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The Micro, Macro-Nutritional and Pharmacological Potential of *Coelogyne suaveolens* Root and Bulb Extracts: A Comprehensive *in-vitro*, *in-vivo* and *in-silico* Assessment

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Abstract

The medicinal potential of an orchid *Coelogyne suaveolens*, subjecting the ethyl acetate fraction of the acetonic extract from its root and bulb to various *in-vitro*, *in-vivo* and *in-silico* bioactivity tests. The micro and macro-nutrients content of ethyl acetate fraction of acetonic extract of *C. suaveolens* bulb and root were assessed by atomic absorption spectroscopic method. The *in-vitro* antioxidant property of the extract was assessed by following the ABTS, DPPH, and H_2O_2 radical scavenging assays. The antidiabetic property of the extract was evaluated using the Alpha-Amylase Inhibitory Assay by DNSA and SI method, while the cytotoxic property was assessed using the brine shrimp lethality bioassay. Thrombolytic activity by clot-lysis and anthelmintic activity were assessed. *In-vivo* antidiarrheal activity by castor oil-induced diarrhea and castor oil-induced intestinal motility (charcoal marker) tests were performed; for antidepressant activity, forced swim and tail suspension tests were performed. Both parts of *C. suaveolens* possess substantial levels of vital minerals such as calcium, magnesium, phosphorus, sodium, potassium, and copper. The outcomes of our investigation demonstrated that the ethyl acetate fraction of *C. suaveolens* acetonic extract showed promising and significant antioxidant activity. The half-maximal inhibitory concentration (IC_{50}) values ranged from 8.43 to 520.29 µg/mL for the bulb extract, and from 47.65 to 195.77 µg/mL for the root extract, in ABTS, DPPH, and H_2O_2 radical scavenging assays respectively. In terms of antidiabetic activity, both the bulb and root extracts showed moderate activity in the Alpha-Amylase Inhibitory Assay by DNSA and SI method. The cytotoxicity of both extracts was found to be significantly toxic. *In-vitro* thrombolytic activity was not significant compared to standard streptokinase. Anthelmintic activity of both extracts was found to be significant, and root is more significant than bulb. Both extracts have no antidiarrheal effect, but it may produce diarrheal or laxative effects. No significant antidepressant activity is produced by either extract. Based on the findings, it is evident that the ethyl acetate fraction derived from the acetonic extract of *C. suaveolens* exhibits remarkable pharmacological potential in various *in-vitro*, *in-vivo* and *in-silico* study models. This orchid may be a good source of antioxidant, antidiabetic, and laxative drugs in the future. Therefore, it has the potential to serve as a viable source for isolating a lead compound to treat a multitude of disorders and can be used as a nutritional supplement.

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Keywords: *Coelogyne suaveolens*, Antioxidant, Antidiabetic, Cytotoxic, anthelmintic, antidiarrheal, antidepressant

1. Introduction:

Despite significant progress in the field of medical treatment, the world is encountering a high occurrence of various hazardous diseases. These encompass conditions like heart ailments (including heart failure) [1], diabetes [2], various forms of cancer [3], Alzheimer's disease [4], infectious diseases [5], and respiratory disorders [6].

Considering this situation, natural remedies have gained renewed attention. These remedies have been utilized for centuries due to their low cost and absence of adverse effects in treating serious medical conditions. The medicinal effects of plants arise from a diverse array of naturally occurring chemical components. Furthermore, science has advanced to the point where numerous phytochemical constituents from therapeutic plants have been successfully identified. Consequently, these compounds are increasingly employed as preventatives, capitalizing on their wide range of physiological effects [7].

Experts began to doubt the reliability of conventional medicine because there was not enough supportive scientific evidence presented by the pharmaceutical industry. However, other traditional medicines with varying effects have been identified over time. In addition, this herbal treatment is cheap, efficient, and has little negative side effects [8]. Nonetheless, several plants are recognized as being poisonous. Several studies are currently being conducted to delve deeper into medicinal plants' pharmacological effects and potential toxicity. The cytotoxicity of the brine shrimp test was considered a useful method for screening potential toxicity [9]. Using this approach, plant extracts with potential bioactivity can be obtained. It is a simple and effective way to monitor the screening and separation of potential novel plant-based bio-active components.

In the food industry and medical research, natural antioxidant preparations from aromatic, medicinal, and other plants were sought [10]. The antioxidative characteristics of medicinal plants are contingent upon a range of factors, encompassing the specific plant type, its variations, environmental factors, seasonal and climatic fluctuations, the geographical region where it grows, the level of maturity it attains, cultivation methods, and various considerations like post-harvest treatments and processing techniques. Moreover, the efficacy of the antioxidant impact is intertwined with the composition and concentration of existing antioxidants, notably phenolic compounds [11]. The phenolic compounds are the most employed in research and have several important nutritional applications [12]. Phenolic substances found in plants include polyphenols, hydrolysable and condensed tannins, as well as phenolic acids and flavonoids. Protecting plant tissues, fruits, and vegetables, as well as human DNA, lipids, and proteins, from oxidative attack is a primary function of these chemicals, which can do so by scavenging free radicals [13]. It is possible that activities that scavenge free radicals help keep inflammation at bay. This is because inflammatory tissue destruction is brought on by active O_2 metabolites released by phagocytic leukocytes, which invade the tissues and damage vital cellular components [14]. Inflammatory illnesses can benefit from compounds that can scavenge these radicals [15].

On the contrary, by 2030, an estimated 552 million individuals worldwide will have diabetes mellitus, up from an estimated 366 million in 2011 [16]. The prevalence of type 2 diabetes is rising worldwide, but it is particularly severe in low and middle-income nations. In 2011, diabetes was responsible for the deaths of 4.6 million people. By the year 2030, it is estimated that around 439 million people are anticipated to be affected by type 2 diabetes. Due to differences in environmental and behavioral risk factors, the occurrence rate of type 2

DM varies widely among regions [17]. Despite advances in our understanding of the disease's biology and the development of promising new treatments, no solution is yet in sight. Therefore, the current study examines the ethnomedicinal plant *C. suaveolens* from a phytochemical, pharmacological, and nutritional perspective.

Orchids are popularly used as ornamental pieces in homes, workplaces, and public spaces due to their well-known beauty. Most people simply enjoy them for their aesthetic value, but others have found useful applications for them. Orchids have been used medicinally for a long time across various regions globally. Inadequate studies into the efficacy and side effects of orchids have led to a gradual decline in their usage in medicine [18]. Orchids' healing abilities were initially discovered by the Chinese, and they continue to be used in modern medicine, most commonly in the form of medicinal tea. *C. suaveolens,* an orchid species found in central China, Assam, the eastern Himalayas, and Thailand, has been chosen to promote the traditional uses of orchids [18]. This study aims to investigate the bioactive phytochemicals, pharmacological, and nutritional effects of this orchid, as there has been no previous research on its antioxidant, mineral content, antidiabetic, cytotoxic, thrombolytic, anthelmintic, antidiarrheal and antidepressant activity.

Figure 1: *Coelogyne suaveolens*

2. Material and Methods

2.1 Chemicals

All the analytical-grade chemicals were obtained from the Department of Pharmacy, Faculty of Biological Science, University of Chittagong.

2.2 Collection and identification of the plant

Coelogyne suaveolens (Lindl.) orchid is newly reported in Bangladesh [18]. Then it was identified by Mr. Md. Owahidul Alam, Assistant horticulturist, Department of Botany, University of Chittagong, under herbarium no- DPCU/2022/011.

2.3 Preparation of crude extracts

It took seven days of drying in the sun in a semi-shed for the plant materials (bulb and root) to be ready for use. The plant materials were dried and then processed into a powder in a mechanical grinder. The plant's bulb and root were ground into a powder, and 250 g of

plant material for each part was soaked in acetone. Both solutions were stirred intermittently for 13 days before being filtered and concentrated using a rotary evaporator at decreased pressure and temperatures below 50 °C. (London) (Stu-art, UK). 15g and 18g concentrated extracts were collected from the bulb and root, respectively. Following the procedure developed by VanWagenen *et al*., crude acetone extracts of the bulb and root of *C. suaveolens* are solvent-solvent partitioned using ethyl acetate solvent [18].

2.4 Micro and macro-nutritional screening

The mineral constitution of *C. suaveolens* was carried out by adopting an Atomic Absorption Spectrophotometer AA -7800 Series. About 1 gram of finely ground bulb and root sample were put in a container. Then, a mixture of concentrated nitric acid and hydrochloric acid in a 1:3 (v/v) ratio, totaling 15 mL, was added to the sample. This mixture was heated while in a fuming hood. After cooling to room temperature, the elements were filtered. The ultimate volume was made up to 100 mL using deionized water. The assimilated sample was examined using an atomic absorption spectrophotometer. To create a calibration curve, functioning standard solutions were prepared from a stock solution with a concentration of 1000 ppm. These working standards had concentrations of 0, 1, 5, 10, 15, 20, 25, and 100 ppm. This experimentation process was performed repeatedly three times to ensure accuracy. The quantity of minerals present in the sample was determined using the regression equation that was formulated based on the standard plot [19].

2.5 Evaluation of antioxidant activity

2.5.1 DPPH free radical scavenging assay

Extracts from the bulbs and roots of plants were tested for their antioxidant activity using the explained technique [20]. Three milliliters of a 0.004% w/v DPPH methanol solution were combined with two milliliters of plant extract at the following concentrations: 250, 500, and 1000 g/ml. To finish the reaction, the tubes containing the mixture were placed in a dark, room-temperature environment for 30 minutes. Using an ultraviolet-visible spectrophotometer (Halo SB-10 single-beam spectrophotometer, Dynamica Scientific Ltd., UK), the absorbance was measured at 517 nm. A positive control was ascorbic acid. The ability to scavenge the DPPH radical was computed using the formula:

$$[(A_0 - A_1)/A_0] \times 100$$

Where A_0 represents the absorbance of the DPPH + methanol control reaction and
A_1 represents the sample absorbance.

2.5.2 ABTS radical scavenging assay

The ABTS radical scavenging technique was employed to evaluate the antioxidant activity, as described by Kim 2024 [21]. To create ABTS radical cation (ABTS +), a 7 mM solution was mixed with 2.45 mM ammonium persulfate and let to stand for 12-16 hours at room temperature and in a dark environment. To conduct the experiment, a solution of ABTS (0.3 ml) was combined with acetonic extract (0.5 ml) of varying concentrations (250, 500, and 1000 g/ml), and the final volume was adjusted to 1 ml with acetone. The percentage of inhibition was determined by measuring absorbance at 745 nm and applying the following formula:

$$\text{Inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Here, A_0 is the absorbance of the control reaction.
A_1 is the absorbance of the sample.

2.5.3 H_2O_2 radical scavenging assay

The standard procedure was employed to determine if the crude extract could scavenge hydrogen peroxide [21]. In a 0.1 M phosphate buffer saline at a pH of 7.4, one milliliter (0.25 milligrams) of the extract was rapidly mixed with two milliliters of a 10 millimoles per million (10 mM) hydrogen peroxide solution. Utilizing a UV spectrophotometer, the absorbance at 230 nm was determined following a 10-minute incubation period compared to a blank (devoid of hydrogen peroxide).

2.6 Antidiabetic Activity

2.6.1 Antidiabetic study by Alpha Amylase Inhibitory Assay by DNSA Method

Using 3,5-dinitrosalicylic acid (DNSA), the α-amylase inhibition experiment was carried out [22]. To achieve concentrations between 10 and 1000 g/ml, the bulb and root extracts were dissolved in a minimum of 10% DMSO, and then we dissolved it in a buffer (Na_2HPO4/NaH_2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9). The extract was combined with 200µl of α-amylase solution (2 units/ml) and left to incubate at 30 °C for 10 minutes. After 3 minutes of incubation, 200µl of the starch solution (1% in water (w/v)) was added to each tube. After boiling in a water bath at 85-90 °C for 10 minutes, the reaction was terminated by adding 200 µl DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitrosalicylic acid solution). A UV-visible spectrophotometer was utilized to record the absorbance reading at a wavelength of 540 nm after bringing the mixture down to room temperature and diluting it with 5 cc of distilled water. By subbing in 200 µl of buffer for the plant extract, a blank with a full complement of enzyme activity was created. A blank reaction was also set up without adding the enzyme solution, employing the plant extract of varying concentrations. Like the reaction with the plant extract, a positive control sample containing acarbose (100 µg/ml–2 µg/ml) was generated and used. Using the following equation, the percentage of inhibition of αamylase was determined. The IC_{50} values were calculated by graphing the percentage of αamylase inhibition versus the extract concentration.

$$\% \ \alpha \text{ amylase inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Here, A_0 was the absorbance of the control (acarbose), and A_1 was the absorbance of plant extracts.

2.6.2 Antidiabetic study by Alpha Amylase Inhibitory Assay by Starch Iodine Method

Screening of plant material for α-amylase inhibitors was carried out according to the starch-iodine test [22]. The total assay mixture composed of 40 µl 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 0.02 units of PPA solution and plant extracts at concentrations from 0.1-1.5 $mgml^{-1}$ (w/v) were incubated at 37°C for 10 min. Then soluble starch (1%, w/v) was added to each reaction well and incubated at 37°C for 15 min. 1 M HCl (20 µl) was added to stop the enzymatic reaction, followed by the addition of 100 µl of iodine reagent (5 mM I_2 and 5 mM KI). The colour change was noted, and the absorbance was read at 620 nm on a microplate reader. The control reaction, which represented 100% enzyme activity, did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls without the enzyme were also included. The known PPA inhibitor, acarbose, was used to control positively at a concentration range of 6.5 - 32.8 μgml^{-1}. A dark blue colour indicates the presence of starch, a yellow colour indicates the absence of starch, while a brownish colour indicates partially degraded starch in the reaction mixture. In the presence of inhibitors from the extracts, the starch added to the enzyme assay

mixture is not degraded and gives a dark-blue colour complex, whereas no colour complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolysed by α-amylase.

2.7 Cytotoxic activity by brine shrimp lethal bioassay

2.7.1 Hatching of brine shrimp

A transparent solution was obtained by dissolving exactly 38 grams of sea salt (without iodine) in 1 L of distilled water and then filtering away the excess solids. Using 1 N NaOH solution, the pH of the seawater was kept between 8.0 and 8.5. Baby brine prawns Upon birth, brine shrimp eggs served as the test organism, or Artemia salina leach, which were procured from Chittagong pet stores. The little aquarium was filled with seawater, and fertilized prawn eggs were inserted. The nauplii would develop from the eggs after two days. During the period of egg hatching, the oxygen pump operated continuously to supply the necessary oxygen. The nauplii were gathered from the well-lit area of the tank because the newly born prawns were drawn to the light (phototaxis). The nauplii were pipetted out of the fish tank.

Ten nauplii were kept in a test tube with 5 ml of seawater and treated with varying doses of plant extract solutions (1000, 800, 500, 250, 125g/ml). After 24 hours, the Petri dishes were examined under a black light with a magnifying glass to count the number of nauplii that had survived. The bioassay considered death to have occurred when the subject no longer made coordinated steps forward after 30s of observation. Using this information, we determined the percentage (%) of brine shrimp nauplii mortality at each concentration using the following equation [20].

% mortality = (Number of brine shrimp dead / Number of brine shrimp introduced) ×100

2.7.2 Estimation of median lethal concentration (LC_{50})

The linear regression technique was used to plot the percentage of mortality against the corresponding concentration of the extracts, yielding the LC_{50} value, which represented the concentration at which fifty percent of the brine shrimp nauplii died after a given exposure time. The connection was close to being linear. Concentration-response data were turned into a straight line using a trend line fit linear regression analysis, and the resulting graph showed a linear relationship between concentration and death rate. From this best-fit line, LC_{50} values were calculated.

2.8 Thrombolytic activity by in-vitro clot-lysis method

The thrombolytic activity test was performed according to the literature study. Ten healthy individuals (n=10) donated whole blood, which was then allowed to form clots in sterile microcentrifuge tubes. The serum was carefully removed, and the weight of the clot was measured. After the clot was dissolved using streptokinase, the fluid was removed and the remaining remnants of the clot, along with the tube, were weighed again. The percentage of clot lysis was determined by calculating the weight difference of the microcentrifuge tubes before and after the clot was dissolved [23].

Percent of clot lysis = (weight of released clot/clot weight) × 100%

2.9 Anthelmintic Activity

The method outlined by Ajaiyeoba *et al.,*was slightly modified to measure the anthelmintic activity of crude extracts [23]. Since the aquarium worm Tubifex tubifex and

other intestinal worms share anatomical similarities, its anthelmintic potential was investigated in this study. The sludge worm utilized in this experiment was gathered from Chittagong's aquarium store. The trial was split up into multiple groups: the test group had various concentrations of crude extracts (5, 8, and 10 mg/mL), while the positive control group received the standard medication Albendazole (50 mg/kg). The negative control group was given only distilled water. Ten to twelve worms in five separate groups were put in each Petri dish for this investigation. Subsequently, the Petri dish was filled with 3 mL of each group's distinct concentration. The worms' initial time, their paralysis time, and their death time were all meticulously observed and documented; the worms' paralysis and death times were used to gauge the experiment's anthelmintic activity. The paralysis time and death time were confirmed when the worm's movement could not be observed following a vigorous shake or after being submerged in slightly heated water.

2.10 Antidiarrheal activity (In vivo)

2.10.1 Castor oil-induced diarrhea

Before the test, the mice were split into four groups (n=5) and allowed free access to water for eighteen hours. Initially, 0.4 mL of castor oil was given to the mice as a screening tool, and only those that displayed diarrhea were chosen for the experiment. The test group received an oral suspension of methanolic leaf extract of *C. suaveolens* at a dose of 200 and 400 mg/kg body weight, respectively, while the control group received only vehicles (distilled water containing 1% Tween-80). The positive control received an oral suspension of the standard anti-motility drug loperamide (5 mg/kg body weight). Following an hour of therapy, each patient received 0.4 mL of castor oil orally via gavage and was placed in individual cages with blotting paper or adsorbent paper at the bottom. The characteristics of diarrheal droppings (wet and dry feces) were noted every hour in four hours of study for each mouse. At the beginning of each hour, the old paper was replaced with a new one [24]

Inhibition (%) = [(A-B)/A] × 100.

Where A = mean number of diarrheal feces of the control group;
B = mean number of diarrheal feces of the treated group.

2.10.2 Gastrointestinal motility test by charcoal marker

Mice were given the same treatment as previously described for diarrhea brought on by castor oil. One milliliter of 10% charcoal and 5% gum acacia solution was administered orally one hour after the initial oral administration. After that, mice were sacrificed for one hour while under strong chloroform anesthesia. The entire length of the small intestine was measured, as well as the distance that charcoal traveled from the pylorus to the cecum [24].

Inhibition (%) = [(A-B)/A] × 100

Where A = Distance travel by the charcoal control group (cm)
B = Distance travel by the charcoal test groups group (cm).
Peristalsis index = (Distance travel by the charcoal meal / Total length of the small intestine) × 100

2.11 Antidepressant Activity

2.11.1 Forced Swim Test (FST)

The test was conducted in a slender glass cylinder measuring 13 cm in diameter by 24 cm in height, filled with water at a temperature of 25ºC to a depth of 10 cm, which prevented

them from escaping. Before the vehicle, standard, or test compounds were given to any of the animals, they were all fasted for three hours. The animals were made to swim thirty minutes later. The animal was given two minutes to acclimate to the new surroundings before a stopwatch was used to measure the duration of immobility, which alternated with periods of increased motor activity, for the remaining four minutes of the six-minute test. The animal floated on the surface with its front paws together during immobility time, moving only as much as was required to stay afloat [25]

2.11.2 Tail Suspension Test (TST)

One hour before testing, the test and standard compounds were given intraperitoneally. With adhesive tape placed approximately 1 cm from the tip of their tails, the mice were suspended on the edge of a shelf 58 cm above the tabletop. A stopwatch was used to record the immobility for a period of six minutes. The mice would stop moving after their initial burst of activity. When the mice were hanging motionlessly and passively, they were said to be immobile [25].

3. Statistical analysis

Statistics were stated using a mean and standard error of the mean (SEM) format. Using "Statistical Package for the Social Science" (SPSS, Version 16.0, IBM Corporation, NY), we ran a one-way analysis of variance (ANOVA) and a post hoc Dunnett's test on the data. Statistical significance was defined as a difference from the control group of at least *p 0.05, **p 0.01, or ***p 0.001.

4. Results

4.1 Evaluation of Micro and Macro-Nutritional content

Table 1 displays the micro and macro-nutrient content of *C. suaveolens* bulbs and roots. Sodium, potassium, and calcium were the macro minerals tested. Magnesium, copper, and zinc were among the microminerals found. The dry weight per gram of the extract is the unit of measure for mineral content. Nevertheless, the mineral concentrations in the roots and the leaves are not the same as shown in Table 1.

Table 1: Mineral content screening report

Minerals (mg/gm)	Bulb	Root
Calcium	0.53	0.5
Magnesium	0.1	0.1
Phosphorus	4.7	5.4
Sodium	53.36	142.6
Potassium	0.782	8.2
Chloride	3.9	1.06
Copper	0.73	0.37
Zinc	0.05	0.13

4.2 Antioxidant Activity

4.2.1 DPPH Radical Scavenging Activity

Antioxidant activity was measured using a range of concentrations of the ethyl acetate fraction of acetonic extract, from 250 to 1000 µg/ml, as shown in Table 2 and Figure 2. *C. suaveolens* phytochemicals are the primary source of exogenous antioxidants. Compared with the standard, they may be a potential source of antioxidant agents.

Table :2 DPPH radical scavenging activity

Sample	Concentration	% Radical Scavenging Activity				IC_{50}
		R1	R2	R3	Mean	
Bulb	250	27.21	26.93	25.28	26.47	577.54
	500	54.14	53.73	52.35	53.41	
	1000	73.48	70.86	72.24	72.19	
Root	250	37.43	37.98	38.54	37.98	407.38
	500	59.53	59.39	60.63	59.85	
	1000	84.95	86.33	85.22	85.50	
Ascorbic Acid (Standard)	250	56.19	61.06	59.73	58.99	258.88
	500	74.77	77.43	71.24	74.48	
	1000	92.4	93.36	91.59	92.45	

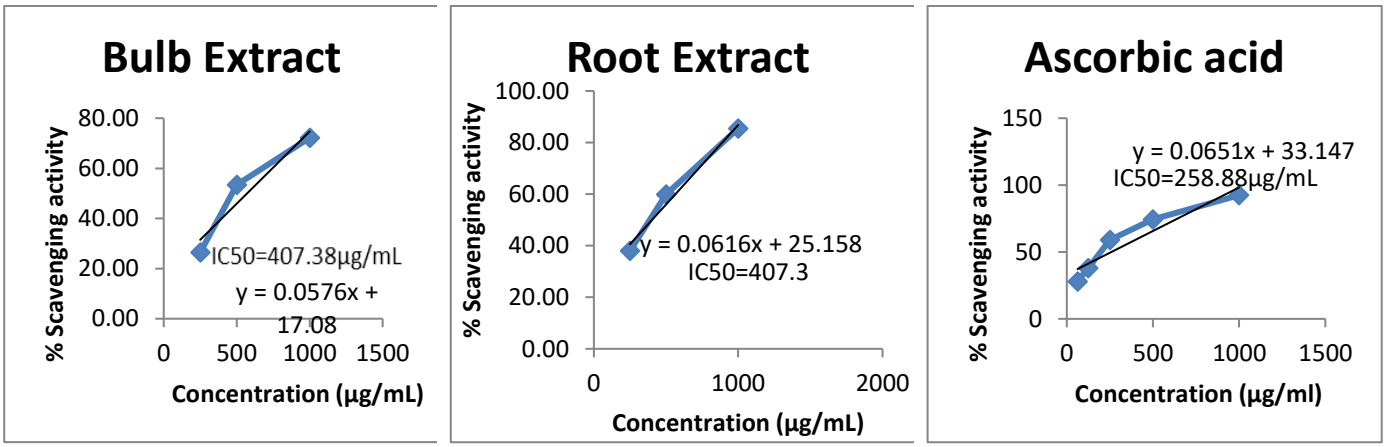

Figure 2: Graphical Presentation of DPPH Radical Scavenging Activity

4.2.2 ABTS radical scavenging method

Percentage inhibition was determined by comparing the extract's ability to scavenge ABTS to that of ascorbic acid and the IC_{50} value of both parts (bulb 8.43 µg/ml, root 47.65 µg/ml) could be comparable with that of the standard, which is ascorbic acid (306.92 µg/ml) as shown in Table 3 and Figure 3.

Table :3 ABTS radical scavenging activity report

Sample	Concentration	% Radical Scavenging Activity				IC50
		R1	R2	R3	Mean	
Bulb	250	61.32	59.20	58.11	59.54	8.43
	500	84.41	79.31	76.44	80.05	
	1000	100	100	100	100	
Root	250	58.65	57.75	55.3	57.15	47.65
	500	77.69	79.48	78.58	78.58	
	1000	97.11	97.5	98.61	97.74	
Ascorbic Acid (Standard)	250	58.65	57.75	55.3	57.15	306.92
	500	77.69	79.48	78.58	78.58	
	1000	97.11	97.5	98.61	97.74	

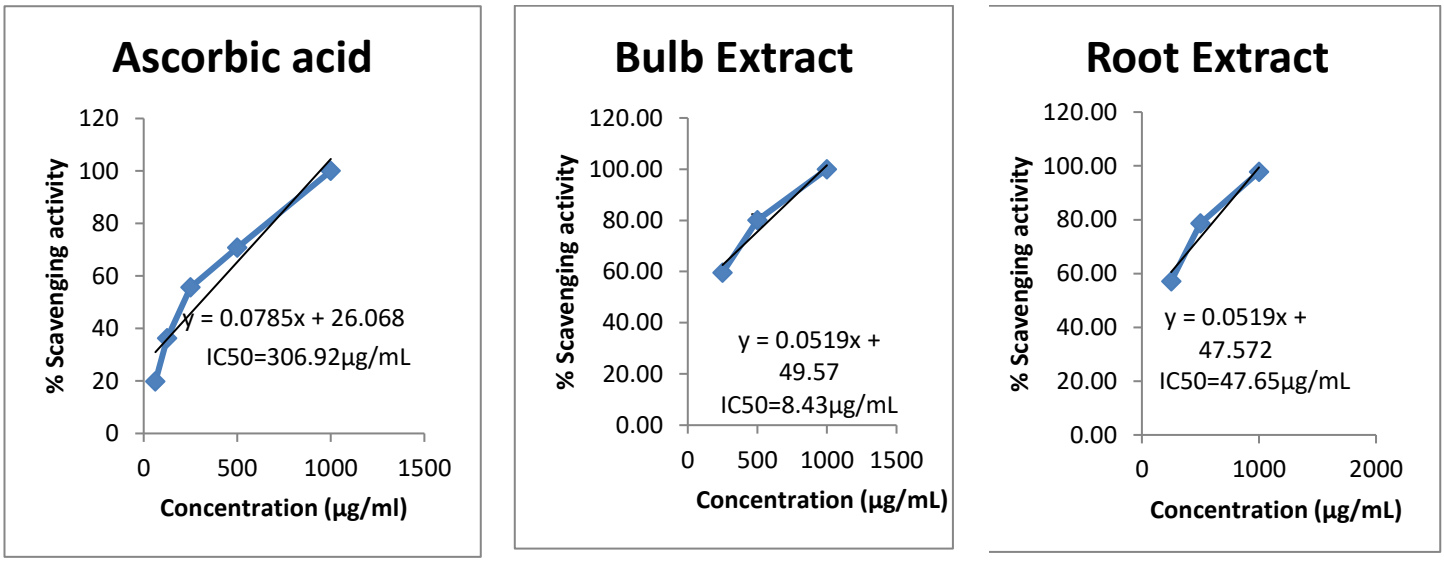

Figure 3: Graphical Presentation of ABTS Radical Scavenging Activity

4.2.3 Hydrogen Peroxide (H_2O_2) Radical Scavenging Assay

H_2O_2 does not seem to be highly reactive on its own, but it can be harmful to cells when it produces hydroxyl radicals. The results of the % inhibition tests showed that root extract (IC_{50} value of 195.77 µg/ml) demonstrated the highest level of antioxidant activity compared to that of the bulb (IC_{50} value of 520.29 µg/ml). This method also used ascorbic acid as standard, showing the IC_{50} value of 6.17 µg/ml, as shown in Table 3 and Figure 4.

Table 4: H_2O_2 radical scavenging activity

Sample	Concentration	%Radical Scavenging Activity				IC50
		R1	R2	R3	Mean	
Bulb	250	26.36	25.36	24.69	25.47	520.29
	500	56.51	57.62	57.84	57.32	
	1000	81.98	80.98	80.31	81.09	
Root	250	49.95	48.72	50.05	49.57	195.77
	500	71.3	70.86	72.3	71.49	
	1000	90.98	90.1	91.88	90.99	
Ascorbic Acid (Standard)	250	61.3	61.41	62.19	61.63	6.17
	500	73.05	73.39	72.94	73.13	
	1000	98.3	97.41	95.19	96.97	

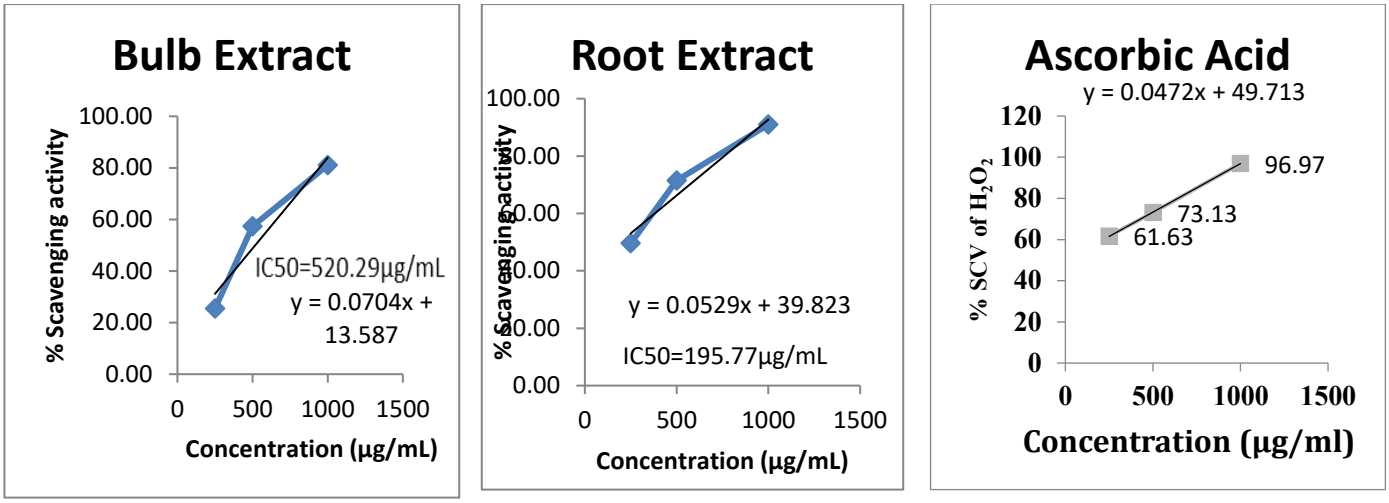

Figure 4: Graphical Presentation of H_2O_2 Radical Scavenging Activity

4.3 Antidiabetic activity

4.3.1 In vitro α-amylase inhibitory assay by DNSA method

The IC_{50} values were derived by plotting the percent α-amylase inhibition against extract concentrations. The IC_{50} for the common positive control, acarbose, was 57.56 µg/ml. Both the bulb and root extracts of *C. suaveolens* demonstrated moderate antidiabetic activity in this investigation, with IC_{50} values of 611.42 and 669.75 µg/ml, respectively, in contrast to the standard (Acarbose) IC_{50} value of 57.56 µg/ml in the Alpha-Amylase Inhibitory Assay using DNSA method, as shown in table 5 and figure 4 represents correlation coefficient (R^2) value of Bulb (0.9984), Root (0.9927) and Standard (0.7871).

Table 5: Antidiabetic activity by Alpha- Amylase Inhibitory Assay by DNSA method

Sample	Concentration	% Inhibition			IC_{50} (µg/ml)
		R1	R2	Mean	
Bulb	250	20	17.42	18.71	611.42
	500	44.28	40	42.14	
	1000	82.85	81.71	82.28	
Root	250	30.57	34	32.285	669.75
	500	40.57	49.14	44.855	
	1000	64	62	63	
Acarbose	1	15.4	13.37	14.385	57.56
	10	42.73	42.15	42.44	
	100	67.44	66.57	67.005	

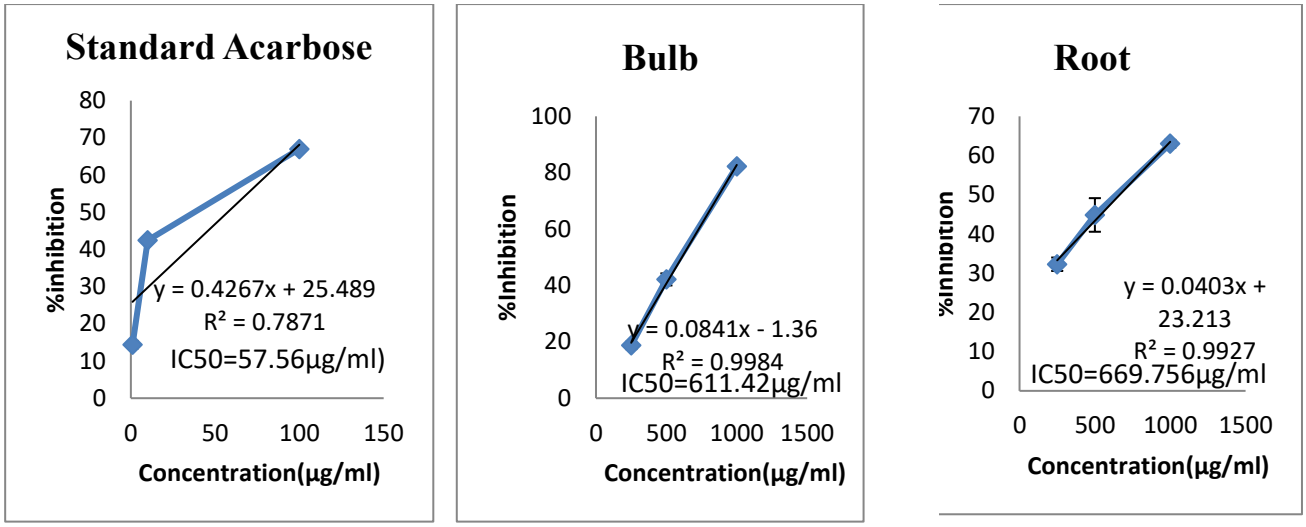

Figure 5: Graphical Presentation of Alpha Amylase Inhibitory Assay by DNSA

4.3.2 Antidiabetic activity by Alpha-Amylase Inhibitory Assay by SI method

The IC_{50} values were determined by graphing the percentage of α-amylase inhibition against the concentrations of the extract by starch iodine method. In this study, both the bulb and root extracts of *C. suaveolens* exhibited moderate antidiabetic activity, with IC_{50} values of 611.42 µg/ml and 669.75 µg/ml, respectively, and 54.36 µg/ml. These values contrast with the standard IC_{50} value of 54.36 µg/ml for acarbose, as shown in Table 6 and Figure 5 displays the correlation coefficient (R^2) values for the Bulb (0.9984), Root (0.9927), and Standard (0.7871).

Table 6: Antidiabetic activity by Alpha- Amylase Inhibitory Assay by SI method

Sample	Concentration	% Inhibition			IC_{50} (µg/ml)
		R1	R2	Mean	
Bulb	250	11.42	22.85	17.135	578.33
	500	45.71	57.14	51.425	
	1000	85.71	80	82.855	
Root	250	11.71	8.85	10.28	736.86
	500	41.42	47.71	44.565	
	1000	66.29	70.28	68.285	
Acarbose	1	13.76	10.12	7.155921	54.36
	10	45.47	41.42	29.28836	
	100	72.06	68.42	48.38025	

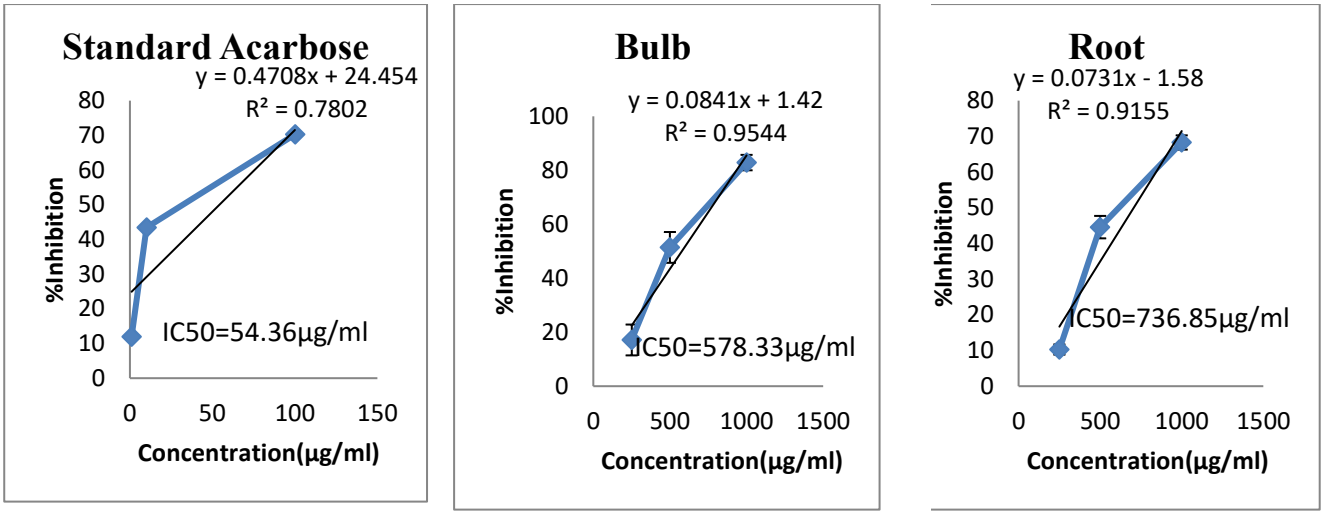

Figure 6: Graphical Presentation of Alpha-Amylase Inhibitory Assay by SI

4.4 Evaluation of cytotoxic activity by brine shrimp lethal bioassay

The accompanying Table 7 shows that the acetonic extract of *C. suaveolens* was cytotoxic, with LC_{50} values of 176.478 and 148.889 µg/ml for the root and bulb, respectively, in comparison to the reference IC_{50} value of 2.351 µg/ml, as shown in Table 7. Vincristine sulfate, used as a positive control, was found to have significant cytotoxic effects.

Table 7: Cytotoxic Activity Test by Brine Shrimp Lethal Bioassay

Sample	Conc. (µg/ml)	Log C	%Mortality	Regression Equation	R2	LC_{50} (µg/ml)
Vincristine Sulphate (Standard)	2.5	0.398	40	Y = 38.053X – 35.869	0.9717	2.351
	5	0.699	70			
	10	1	90			
	30	1.477	100			
	50	1.699	100			
Bulb	125	2.097	40	Y = 67.186X – 95.986	0.961	148.889
	250	2.398	70			
	500	2.699	90			
	800	2.903	100			
	1000	3	100			
Root	125	2.097	40	Y = 69.747X – 106.7	0.99	176.478
	250	2.398	60			
	500	2.699	80			
	800	2.903	100			
	1000	3	100			

4.5 Thrombolytic activity by in-vitro clot-lysis method

The thrombolytic activity of *C. suaveolens* was investigated by following the *in-vitro* clot-lysis method. The findings are summarized in Table 8 and Figure 6. The results are not significant compared to standard streptokinase.

Table 8: Thrombolytic activity by in-vitro clot-lysis method

Treatment (mg/ml)	% of Clot lysis
Control	10.98 ± 0.93
Streptokinase (SK)	72.86 ± 1.57
Bulb 5	16.99 ± 1.97
Bulb 10	15.41 ± 1.46
Bulb 20	12.18 ± 1.77
Root 5	17.93 ± 1.46
Root 10	15.22 ± 1.98
Root 20	10.10 ± 1.59

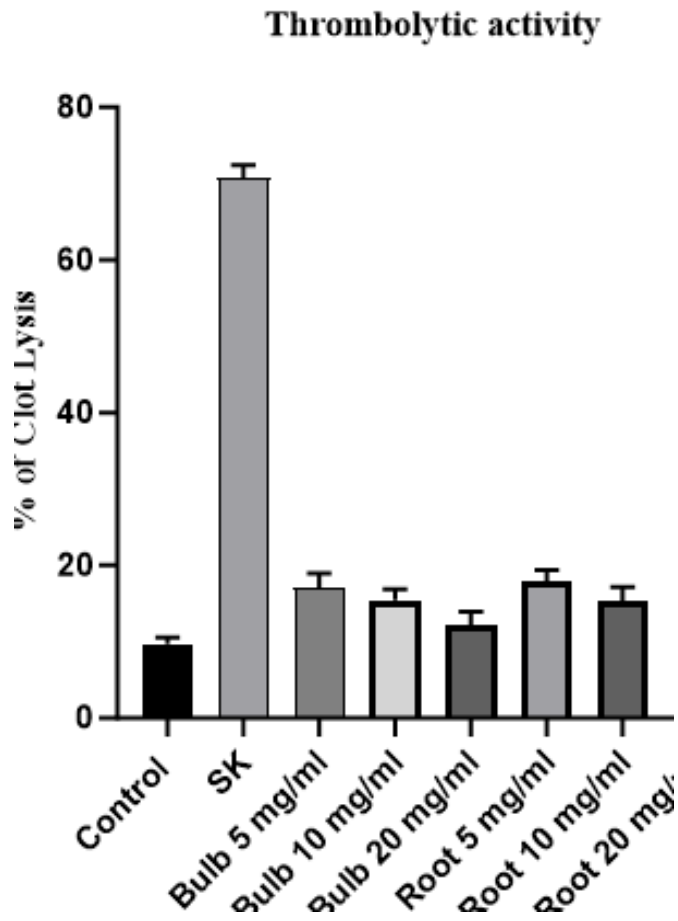

Figure 7: Thrombolytic activity by in-vitro clot-lysis

4.6 Anthelmintic Activity

The anthelmintic activity of *C. suaveolens* was investigated against *Tubifex tubifex* worms, the findings of which are summarized in Table 9 and Figure 7. In this investigation, at the 5, 8, 10 mg/mL concentrations the extract manifested significant paralysis time in Bulb 19.83 ± 0.27, 14.98 ± 0.32, 9.58 ± 0.21 min; and death time 32.8 ± 0.28, 23.5 ± 0.76, 16.6 ± 0.33 min,

and in Root paralysis time 6.16 ± 0.88, 4.27 ± 0.28, 3.04 ± 0.03 and death time 18.3 ± 0.88, 14.16 ± 0.44, 12.01 ± 0.01, respectively. Whereas the standard drug albendazole showed paralysis and death time 3.82 ± 0.189 and 6.35 ± 0.15 min, respectively, at 50 mg/kg. The result indicated that the effect of anthelmintic was directly proportional to the concentrations of crude extract. On the other hand, root extract showed a more significant effect than bulb extract.

Table 9: Anthelmintic effect of Coelogyne suaveolens (Bulb and Root Extract)

Treatment (mg/ml)	Paralysis Time (Min)	Death Time (Min)
Control	---	---
Levamisole 1	3.17 ± 0.18	6.50 ± 0.38
Bulb 5	19.83 ± 0.27	32.50 ± 0.28
Bulb 8	14.98 ± 0.32	23.50 ± 0.76
Bulb 10	9.58 ± 0.21	16.66 ± 0.33
Root 5	6.16 ± 0.08	18.16 ± 0.08
Root 8	4.70 ± 0.28	14.16 ± 0.04
Root 10	3.04 ± 0.03	12.01`± 0.01

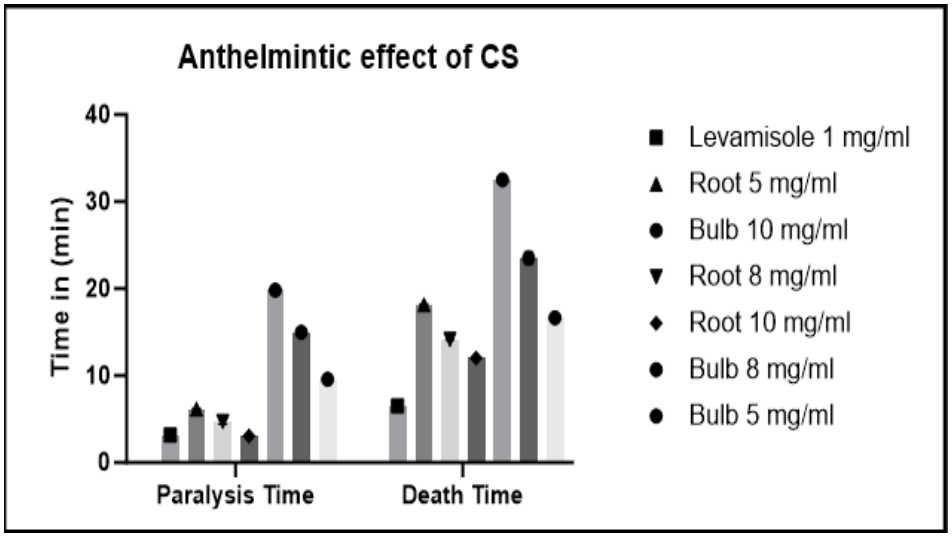

Figure 8: Anthelmintic effect of *Coelogyne suaveolens* (Bulb and Root Extract)

4.7 Antidiarrheal activity

4.7.1 Castor oil-induced mice model

Antidiarrheal activity assay was performed in castor oil-induced mice model, and the results are summarized in Table 10. We performed the test to find out antidiarrheal activity, but the results were more significant in producing diarrhea.

Table 10: Antidiarrheal activity by Castor oil-induced mice model

Treatment (mg/kg)	Number of feces	Inhibition of defecation (%)	Number of diarrheal feces	Inhibition of diarrheal (%)
Negative Control (0.1 mL/mice)	14.33 ± 0.33	---	6 ± 0.57	---
Loperamide (5)	5.33 ± 0.33	62.80	2.66 ± 0.88	55.66
CSB 200	8 ± 0.57	44.17	3.33 ± 0.33	44.5
CSB 400	13 ± 0.57	9.28	4.33 ± 0.33	27.83
CSR 200	8.66 ± 0.88	39.56	3.66 ± 0.33	39
CSR 400	12.66 ± 0.88	11.65	4.66 ± 0.33	22.33

4.7.2 GIT Motility by Castor oil induced mice model (charcoal marker)

A gastrointestinal motility test was performed for more information to screen diarrheal activity using the castor oil-induced mice model. A charcoal marker was used to determine the following parameters tabulated in Table 11.

Table 11: Castor oil-induced GIT motility in mice model (charcoal marker)

Treatment (mg/kg)	Total Length of Intestine (cm)	Distance Travel by Charcoal (cm)	Peristalsis Index (%)	Inhibition (%)
Control	50.33 ± 0.33	42.33 ± 1.20	84.10 ± 2.31	---
Loperamide (5)	52.33 ± 0.33	22.33 ± 0.33	42.66 ± 0.36	47.24
CSB 200	59.66 ± 0.33	28 ± 0.57	46.91 ± 0.75	33.33
CSB 400	61 ± 0.57	36 ± 1.15	59 ± 1.69	14.28
CSR 200	59 ± 0.57	31.66 ± 0.88	73.48 ± 2.20	25.2
CSR 400	60.33 ± 0.33	37 ± 1.15	80.66 ± 1.45	11.9

4.8 Antidepressant activity

4.8.1 Force swim test

Forced swim test model used for preliminary screening test for characterizing potential antidepressant drugs. In these models, *C. suaveolens* bulb and root at doses of 200 mg/kg and 400 mg/kg, showed a decrease in the motor activity of mice, which demote depressed mood by increasing the immobility time of mice. The parameters observed in this model are the immobility time of mice. Results can be found in Table 12 and Figure 9.

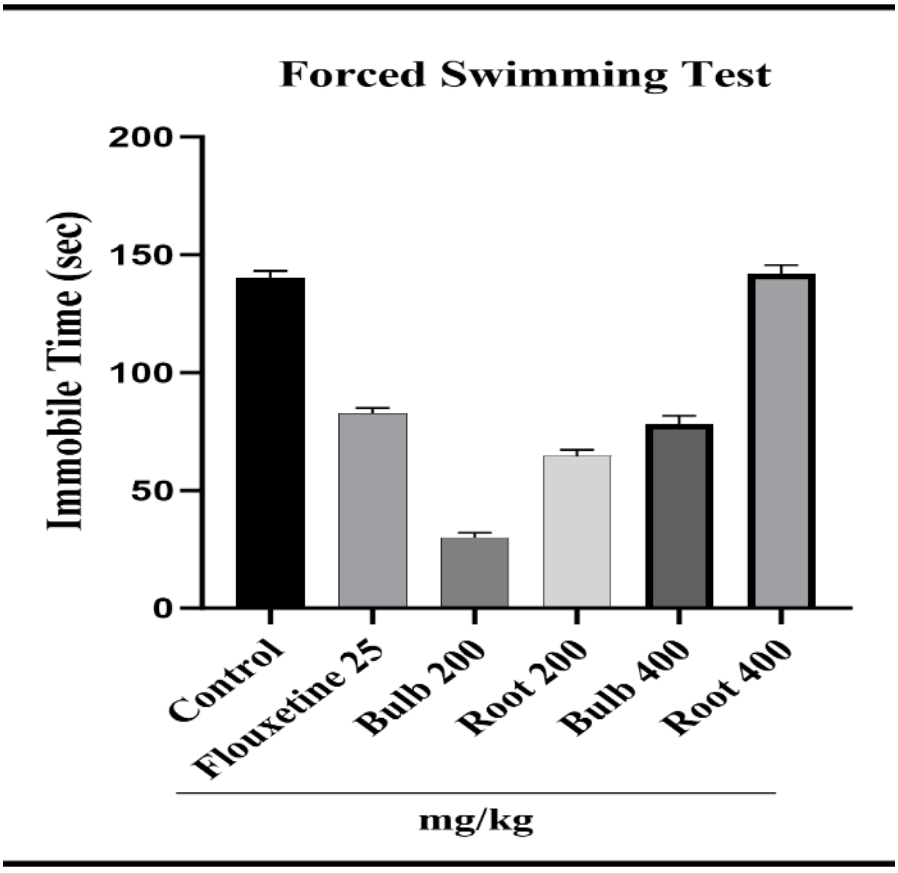

Figure 9: Antidepressant activity

Table 12: Antidepressant activity CS Root and Bulb

Group	Immobility Time
Control	140.2 ± 1.67
Flouxetine (25 mg/kg)	82.6 ± 1.37
CSB 200 (mg/kg)	30 ± 1.15
CSB 400 (mg/kg)	78 ± 2.08
CSR 200 (mg/kg)	64.66 ± 1.45
CSR 400 (mg/kg)	142 ± 2.08

4.8.2 Tail suspension method

The tail suspension test is the most used preliminary screening test for characterizing potential antidepressant drugs. In these models, CS Bulb and Root, at doses of 200 mg/kg, and 400 mg/kg, showed a decrease in the motor activity of mice, which demoted depressed mood by increasing the immobility time of mice. The parameters observed in this model are the immobility time of mice. Results are found in Table 13 and Figure 10.

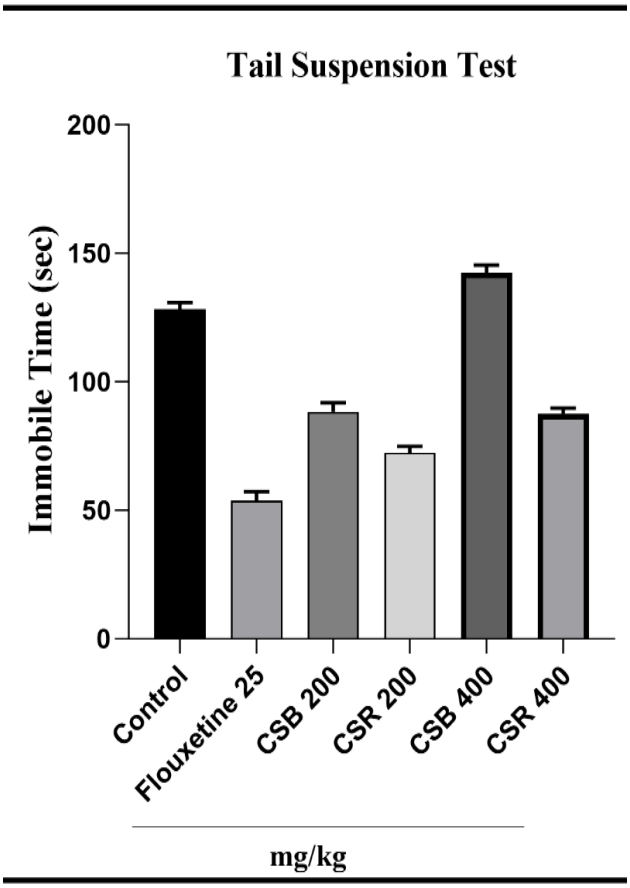

Figure 10: Antidepressant Screening by Tail Suspension method

Table 13: Antidepressant screening by Tail Suspension method

Group	Immobility Time
Control	128.2 ± 1.57
Flouxetine (25 mg/kg)	53.6 ± 2.09
CSB 200 (mg/kg)	88.33 ± 2.02
CSB 400 (mg/kg)	142.33 ± 1.76
CSR 200 (mg/kg)	72.33 ± 1.45
CSR 400 (mg/kg)	87.66 ± 1.20

5. Discussion

Coelogyne suaveolens roots and bulbs acetone extract and ethyl acetate fraction were tested in this ethnopharmacological experiment for preliminary screening of phytochemicals, micro and macro-nutrients content, antioxidant, anti-diabetic properties, cytotoxic activity, thrombolytic, anthelmintic, antidiarrheal and antidepressant activity. The early phytochemical investigation revealed that the ethyl acetate fraction of acetonic extract of *C. suaveolens* roots and bulbs includes a variety of phytochemical substances [18]. It's possible that these substances have the potential to perform a function in a wide range of biological and medicinal procedures. Therefore, this species is expected to be useful in numerous medicinal contexts. The ethyl acetate fraction of the orchid's acetonic extract was subjected to numerous pharmacological tests to evaluate its analgesic, anxiolytic, and sedative effects on Swiss albino mice and potentials supported by in-silico study.

Evaluation of micro and macro-nutritional content of *C. suaveolens* bulbs and roots reveals that both extracts contain sodium, potassium, and calcium as the macro minerals, and magnesium, copper, and zinc were among the microminerals found. Nevertheless, the mineral concentrations in the roots and the leaves are not the same, as shown in Table 1. The nutritional analysis of *C. suaveolens* bulbs and roots reveals their potential as a valuable food supplement due to their rich mineral content. Both parts contain essential minerals such as calcium, magnesium, phosphorus, sodium, potassium, and copper. However, the root appears to be particularly rich in beneficial minerals compared to the bulb. The root contains higher levels of sodium and phosphorus than the bulb, while the bulb has significantly higher calcium and slightly more potassium. The prospects for *C. suaveolens* as a food supplement include potential use as a micro and macro-nutritional supplement, further exploration of health benefits, and continued research on mineral content variations on animal models.

The *in-vitro* study that was done recently on this plant revealed that the crude extracts expose powerful antioxidant characteristics, as well as ordinary anti-diabetic and cytotoxic effects. Several approaches have been taken in the quest for antioxidants in various kinds of medicinal plants, and many different sorts of research have been carried out. The antioxidant activity against free radicals and other reactive oxygen species is increased by the abundance of phenolic compounds, which are the most prevalent secondary metabolites. As a defense mechanism against the radicals produced by their highly oxidative surroundings, plants have evolved an antioxidant system based on the synthesis of phenolic substances like flavonoids, tannins, and other secondary metabolites [26]. Several concentrations of the *C. suaveolens* extracts were tested for their antioxidant capacity to see how well they neutralize free radicals. This quality arises from secondary metabolites' ability to counteract the damaging effects of free radicals and oxygen reactive species in the body [26]. This activity may be related to the existence of flavonoids and phenols, which are known to be effective antioxidants, as shown by the phytochemical analysis in the present study. This activity may be related to the presence of flavonoids and phenols, which are known to be effective antioxidants, as shown by the phytochemical analysis in the present study [27]. To identify the scavenging effect of this plant extract, ABTS, DPPH, and H_2O_2 radical scavenging assay were used in this study. Low IC_{50} values indicate high-activity antioxidants [28] and the acetonic extract of this plant showed encouraging antioxidant capacity (which can vary from 8.43 to 520.29 µg /mL for the bulb extract and from 47.65 to 195.77 µg /mL for the root extract). This suggests that the extract contains ingredients with high antioxidant activity. Antioxidant properties of herbal extracts have been shown to play a crucial part in preventing the formation of calcium oxalate monohydrate papillary calculi, so this extract will be even

more evaluated for its antiurolithiatic perspective in upcoming studies [29]. It is important to verify these *in vitro* findings *in vivo* before moving further with the development of a robust antioxidant derived from this plant, as this property of the extract will be useful in warding off oxidative stress and the myriad ailments it may cause. The extract could then be fractionated more, and its constituent parts examined to determine the precise active component responsible for this result.

It is hypothesized that free radicals produced during glucose oxidation and the subsequent oxidative destruction of glycated proteins contribute to developing diabetes problems. Because of this, it is often advised that diabetics take anti-diabetic medicines alongside antioxidants. The extract's antioxidant properties allowed us to test its ability to combat diabetes. The % alpha-amylase Inhibitory Assay using DNSA and SI technique was used to determine the anti-diabetic action. These substances, referred to as α-amylase inhibitors or starch blockers, function by hindering the breakdown of starch and other oligosaccharides. This hindrance occurs in the hydrolysis process of the 1,4-glycosidic bonds, preventing the formation of maltose, maltriose, and other basic sugars, hence reducing or preventing starch absorption into the body [30]. Compared to the reference (Acarbose, a frequently prescribed and commercialized anti-diabetic medicine), the crude extract of bulb and root in the current investigation showed moderate activity. The reported anti-diabetic action may be due to the presence of alkaloids, carbohydrates, flavonoids, tannins, or terpenes in this plant of *C. suaveolens*, either as single ingredients or in combination [31].

Based on the results of the current study, vincristine sulfate, a well-known anticancer drug, was shown to have the lowest LC_{50} value, demonstrating its highly effective cytotoxic potential. In this research, it was found that the ethyl acetate fraction of the acetonic extract of *C. suaveolens* exhibited significant cytotoxic activity compared with that of the standard. Thus, they might be examined further for their potential as anticancer drugs. In the investigated extracts, flavonoids, glycosides, saponins, and alkaloids were found during the initial phytochemical assessment, which may have contributed to their cytotoxic actions [32]. The cytotoxic effects of flavonoids arise from their ability to stimulate cellular ROS generation [33]. To put it another way, saponins trigger cancer cell death [34]. Previous research revealed that alkaloids can suppress the growth of various cell mutations that cause cancer [32].

The thrombolytic potential of *C. suaveolens* was examined using an *in-vitro* clot-lysis technique. The findings did not show any significant difference when compared to the standard streptokinase.

The efficacy of *C. suaveolens* as an anthelmintic was examined in relation to *Tubifex tubifex* worms. The findings of this study are presented in Table 9 and Figure 8. During the investigation, the extract demonstrated noteworthy paralysis and death time in the bulb and root when compared to the standard drug levamisole. The results suggest that the anthelmintic effect was directly influenced by the concentrations of the crude extract. Furthermore, the root extract exhibited a more pronounced effect compared to the bulb extract. Further research is required to prove the mechanism of action, and we hope that this plant may be a potential anthelmintic drug candidate in future.

Castor oil-induced mice model is a common model employed to screen antidiarrheal activity. This model was also employed to assess the antidiarrheal activity of *C. suaveolens* bulb and root extract. Two techniques using a charcoal marker, castor oil-induced diarrhea, and GI

motility tests. Both results are not significant as antidiarrheal activity but more significant as diarrheal activity. That means *C. suaveolens* bulb and root extract exhibit diarrheal action. We think that this extract may produce laxative action like castor oil. Further exploration is required to prove the mechanism of action as laxative use, and we hope that this plant may be a potential laxative drug candidate in future.

The two most popular initial screening tests for evaluating possible antidepressant medications are the tail suspension test and the forced swim test. In these models, *C. suaveolens* bulb and root at varying doses demonstrated a reduction in mice's motor activity, which worsened their mood by prolonging their periods of immobility. The parameters that are observed in this model are the mice's immobility times. Medication that prolongs immobility causes mice's motor activity to decline, which in turn causes depression to develop prematurely since the mice in these studies swim and have their tails suspended. The results of this investigation are not significant.

6. Conclusion:

In summary, this research investigated the potential presence of macro and micro-nutrients like calcium, magnesium, phosphorus, sodium, potassium, copper, and zinc in the extract of *C. suaveolens*. These minerals are essential to many bodily functions and can positively affect health. Our laboratory results demonstrated that *C. suaveolens* has potent antioxidant and moderate antidiabetic, and significant cytotoxic properties. *In-vitro* thrombolytic activity was not significant compared to standard streptokinase. Anthelmintic activity of both extracts was found to be significant, and the root is more significant than the bulb. Both extracts have no antidiarrheal effect, but they may produce diarrheal or laxative effects. No significant antidepressant activity is produced by either extract. Based on these findings, the plant warrants further investigation as a possible natural product source for the creation of novel antioxidant, anticancer, antidiabetic, and laxative medicines as well as potential food supplements. Still, more research is needed to elucidate the mechanism of action and determine the bioactive chemicals responsible for the observed activity. Clinical trials on humans are also required to assess the safety and efficacy of *C. suaveolens.* Taken together, our results show that *C. suaveolens* is worth exploring further as a natural origin of critical minerals for public health and a possible source of novel medicines with anticancer, antidiabetic, and antioxidant characteristics.

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Ethical approval:

The study protocol was authorized in accordance with government directives under Pharm/P&D/CUDP-16,2023:10 by the departmental ethical review committee, University of Chittagong, Chittagong, Bangladesh.

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