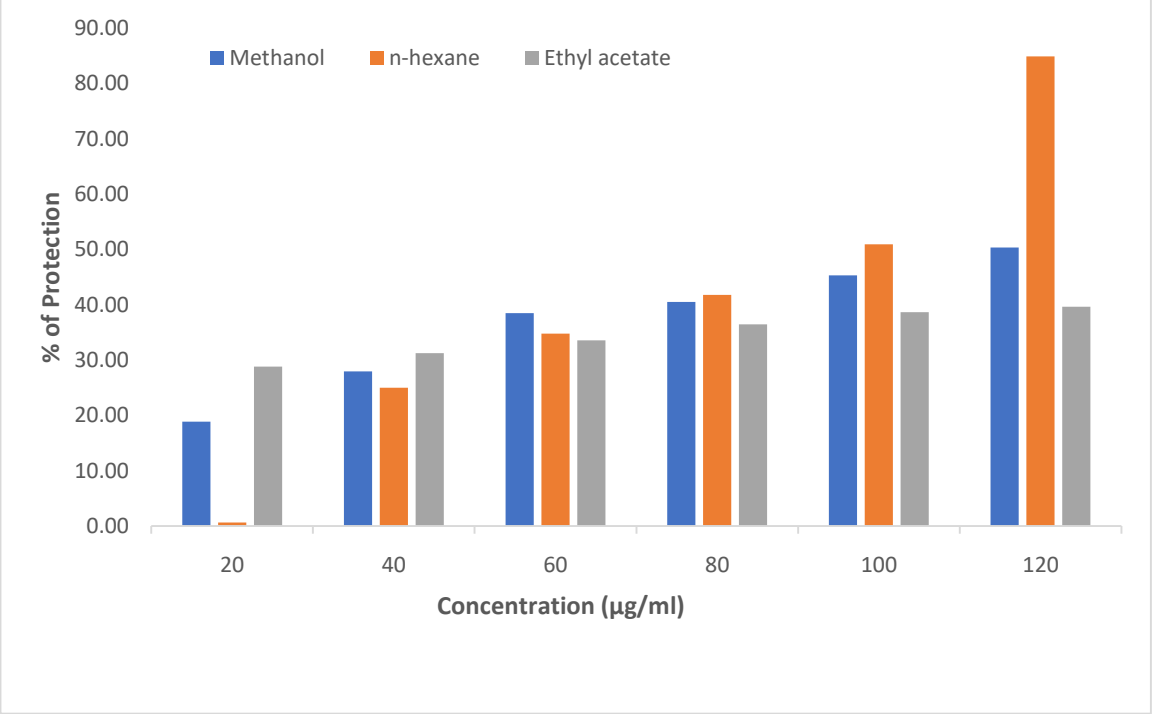


**Fig 6:** Percentage of protection showing by the concentration series of rhizome extract of *Hedychium coronarium.*




**4.6 Evaluation of α-Amylase inhibiting activity**

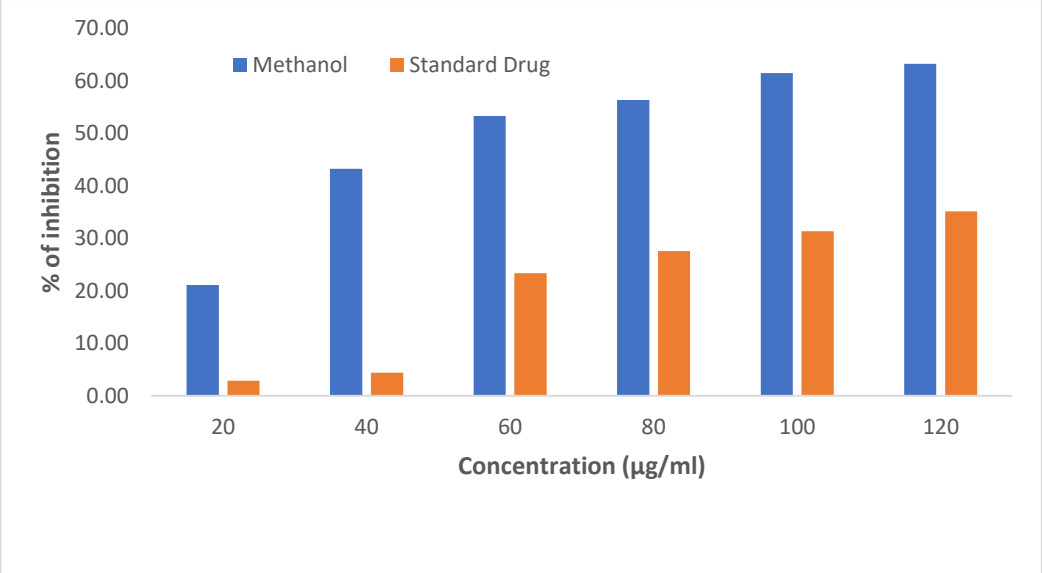
In this study, *H. coronarium* were investigated for their α-Amylase inhibition using a Spectroscopic method. The α-Amylase inhibitory potentials of the plant sample were compared with standard acarbose under specific assay conditions. As shown in (Table 7), the entire sample had inhibitory action on α-Amylase enzyme which catalyzes the hydrolysis of starch into sugar. For standard acarbose serial dilutions were carried out to obtain concentration range from (10 µg/ml to 60 µg/ml) and shows inhibitory activity at 35.16% to 60 µg/ml concentrations and for the methanolic rhizome extract concentration range from (20 µg/ml to 120 µg/ml) and shows maximum inhibitory activity at 63.25±0.67% to 120µg/ml concentrations. The consecutive concentration of methanol extract of *H. coronarium* rhizome extract (63.25±0.67% at 120µg/ml) and standard acarbose (35.16% - 60µg/ml) produced inhibition of α-Amylase activity (Table 7) showed maximum α-Amylase inhibitory activity compared to inhibition exhibited by acarbose. The $IC_{50}$ of the rhizome extract was (94.87 µg/ml concentrations). These results indicated that these plant extract may contain bioactive phytoconstituents which are effective to reduce the rate of digestion and absorption of carbohydrates and thereby contribute for effective management of diabetes. Future studies will provide an insight for the mode of action/mechanisms by which these active compounds regulate glucose level.

As shown in (Figure 7), the events observed during the α-Amylase inhibition assay and compared with the standard drug Acarbose. In this test, Methanol fraction revealed prominent inhibition of α-Amylase activity, the extract of *H. coronarium* rhizome (63.25±0.67% at 120µg/ml) and standard acarbose (35.16% - 60µg/ml) produced inhibition of α-Amylase activity (Table 7). The methanolic rhizome extract showed maximum α-Amylase inhibitory activity compared to inhibition exhibited by acarbose. The standard drug, acarbose at the concentration range 60µg/ml shows 35.16% inhibition (Figure 7) and the rhizome extract have excellent anti-diabetic potential via α-Amylase activity at the concentration range 120µg/ml shows 63.25±0.67% inhibition. The $IC_{50}$ of the rhizome extract was (94.87 µg/ml concentrations). Hence this study concludes methanol rhizome extract of *H. coronarium* to explore its active principles and *H. coronarium* have significant α-Amylase inhibition activity tabulated in (Table 7). The anti-diabetic activity may be due to alkaloid or steroid present in the alcoholic extract of *H. coronarium*

**Table 7:** α-Amylase inhibitory activity and $IC_{50}$ values of the methanolic extracts of *Hedychium coronarium.*

| Solvent System | Concentration (µg/ml) | Inhibition (%) | IC50 (µg/ml) |
|---|---|---|---|
| Methanol | 20 | 21.13±1.33 | 94.87 |
| | 40 | 43.28±0.59 | |
| | 60 | 53.36±0.02 | |
| | 80 | 56.37±0.89 | |
| | 100 | 61.51±1.58 | |
| | 120 | 63.25±0.67 | |

**Fig 7:** α-Amylase inhibitory activity of the methanolic rhizome extracts of *Hedychium coronarium* with the Standard drug Acarbose.




## 5. Discussion

The phytochemical analysis of the methanolic extracts of *H. coronarium* revealed the presence of alkaloids, flavonoids, steroids, phenolic substances, saponins, tannins which could be responsible for the medicinal properties of this plant. Antioxidants delay the oxidation of cellular molecules by mopping up free radicals involved in the oxidative chain reaction that leads to oxidative damage of cells, which would result in the development of chronic diseases. Many plant-derived phenolics are potent antioxidants [48]. Plants such as *Tamarindus indica* L. (Fabaceae), *Mitragyna inermis* (willd) O ktze (Rubiaceae) and *Holarrhea floribunda* (G. Don) T. Durand & Schinz (Apocynaceae) have been shown to contain phytochemicals with antidiabetic properties and their use as ethnomedicines for the treatment of diabetes mellitus has been scientifically validated [30]. The World Health Organization has developed the Traditional Medicine Strategy (2014–2023) to promote the use of ethnomedicines, of proven quality, safety and efficiency [49]. Thus, it is important to continue to screen and validate the medicinal potential of plants commonly used in ethnomedicine thereby further unlocking their potential uses. To explore the actual potential in treating inflammation and diabetes, the medicinal plant *Hedychium coronarium* taken and studied. Presence of these biological contents makes this herb as a valuable drug. Presence of these valuable Phyto-compounds, *Hedychium coronarium* possesses various biological actions like analgesic, anti-inflammatory, antioxidant, anti-inflammatory, antitumor, anti-diabetic and cytotoxic. It also has high ethnomedicinal significance and used as traditional medicine throughout the world. Due to high medicinal demand and important biological properties, it was interesting to work on this plant and decided to work out with *Hedychium coronarium* for the scientific exploration of traditional knowledge of this plant in therapeutic area.

Polyphenols are carbon-based aromatic phenyl ring compounds which oxidise to quinones by reactive oxygen species, characteristic responsible for the antioxidant activity and anti-inflammatory activity [50] in most plant extracts. Since human red blood cell (HRBC) membranes are similar to these lysosomal membrane components, the prevention of Hypotonicity induced HRBC membrane lysis was taken as a measure in

estimating the anti-inflammatory property of various extracts of *Hedychium* coronarium. Thus, human blood cell membrane stabilization (HRBC method) [10] has been used as a method in estimating the anti-inflammatory property. Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. Here anti-inflammatory activity was performed based on the information using HRBC method. It was selected for the invitro evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane [51] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release.

Inflammation is caused by release of chemicals from tissues and migrating cells. Most strongly implicated are the prostaglandins (PGs), leukotrienes (LT5), histamine, bradykinin, and, more recently, platelet-activating factor (PAF) and interleukin-1. Evidence for their involvement comes from studies with competitive antagonists for their receptors and inhibitors of their synthesis. H1 histamine antagonists are effective for hay fever and some skin allergies such as urticaria, which indicates the importance of histamine in these conditions. Symptoms of rheumatoid arthritis are alleviated by the aspirin like anti-inflammatory drugs, which inhibit the cyclo-oxygenase enzyme and reduce synthesis of prostanoids. Corticosteroids prevent the formation of both PGs and LTs by causing the release of lipocortin, which by inhibition of phospholipase A2 reduces arachidonic acid release. They suppress the inflammation of rheumatoid arthritis and asthma. Currently, high doses of nonsedating H1 antihistamines and PAF antagonists are being tested for the treatment of allergic asthma [52].

Many other aspirin like drugs are now available - the nonsteroid anti-inflammatory drugs (NSAIDs). We also have the anti-inflammatory steroids, but none of these substances is a cure or stops the progression of arthritis or chronic inflammation. Inflammation covers a host of pathophysiological events and means different things to different people. The result indicted that the rhizome extract of *H. coronarium* at various concentrations has significant anti-inflammatory property. This significant anti-inflammatory effect may be due to the inhibition of any inflammatory substance by the alkaloids and steroids [53] present in the extract. The result indicates the efficiency of an effective therapeutic agent in the treatment of inflammations. This study asthenies the folk lore information on the anti-inflammatory property of the rhizome extract of *H. coronarium.* Further and detailed studies are in process for the isolation of active constituent responsible for this property and to identification of the possible mechanism of its anti-inflammatory property.

The present study provides that HRBC membrane stabilization was observed at various concentration range. At the concentration range of *H. coronarium* methanolic rhizome extract shows 81.13% inhibition of RBC hemolysis and Ethyl acetate fraction shows 71.13 % inhibition and compared to 99.31% of inhibition shown by the n-hexane rhizome extract and this is the maximum inhibition than the standard drug, aspirin 96.77% of inhibition of RBC hemolysis (Table 6 and Figure 5). *H. coronarium* shows a protection percentage of 18.86% to 50.38% in methanolic rhizome extract, 28.86% to 39.63% in Ethyl acetate fraction and whereas the protection percentage of 0.68% to 84.87% for n-hexane rhizome extract shows maximum protection compared to other fractions and also to the standard drug, aspirin at the same concentration range shows 3.22% to 27.13% (Table 6 ang Figure 6). In this study shown some preliminary in vitro screening that alcoholic extract of *H. coronarium* rhizome possess high anti-oxidant activity and effective protection and healing of inflammation. This contributes to the validation of *H. coronarium* use in anti-inflammatory treatment. Work aimed at isolating the active molecules is also needed. However, a need to explore the anti-inflammatory potential of the plant in vivo.

Diabetes mellitus is postulated to a state of increased free radical activity. Increased free oxygen radical activity can initiated peroxidation of lipids, which in turn stimulates glycation of proteins, inactivation of enzymes and alteration in the structure and functions of collagen, basement and other membranes and play a role in the long-term complication of diabetes [54]. Glucose auto-oxidation is thought to be the cause of the increase oxidative stress associated with diabetes mellitus

[55]. Type 2 diabetes mellitus is a chronic disease characterized by hyperglycemia, insulin resistance, and oxidation of cellular lipids and proteins [30]. Two – electron reduction of alloxan gives dialuric acid. It is unstable at aqueous solution and oxidized to back alloxan. This process is accompanied by reduction of oxygen to $O_2$ which is converted to $H_2O_2$ and possibly the hydroxyl radical in the presence of traces of transitional metal ions [56,57].

The present work showed that the methanolic extracts of *H. coronarium* rhizome extract inhibited α-Amylase in a concentration dependent manner (Table 7). It exhibited a higher degree of inhibition in different concentrations compared to the standard drug Acarbose. At a concentration of 120 µg/ml the methanolic rhizome extract showed 63.25±0.67 % inhibition of α-Amylase while at the 60 µg/ml concentration, the control had a 35.16 % inhibition. *H. coronarium*, which is used in ethnomedicine for the treatment of diabetes. The $IC_{50}$ of the rhizome extract was (94.87 µg/ml concentrations) (Figure 7). In this study, the methanolic extracts of *H. coronarium* are good inhibitors of α-Amylase and have potential to manage diabetes and serve as antidiabetic treatments. The extracts showed a concentration dependent increase in the percentage inhibitory activity against α-Amylase (Table 7). In this study shown some preliminary in vitro screening that methanolic extracts of *H. coronarium* rhizomes possess high free radical scavenging activity and effective inhibition of α-Amylase. This contributes to the validation of *H. coronarium* use in antidiabetic treatment. Work aimed at isolating the active molecules is also needed However, a need to explore the antidiabetic potential of the plant in vivo.

## 6. Conclusion

The findings of the phytochemical screening, antioxidants assays and other activities of *H.coronarium* rhizome extract was recorded and presented here. The following conclusion obtained from the studies that the plant *H.coronarium* rhizome contains a rich source of phytochemicals and natural antioxidants, which possesses a wide range of biological activity including antioxidant, anti-inflammatory, anti-proliferative, anti-diabetic and anticancer activities. Many diseases and illness caused due to the poor nutrition and food habits. These type of herbal plants helps in maintaining the proper cell cycle and growth in humans. Further clinical studies are required to confirm its therapeutic efficacy and food value of the plant to control many problems causing by the microbes, bacteria, fungi and other microorganism and also serves as a food material. Rhizomes of *H. coronarium* were used by Talaandig tribe for post-partum, tonic and birth control. Furthermore investigations are achieved only through the analysis and various laboratory tests conducted on this plant This study reports that the evidences provides convincing phytochemicals and ethno-medical approach proving the relevance of *H.coronarium* rhizome usage and thus providing scientific validity to its traditional consumption by the local populace of south India and all over the world.

The medicinal plants have action not only at one target site but may act at several potential targets. Therefore, various tests and assays assist with proper elucidation of the mode of action of the selected sample plant. Internal toxics and damage accelerate to create pathogenesis in so many human diseases. Hence naturally available therapies and medicines particularly from plant origin having considerable number of phenolic compounds having good antioxidant, anti-inflammatory activity and anti-diabetic properties. The rhizome extract indicated good anti-inflammatory activity as determined by Human Red Blood Cell Membrane stabilization assay and the agents showing inhibition activity are as safer medicines with minimum or no side effects invitro. The rhizome extract screened are a promising factor for develop the anti-inflammatory agents, that assist in various processes of inflammation, coupled with the excellent α-Amylase activity in treating diabetes. Overall, the study results do present some valuable anti-inflammatory and antidiabetic activities in the selected medicinal plant *H. coronarium* that can be used for the healthcare benefit of humankind. The results lend scientific evidence in support of the traditional medicinal use particularly on inflammation and to treat diabetes. In addition, the use of antioxidant agents presents in the plant for treating many microbial and pathological diseases. Further work on isolation, identification and characterization of the pharmaceutically important active component responsible for this activity are need. Once it was isolated the active compounds can further studied to determine and examine the mechanism of activity of the plant. Overall results indicate that rhizome extracts of the plant have valuable capacity and potential in treating inflammation and diabetes.

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