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In-Vitro assessment of anti-asthmatic and antioxidant activities of hydroalcoholic extract of *Corallocarpus Epigaeus* rhizome (HAECE$_R$)

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Abstract
Corallocarpus epigaeus has been traditionally used in southern India for the treatment of respiratory ailments such as asthma. However, scientific validation of its pharmacological activity remains limited. The present study aimed to investigate the *in vitro* anti-asthmatic and antioxidant potential of the hydroalcoholic extract of *Corallocarpus epigaeus* rhizome (HAECE$_R$) and to substantiate its ethnomedicinal claims.
Methods: The rhizomes were shade-dried, pulverized, and extracted using a hydroalcoholic solvent (75:25). The *in vitro* anti-asthmatic activity was evaluated using isolated goat tracheal chain preparation against histamine-induced contractions, with chlorpheniramine maleate (100 µg/mL) serving as the standard. Antioxidant activity was assessed using five assays—DPPH radical scavenging, hydrogen peroxide scavenging, nitric oxide scavenging, ferric reducing antioxidant power, and total antioxidant capacity—compared with ascorbic acid as reference.
Results: HAECE$_R$ produced a dose-dependent inhibition of histamine-induced tracheal contractions, reducing responses from 3.3 % at 0.1 mL to 56.1 % at 0.32 mL, indicating H_1-receptor antagonism comparable to chlorpheniramine maleate. In antioxidant assays, HAECE$_R$ demonstrated strong free-radical-scavenging activity with IC_{50} values of 4.56 µg/mL (DPPH), 4.45 µg/mL (H_2O_2), 7.29 µg/mL (NO), 2.30 µg/mL (FRAP), and 3.05 µg/mL (TAC), comparable to or superior to ascorbic acid. The potent antioxidant capacity suggests the presence of phenolic and flavonoid compounds responsible for the extract's reducing power and radical-neutralizing effects.
Conclusion: The study confirms that HAECE$_R$ possesses significant *in vitro* anti-asthmatic and antioxidant properties. These findings validate the traditional use of *Corallocarpus epigaeus* in treating respiratory disorders and highlight its potential as a natural therapeutic candidate for oxidative stress-related conditions such as asthma. Further *in vivo* and molecular studies are recommended to isolate and characterize the active constituents.

Keywords: Anti-asthmatic, antioxidant, *Corallocarpus epigaeus,* Goat tracheal chain, histamine antagonism, hydroalcoholic extract, phytochemicals

Introduction
Traditional medicine, as defined by the World Health Organization, is the sum total of the knowledge, skills and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness [1]. Herbal medicine is the most widely used system of medicine in the world today. They are made exclusively from plants [2]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants [3]. Plant drug have been the major source for treatment of disease for a long time. They have been used in traditional medicine on basis of experience and practices [4]. Plants are potential sources of natural antioxidants [5].
Corallocarpus epigaeus (Rottl.) Hook.f. is a species in the Cucurbitaceae family, characterized as an herbaceous plant with a trailing or climbing growth habit, commonly found in tropical and subtropical regions. It is widely distributed across India—including states like Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, and West Bengal—as well as in tropical Africa and parts of the Persian Gulf [6]. The phytochemical reported the presence of carbohydrates, flavonoids, alkaloids, mucilage, proteins and amino acids [2]. In folk medicine this rhizome is especially used for the treatment of various ailments, including, lateral stage of dysentery, enteritis, laxative, rheumatism, syphilis and venereal complaints. It is used as a remedy for snake bites [7]. It exhibited Analgesic, Antipyretic, Anti-inflammatory [8, 9], Anthelmintic [10, 11], Anti-fungal [12], Anti-diabetic [13, 14], Anti-bacterial [15, 16], Hepatoprotective [17]. In recent years much attention has been devoted to natural antioxidant and their association with health benefits [18]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [19].
Asthma word derived from a Greek word meaning 'Breathless'. Asthma is a chronic inflammatory disease of the airways. This chronic inflammation is associated with an exaggerated airway-narrowing response to specific triggers such as viruses, allergens and exercise that leads to recurrent episodes of wheezing, breathlessness, chest tightness and/or coughing that can vary over time and in intensity. Asthma has been a major cause of morbidity and mortality in

various countries. Bronchial hyper responsiveness is a characteristic feature in most asthmatic patients. Asthma is characterized by airway inflammatory cells, including eosinophils, macrophages, mast cells, epithelial cells and activated lymphocytes that release various cytokines, adhesion molecules and other mediators. Inflammation results in an acute, sub-acute or chronic process that alters airway tone, modulates vascular permeability, activates neurons, increases secretion of mucus, and alters airway structure reversibly or permanently. The currently available treatment for asthma most medications work by relaxing bronchospasm (bronchodilators) or reducing inflammation (corticosteroids). Though the available treatment is not efficient for treating asthma finally as they have many toxic side effects [20].

Hence, the present study was aimed to investigate the *in vitro* anti-asthmatic and antioxidant potential of the hydroalcoholic extract of *Corallocarpus epigaeus* rhizome ($HAECE_R$).

Materials and Methods

Collection of rhizome and Authentication

Rhizomes were collected from the foot of Sirumalai, Sadaiyandipuram village, Dindigul district, Tamil Nadu in the month of March 2025. The Rhizome was identified and authenticated by Dr. Stephen, Professor, Department of Botany, American College, Madurai-625 002.

Powder preparation

The dust and debris on the collected rhizomes were washed using running tap water followed by distilled water. The rhizome skin was peeled off. The peeled rhizome was chopped into small pieces and then they were dried well in shade and converted into moderately coarse powder by mechanical grinder. Then the powder was sieved with a commercial sieve of mesh size approximately 60mm to make the particle size uniform. Finally, stored in air tight containers for further use.

Preparation of hydroalcoholic extract of *Corallocarpus epigaeus* rhizome ($HAECE_R$)

About 30g of air-dried coarse powder of rhizome of *Corallocarpus epigaeus* was macerated with 300ml of hydro alcoholic solvent (75:25) in the closed flask for 72hrs. The flask was shaken frequently. The extracts were then filtered through whatmann filter paper No.42 (125 mm) to remove all non- extractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness and stored in sterile bottles for further use.

Anti-Asthmatic Activity

In vitro- isolated goat tracheal chain preparation

Materials

- Fresh goat trachea (from an approved, ethical source)
- Organ bath (10–20 mL) with isometric transducer and data system (or kymograph)
- Aeration: Carbogen (95% O_2: 5% CO_2)
- Temperature: 37 ± 0.5 °C
- Tyrode's solution (pH 7.4)
- Histamine dihydrochloride stock (prepare serial dilutions)
- Test drug: Chlorpheniramine 0.3 µM for a competitive antagonism demo)
- Thread, fine scissors, tissue hooks, stopwatch, pipettes

Tyrode's solution (per liter; pH 7.4 when bubbled with carbogen)

NaCl- 8.0 g, KCl- 0.2 g, $CaCl_2 \cdot 2H_2O$- 0.2 g, $MgCl_2 \cdot 6H_2O$- 0.1 g, $NaHCO_3$- 1.0 g, $NaH_2PO_4 \cdot 2H_2O$- 0.05 g, Glucos-e 1.0 g.

Procedure

- **Preparation of tracheal chain**

Rinse trachea in cold Tyrode's. Cut 4–6 cartilaginous rings, slit opposite the trachealis muscle, and tie as a chain keeping the smooth muscle in line with the axis of pull.

- **Mounting**

Mount the chain in the organ bath containing Tyrode's at 37 °C, aerated with carbogen. Attach to isometric transducer. Apply 1 g resting tension; allow 45–60 min equilibration, washing every 10–15 min.

- **Control CRC to histamine**

Record cumulative concentration–response to histamine Allow plateau after each addition (≈60–90 s). Define the maximal response at 0.32 ml as 100% for that tissue.

- **Test drug**

Wash tissue and re-equilibrate 20 min. Add Plant extract 100 µg/mL Reconstruct the cumulative histamine CRC under identical settings.

- **Replicates**

Repeat on n = 4 tissue preparations (from the same trachea where possible).

- **Calculations**

Express responses as % of the control maximal contraction [21, 22].

Antioxidant Assay

1. DPPH Radical Scavenging Assay

The antioxidant activity of the hydroalcoholic extract of Corallocarpus epigaeus rhizome was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of $HAECE_R$ of varying concentrations (5, 10, 15, and 20 µg/ml). Corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

$$\% \, Inhibition = \frac{Ac - As}{Ac} \, X \, 100$$

Where Ac is the absorbance of the control as is the absorbance of the sample [23]. IC_{50} (minimum concentration at which 50% of radicals were scavenged) values were estimated graphically using a non-linear regression a logarithm.

2. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of hydroalcoholic extract of *Corallocarpus epigaeus* rhizome was determined by monitoring the reduction of H_2O_2. 1ml of $HAECE_R$ of varying concentrations (5, 10, 15, and 20 µg/ml). Corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. And then add 0.6mL of 40 mM H_2O_2 solution and made up to 2 mL using 50 mM sodium phosphate buffer (pH 7.4) and incubated for 40 min at 30 °C and the absorbance was read at 230 nm. The percentage of inhibition of H_2O_2 was calculated as follows.

$$\%\ Inhibition = \frac{Ac - As}{Ac}\ X\ 100$$

Where Ac is the absorbance of the control as is the absorbance of the sample [24]. IC_{50} (minimum concentration at which 50% of radicals were scavenged) values were estimated graphically using a non-linear regression a logarithm.

3. Nitric oxide radical scavenging activity

Nitric oxide evolved from nitroprusside was measured by greiss reaction. Sodium nitropruside is decomposed to generate NO in aqueous solution at physiological pH. This NO interacts with oxygen to produce stable nitrate and nitrite ions, the amount of which can be estimated by greiss reaction. Briefly, 2 ml of 10 mM sodium nitropruside in phosphate buffered saline was mixed with $HAECE_R$ at distinct concentrations (5, 10, 15 and 20 µg/ml) and allowed to be incubated at room temperature for 150 min. Then, 0.5 ml of greiss reagent (1 ml of o.33% sulfanilic acid reagent in 20% glacial acetic acid) was added to the test solutions and kept at room temperature for 5 min. Subsequently, 1 ml of 0.1% napthylethylenediamine dichloride was added and allowed to be incubated again for 30 min at room temperature. The absorbance was then measured at 546 nm. L-Ascorbic acid (1-100 µg/ml) was considered to be the reference standard. The percentage of inhibition was calculated as follows.

$$\%\ Inhibition = \frac{Ac - As}{Ac}\ X\ 100$$

Where Ac is the absorbance of the control as is the absorbance of the sample [25]. IC_{50} (minimum concentration at which 50% of radicals were scavenged) values were estimated graphically using a non-linear regression a logarithm.

4. Ferric reducing anti-oxidant power assay

Different concentrations of $HAECE_R$ and its various fractions (5, 10, 15 and 20 µg/mL) was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K3Fe (CN)6] solution. The reaction mixture was vortexed well and then incubated at 50°C for 20 min using vortex shaker. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride. The coloured solution was read at 700 nm against the blank with reference to standard using UV Spectrophotometer. Here, ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard. The percentage of inhibition was calculated as follows.

$$\%\ Inhibition = \frac{Ac - As}{Ac}\ X\ 100$$

Where Ac is the absorbance of the control as is the absorbance of the sample [26]. IC_{50} (minimum concentration at which 50% of radicals were scavenged) values were estimated graphically using a non-linear regression a logarithm.

5. Total antioxidant capacity

The total antioxidant capacity of the extracts was determined by the phosphomolybdate method. Briefly, 0.1 ml aliquot of various concentration of the plant extracts (5, 10, 15 and 20 µg/ml) was mixed with 1 ml of reagent solution (600 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate, 1:1:1). The test tubes were covered with aluminium foil and incubated in a water bath at 95° C for 90 min. Then the extracts were cooled to room temperature and the absorbance of the mixture was determined at 765 nm against a blank containing 1 ml of the reagent solution. Ascorbic acid was used as standard. The assay was carried out in triplicate. The total antioxidant capacity (TAC) was expressed as mg equivalents of ascorbic acid per gram (EAA/g). The antioxidant capacity was estimated by using the following formula:

$$\%\ Inhibition = \frac{Ac - As}{Ac}\ X\ 100$$

Where Ac is the absorbance of the control as is the absorbance of the sample [27]. IC_{50} (minimum concentration at which 50% of radicals were scavenged) values were estimated graphically using a non-linear regression a logarithm.

Result and Discussion

1. Anti-Asthmatic Activity of $HAECE_R$

***In vitro*- isolated goat tracheal chain preparation**

Table 1: Effect of $HAECE_R$ on Histamine induced contraction of isolated goat tracheal chain preparation

Histamine 10µg/ml	% Response		
	Control	CPM (100µg/ml)	$HAECE_R$ (100 µg/mL)
0.1 ml	8.5	2.1	3.3
0.2 ml	22.9	5.9	13.8
0.4 ml	65.8	15.8	25.1
0.8 ml	84.7	25.4	32.2
0.16 ml	94.3	38.6	48.0
0.32 ml	100	49.7	56.1

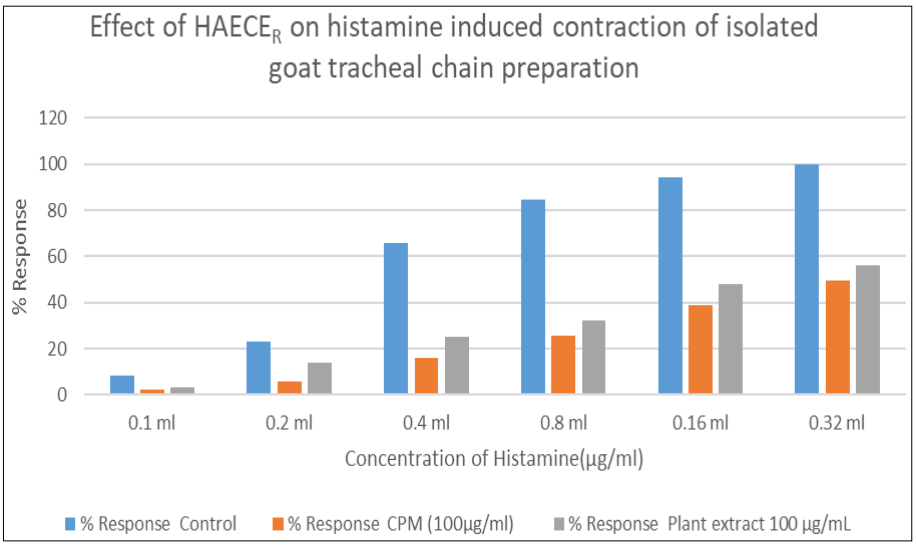

Fig 1: Graphical presentation of effect of HAECE$_R$ on histamine induced contraction of isolated goat tracheal chain preparation

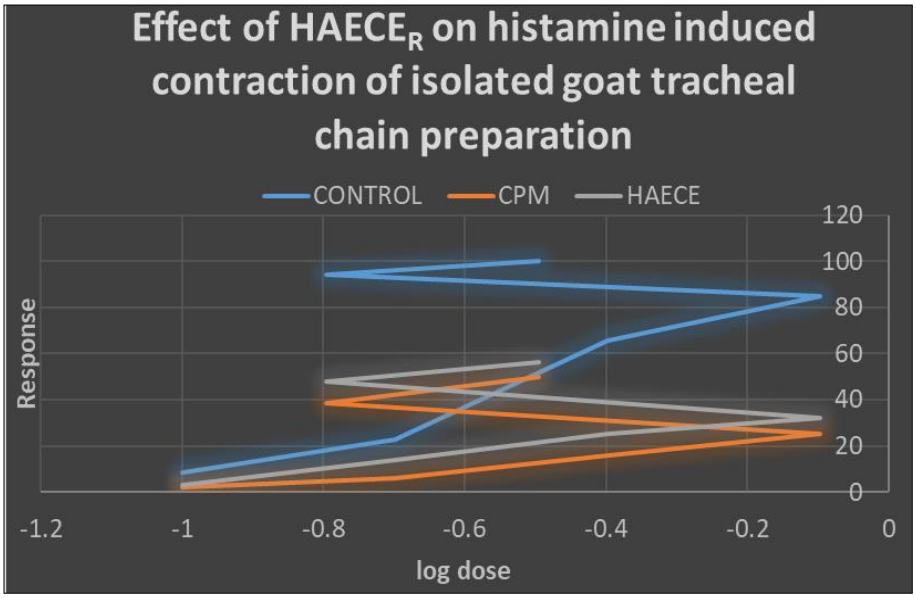

Fig 2: Graphical presentation of dose- response curve of histamine induced isolated goat tracheal chain preparation

The cumulative dose–response effect of histamine (10 µg/mL) was recorded on isolated goat tracheal tissue, both in the absence (control) and presence of chlorpheniramine maleate (CPM, 100 µg/mL) and hydroalcoholic extract of *Corallocarpus epigaeus* rhizome (HAECE$_R$, 100 µg/mL). In the control group, histamine produced a concentration-dependent contraction, reaching 100% response at 0.32 mL. In the presence of CPM, a standard H_1 receptor antagonist, there was a marked inhibition of the histamine-induced contractions, with responses reduced to 2.1% at 0.1 mL and 49.7% at 0.32 mL compared to control. Similarly, HAECE$_R$ at 100 µg/mL also significantly reduced histamine-induced tracheal contraction, with responses ranging from 3.3% at 0.1 mL to 56.1% at 0.32 mL. Although the inhibitory effect of HAECE$_R$ was slightly lower than that of CPM, the extract exhibited a clear concentration-dependent antagonism of histamine response. Although the effect of HAECE$_R$ was slightly lower than that of CPM, the dose-dependent response suggests promising anti-asthmatic potential.

2. Anti-Oxidant Activity

In vitro antioxidant activity of HAECE$_R$ were determined by five methods and the results are depicted below.

2.1 Dpph Free Radical Scavenging Assay

Table 2: Determination of DPPH free radical scavenging assay of HAECE$_R$

Concentration	% Inhibition of Ascorbic Acid(µg/ml)	% Inhibition of HAECE$_R$ (µg/ml)
0	0	0
5	29.92963	34.3209
10	34.15499	34.4856
15	43.83811	46.6666
20	50.00009	48.7654
IC_{50}	4.616627	4.560912

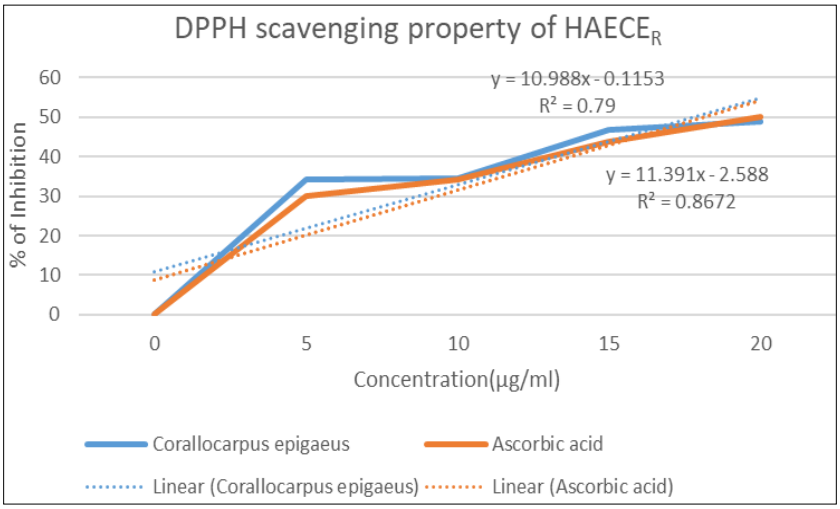

Fig 3: Graphical data of DPPH free radical scavenging assay of HAECE$_R$

The DPPH assay measures the ability of antioxidants to scavenge free radicals by donating hydrogen atoms. The percentage inhibition of HAECE$_R$ increased with concentration, showing significant radical scavenging activity. The IC_{50} value of HAECE$_R$ (4.56 µg/mL) was almost equivalent to that of ascorbic acid (4.61 µg/mL), indicating strong hydrogen-donating capacity. This suggests that the extract possesses potent free radical quenching ability due to the presence of phenolic and flavonoid constituents.

2.2 Hydrogen Peroxide Scavenging Activity

Table 3: Determination of H_2O_2 scavenging activity of HAECE$_R$

Concentration	% Inhibition of Ascorbic Acid(µg/ml)	% Inhibition of HAECE$_R$ (µg/ml)
0	0	0
5	29.92963	24.16361
10	32.03546	30.51282
15	47.23119	30.84249
20	50.08858	64.94505
IC_{50}	4.544314	4.457641

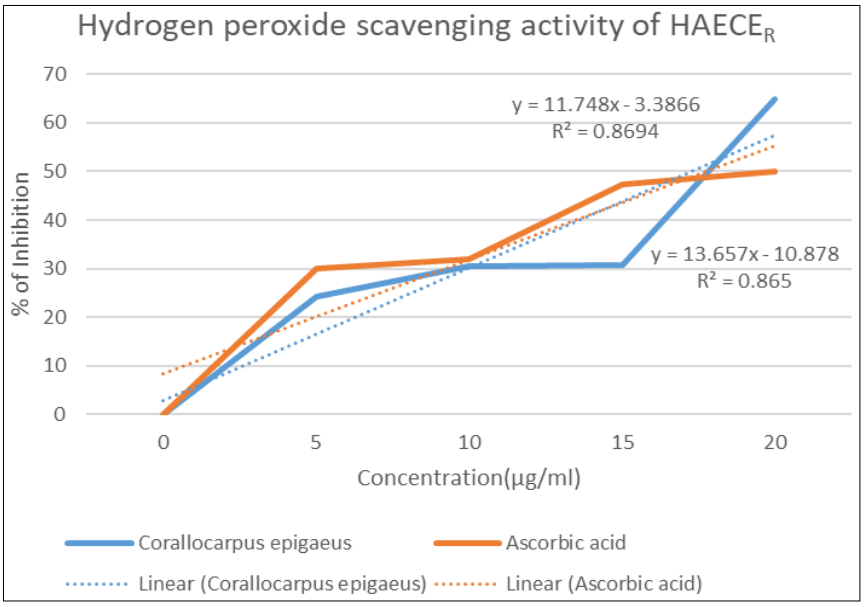

Fig 4: Graphical data of H_2O_2 scavenging activity of HAECE$_R$

In the hydrogen peroxide scavenging assay, HAECE$_R$ exhibited concentration-dependent scavenging activity. The IC_{50} value of the extract (4.45 µg/mL) was comparable to that of ascorbic acid (4.54 µg/mL), demonstrating an efficient capacity to neutralize hydrogen peroxide radicals. This indicates that the extract contains compounds capable of converting reactive oxygen species into less harmful products, thereby preventing oxidative damage to biomolecules.

2.3 Nitric Oxide Scavenging Assay

Table 4: Determination of Nitric oxide scavenging assay of HAECE$_R$

Concentration	% Inhibition of Ascorbic Acid(µg/ml)	% Inhibition of HAECE$_R$ (µg/ml)
0	0	0
5	27.25669	15.1981
10	32.03546	25.9152
15	38.40715	28.4492
20	44.24787	28.6905
IC_{50}	5.168737	7.296834

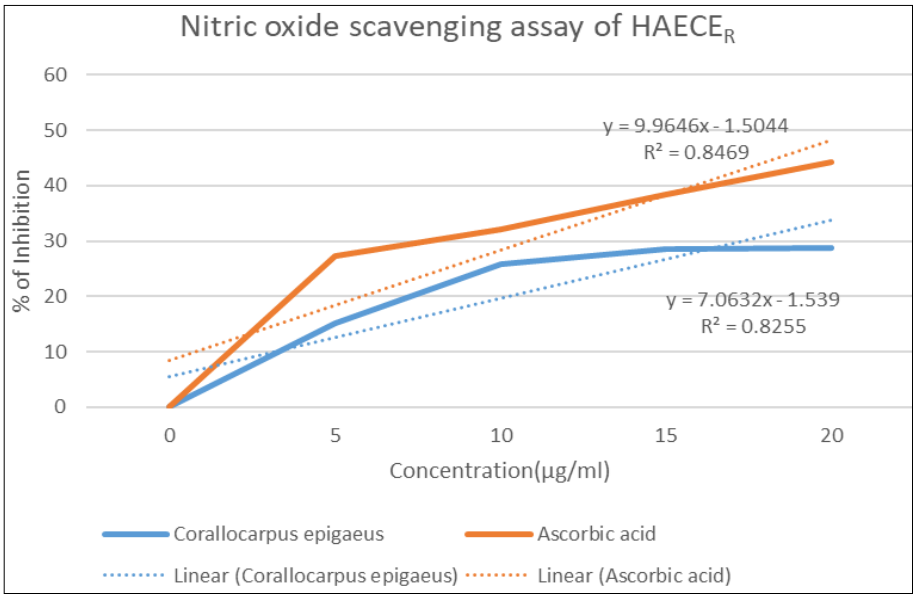

Fig 5: Graphical data of Nitric oxide scavenging assay of HAECE$_R$

Nitric oxide is a reactive free radical involved in various inflammatory processes. The HAECE$_R$ extract exhibited a moderate inhibitory effect against nitric oxide generation with an IC_{50} value of 7.29 µg/mL, compared to 5.16 µg/mL for ascorbic acid. Although the activity was slightly lower than the standard, the result still indicates a considerable capacity of the extract to scavenge nitric oxide radicals, possibly attributed to its secondary metabolites that act as electron donors.

2.4 Ferric Reducing Power Assay

Table 5: Determination of Ferric reducing power assay of HAECE$_R$

Concentration	% Inhibition of Ascorbic Acid(µg/ml)	% Inhibition of HAECE$_R$ (µg/ml)
0	0	0
5	29.92963	73.04527
10	35.56345	74.75995
15	40.49303	76.81756
20	45.24656	88.68313
IC_{50}	4.954522	2.301005

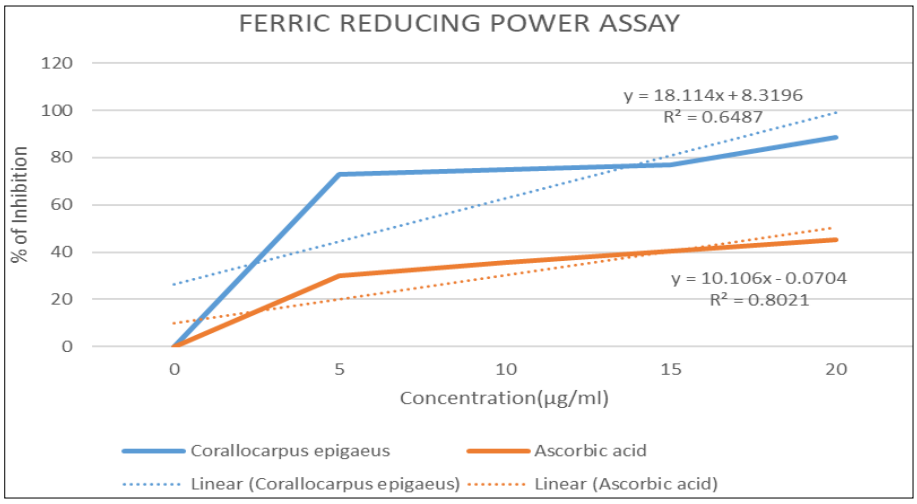

Fig 6: Graphical data of Ferric reducing power assay of HAECE$_R$

The ferric reducing antioxidant power (FRAP) assay determines the electron-donating potential of antioxidants, which correlates with their reducing ability. $HAECE_R$ showed remarkably higher reducing power (IC_{50} = 2.30 µg/mL) than ascorbic acid (IC_{50} = 4.95 µg/mL). This strong reducing ability indicates that $HAECE_R$ contains potent reductones that contribute to the termination of free radical chain reactions by donating electrons.

2.5 Total Antioxidant Capacity

Table 6: Determination of Total antioxidant capacity of $HAECE_R$

Concentration	% Inhibition of Ascorbic Acid(µg/ml)	% Inhibition of $HAECE_R$ (µg/ml)
0	0	0
5	28.23	39.53
10	36.47	55.69
15	53.23	65.8
20	73.67	83.4
IC_{50}	3.67773	3.057803

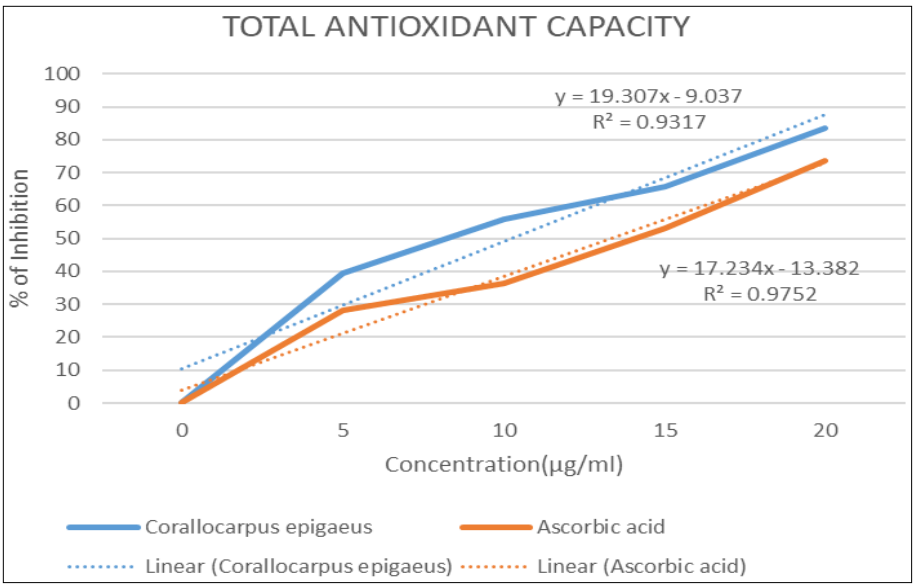

Fig 7: Graphical data of Total antioxidant capacity of $HAECE_R$

The total antioxidant capacity assay revealed that $HAECE_R$ had higher activity (IC_{50} = 3.05 µg/mL) compared to ascorbic acid (IC_{50} = 3.67 µg/mL). This suggests that the extract has excellent overall antioxidant potential, which may result from synergistic effects of diverse phytochemicals such as phenolics, flavonoids, alkaloids, and saponins present in the hydroalcoholic extract.

Across all assays, $HAECE_R$ demonstrated strong antioxidant activity comparable to, and in some cases exceeding, that of ascorbic acid. The low IC_{50} values obtained indicate a high efficiency of the extract in scavenging different types of free radicals. The results confirm that *Corallocarpus epigaeus* rhizome is a rich source of natural antioxidants and could play a significant role in mitigating oxidative stress-related disorders such as asthma.

Conclusion

The present study revealed that the Hydroalcoholic Extract of *Corallocarpus epigaeus* rhizome ($HAECE_R$) exhibits significant anti-asthmatic and antioxidant activities *in- vitro*. In the isolated goat tracheal chain model, the extract showed a dose-dependent inhibition of histamine-induced tracheal contraction, demonstrating its potential H_1-receptor antagonistic activity comparable to the standard drug, chlorpheniramine maleate. Furthermore, the extract displayed remarkable antioxidant potential across all five assays—DPPH, hydrogen peroxide, nitric oxide scavenging, ferric reducing power, and total antioxidant capacity—with IC_{50} values comparable to or even lower than those of ascorbic acid. These findings suggest that the strong antioxidant efficacy of the extract may be attributed to the presence of bioactive phytoconstituents such as phenolics and flavonoids, which play a key role in neutralizing free radicals and reducing oxidative stress.

Overall, the study substantiates that *Corallocarpus epigaeus* rhizome possesses promising anti-asthmatic potential possibly mediated through its antioxidant mechanism, supporting its traditional use in the management of respiratory ailments. Further *in- vivo* and molecular studies are recommended to elucidate the precise mechanisms and to isolate the active principles responsible for these effects.

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