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# Phytochemical Assessment and Pharmacological Evaluation of *Curcuma zedoaria* (Christm.) Roscoe Methanolic Extract – Preliminary Study

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***Curcuma zedoaria* (Christm.) Roscoe, a traditional herb used to treat helminthiasis, cardiovascular issues, and cancer, lacks scientific evidence to support its therapeutic value. This study intended to explore the scientific rationale for its traditional usage, by phytochemical assessment and pharmacological evaluation of its methanolic extract (ME). The phytochemical analysis of ME was performed using conventional approaches, for qualitative detection. In vitro antioxidant capacity was then measured using DPPH free radical scavenging assay, and the percentage of inhibition (IC50) of both standard ascorbic acid (AA) and ME was calculated. The anthelmintic activity was investigated at doses 50, 100, and 150 mg/ml by comparing the effects of ME and albendazole on the paralysis and death of earthworms. Furthermore, cyclophosphamide (CP) induced cardiotoxic rats were used to evaluate the in vivo cardioprotective ability of ME at doses of 150 and 300 mg/kg body weight. Subsequently, serum cardiac biomarkers including aspartate aminotransferase, creatine kinase-MB, lactate dehydrogenase, as well as lipid profiles (triglycerides, and total cholesterols) were assessed. The phytochemicals assessment demonstrated the diversity of compounds' presence. The antioxidant assay demonstrated a significant ($p<0.01$) dose-response relationship of ME (IC50 33.132 $\mu$g/ml) compared to AA (IC50 20.276 $\mu$g/ml). Besides, the anthelmintic study thereafter revealed a significant ($p<0.01$) dose-dependent paralysis (2.824 ± 0.037 minutes) and mortality (3.732 ± 0.031 min.) of worms at 50 mg/ml dose compared to the standard control. Furthermore, the cardioprotective indicators and lipid profiles which CP elevated, have significantly ($p<0.001$) returned to nearly normal levels in serum due to the ME treatment at both 150 and 300 mg/kg doses. In addition, rats' CP intoxicated heart weight (0.904 ± 0.019 gm) was significantly ($p<0.01$) reduced (0.860 ± 0.016 gm) by ME at 150 mg/kg dose. Therefore, C. zedoaria rhizome possesses powerful antioxidant, anthelmintic, and cardioprotective efficacy. The outcomes paved the way for more exploration to develop novel therapies from this herb.**

**Keywords:** Anthelmintic; cardioprotective; Curcuma zedoaria; methanolic extract; phytochemicals.

The significance of plants as reservoirs of medicinal substances has been widely recognized throughout history, especially in the context of traditional medicine in various civilizations. Usually, the bioactive components synthesized during the secondary metabolism of the plants are considered the source of their therapeutic potential.[1,2] According to the World Health

Organisation (WHO), a substantial number of people in countries like Bangladesh, Burma, and India rely on medicinal plants to preserve their health and treat a variety of disorders.[3] Additionally, it has become more crucial due to the side effects of various modern medications and the absence of effective remedies for several emerging disorders.[4] However, using phytomedicines to treat numerous complications has been demonstrated to be very safe and have minimal to no adverse effects.[5]

Nowadays, numerous life-threatening ailments are plaguing people worldwide. Cardiovascular diseases are not only the world's predominant cause of mortality, but they also significantly deteriorate health and drive up the expense of medical care.[6] A large proportion of these deaths take place in developing countries like Bangladesh, whose populations are severely affected.[7] The underlying risk factors encompass hypertension, dyslipidemia, diabetes, obesity, sedentary behavior, advanced age, and smoking.[8] Besides, owing to the noteworthy rate of chemotherapy resistance, cancer is still the second foremost reason for deaths around the world.[9] Fourteen million new cases of cancer were reported in 2012; by 2035, that number is predicted to rise to nearly 24 million new cases worldwide which directs a rapid growth in the cancer burden.[10] Additionally, one of the most prevalent health issues worldwide, especially in underdeveloped states, is infection with intestinal parasites. It still poses a serious threat to public health, mostly in developing countries, and wreaks havoc on vulnerable rural populations' socioeconomic conditions.[11] Therefore, the demand for more advanced therapies and/or technologies to combat these hurdles is continuously growing. Moreover, exploring medicinal plants is becoming more popular as well since using synthetic drugs improperly can lead to resistance and other complications.

*Curcuma zedoaria* (Christm.) Roscoe rhizome or white turmeric, a perennial herb of the Zingiberaceae family, has a long-standing traditional usage in multiple regions such as India, Bangladesh, Indonesia, China, Vietnam, Malaysia, and Japan.[12] Globally, there are around 133 species in the genus Curcuma, and herbs of this genus are mostly inhabited in Australia, Brazil, and Southeastern Asia.[13,14] Asians have traditionally utilized this rhizome as a carminative and to cure arthritis, digestive disorders, liver diseases, anorexia, helminthiasis, gastritis, stroke, cardiovascular complications, and even cancer.[15-17] Contrary to some other species in this genus that have received continual focus, *C. zedoaria* was attracting more attention because of its traditional use and some newly revealed biological properties. Still, there is a dearth of research regarding the antioxidant, anthelmintic, and cardioprotective activities. Although the traditional uses are well recorded, scientific data supporting its medicinal abilities remains scarce. To bridge this knowledge gap, this study embarks on an ethnopharmacological journey, exploring the diverse pharmacological properties of this easily accessible herb. The primary objective was preserving and documenting vital cultural knowledge before it disappeared into obscurity. Additionally, we intended to evaluate the antioxidant, anthelmintic, and cardioprotective potential of *C. zedoaria* rhizome along with phytochemical assessment to provide scientific evidence of the herb's traditional applications and support future research for novel therapies.

## MATERIALS AND METHODS

### Collection and preparation of plant materials

Having collected from Bangladesh's Tangail District, the rhizome of *C. zedoaria* was verified and recognized by the National Herbarium, Dhaka, Bangladesh (Accession number: DACB87211). We collected 6 kg of *C. zedoaria* rhizome and removed undesirable components. They were then carefully cleaned with double-distilled water, chopped into tiny pieces, and let to shed dry for a week. The dried rhizomes were further crushed into fine particles using a suitable grinder.

### Preparation of methanolic extract

Crushed material (rhizome) weighing 500 g was soaked with 1500 ml of methanol (95%) in a flat-bottomed fresh glass vessel, firmly sealed, and left for 21 days with infrequent shaking and stirring. The entire mixture was filtered through a sterilized piece of white cotton material and then run through Whatman (Grade 2) filter paper.[18] A sticky black concentrate obtained was designated as methanolic

extract (ME) after the resulting filtrate was allowed to evaporate in normal conditions. Then the yield value (%) was calculated by Equation 1.

$$\text{Yield (g/100 g)} = (W_1 \times 100)/W_2 \quad ...(1)$$

Notes: $W_1$=Weight of the extract residue (ME) obtained after solvent elimination, $W_2$=Weight of powder taken for extract preparation.

In the study, the % of yield = (11×100)/500
= 2.2%

**Phytochemical assessment**

Using conventional approaches, the primary phytochemical assessment was carried out for the qualitative detection of alkaloids, flavonoids, polyphenols, tannins, saponins, carbohydrates, proteins, amino acids, terpenoids, and phytosterols. An analytical response to these qualitative investigations was the color intensity or precipitate formation.[19]

**Antioxidant activity assay**

Through slightly modified protocols, the extract's *in vitro* antioxidant capacity was assessed using the DPPH free radical scavenging test.[20,21] 0.004% DPPH solution was utilized as the control, and the concentration levels of ME and standard ascorbic acid (AA) were 500, 400, 200, 100, 50, 25, 12.5, and 6.25 ìg/ml. Two ml of 0.004% DPPH solution was added with two ml of ME and AA respectively followed by proper mixing and allowed to complete the reaction by incubating for 30 minutes. Afterward, the percentage of inhibition ($IC_{50}$) of ME and the standard was calculated by measuring the solution's absorbance at 517 nm in a UV-Vis Spectrophotometer. Concentration was determined by Equation 2, which was then plotted against the percentage of inhibition to estimate the $IC_{50}$.

$$\text{Concentration} = \{(A_0-A_1)/A_0\} \times 100 \quad ...(2)$$

Notes: $A_0$ = Absorbance of the control, $A_1$ = Absorbance of the ME/AA

**Anthelmintic activity assay**

With minor adjustments, the reported procedure was used to conduct the *in vitro* anthelmintic assay.[22] This study employed adult earthworms (*Pheretima posthuma*) owing to their physiological and anatomical resemblance to the human abdominal roundworm parasite. They were split up into nine groups, each with five worms, and the first three groups (I, II, and III) were used as controls and given normal saline water. Furthermore, Albendazole (standard) and ME were administered to Groups (IV, V, VI) and Groups (VI, VIII, IX) at doses of 50, 100, and 150 mg/ml, respectively. The parasites were frequently observed for their spontaneous movement and evoked responses, immediately after incubation, which lasted for 130 minutes in total. Worm's mortality or paralysis was detected by the inhibitions of their movement. Paralysis was defined as the worms' inability to move after being violently shocked; whereas death time was established by their failure to move after being vigorously shaken or submerged in warm water (50°C). In this assay, the earthworm parasites' paralysis and death were visually observed with the naked eye.

**Animal selection**

This study involved male Long Evans rats (age 9 weeks), weighing 160-200 gm, obtained from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB). Before the experiment, they were adopted for seven days under standard conditions (25±2°C) and humidity with a regular 12-hour light/dark cycle and allowed free access to normal food and water.[23]

**Cardioprotective activity test**

We evaluated ME's *in vivo* cardioprotective ability in cyclophosphamide (CP) induced acute cardiotoxic rats by slightly altering published protocols.[24,25] However, this preliminary *in vivo* study aimed to compare biomarker levels of different animal groups with their normal physiological conditions without using a conventional standard. For this study, earlier approval was taken from the institutional ethical review committee that oversees animal experimentation (Ref: MBSTU/ERC/ECC/157/2024/9). In all, 20 male Long Evans rats were divided into four distinct groups of 5 animals each, and every group received the usual diet along with the following treatment orally for thirteen consecutive days. The first group, which served as the normal control, received oral saline one ml/kg body weight (BW) whereas the second group received CP 150 mg/kg BW intraperitoneally during trial days $9^{th}$ through $12^{th}$. Furthermore, animals in the third and fourth groups received ME

orally for eight days at doses of 150 (D1), and 300 (D2) mg/kg BW respectively. Afterward, CP (150 mg/kg BW) was administered intraperitoneally to both groups on the 9th to 12th day of the experiment. The animals were sacrificed on day 13th after a 24-hour period. Following their dissection, blood was drawn from the heart's vein to measure the serum levels of creatine kinase-MB (CK-MB), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), troponin I, and lipid profiles such as total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), & Triglycerides (TGs). Furthermore, their heart weights were also recorded.

**Statistical analysis**

One way analysis of variance (ANOVA) and Dunnett's t-tests using SPSS software were employed for statistical analysis. Values are expressed as mean ± standard error of the mean (SEM). With Microsoft Excel, the logarithmic equation of the graph was utilized to determine the median inhibitory concentration ($IC_{50}$) of the test samples.

## RESULTS AND DISCUSSION

**Phytochemical assessment**

The preliminary phytochemical analysis revealed various constituents present in the ME of *C. zedoaria* rhizome (Table 1). Among these, multiple tests have identified the presence of alkaloids, carbohydrates, and flavonoids.

**Antioxidant activity assay**

The antioxidant capacity of the ME has been expressed as a median inhibitory concentration ($IC_{50}$) which represents the dose of sample needed to inhibit 50% of the DPPH free radical after a certain exposure time. The extract's potential as an antioxidant increases with the percentage of its scavenging action. As compared to reference standard AA ($IC_{50}$ 20.276 ìg/ml), the ME ($IC_{50}$ 33.132 ìg/ml) exhibited a significant ($p<0.01$) antioxidant capability in this investigation (Table 2, Table 3).

**Anthelmintic activity assay**

In this experiment, the earthworms died with regular saline after more than 130 minutes, and the test was conducted up to that point. The death time of worms by ME at doses 50, 100, and 150 mg/ml were 3.732 ± 0.031, 2.950 ± 0.107, 2.041 ± 0.064 minutes respectively. Furthermore, the death times were 3.562 ± 0.040, 2.803 ± 0.137, and 2.027 ± 0.083 minutes, respectively, at the same doses of the conventional drug Albendazole (standard). The anthelmintic activity of each group that received ME was compared to the standard. Regarding the paralysis and death times, the ME showed (Fig. 1) an impressive dose-dependent activity.

**Cardioprotective activity test**

This experiment used biomarkers to correlate outcomes from three distinct groups of animals in their normal, diseased, and test treatment conditions without using a standard drug. Since we attempted to see how the values varied with ME therapy. Therefore, we assessed the degree of protection provided by ME against CP-induced cardiotoxic rats at 150, and 300 mg/kg BW doses using the aforementioned markers (CK-MB, AST, LDH, Troponin I), lipid profile, and heart weight. CP administration resulted in a decline in body weight and an rise in heart weight, which was a measure of relative heart weight elevation, however, these issues have been resolved by the ME therapy (Table 4). Additionally, ME treatment caused a dose-dependent reduction ($p<0.001$) in serum biomarkers (CK-MB, AST, LDH, & Troponin I) and lipid indicators (TC, TGs, & LDL-C) that had been significantly ($p<0.001$) elevated during CP intoxication (Fig. 2, Fig. 3).

Medicinal floras are presumed to be a promising supplier of novel compounds. Published literature has mentioned that the Curcuma herbs are found to possess a diverse range of primary and secondary metabolites. Accordingly, our phytochemical analysis has also confirmed the existence of alkaloids, carbohydrates, flavonoids, tannins, phytosterols, polyphenols, saponins, terpenoids, proteins, and amino acids in *C. zedoaria* ME. Meanwhile, a key sign of medicinal plants' ability to have beneficial effects is their flavonoid content. The antioxidant activity assay of this study showed that at a dose of 500 ìg/ml, ME possesses the strongest capacity to scavenge free radicals and has a 90.102% inhibition, compared to 96.075% for standard AA at the same dose (Table 2, Table 3). The outcome therefore indicates the significant ($p<0.01$) dose-dependent antioxidant properties of ME (Table 3), and the components, especially flavonoids and polyphenols, found in the ME may be accountable for the scavenging

action. Similarly, published study revealed that ME of *C. zedoaria* had an intense scavenging effect that was significantly stronger in comparison to other solvent extracts.[26] The hydrogen atoms from several hydroxyl groups in polyphenols' structures may interact with the free radical DPPH resulting in an antioxidant response.[27] Further exploration is necessary since it is unclear which particular compounds have the mode of action as antioxidants.

The current investigation revealed that ME has substantial dose-dependent anthelmintic activity since the paralysis and death times of the parasites are mostly near to the reference drug Albendazole (Fig. 1). Reported studies claimed the significant anthelmintic activities of other Curcuma species notably *C. longa*, *C. amada*, *C. caesia,* and *C.* aromatic.[28-30] But as far as we are aware, this is the first *in vitro* study that confirms the anthelmintic activity of *C. zedoaria* crude ME against earthworms (*Pheretima posthuma*). Acetylcholinesterase (AChE), a cholinergic enzyme predominantly found at postsynaptic neuromuscular junctions, has the principal physiological role is halting transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine. Desensitization of the muscle receptor caused by the blocking AChE leads to paralysis and eventually death.[31,32] Cholinergic antagonists, such as monoterpenes and sesquiterpenes, impede the contraction of worm muscles and can induce the aforementioned effects.[33,34] Moreover, the effect of polyphenols as AChE inhibitors was documented in some

**Table 1.** Phytochemical constituents of *C. zedoaria* ME

| Phytochemicals | Name of the Test | ME |
|---|---|---|
| Alkaloids | a) Mayer | + |
| | b) Wagner | + |
| | c) Dragendorff | + |
| Carbohydrates | a) Molisch | + |
| | b) Benedict | + |
| | c) Fehling | + |
| Saponins | a) Foam | + |
| Terpenoids | a) Salkowski | + |
| Phytosterols | a) Liebermann-Burchard | + |
| Polyphenols | a) Ferric Chloride | + |
| Tannins | a) Ferric Chloride | + |
| Flavonoids | a) Alkaline Reagent | + |
| | b) Lead Acetate | + |
| Proteins and Amino Acids | a) Xanthoproteic | + |

Notes: (+) = Present and (-) = Absent of Phytochemicals

**Table 2.** DPPH free radical scavenging ability ($IC_{50}$ value) of AA

| Concentration (µg/ml) | Absorbance of AA (Mean± SEM) | Absorbance of Blank | % of Inhibition | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|
| 500 | 0.023± 0.00231 | 0.586 | 96.075 | 20.276 |
| 400 | 0.042± 0.00233 | 0.586 | 92.833 | |
| 200 | 0.113 ± 0.00260 | 0.586 | 80.717 | |
| 100 | 0.164 ± 0.00173 | 0.586 | 72.014 | |
| 50 | 0.227 ± 0.00231 | 0.586 | 61.263 | |
| 25 | 0.277 ± 0.00231 | 0.586 | 52.730 | |
| 12.5 | 0.338± 0.00410 | 0.586 | 42.321 | |
| 6.25 | 0.378± 0.00353 | 0.586 | 35.495 | |

research.[35] The early phytochemical assessment of *C. zedoaria* revealed the existence of alkaloids, polyphenols, flavonoids, and terpenoids in the ME, which can be suspected to have anthelmintic effects. More studies therefore needed to clarify which specific component affects nematodes in this way.

In this study, we attempted to ascertain the degree of protection provided by ME against CP-intoxicated rat hearts and lipid profiles at different dosages. CP is a cardiotoxic agent that can destroy myocardial cells, consequently, cardiac tissue damage can diagnosed by the release of CK-MB, AST, LDH, and Troponin I into the circulation.[36] As expected, the findings (Table 4) also displayed that CP administration significantly ($p<0.001$) raised heart weight, lipid profiles, and serum cardiac biomarkers (CK-MB, AST, LDH, and Troponin

**Table 3.** DPPH free radical scavenging ability ($IC_{50}$ value) of ME

| Concentration (µg/ml) | Absorbance of ME (Mean± SEM) | Absorbance of Blank | % of Inhibition | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|
| 500 | $0.058 \pm 0.00231^{a***}$ | 0.586 | 90.102 | 33.132 |
| 400 | $0.072 \pm 0.00233^{a***}$ | 0.586 | 87.713 | |
| 200 | $0.123 \pm 0.00233^{a*}$ | 0.586 | 79.010 | |
| 100 | $0.179 \pm 0.00260^{a**}$ | 0.586 | 69.454 | |
| 50 | $0.233 \pm 0.00260^{ns}$ | 0.586 | 60.239 | |
| 25 | $0.303 \pm 0.00393^{a**}$ | 0.586 | 48.294 | |
| 12.5 | $0.377 \pm 0.00296^{a***}$ | 0.586 | 35.666 | |
| 6.25 | $0.480 \pm 0.00463^{a***}$ | 0.586 | 18.089 | |

Notes: [a]Compared the absorbance of ME with AA; [ns]=Not significant; Values are expressed as Mean ± SEM (n=3). Three thresholds of P values are used. *p<0.05, **p<0.01, ***p<0.001 significant when compared with the corresponding value of the standard group.

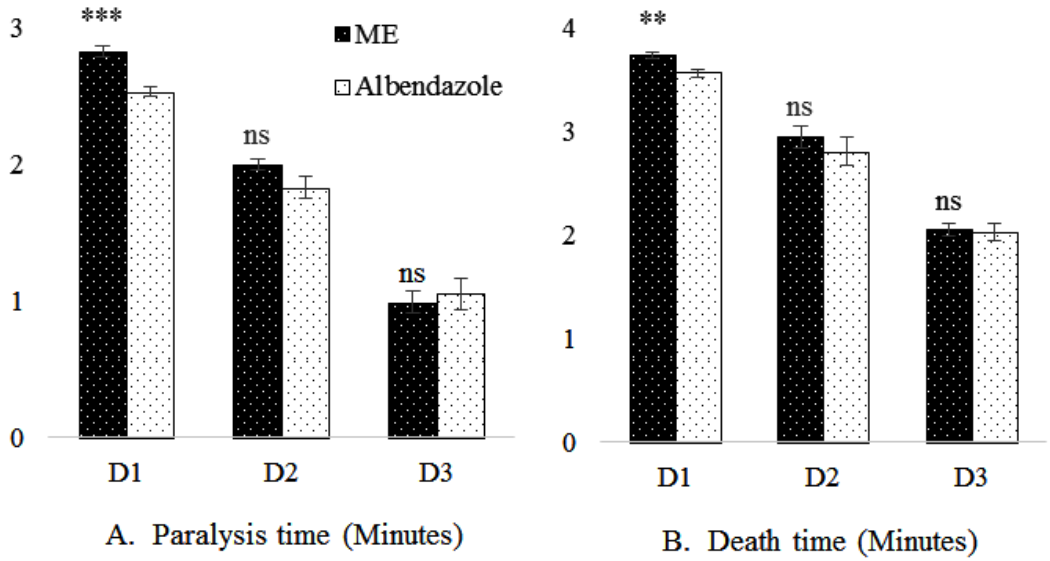


**Fig. 1.** Comparative anthelmintic activity (A. Paralysis time; B. Death time) of ME & albendazole. All values are expressed as Mean ± SEM (n=5); ns=Not significant; D=Dose; D1=50, D2=100, D3=150 mg/ml. Three thresholds of P values are used. *p<0.05, **p<0.01, ***p<0.001 significant when compared with the corresponding value of the standard group

I) (Fig. 2, Fig. 3). Myofibrillar degeneration, sarcoplasmic reticulum enlargement, myocyte disruption, cytoplasmic vacuolization, and fibrosis are possible effects of CP-induced cardiotoxicity which may be responsible for this increase in heart weight.[37] However, in this study the ME-pretreated rats had significantly ($p<0.01$) lower relative heart weight compared to the positive control (Table 4) in a dose-dependent fashion. The elevated serum cardiac biomarker enzyme levels in CP-treated rats are possibly linked to the overproduction of reactive oxygen species (ROS) which damages the myocardial membranes through lipid peroxidation thus losing integrity and functionality.[38] As stated by reported assay, acrolein, CP's toxic metabolite, is also accountable for this complication.[39] Furthermore, the higher plasma levels of TC, LDL-C, and TGs in the CP-administered group might be explained by the drug-induced boost in their biosynthesis, and suppression of usage. Especially the enzyme lipoprotein lipase (LPL), which transforms TGs into fatty acids, may have been inhibited by CP, therefore increasing blood TGs levels.[40] Because of the ME therapy, the blood levels of cardiac indicators and lipid profiles that CP raised have dropped significantly ($p<0.001$) in a dose-dependent manner, restoring them to almost normal levels (Fig. 2, Fig. 3). Compared to our findings, reported study on hydroethanolic extract of *C. zedoaria* has less anti-hyperlipidemic activity.[41] A similar experiment has found that the crude ethanolic extract (EE) at doses of 200, 400, and 800 mg/kg BW reduced serum CK-MB and Troponin T levels in a dose-dependent way.[42] However, ME's CK-MB enzyme-lowering ability in the current study is superior to the EE. Furthermore, the cardioprotective role of curcumin derivatives which are commonly found in the

**Table 4.** Effect of ME on the body, heart, and relative heart weight

| Groups | Initial body weight (gm) | Final body weight (gm) | Heart weight (gm) | Relative heart weight (%) |
|---|---|---|---|---|
| Control | 174.260 ± 4.201 | 175.768 ± 3.208 | 0.638 ± 0.035 | 0.393 ± 0.023 |
| CP Treated | 174.732 ± 1.710 | 159.950 ± 2.874$^{a*}$ | 0.904 ± 0.019$^{a***}$ | 0.509 ± 0.004$^{a***}$ |
| CP+ME (D1) | 179.362 ± 1.406 | 180.474 ± 2.500$^{b**\ a\ ns}$ | 0.860 ± 0.016$^{a***\ b\ ns}$ | 0.477 ± 0.011$^{a**\ b\ ns}$ |
| CP+ME (D2) | 178.716 ± 2.55 | 178.244 ± 4.333$^{b**}$ | 0.800 ± 0.026$^{a**b\ ns}$ | 0.419 ± 0.009$^{a\ ns\ b**}$ |

Notes: [a]Compared CP treated group & CP+ME pre-treated group with the normal control group; [b]Compared CP treated group with CP+ME pre-treated group; ns=Not significant; Values are expressed as Mean ± SEM (n=3). Three thresholds of P values are used. *p<0.05, **p<0.01, ***p<0.001 significant when compared with the corresponding value of the standard group.

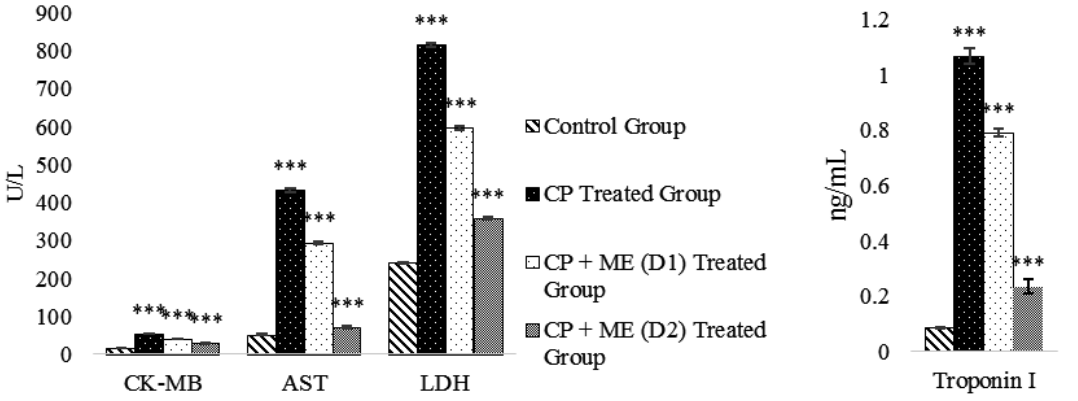


**Fig. 2.** Effect of ME on serum cardiac marker enzymes (CK-MB, AST, LDH, and Troponin I, Level). All values are expressed as Mean ± SEM (n=5); D=Dose; D1=150, D2=300 mg/kg. Three thresholds of P values are used. *p<0.05, **p<0.01, ***p<0.001 significant when compared with the corresponding value of the standard group

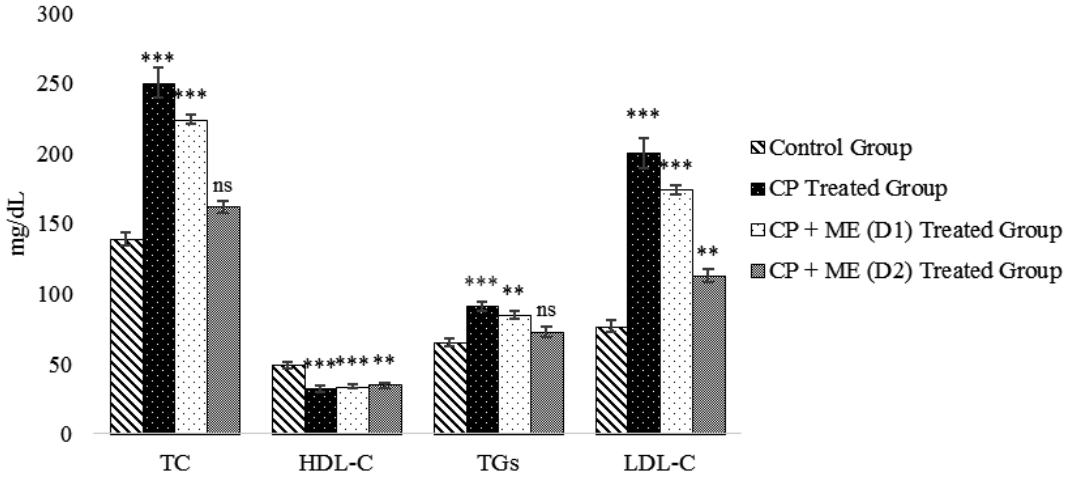


**Fig. 3.** Effect of ME on lipid profile (TC, HDL-C, TGs, and LDL-C). All values are expressed as Mean ± SEM (n=5); ns=Not significant; D=Dose; D1=150, D2=300 mg/kg. Three thresholds of P values are used. *p<0.05, **p<0.01, ***p<0.001 significant when compared with the corresponding value of the standard group. [a]Compared CP treated group & CP+ME pre-treated group with the normal control group; [b]Compared CP treated group with CP+ME pre-treated group

Curcuma herbs has been mentioned by reported assay.[43]

These suggested that *C. zedoaria* has conserved the structural integrity of the myocardial membranes, while concurrently hindering the cardiac indicators from excessive production and discharge in the bloodstream. Besides, it is plausible that this rhizome has lipid-lowering abilities as well because of the polyphenolic compounds it contains, which can bind to bile acids to facilitate their excretion, restrict the formation of hepatic cholesterol, and induce the LPL enzyme.[44,45] Furthermore, ME's antioxidant potential may have contributed to its cardioprotective ability since free radicals are intrinsically linked with oxidative stress in cardiac damage. However, to assess myocardial damage and identify the precise phytoconstituents that provide *C. zedoaria* ME's cardioprotective action, cardiac tissue histopathology and compound isolation are needed.

Although we didn't utilize a standard drug in this preliminary *in vivo* investigation, and as the promising activity of ME has been obtained, our further continuation of this research will employ it. Besides, due to insufficient funding, the separation of specific bioactive molecules and histopathologic analysis were not performed. Nonetheless, our future research will focus on uncovering lead compounds from this plant extract through *in silico* correlation with sophisticated phytochemical screening and pharmacological assessment.

## CONCLUSION

This study was contrived to explore the biological activities of *C. zedoaria* ME. Our initial assessment for phytochemicals ensured a diversity of components. Subsequent *in vitro* and *in vivo* pharmacological investigations exhibited remarkable dose-dependent antioxidant, anthelmintic, and cardioprotective abilities. Since the extract was enormously effective in various treatments in this study, it might be a potential source of novel drugs to treat life-threatening illnesses, especially cancer and cardiac disorders. Therefore, more sophisticated research including compound isolation and histopathology of cardiac tissue is necessitated before coming to a definite conclusion on the findings of the present study.

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**Conflict of Interest**

The author(s) do not have any conflict of interest.

**Data availability statement**

The manuscript incorporates all datasets produced or examined throughout this research study. The derived data supporting the findings of this study are also available within the article as supplementary materials.

**Ethical approval statement**

This research involved animal experimentation, and therefore, prior approval was taken from the ethical review committee (ERC) of Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh (Ref: MBSTU/ERC/ECC/157/2024/9).

**Informed consent statement**

This study did not involve human participants, and therefore, informed consent was not required.

**Clinical Trial Registration**

This research does not involve any clinical trials

**Authors' contribution**

Fairuz Fatema Priya: Investigation, Data Collection, Writing–original draft; Abu Zobayed: Data analysis, Writing–review and editing; Md. Abu Sayeed: Conceptualization, Methodology; Md. Mizanur Rahman Moghal: Resources, Funding acquisition, Project administration, Supervision.

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