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Assessment of Phytochemical Screening, Antioxidant, Anti-inflammatory, Hypoglycemic, Antidiarrheal, and Anxiolytic Effects of *Curculigo orchioides* Leaf Extracts

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Abstract: *Curculigo orchioides* Gaertn., commonly known as Talamuli in Bangladesh and widely distributed across Asia, is traditionally used in Ayurvedic and Chinese medicine as an aphrodisiac and for treating asthma and jaundice. Our study aimed to perform a comparative phytochemical analysis and evaluation of some biological effects using *in vitro* and *in vivo* approaches of its leaf's ethanol (ECO), n-hexane (HCO), and chloroform (CCO) fractions. After extraction and fractionation, a phytochemical analysis was followed by different tests for anti-bacterial, antioxidant, anti-inflammatory, anti-diabetic, anti-diarrheal, and anxiolytic effects. Phytochemical screening revealed high concentrations of alkaloids, glycosides, tannins, and reducing sugars. Acute toxicity tests confirmed ECO's safety up to 3000 mg/kg in Swiss mice. Antibacterial assays indicated ECO and CCO exhibited moderate inhibition against tested bacteria, while HCO showed no activity. HCO demonstrated superior antioxidant, α-amylase inhibitory, and anti-diarrheal effects, whereas CCO effectively suppressed protein denaturation, stabilized membranes, and exhibited significant hypoglycemic and anxiolytic effects. Taken together, *C. orchioides* might be a good source of phytotherapeutic agents.

Keywords: *Curculigo orchioides*; Leaf extract; Pharmacological investigations; Phytochemical analysis; Talamuli

1. Introduction

To treat various ailments or to maintain sound health, the use of plant-based products has dramatically increased throughout the world (Sasidharan et al., 2011; Bhuia et al., 2025c). More than 80% of people around the world use medicinal plants and their derivatives as self-medications for various health benefits (Duraipandiyan et al., 2006; Al Hasan et al., 2025). Interestingly, the intake of herbal supplements is high among men and women of all ages. According to a survey, global consumption of herbal medicines was estimated to be worth around USD 230.03 billion in 2021. By 2028, the market is expected to grow to USD 430.05 billion (Kushalan et al., 2022). This might be due to the few adverse effects and excellent safety of experimental animals and humans (Di Lorenzo et al., 2015). Plants have medical value because they contain certain chemicals that have a clear physiological impact on humans, such as alkaloids, glycosides, phenolics, flavonoids, tannins, lignins, and reducing sugars (Edeoga et al., 2005; Dass & Mathur, 2009; Bithi et al., 2025).

Hypoxidaceae plant *C. orchioides* Gaertn. (*C. orchioides*) is also known as "Talamuli" in Bangladesh, "Kali musli" in India, and "Xian mao" in China. It is widely distributed in large quantities in China, India, Nepal, Bangladesh, Japan, Malaya, and Australia (Chaturvedi et al., 2016). **Fig. 1** shows a plant's leaf and flower.

C. orchioides is essential in Indian and Chinese traditional medicine systems for its valuable bioactives. Its leaves are employed as Rasayana (anti-aging), Vrushya (aphrodisiac), Brimhana (weight loss), alterative, appetizer, fattening, and beneficial in piles, jaundice, menstrual irregularities, menstrual cramps, colic disorders, asthma, and amenorrhea. The leaf juice also strengthens the bones, spleen, and kidneys in humans (Pandit et al., 2008; Nie et al., 2013). To date, a total of 61 phytoconstituents have been isolated under the gross phytochemical groups of glycosides, lignans, polysaccharides, alkaloids, saponins, triterpenes, and aliphatic compounds (Kushalan et al., 2023). Different extracts of the plant have diverse biological effects, including antioxidant (Hejazi et al., 2018), anti-hypertensive (Cao et al., 2008; Chauhan & Dixitet, 2007; Madhavan et al., 2007; Singh & Gupta, 2008; Jiao et al., 2009; Joshi et al., 2012; Liu et., 2012; Wang et al., 2012; Zhao et al., 2014; Murali & Kuttan

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et al., 2015; Zhu et al., 2015; Kayalvizhi et al., 2016; Xia et al., 2016; Wang et al., 2017), anti-microbial (Nagesh & Shanthamma, 2009), anti-inflammatory (Agrahari et al., 2010; Ku et al., 2012), immunostimulant (Bafna & Mishra, 2006), neuroprotective (Jiang et al., 2011; Ramchandani et al., 2014; Tian et al., 2012; Wang et al., 2016), hepatoprotective (Venukumar & Latha, 2002), aphrodisiac (Chauhan & Dixit, 2008; Thakur et al., 2009; Thakur et al., 2012; Thakur & Dixit, 2007; Vijayanarayana et al., 2007), anti-arthritic (Jiao et al., 2009; Tan et al., 2019), anti-asthmatic (Pandit et al., 2008; Venkatesh et al., 2009), anticancer (Hejazi et al., 2018), antidiabetic (Gulati et al., 2015), and hearing improvement (Hong et al., 2011) properties.

This study explores the largely unexamined pharmacological potential of *C. orchioides* leaf extracts, unlike previous research focused on its rhizomes. The current study aims to perform a preliminary comparative phytochemical screening and investigate some biological properties of the ethanol (ECO), n-hexane (HCO), and chloroform (CCO) leaf extracts of *C. orchioides*.

Fig. 1. *C. orchioides* (leaf and flower)

2. Materials and methods

2.1. Collection, identification, drying and grinding of plant materials

Fresh *C. orchioides* leaves were collected from the Bangladesh Forest Research Institute (BFRI), Chittagong, and were identified by a taxonomist from the same institution's Herbarium Section (voucher specimen number: BFRIH-37928). The plant parts were collected, separated from adulterants (e.g., dust, other plant parts), and washed with running tap water. The leaves were cut into small pieces and dried for a week at room temperature in the shade (25-35 ºC). Finally, the small pieces of rhizomes were put into an electric oven for 30 minutes at 35 ºC and ground into a coarse powder using a mechanical grinder. The powder was kept in a dry, cool, and amber-colored airtight glass container until the extraction commenced.

2.2. Extraction and fractionation

Approximately 345 g of coarse powder was taken in a Soxhlet apparatus and it was extracted with 800 ml of absolute ethanol for 8 h. A rotary evaporator was used to make the concentrate of the extract at a temperature below 40°C. The viscous concentrate was taken in a clean, airtight container, weighed, properly labeled, and kept in a refrigerator at 4°C. The solvent-solvent fractionation procedure was followed as below (**Fig. 2**).

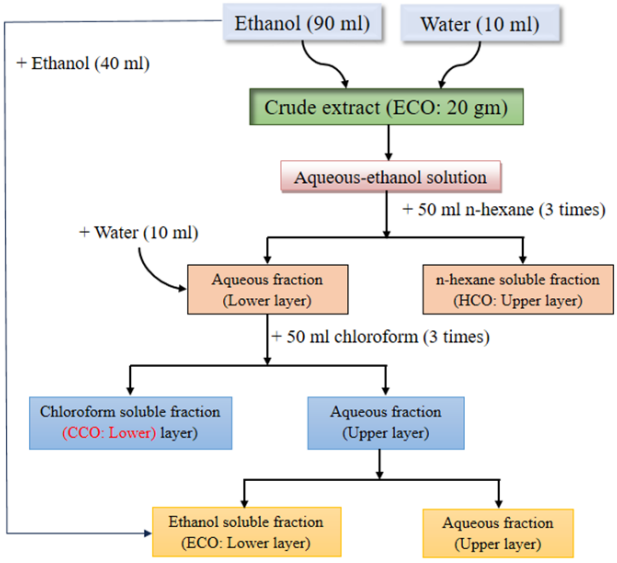

Fig. 2. Schematic representation of fractionation of the crude extract of *C. orchioides*

2.3. Reagents and chemicals

Ethanol, chloroform, n-hexane, Tween 80, ascorbic acid, and alpha amylase were purchased from Merck India, while nutrient agar medium was purchased from Himedia Laboratories Ltd., India. All the reference standards were kindly provided by different pharmaceutical industries in Bangladesh.

2.4. Experimental animals

The eight-week-old young *Swiss* albino male mice with an average weight of 24-30 gm were purchased from the animal research branch of the Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram, Bangladesh. The animals were maintained in standard laboratory conditions (12 h light/dark cycle; room temperature 25 ± 2 °C; relative humidity 55–60%) and fed a standard diet and water *ad libitum*. Prior to executing the studies, the animals were acclimatized to the laboratory environment for 7 days. Before the studies, the animals were starved overnight (12 h). All experimental animals were cared for in accordance with the *Swiss* Academy of Medical Sciences and the *Swiss* Academy of Sciences Ethical Principles and Guidelines for Scientific Experiments with Animals (1995). The Institutional Ethics Committee (SUB/IAEC/12.01) authorized all experimental protocols.

2.5. Phytochemical investigation

Preliminary phytochemical screenings were carried out according to the method described by Islam (2017) to investigate the plant secondary metabolites present in the crude extract of the plant by adopting the standard procedure (Islam, 2017). Three types of solutions of extract were made first for this test: A 5% w/v solution of extract was prepared for each fraction.

2.6. Acute toxicity analysis and dose determination

The test dose for this study of crude extracts was selected by the acute toxicity study following the OECD guidelines using *Swiss* albino mice. Briefly, the ECO was administered at doses of 500, 1000, 2000, and 3000 mg/kg orally. The animals were then frequently observed for behavioral changes, toxicological symptoms, and death for 2 days (Thangjam et al., 2020).

2.7. Antibacterial activity test (Disc diffusion assay)

This test was performed according to the method described by Razmavar et al. (2014) with slight modifications (Razmavar et al., 2014). Briefly, the test samples were prepared by dissolving the extracts of the samples in ethanol (ECO), chloroform (CCO), and n-hexane (HCO). All the samples were tested at 250 µg/disc. Azithromycin (AZT) was taken as a reference antibiotic and was tested at 25 µg/disc. For this study, we used 5 gram-positive (+) bacteria (*Lactobacillus casei, Lactobacillus coryniformis, Bacillus cereus, Staphylococcus aureus, Bacillus azotoformans*) and 5 gram-negative (−) bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Vibrio cholerae*) (**Table 2**). After the inoculation of the test bacterial strain and after allowing the plates to solidify, the respective paper discs containing the test sample or standard drug were subsequently impregnated centrally into the agar gel separately with the help of sterile forceps to assure complete contact with the previously cultured medium surface. Finally, the plates were incubated at 37°C for 24 hours. The antibacterial sensitivities of *C. orchioides* leaf extracts were determined by comparing the zone of inhibition (mm) with the standard drug AZT (25 µg/µl).

2.8. Free radical scavenging test (DPPH free radical scavenging assay)

The free radical scavenging capacity of the extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay described by Blois (1958) with slight modifications (Blois, 1958). Briefly, a 0.004% w/v DPPH solution was prepared in ethanol. Immediately, its absorbance was taken. To 1 ml of extract solution of different concentrations (20, 40, 60, 80, and 100 µg/ml), 2 ml of DPPH solution was added, mixed properly, and allowed to stand in a dark condition for 30 minutes to complete the reaction. The same concentrations of ascorbic acid (AA) were used as a reference radical scavenger, while ethanol was used as a blank. After the reaction time, the absorbance was measured at 517 nm using a UV spectrophotometer. Then the percent inhibition was calculated using the following formula:

$$\%\text{Radical scavenge} = [\text{Absorbance}_{\text{Before}} - \text{Absorbance}_{\text{After}}) \div \text{Absorbance}_{\text{Before}}] \times 100$$

The half-minimal inhibitory concentration (IC_{50}) was determined using non-linear regression analysis using Graph Pad Prism software.

2.9. Anti-inflammatory activity test

2.9.1. Egg album assay

This test was performed by checking inhibitory effects on egg albumin using the method described by Banerjee and Chanda (Banerjee et al., 2014). Briefly, 0.2 ml of egg albumin (from a fresh hen's egg) was mixed with 2.8 ml of isosaline (0.9% NaCl, pH 6.4) and 2 ml of a test sample or standard drug. Distilled water and diclofenac-Na served as control and positive controls, respectively. The sample and standard were tested at 20, 40, 60, 80, and 100 µg/ml. After incubation at 37 ± 2°C for 15 minutes, the reaction mixtures were then heated at 70°C for 5 minutes in a water bath. The mixtures were then cooled and filtered through Whatmann filter paper no. 1. The absorbance was measured at 660 nm using a colorimeter. The percentage inhibition of protein denaturation was estimated using the following formula:

$$\%\text{Inhibition of protein denaturation} = 100 \times ([Vt/Vc] - 1)$$

Where, Vt and Vc stand for the absorbance of the test sample and the control, respectively. The IC_{50} values were determined as mentioned above.

2.9.2. Membrane stabilizing test (HRBC assay)

This test was done according to the method described by Gandhidasan and his coworkers (Gandhidasan et al., 1991) with slight modifications. Briefly, 0.5 ml of 10% RBC suspension was mixed with 5 ml of hypotonic solution (0.5% NaCl solution, pH 7.4) and 1 ml of different concentrations of test extracts or standard solution. After incubation at 37 ± 2 °C for 10 min, all the reaction mixtures were centrifuged for 10 min at 3000 rpm. The mixtures were then filtered using Whattmann filter paper no. 1. Then, the absorbance was measured at 546 nm using a colorimeter. Hemolysis was compared with the control group, and the percentage inhibition of hemolysis was determined using the following formula:

$$\%\text{ Haemolysis inhibition} = [(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}) \div \text{Absorbance}_{\text{Control}}] \times 100$$

2.10. Hypoglycemic test

2.10.1. Alpha-amylase inhibition assay (*in vitro*)

This test was performed according to the method described by Wickramaratne et al. (2016) with slight modifications in *in vitro*

(Wickramaratne et al., 2016). Briefly, 80 µl of alpha amylase solution (0.050 mg/ml) was mixed with 5.2 ml of Tris-HCl buffer (0.01 M containing 0.006 M NaCl, pH 6.8) and 320 µl of different concentrations of test samples or standard solutions (20-100 µg/ml). Acarbose was used as a reference drug for this assay. After incubation at 37ºC for 20 min, 400 µl of the starch solution (0.1%) was added, and the mixture was re-incubated at 37°C for 20 min, after which 8 ml of 0.01% acidic iodine solution was added to the reaction mixtures. After filtration, the absorbance of the filtrate was taken at 578 nm using a colorimeter. Alpha-amylase inhibitory activity was determined as below.

%Amylase activity inhibition = [(Absorbance$_{Control}$ − Absorbance$_{Sample}$) ÷ Absorbance$_{Control}$] × 100

The IC_{50} values were determined as mentioned above.

2.10.2. Glucose tolerance test (*in vivo*)

For this study, we followed the method described by Babu et al. (2002)with slight modifications (Babu et al., 2002). After 12 hours of fasting, the animals were randomly divided into different groups (n = 6). Baseline blood glucose levels were measured by using a glucometer. Blood was collected by cutting the tail aseptically with a surgical blade. The control group received 0.1% Tween-80 dissolved in a 0.9% NaCl solution, while the positive control group received the standard hypoglycemic drug glibenclamide (GBC) at 10 mg/kg. The extracts were tested at 500 mg/kg. All the treatments were given orally (p.o.). After 30 minutes, the animals were treated with a 10% glucose solution at 10 g/kg body weight. Then, the blood glucose level was measured similarly at the 30th, 90th, and 120th minutes after the standard glucose solution administration. A glucose solution, test sample, and controls were given orally.

2.11. Antidiarrheal activity test (*castor oil-induced diarrheal model*)

For this study, we followed the method described by Awouters (Awouters et al., 1978) with slight modifications. After 12 hours of fasting, the animals were randomly divided into different groups (n = 6). The control group received 0.1% Tween-80 dissolved in a 0.9% NaCl solution, while the positive control group received the standard antidiarrheal drug loperamide (LOP) at 3 mg/kg. The extracts were tested at 500 mg/kg. All the treatments were given orally (p.o.). After 30 minutes, the animals were treated with 0.4 ml of castor oil. Then, each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. The latency period (first diarrheal stooling time after the administration of castor oil) and the total number of feces (hard and soft) excreted by the animals were recorded during the observation period (4 hours). After the study, we also observe for an additional 20 hours to check for serious illness or mortality.

2.12. Anxiolytic activity study

2.12.1. Open-field test

This test was performed according to the method described by Molla (Molla et al., 2022) with slight modifications. The animals (n = 6) were grouped as per the previous study, and the treatments were given. In this case, we used diazepam (DZP) at 3 mg/kg as a reference drug. All the treatments were given orally (p.o.). After 25 minutes of sample or drug administration, the number of squares crossed by each mouse was counted for 5 minutes.

2.12.2. Hole-cross test

This study was performed according to the method described by Takagi (Takagi et al., 1971). The animals from the open-field test were given a 2-minute rest and then submitted to this study. In this case, we counted the number of squares crossed by each mouse within 5 minutes.

2.13. Statistical analysis

Values are expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was followed by Newman-Keuls *post hoc t-student tests* using the GraphPad Prism software (version 6.5), considering $p < 0.05$ at 95% confidence intervals (Islam et al., 2024).

3. Results

3.1. Extraction and phytochemical investigation

The ECO was a viscous concentrate. The yield values of ECO, CCO, and HCO were 11.14, 0.87, and 2.03%, respectively. Preliminary phytochemical screening showed that the ECO contains alkaloids, glycosides, tannins, flavonoids, saponins, reducing sugar, gums, and amides. On the other hand, CCO contains alkaloids, glycosides, steroids, tannins, saponins, reducing sugar, and amides. The HCO contains alkaloids, glycosides, steroids, tannins, flavonoids, saponins, reducing sugar, gums, and amides (**Table 1**).

Table 1. Phytochemical groups detected in *C. orchioides* leaf extracts

Chemical groups	Fractions and their color intensities		
	ECO	CCO	HCO
Alkaloids	+++	+++	+++
Glycosides	++	+	+
Steroids	−	+	+
Tannins	++	++	+++
Flavonoids	+	−	+
Saponins	+	+	+
Reducing sugar	++	++	++
Gums	+	−	+
Amides	+	+	+

+ = Positive (Present); − = Negative (Absent); multiplication of plus (+) sign means observed intensity; ECO: Ethanolic Curculigo orchioides extract; CCO: Chloroform Curculigo orchioides extract; HCO: n-Hexane Curculigo orchioides extract

3.2. Pharmacological activities

3.2.1. Acute toxicity analysis and dose determination

The findings of the acute toxicity study demonstrate that the ECO did not cause serious illness or death in the *Swiss* mice up to an oral dose of 3000 mg/kg. Thus, we selected 1/6th, i.e., 500 mg/kg, as a test dose for *in vivo* studies.

3.2.2. Antibacterial activity

Table 2 suggests that ECO exhibited antibacterial effects against all the tested gram (+) bacteria (zone of inhibition: 9.90 ± 0.14 to 12.22 ± 2.04 mm) and 4 species of gram (−) bacteria (zone of inhibition: 9.89 ± 3.56 to 12.53 ± 1.50 mm). However, it remained inactive against *Salmonella typhi*. On the other hand, the CCO was found to act against *Lactobacillus casei*, *Lactobacillus coryniformis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* between the zones of ingestion 11.33 ± 1.16 and 13.00 ± 1.00 mm. It remained insensitive at the test concentration (250 µg/disc) towards *Bacillus azotoformans*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Vibrio cholera*. The HCO fraction did not act against any test bacteria at 250 µg/disc. In contrast, the standard antibiotic AZT exhibited significantly higher efficacy, with inhibition zones ranging from 17.33 ± 0.58 mm to 21.67 ± 0.58 mm at a much lower concentration (25 µg/disc), demonstrating superior antibacterial potency against all tested pathogens. These findings suggest that while C. orchioides extracts possess some antibacterial potential, their activity is considerably weaker than the standard drug, highlighting the need for further refinement or combination strategies to enhance their therapeutic value.

3.2.3. Free radical scavenging capacity

Table 3 suggests that the control group exhibited negligible (2.17 ± 0.37%) inhibition of DPPH free radicals. Both the test sample (all fractions) and the standard drug AA exhibited concentration-dependent radical scavenging capacities. AA produced better activities than the test subjects. Among the extracts, ECO exhibited better radical scavenging capacity. The IC_{50} values calculated for the AA, ECO, CCO, and HCO are 16.11 ± 0.08, 34.01 ± 0.11, 17.82 ± 0.12, and 54.01 ± 0.11 µg/ml, respectively.

Table 2. Antibacterial activity of *C. orchioides* leaf extracts and the reference antibacterial drug

Test bacteria	Zone of inhibition (mm)			
	Leaf extract (250 µg/disc)			AZT (25 µg/disc)
	ECO	**CCO**	**HCO**	
Gram (+)ve species				
Lactobacillus casei	11.50 ± 2.58	13.00 ± 1.00	NI	18.33 ± 0.58
Lactobacillus coryniformis	9.90 ± 0.14	11.33 ± 1.16	NI	17.33 ± 0.58
Bacillus cereus	12.22 ± 2.04	12.22 ± 0.75	NI	19.00 ± 1.00
Staphylococcus aureus	10.29 ± 2.47	11.50 ± 1.53	NI	20.00 ± 1.00
Bacillus azotoformans	10.33 ± 3.22	NI	NI	19.00 ± 1.00
Gram (-)ve species				
Escherichia coli	9.89 ± 3.56	12.17 ± 1.89	NI	19.67 ± 1.53
Pseudomonas aeruginosa	11.56 ± 1.50	NI	NI	18.67 ± 0.58
Salmonella typhi	NI	NI	NI	19.67 ± 0.58
Klebsiella pneumoniae	12.33 ± 2.52	12.00 ± 2.65	NI	18.33 ± 0.58
Vibrio cholera	12.53 ± 1.50	NI	NI	21.67 ± 0.58

Values are mean ± SEM (standard error of mean) (n = 3); NI = No inhibition; Zone of inhibition ≤7 mm was discarded; AZT: Azithromycin; ECO: Ethanolic Curculigo orchioides extract; CCO: Chloroform Curculigo orchioides extract; HCO: n-Hexane Curculigo orchioides extract

Table 3. DPPH free radical scavenging potential of *C. orchioides* leaf extracts and controls

Parameters		Percentage radical scavenging			
		AA	**ECO**	**CCO**	**HCO**
Conc. (µg/ml)	20	55.67 ± 0.27*	40.89 ± 0.36*	54.52 ± 0.27*	35.96 ± 0.29*
	40	68.97 ± 0.19*	58.46 ± 0.25*	61.41 ± 0.23*	43.02 ± 0.34*
	60	78.00 ± 0.14*	64.86 ± 0.21*	68.14 ± 0.19*	57.96 ± 0.26*
	80	85.55 ± 0.08*	78.65 ± 0.13*	72.91 ± 0.17*	67.98 ± 0.24*
	100	93.27 ± 0.04*	83.42 ± 0.11*	79.64 ± 0.12*	75.86 ± 0.18*
IC_{50} (µg/ml)		16.11 ± 0.08	34.01 ± 0.11	17.82 ± 0.12	54.01 ± 0.11
CI (µg/ml)		9.11–28.47	26.97–42.66	11.47–22.70	44.68–61.57
r^2		0.96	0.84	0.84	0.83
Control		2.17 ± 0.37			

Values are mean ± SEM (standard error of mean) (n = 3); *$p < 0.05$ when compared to the control (0.05% Tween 80 dissolved in 0.9% NaCl solution) group. AA: Ascorbic acid; ECO: Ethanolic Curculigo orchioides extract; CCO: Chloroform Curculigo orchioides extract; HCO: n-Hexane Curculigo orchioides extract; IC_{50}: Half minimum inhibitory concentration; CI: Confidence of intervals; r^2: Coefficient of determination

3.2.4. Anti-inflammatory activity

Egg albumin denaturation inhibition assay

According to **Table 4**, the control group exhibited negligible (2.23 ± 0.29%) inhibition of egg protein denaturation capacity. All the fractions and the standard drug DCN exhibited concentration-dependent stabilization effects on the egg protein. DCN produced better activities than the test subjects. Among the extracts, CCO exhibited better protein protection capacity. The IC_{50} values calculated for the AA, ECO, CCO, and HCO are 43.82 ± 0.05, 89.05 ± 0.07, 62.94 ± 0.04, and 109.65 ± 0.04 µg/ml, respectively.

HRBC membrane stabilization assay

Table 5 shows that the control group exhibited a negligible (1.39 ± 0.11%) cytoprotective effect on the HRBC suspension. All the fractions and the standard drug ASA exhibited concentration-dependent stabilization effects on the HRBC suspension. ASA produced better activities than the test subjects. Among the extracts, CCO exhibited better membrane stabilizing capacity. The IC_{50} values calculated for the AA, ECO, CCO, and HCO are 38.35 ± 0.03, 95.05 ± 0.04, 87.52 ± 0.05, and 107.38 ± 0.07 µg/ml, respectively.

Table 4. Egg protein protection capacity of *C. orchioides* leaf extracts and controls

Parameters		Percentage inhibition of protein denaturation			
		DCN	**ECO**	**CCO**	**HCO**
Conc. (µg/ml)	20	31.37 ± 0.21*	27.86 ± 0.33*	15.44 ± 0.29*	09.80 ± 0.21*
	40	49.75 ± 0.13*	38.73 ± 0.23*	18.22 ± 0.27*	20.92 ± 0.31*
	60	69.04 ± 0.15*	40.36 ± 0.25*	49.59 ± 0.34*	23.53 ± 0.23*
	80	84.23 ± 0.18*	46.08 ± 0.17*	55.23 ± 0.36*	38.73 ± 0.33*
	100	97.06 ± 0.25*	62.91 ± 0.19*	63.56 ± 0.32*	46.08 ± 0.15*
IC_{50} (µg/ml)		43.82 ± 0.05	89.05 ± 0.07	62.94 ± 0.04	109.65 ± 0.04
CI (µg/ml)		33.04–59.65	78.99–97.93	52.62–63.31	97.68–119.42
r^2		0.93	0.82	0.94	0.95
Control		2.23 ± 0.29			

Values are mean ± SEM (standard error of mean) (n = 3); *p < 0.05 when compared to the control (0.05% Tween 80 dissolved in 0.9% NaCl solution) group; DCN: Diclofenac-Na; ECO: Ethanolic Curculigo orchioides extract; CCO: Chloroform Curculigo orchioides extract; HCO: n-Hexane Curculigo orchioides extract; IC_{50}: Half minimum inhibitory concentration; CI: Confidence of intervals; r^2: Coefficient of determination

Table 5. RBC membrane stabilizing capacity of *C. orchioides* leaf extracts and controls

Parameters		Percentage inhibition of membrane lysis			
		ASA	**ECO**	**CCO**	**HCO**
Conc. (µg/ml)	25	39.17 ± 0.23*	18.71 ± 0.31*	20.39 ± 0.31*	08.78 ± 0.29*
	50	69.36 ± 0.26*	38.73 ± 0.23*	40.36 ± 0.27*	20.92 ± 0.11*
	100	90.20 ± 0.19*	55.23 ± 0.29*	63.56 ± 0.23*	49.75 ± 0.13*
IC_{50} (µg/ml)		38.35 ± 0.03	95.05 ± 0.04	87.52 ± 0.05	107.38 ± 0.07
CI (µg/ml)		20.20–48.76	87.35–101.50	76.58–98.6	97.51–119.70
r^2		0.98	0.98	0.97	0.94
Control		1.39±0.11			

Values are mean ± SEM (standard error of mean) (n = 3); *p < 0.05 when compared to the control (0.05% tween 780 dissolved in 0.9% NaCl solution) group; ASA: Acetyl salicylic acid; ECO: Ethanolic Curculigo orchioides extract; CCO: Chloroform Curculigo orchioides extract; HCO: n-Hexane Curculigo orchioides extract; IC_{50}: Half minimum inhibitory concentration; CI: Confidence of intervals; r^2: Co-efficient of determination

3.2.5. Hypoglycemic activity test

Alpha-amylase inhibitory effect

Table 6 shows that the control group exhibited a negligible (2.39 ± 0.08%) alpha amylase inhibitory effect. All the fractions and the standard drug ACB exhibited significant (*p < 0.05) and concentration-dependent alpha-amylase inhibitory effects as compared to the control group. ACB produced better activities than the test subjects. Among the extracts, HCO exhibited a better alpha-amylase inhibitory effect. The IC_{50} values calculated for the AA, ECO, CCO, and HCO are 43.63 ± 0.06, 67.69 ± 0.23, 268.94 ± 0.06, and 121.82 ± 0.03 µg/ml, respectively.

Glucose tolerance test

Table 7 suggests that the standard hypoglycemic drug GBC reduced peripheral blood glucose levels effectively after 30 minutes. In comparison to the control group, all the extracts also significantly (*p < 0.05) reduced peripheral blood glucose levels after 30 minutes. CCO at 500 mg/kg exerted strong hypoglycemic effects in comparison to the control group.

Table 6. Alpha amylase inhibition capacity of *C. orchioides* leaf extracts and controls

Parameters		Percentage amylase activity inhibition			
		ACB	**ECO**	**CCO**	**HCO**
Conc. (µg/ml)	25	42.31 ± 0.14*	21.43 ± 0.27*	10.16 ± 0.23*	12.50 ± 0.27*
	50	57.69 ± 0.21*	41.43 ± 0.17*	20.31 ± 0.21*	28.13 ± 0.33*
	100	69.23 ± 0.23*	58.57 ± 0.13*	32.01 ± 0.17*	46.88 ± 0.31*
	200	80.77 ± 0.34*	72.14 ± 0.29*	46.88 ± 0.14*	62.50 ± 0.27*
	400	96.15 ± 0.28*	85.71 ± 0.31*	63.28 ± 0.11*	78.13 ± 0.24*
IC_{50} (µg/ml)		43.63 ± 0.06	67.69 ± 0.23	268.94 ± 0.06	121.82 ± 0.03
CI (µg/ml)		31.74–52.01	58.56–82.83	246.51–289.18	111.67–134.45
r^2		0.96	0.98	0.96	0.99
Control		2.39 ± 0.08			

Values are mean ± SEM (standard error of mean) (n = 3); *p < 0.05 when compared to the control (0.05% Tween 80 dissolved in 0.9% NaCl solution) group; ACB: Acarbose; ECO: Ethanolic Curculigo orchioides extract; CCO: Chloroform Curculigo orchioides extract; HCO: n-Hexane Curculigo orchioides extract; IC_{50}: Half minimum inhibitory concentration; CI: Confidence of intervals; r^2: Coefficient of determination

Table 7. Glucose level (mmol/L) of peripheral blood at observation time for *C. orchioides* leaf extracts and controls

Treatments (p.o.)		Glucose level (mmol/L)			
		0 min	**30 min**	**60 min**	**120 min**
Control (10 ml/kg)		22.25 ± 0.18	18.35 ± 1.09	15.35 ± 1.08	12.55 ± 1.02
GBC (10 mg/kg)		14.65 ± 0.07*	6.15 ± 1.48*	3.75 ± 0.49*	3.33 ± 0.45*
500 mg/kg	**ECO**	15.20 ± 1.56*	7.35 ± 1.91*	4.20 ± 1.27*	4.05 ± 0.64*
	CCO	16.75 ± 0.64*	7.85 ± 1.20*	5.15 ± 0.07*	3.55 ± 0.07*
	HCO	18.25 ± 0.07*	8.90 ± 0.28*	5.55 ± 0.78*	4.35 ± 0.07*

Values are mean ± SEM (standard error of mean) (n = 5); One-way ANOVA followed by t-student test; *p < 0.05 when compared to the control group. GBC: Glibenclamide; ECO: Ethanolic Curculigo orchioides extract; CCO: Chloroform Curculigo orchioides extract; HCO: n-Hexane Curculigo orchioides extract

3.2.6. Antidiarrheal activity

According to **Table 8**, the standard drug LOP at 3 mg/kg significantly (*p < 0.05) augmented the latency period and reduced the total diarrheic secretions in animals. In comparison to the control group, all the extracts significantly (*p < 0.05) increased the latency period and reduced the total diarrheic secretions in animals. ECO and HCO at 500 mg/kg exerted better antidiarrheal effects than CCO.

3.2.7. Anxiolytic activity

Table 9 suggests that the standard drug DZP at 3 mg/kg significantly (*p < 0.05) reduced the number of square crossing and hole crossing behaviors in animals. Among the test extracts, CCO was found to reduce the square crossing and hole crossing behaviors in animals significantly. However, in comparison to the control group ECO and HCO groups also exerted significant (*p < 0.05) calming effects in animals.

Table 8. Latency and number of diarrheic secretions observed in *C. orchioides* and controls

Treatment groups (p.o.)		Observed parameters	
		Latency (min)	**Diarrheic secretions**
Control (10 mL/kg)		28.00 ± 3.00	22.33 ± 2.52
LOP (3 mg/kg)		50.33 ± 1.53*	6.33 ± 2.52*
500 mg/kg	**ECO**	42.67 ± 2.89*	8.00 ± 1.00*
	CCO	39.33 ± 2.52*	9.67 ± 1.53
	HCO	40.67 ± 2.08*	8.33 ± 2.08*

Values are mean ± SEM (standard error of mean) (n = 5); One-way ANOVA followed by t-student test; *p < 0.05 when compared to the control group. DZP: Diazepam; p.o.: Per oral; ECO: Ethanolic Curculigo orchioides extract; CCO: Chloroform Curculigo orchioides extract; HCO: n-Hexane Curculigo orchioides extract

Table 9. Number of square crossings and hole crossings observed in *C. orchioides* leaf extracts and control groups

Treatment groups		Observed parameters	
		Square crossing	**Hole crossing**
Control (10 mL/kg, p.o.)		90.00 ± 2.00	10.00 ± 1.00
DZP (3 mg/kg, i.p.)		49.00 ± 4.58*	3.67 ± 0.58*
500 mg/kg (p.o.)	**ECO**	74.67 ± 2.52*	5.33 ± 1.53*
	CCO	64.67 ± 2.52*	4.00 ± 3.60*
	HCO	81.33 ± 3.06*	6.00 ± 2.00*

Values are mean ± SEM (standard error of mean) (n = 5); One-way ANOVA followed by t-student test; *$p < 0.05$ when compared to the control group; DZP: Diazepam; p.o.: Per oral; ECO: Ethanolic Curculigo orchioides extract; CCO: Chloroform Curculigo orchioides extract; HCO: n-Hexane Curculigo orchioides extract

4. Discussion

The traditional use of a plant provides indirect hints to investigators that the plant is the source of targeted phytochemicals (Tanim et al., 2025). This information has been passed down from generation to generation, and in recent decades, scientists have been taking these local usage practices into great consideration. It has been estimated by the World Health Organization (WHO) that herbal remedies are relied upon by roughly 80% of people worldwide for some aspects of their primary healthcare requirements. The WHO has stated that there are around 21,000 plant species that possess medicinal properties. Based on available data, it has been concluded that over three-quarters of the global population primarily depends on plants and plant extracts for their healthcare needs (Petersen & yamamoto, 2005).

C. orchioides, the origin of this plant, is traced back to India, where it is recognized as a powerful adaptogen and aphrodisiac in the Ayurvedic medical tradition. It is widely used in various Ayurvedic remedies and is believed to possess hepatoprotective, anticancer, antioxidant, antidiabetic, aphrodisiac, and immunostimulatory properties (Chauhan et al., 2010). Its rhizomes are well used traditionally rather than its leaf. The rhizomes of this plant have been utilized for numerous purposes in traditional practices, such as treating skin ailments, promoting wound healing, providing a cooling effect, combating bacterial infections, enhancing sensual experiences, managing jaundice and respiratory conditions like asthma and bronchitis, preventing cancer, and serving as a tonic for leucorrhea, hemorrhoids, pruritis, and sweetness, among others (Asif, 2012).

Plants contain a wide range of components that possess various bioactivities in both plant and animal systems. These components can regulate biological signaling pathways and are therefore potentially useful in treating and managing a wide range of diseases and disorders (Lee et al., 2021). *C. orchioides* contains a diverse range of phytoconstituents, as indicated by qualitative analyses of extracts obtained from both the plant's rhizomes and the whole plant. These extracts contain saponins, phenolics, alkaloids, steroids, flavonoids, triterpenes, and polysaccharides (Kushalan et al., 2022; Wang et al., 2019). Major glycosides identified from the *C. orchioides* plant includes syringic acid; glucopyranoside; curculigosaponins E, F, G, H, I, and J; curculigoside B; curculigines P and Q; curculigine B and C; orcinosides I-J; 2-beta-Dglucopyranosyloxy-5-hydroxybenzyl-2'-methoxy-6'-hydroxybe nzoate; 2,6-dimethoxyl benzoic acid; orcinol-β-D-glucoside (Chen et al., 2017; Deng et al., 2021; Pham et al., 2022; Wang et al., 2012; Zuo et al., 2010).

In our study, we found that *C. orchioides* contains flavonoids, sugar moieties, and glycosides in its ECO, CCO, and HCO extractions. The issue of antibiotic resistance is a significant challenge in the contemporary era, resulting in the failure of conventional antibiotics, chronic ailments, treatment complexities, and escalated costs. Multidrug-resistant bacteria can cause severe health consequences for both humans and animals. Phytochemicals offer a promising alternative by providing a diverse range of bioactive groups such as alkaloids, glycosides, polyphenols, and flavonoids, which are capable of combating various pathogens, including multidrug-resistant ones, by targeting their efflux pumps, biofilms, membrane-protein dysregulation, interapting cellular communications, and other biological processes (Suganya et al., 2022). It has evidence that *C. orchioides* has an anti-*Streptococcus pyogenes* effect with a minimum inhibitory concentration (MIC) value of 49 µg/ml (Marasini et al., 2015). Our research findings indicate that at a dosage of 250 µg/disc, the herb's CCO exhibited antibacterial properties against a range of gram-positive and gram-negative pathogenic bacteria. This confirms earlier reports of the plant's antibacterial effects.

Unbound radicals are chemical compounds containing atoms, molecules, or ions that contain one or more unpaired electrons in their outer shells, rendering them extremely responsive. Usually, these molecules play a double duty in our organisms: on one hand, they can inflict oxidative harm and tissue malfunction as poisonous byproducts of oxygen metabolism, while on the other hand, they also act as molecular signaling agents that promote advantageous stress cascades (Di Meo et al., 2020; Bhuia et al., 2025a). An overabundance of unbound radicals can inflict harm on a living system. Antioxidants counteract these radicals in a living system via various means. Medicinal plants and their byproducts, boasting a range of chemical groups, have the potential to act against an excess of hazardous unbound radicals that may affect our bodies (Khan et al., 2019).

As an antioxidant, *C. orchioides* is believed to possess the ability to scavenge radicals, according to previous reports. Furthermore, another study indicated that the antioxidant impact of *C. orchioides* is associated with its ability to protect the liver in experimental animals (Venukumar & Latha, 2002; Tacchini et al., 2015). In our investigation, it was discovered that every fraction of *C. orchioides* leaf could effectively and proportionally remove DPPH free radicals at a concentration range of 20−100 µg/ml. The overproduction of harmful free radicals is related to the destruction of important cellular macromolecules such as carbohydrates, proteins, lipids, and genetic molecules inside an organism. This phenomenon can stimulate both pro-inflammatory and inflammatory reactions, which may trigger the liberation of various mediators (Mason, 2016).

Nonetheless, microbial invasions can cause both oxidative stress and inflammatory responses within our bodies (Hawiger & Zienkiewicz, 2019; Bhuia et al., 2025d). Though leaf extract was not previously studied, in a previous study conducted by Ku et al. (2012), rhizome extracts of the plant, when administered at 150, 300, and 600 mg/kg doses, had a significant impact on reducing the levels of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6 when compared to the control groups in rats (Ku et al.,

2012). Our investigation revealed that all the *C. orchioides* leaf extracts exhibited dose-dependent inhibition of egg protein denaturation and safeguarded HRBC within the range of 20 to 100 µg/ml. The improper management of blood sugar levels continues to pose a significant health challenge globally, leading to a surge in the prevalence of diabetes mellitus and related fatalities. Although conventional antidiabetic medications have been successful, they often come with inevitable side effects. Hence, alternative options, such as the development of novel and safe antidiabetic drugs, may be necessary to address this issue (Salehi et al., 2019).

As per several prior studies, *C. orchioides* possesses antidiabetic characteristics (Thakur et al., 2012; Umar et al., 2021). According to Umar, *C. orchioides* is capable of inhibiting alpha-glucosidase. In diabetic patients, enzymes such as alpha-amylase and alpha-glucosidase decompose carbohydrates and lead to elevated glucose levels in the blood after meals (Umar et al., 2021). Thus, compounds that can restrict these enzymes could regulate postprandial hyperglycemia, minimizing the likelihood of diabetes development (Anza et al., 2015). Our research indicates that all the segments of *C. orchioides* leaves were able to dose-dependently restrain alpha-amylase. In addition, they also reduced the oral glucose level in experimental animals 30 minutes after extract administration at a 500 mg/kg oral dose. Diarrhea is a medical condition in which a person experiences frequent bowel movements that are unusually watery or loose in texture. The condition is triggered by a combination of factors, including osmotic and active secretion, impaired peristalsis, and exudation (Anza et al., 2015). It has been estimated that diarrhea leads to about 1.7 billion cases annually in children and is responsible for almost 5.5 million child deaths below the age of 5. In South Asia, diarrhea causes nearly 2.75 million deaths among children under the age of 5. Luckily, diarrhea can be prevented and cured. Nevertheless, traditional antidiarrheal medications are hazardous, develop resistance, become ineffective, and are not recommended for numerous pathological conditions and therapeutic approaches (Sarath et al., 2020).

The use of local medicinal plants for diarrhea treatment has a long history in many countries across the globe. As far as we know, this study represents the first antidiarrheal activity test conducted using the castor oil-induced diarrhea model in *Swiss* mice. Castor oil prevents water reabsorption, leading to an increase in the volume of intestinal contents and causing diarrhea in animals. The ricinoleic acid present in castor oil mediates irritation and inflammation of the intestinal mucosa, resulting in prostaglandin secretion that alters the transport of mucosal fluid and electrolytes, thereby preventing the reabsorption of NaCl and water. In experimental animals, ricinoleic acid was found to significantly increase the PGE2 content in the gut lumen, leading to the release of excessive water and electrolytes into the small intestine. Additionally, castor oil has the ability to synthesize nitric oxide (NO), which increases the permeability of the epithelial layer to calcium ions, resulting in increased intracellular Ca^{2+} levels and stimulation of NO synthase activity. As a result, both prostaglandins and NO can induce intestinal secretion and trigger inflammatory cascades (Sharma et al., 2010; Bhuia et al., 2025a).

Bacterial diarrhea is commonly caused by specific pathogens, including *E. coli* and *Salmonella* sp. According to Marasini et al. (2015) study (Marasini et al., 2015) and our own findings, there may be a correlation between the antibacterial effects observed and the efficacy against *E. coli* and Salmonella sp. In our research, we found that administering CCO at a dose of 500 mg/kg and the standard antidiarrheal drug LOP at 3 mg/kg resulted in similar latency and diarrhea episodes in the castor oil-induced diarrhea model in mice. According to Islam (Islam, 2017; Ghosh et al., 2025), there are more than 600 known neurological diseases and disorders. Anxiety is a common mood disorder caused by stress that can cause disability and even premature death. Around 20% of adults experience anxiety at some point in their lives (Dang et al., 2009; Bhuia et al., 2025b). The neurotransmission systems involving gamma-aminobutyric acid (GABA) and serotonin play a role in anxiety. Although SSRIs are effective in treating anxiety, they are mainly used as antidepressants (Sugimoto et al., 2010).

Benzodiazepines are commonly used to treat anxiety, but they have many unwanted effects like muscle dependence, relaxation, memory problems, sedation, and interaction with other treatments. Therefore, plant-based compounds can be a safe and effective source of anxiolytic agents (Bishwas et al., 2025; Bhuia et al., 2025b). Our research indicates that CCO can be used as an alternative to benzodiazepines to treat anxiety (Costa et al., 2014). The study found that CCO showed similar anxiolytic effects as the positive control group (DZP 3 mg/kg, i.p.) in *Swiss* mice at 500 mg/kg (p.o.). All the extracts significantly reduced the number of square and hole-crossing behaviors in animals compared to the control group ($p < 0.05$). Various solvents are used for extracting compounds from plants, such as ethanol, chloroform, n-hexane, methanol, and ethyl acetate, depending on the type of compound. Ethanol, methanol, and ethyl acetate are used for extracting hydrophilic compounds, while chloroform, dichloromethane, or a mixture of dichloromethane and methanol are used for lipophilic compounds. Moreover, n-hexane is used to remove chlorophyll from plant extracts (Cosa et al., 2006). To obtain initial extraction, dried plant materials are usually extracted with chloroform. This solvent is often utilized to extract glycosidic contents from crude samples as well (Onwukaeme et al., 2007). A preliminary phytochemical study suggests that CCO exhibited a higher intensity of glycoside contents in the leaf extract, which is consistent with previous studies that isolated glycosides from the plant. Furthermore, according to a study, the polysaccharides of this herb are evidently enhancing the rate of osteogenic mineralization in MC3T3-E1 cells. Osteoarthritis is related to oxidative stress and inflammation, which are promising factors (Wang et al., 2019; Minguzzi et al., 2018).

C. orchioides has been previously reported to have anti-arthritis effects (Wang et al., 2017; Gong et al., 2022; Liu et al., 2023). Gong attributed this effect to orcinol glucoside, present in *C. orchioides*, which attenuated oxidative stress and autophagic cascades in SAMP6 mice (Gong et al., 2022) . The development of arthritis is associated with chronic inflammation of the synovial membrane and bone erosion, caused by autoantibodies and proinflammatory cytokines like TNF-α and IL-6 (Smolen & Aletaha, 2015; Akhi et al., 2025). *C. orchioides* rhizome extract has been found to be active against TNF-α and IL-6 (Ku et al., 2012), and it also exhibits immunostimulatory effects in mice (Bafna & Mishra, 2006). The CCO and HCO fractions of the plant contain bioactive sugars that might be responsible for their observed free radical scavenging and anti-inflammatory effects.

5. Conclusion

All the fractions showed high intensity for the phytochemical groups alkaloids, glycosides, tannins, and reducing sugars. Only ECO and CCO showed sensitivity against the test bacteria at 250 µg/disc. HCO exhibited better radical scavenging, alpha-amylase inhibition, and anti-diarrheal effects. On the other hand, CCO showed better inhibition of protein denaturation and membrane lysis, hypoglycemic, and anxiolytic effects. We suppose that *C. orchioides* leaf extract-mediated biological activities might be linked to the presence of high amounts of alkaloids, glycosides, tannins, and reducing sugars. The *in vitro* results (e.g., antioxidant, anti-inflammatory, and alpha-amylase inhibitory) were significant and concentration-dependent. All the extracts also showed significant hypoglycemic, antidiarrheal, and anxiolytic effects in

Swiss mice at 500 mg/kg. Further studies are required to isolate and characterize its active principles and elucidate their bioactivities with possible molecular mechanisms.

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Ethical statement

The Institutional Ethics Committee (SUB/IAEC/12.01) authorized all experimental protocols.

Conflict of interest

There is no conflict of interest.

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