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Evaluation of Pharmacological Properties of *Curcuma zedoaria* Rhizome via Ethnopharmacological Approaches

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ABSTRACT

Phytochemicals or plant secondary metabolites secure plant cells in stressful circumstances and offer human well-being and hence are used in traditional medicine. *Curcuma zedoaria* (Christm.) Roscoe of the family Zingiberaceae has numerous traditional uses, however its pharmacological effectiveness has not yet been fully scrutinized. This study intended to explore the pharmacological properties of the plant using the crude methanolic extract (ME) of its rhizome. *In vitro* and *in vivo* assessments were performed to ascertain the hepatoprotective, antimicrobial, and cytotoxic effects. A promising dose-dependent hepatoprotective effect was observed in paracetamol (2 g/kg body weight) induced hepatotoxic rats, pretreated with the ME (150 & 300 mg/kg body weight). There was a significant decline ($p<0.001$) in the level of the serum biomarkers of hepatic necrosis (Alanine Aminotransferase, Alkaline Phosphatase, Aspartate Aminotransferase, and Total Bilirubin). Antibacterial potency of the ME was evaluated against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Staphylococcus aureus* employing the disc diffusion method, where a dose of 800 µg demonstrated notable effects ($p<0.01$) compared to the reference standards, Kanamycin and Norfloxacin. Finally, a mild dose-dependent response was observed compared to the reference standard vincristine sulfate in *in vitro* brine shrimp lethality bioassay, a test taken as an indicator of cytotoxicity. To conclude, the findings of this study opened an avenue for more extensive research to find new phytochemicals with hepatoprotective, antibacterial, and anticancer potential.

1. Introduction

Every disease that may affect humans has a remedy in the natural resources existing on this earth. Plants have historically been considered one of the major search areas for potential therapies for different diseases (Sofowora *et al.*, 2013). Nowadays, people are returning back from modern medicine to botanical therapies for a number of reasons including drug resistance, adverse effects, and lack of appropriate therapies for certain emerging illnesses like various types of cancers, and infections (Nasim *et al.*, 2022). Bangladesh is a habitat of more than 500 medicinal plant species, of which around 250 are used in traditional medicine (Rahman *et al.*, 2022). Lots of novel medications for life-threatening ailments may be discovered through extensive research on these plants. However, the bulk of these plants has not yet been the subject of pharmacological, toxicological, or chemical studies to identify their bioactive components.

Globally, the second predominant cause of death is cancer, particularly due to chemotherapeutic resistance (Bray *et al.*, 2018; Jin *et al.*, 2022). Exploration of the cytotoxic or anticancer properties of various plant species is getting progressively more important because some anticancer agents have already been derived from plant sources (Reddy *et al.*, 2009; Nirmala & Durai, 2019). On the other hand, infectious diseases associated with bacteria are causing a serious global threat to public health because of the emergence of resistance that has lately been observed to even the best available antibiotics (Kumarasamy, *et al.*, 2010). Consequently, a growing number of diseases, such as salmonellosis, tuberculosis, gonorrhea, and pneumonia, are getting more complicated to treat and this in turn is raising mortality, healthcare expenses, & hospital stays. In 2019, a total of 33 selected bacterial strains were responsible for 7.7 million deaths worldwide, among them 55% were caused by five microbes namely *Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Brüssow, 2024). Scientists are actively working across the world on natural resources to find new antibiotics to combat these

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pathogens (Newman & Cragg, 2020; Miethke *et al.*, 2021). Liver disease is one of the more devastating illnesses that affects people. Hepatitis, liver cancer, and liver cirrhosis cause more than two million deaths a year or 4% of all fatalities globally (Devarbhavi *et al.*, 2023). There are few medications for these disorders, and most of them have serious adverse effects (Fried, 2002). However, a variety of herbals have shown promising effects in the treatment of liver diseases especially cirrhosis (Farzaei *et al.*, 2018; Kyung *et al.*, 2018).

Curcuma zedoaria (Christm.) Roscoe, also known as white turmeric (local name Sotthi), belonging to the Zingiberaceae family, has both medicinal and culinary uses, and phytochemical studies have identified several bioactive components in the plant (Rahmatullah *et al.*, 2009; Karim *et al.*, 2011). Southeastern Asia, Brazil, and Australia are the primary habitats for plants of the genus Curcuma (Chen *et al.*, 2011). The rhizome of *C. zedoaria* has long been used by Asians as a carminative and to treat food poisoning, cancer, acute stomach pain, loss of appetite, inflammation, vomiting, cough, bacterial infections, menstrual disorders, and more (Bantawa & Rai, 2009; Dhal *et al.*, 2011). Dysentery, diarrhea, hepatocirrhosis, and other liver diseases have also traditionally been treated by *C. zedoaria* in several nations (Kanase & Khan, 2018; Subositi & Wahyono, 2019). Although *C. zedoaria* rhizome has been used traditionally in Bangladesh since ancient times, not much has been revealed about its pharmacological properties. According to recent studies, its ethanolic extract (EE) demonstrated a protective role in drug/chemical-induced hepatotoxic rats through the reduction of elevated serum hepatic biomarker enzyme levels (Sari *et al.*, 2022; Sumarheni *et al.*, 2023). However, its effect on serum bilirubin has not been clarified. The previous phytochemical screening studies on the plant's methanolic extract (ME) have recognized it as a rich source of antioxidants like flavonoids, tannins, and saponins which may contribute to the plant's capacity to shield hepatocytes (Sumathi *et al.*, 2013; Setyani *et al.*, 2020). Still, there is a lack of conclusive research on ME's hepatoprotective benefits. Though published antimicrobial activity tests on EE showed its efficacy against some common pathogenic microbes (Chachad *et al.*, 2016; Islam *et al.*, 2017), the potency of ME has not been thoroughly assessed. Moreover, the dose-response relationship of these biological activities hasn't been clearly demonstrated. So, comprehensive research is warranted on its therapeutic effects to address this gap. In this backdrop, this study intended to explore the antibacterial, cytotoxic, and hepatoprotective abilities of this rhizome. The work is also expected to provide scientific evidence of the herb's traditional applications and potentially uncover new therapeutic options for these diseases.

2. Materials and Methods

2.1. Collection of Plant Material & Reagents

C. zedoaria rhizomes were collected from Tangail, Bangladesh, and subsequently the identity was verified by Bangladesh National Herbarium, Mirpur, Dhaka (Accession number: DACB87211). All the reagents used in the study were of analytical grade and purchased from the USA and Europe by authentic vendors. All the solutions were made on the same day of the experiment.

2.2. Preparation of Plant Extract

A clean, flat-bottomed glass container was filled with 500 g of powdered material and soaked in 1500 ml Methanol (95%). After sealing, the container was stored for 21 days with occasional shaking and stirring. Later, the entire mixture was coarsely filtered through a piece of clean, white cotton material and then Whatman filter paper (Grade 2) was used to filter it further (Rashid *et al.*, 2016). The filtrate obtained was evaporated under normal environmental conditions and formed a black sticky concentrate, designated as a crude ME. Then the yield value (% yield) was calculated by Equation 1.

$$Yield\ (g/100\ g) = (W1 \times 100)/W2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Notes: W1=Weight of the extract residue (ME) obtained after solvent removal, W2=Weight of powder taken for extract preparation

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

So, the yield value of *C. zedoaria* ME was 2.2%.

2.3. Collection of Study Animals, Test Microorganisms, and Brine Shrimp Eggs

Male *Long evans* rats (145 to 175 gm weight) were obtained from the animal research facility of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB). After acclimatization, they were divided into various experimental groups. For the animal study, prior approval was taken from the institution's Ethical Review Committee that oversees and controls animal experimentation. Pure cultures of one gram-positive bacterium (*S. aureus*) and three gram-negative bacteria (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) were collected from the laboratory of the Biotechnology & Genetic Engineering Department of Mawlana Bhashani Science and Technology University (MBSTU). Brine shrimps (*Artemia salina* Leach) were utilized for cytotoxic activity tests because they are easy to screen and fractionate when exploring novel bioactive natural compounds (Lieberman, 1999). Freeze-dried brine shrimp eggs were collected from aquarium stores because they lasted for several years and could be hatched without special equipment.

2.4. Hepatoprotective Activity Assay

Cirrhosis or liver disease is marked by elevated blood levels of bilirubin along with hepatic enzymes like alanine aminotransferase (ALT/SGPT), aspartate aminotransferase (AST/SGOT), and alkaline phosphatase (ALP) (Shivaraj *et al.*, 2009). This study aimed to assess *C. zedoaria*'s hepatoprotective effect against paracetamol (PCM) induced liver damage in rats. After splitting into four groups with 5 rats in each, a total of 20 rats were given the following treatments orally. For 7 days, all groups were

fed a regular diet. In addition, Group I (control) received oral doses of 1% Na-CMC, 1 ml/kg and normal saline 1 ml/kg body weight (BW). Group II received PCM at a dose of 2 g/kg B W orally on the 5th day. Group III and Group IV were administered 150 and 300 mg/kg of the ME of the plant, respectively over a period of 7 days. In addition, both groups were also given oral PCM at a dose of 2 g/kg BW on the 5th day. Rats were sacrificed 24 hours after the last dose, blood was drawn from a vein, let to clot at room temperature for 1 hour, and serum was extracted by centrifugation for 15 minutes at 30°C and 2500 rpm. Finally, the serum was collected and subjected to analysis for biochemical parameters (Takate *et al*., 2010; Parmar *et al*., 2010). Rats were weighed before and after each treatment's commencement to measure their initial and final body weights. Furthermore, the liver weight was measured immediately after the rats' sacrifice and dissection. The values were then recorded and subjected to calculate the relative liver weight by following Equation 2.

Relative liver weight=WL/WB x100 …….....……(2)

Notes: WL=Weight of liver, WB=Final (total) body weight

2.5. Evaluation of Antibacterial Activity

Antibacterial potencies of the plant extract were measured at the doses of 800, 600, 400, and 200 µg against *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* using Agar disc diffusion method (Senthamilselvi *et al*., 2012). Using DMSO as a negative control (blank discs), the test discs were impregnated with *C. zedoaria* ME. Kanamycin (30 µg) & Norfloxacin (10 µg) were the reference standards. After 24-hour incubation at 37°C, the zones of inhibition were measured.

2.6. Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay is considered as a very useful bench-top method for cytotoxicity study because of its commercial availability of shrimp eggs, low cost, and ease of performing the experiment (Meyer *et al*., 1982). For screening cytotoxic compounds from plant extracts, this method serves as a guidance where the mortality rate is observed as one of the simplest biological responses (Mohtasheem *et al*., 2001). Meyer's technique was adopted to conduct the cytotoxic test on brine shrimp nauplii with slight modification (Meyer *et al*., 1982; Otang *et al*., 2013). Nauplii or mature shrimps, were grown by hatching brine shrimp eggs in simulated seawater (3.8% NaCl solution) at 37°C for 48 hours. Utilizing DMSO as the solvent, the test sample (ME) solutions were administered at doses 800, 400, 200, 100, 50, 25, 12.5, and 6.25 µg/ml, as well as the positive control (vincristine sulfate) solutions were administered at doses 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 and 0.078125 µg/ml. The test sample and positive control solutions were added at 1 ml to their respective pre-marked glass vials containing 10 living brine shrimp nauplii in 5 ml simulated seawater. Then three pre-marked vials containing 5 ml of simulated seawater, 10 shrimp nauplii, and 100 µl of DMSO were used as negative control groups. After 24-hour incubation period at 37°C, the number of survivors (nauplii) was counted under a magnifying glass, and Equation 3 was used to determine the percent (%) mortality (Rumzhum *et al*., 2008).

Percent (%) mortality=(Nt-Na)/Nt x100…….....….. (3)

Notes: Nt = Number of nauplii taken, Na = Number of nauplii alive.

The percent (%) mortality was calculated for each dilution. Afterward, linearity graphs were drawn by plotting the percentages of mortality observed with different doses or concentrations of ME and vincristine sulfate (reference standard) against their corresponding concentrations. Finally, the median lethal concentration (LC_{50}) of all test samples was calculated from the logarithmic equation of the graph.

2.7. Statistical Analysis

One Way ANOVA and the Dunnett t-test were employed for the statistical analysis and values were expressed as the Mean ± SEM. The median lethal concentrations (LC_{50}) of the test samples were determined, as an indication of the plant extract's toxicity, from the logarithmic equation of the graph using Microsoft Excel.

3. Results

3.1. Hepatoprotective Activity Assay

The findings from this study demonstrate that, at an intoxication dose of 2 g/kg, PCM increased the rats' body weight and, also their liver weight (Table 1). Additionally, the serum levels of the hepatic enzymes (ALT, ALP & AST) and total bilirubin (TB) were also substantially raised (Figure 1). However, the ME of *C. zedoaria* was capable of reducing the elevation in the liver weight, serum hepatic enzyme levels, and total bilirubin in a dose-dependent manner.

Table 1. Effect of *C. zedoaria* ME on Rats' Body and Liver Weight

Groups	Initial Body Weight (gm) (Mean ± SEM)	Final Body Weight (gm) (Mean ± SEM)	Liver Weight (gm) (Mean ± SEM)	Relative Liver Weight (Mean ± SEM)
I (Control)	166.308 ± 2.005	168.476 ± 1.497	4.078 ± 0.078	2.420 ± 0.037
II (PCM 2 g/kg)	165.386 ± 2.985	180.568 ± 2.082^{a**}	6.324 ± 0.289^{a***}	3.496 ± 0.135^{a***}
III (PCM+ ME 150 mg/kg)	156.052 ± 3.108	161.936 ± 2.378^{b***}	5.884 ± 0.178^{a***}	3.634 ± 0.098^{a***}
IV (PCM+ ME 300 mg/kg)	167.908 ± 1.600	171.612 ± 1.886^{b**}	4.544 ± 0.182^{b***}	2.646 ± 0.085^{b***}

*Note: PCM= Paracetamol, ME= Methanolic Extract, SEM=Standard Error of Mean. Three threshold 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, done by independent sample test; [a] Compared PCM & PCM + ME Pre-treated group with normal control group; [b] Compared PCM treated group with PCM + ME pre-treated group n=5.*

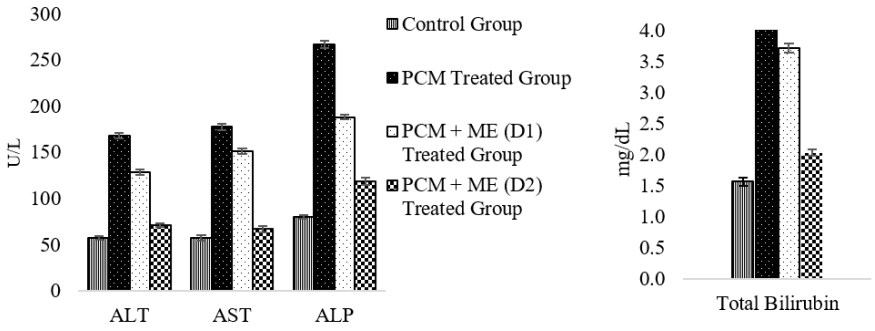

Figure 1. Effect of *C. zedoaria* ME on the Hepatic Enzymes and Total Bilirubin
Note: D1= 150 mg/kg, D2= 300 mg/kg. Values are expressed as Mean ± SEM, n=5.

3.2. Evaluation of Antibacterial Activity

The zones of inhibition (ZOI) are shown in Table 2 along with the disc diameter (6 mm). To determine the level of significance, the ZOI values of the crude ME were compared to those of standard Kanamycin and Norfloxacin. Promising antibacterial activity was found at the maximum dose of 800 µg. A graded dose-response relationship was observed against all of our selected microorganisms (Table 2).

Table 2. Antibacterial Activity of *C. zedoaria* ME

Test Organisms	Dose (µg) of ME	Zone of Inhibition Including Disc Diameter (6 mm)			
		Methanolic Extract	Kanamycin (30 µg)	Norfloxacin (10 µg)	DMSO
Escherichia coli	200	6.620 ± 0.112 a***b***	14.75 ± 0.479	24.50 ± 0.957	6.0
	400	8.420 ± 0.137 a*** b***			
	600	12.105 ± 0.113 a*b***			
	800	19.250± 0.629a**b**			
Pseudomonas aeruginosa	200	12.220 ± 0.127 a*** b***	21.75 ± 0.479	23.00 ± 0.408	6.0
	400	14.365 ± 0.162 a*** b***			
	600	17.225 ± 0.235 a*** b***			
	800	25.250 ± 0.854a** b ns			
Klebsiella pneumoniae	200	7.240 ± 0.049 a***b***	16.00 ± 0.707	19.25 ± 0.629	6.0
	400	9.528 ± 0.069 a*** b***			
	600	10.875 ± 0.118 a*** b***			
	800	13.250 ± 0.479a*b***			
Staphylococcus aureus	200	8.820 ± 0.068 a***b***	23.00 ± 0.408	30.25 ± 0.854	6.0
	400	11.483 ± 0.145 a*** b***			
	600	14.180 ± 147 a*** b***			
	800	21.00 ± 0.707 a ns b***			

*Note: ME= Methanolic Extract, SEM= Standard Error of Mean, 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, done by independent sample test; [a] Compared the antibacterial activity of ME with Kanamycin; [b] Compared the antibacterial activity of ME with Norfloxacin; ns = Not significant; Values are expressed as Mean ± SEM (n=4).*

3.3. Brine Shrimp Lethality Bioassay

The LC_{50} (concentration that kills 50% Nauplii) values of *C. zedoaria* ME and vincristine sulfate were 35.187 and 0.951 µg/ml respectively (Table 3 and Table 4). The degree of lethality was found directly proportional to the concentration of the dose (Figure 2).

Table 3. Cytotoxic Effect of *C. zedoaria* ME on Brine Shrimp Nauplii

Concentration (C) (µg/ml)	Log C	No. of Nauplii	Alive	Dead	Mortality (%) (Mean ± SEM)	LC_{50} (µg/ml)
800	2.903	10	1	9	93.33 ± 3.33	35.187
400	2.602	10	2	8	76.67 ± 3.33	
200	2.301	10	3	7	70.00 ± 0.00	
100	2.000	10	4	6	63.33 ± 3.33	
50	1.699	10	5	5	53.33 ± 3.33	
25	1.398	10	5	5	46.67 ± 3.33	
12.5	1.097	10	6	4	40.00 ± 5.77	
6.25	0.796	10	7	3	26.67 ± 3.33	

Note: Values are expressed as Mean ± SEM (n=3).

Table 4. Cytotoxic Effect of Vincristine Sulphate on Brine Shrimp Nauplii

Concentration (C) (µg/ml)	Log C	No. of Nauplii	Alive	Dead	Mortality (%) (Mean ± SEM)	LC_{50} (µg/ml)
40	1.602	10	0	10	100.00 ± 0.00	0.951
20	1.301	10	1	9	93.33 ± 3.33	
10	1.000	10	2	8	76.67 ± 3.33	
5	0.699	10	3	7	63.33 ± 3.33	
2.5	0.398	10	5	5	53.33 ± 3.33	
1.25	0.097	10	5	5	50.00 ± 0.00	
0.625	-0.204	10	6	4	40.00 ± 5.77	
0.3125	-0.505	10	6	4	36.67 ± 3.33	
0.15625	-0.806	10	6	4	33.33 ± 3.33	
0.078125	-1.1072	10	7	3	26.67 ± 3.33	

Note: Values are expressed as Mean ± SEM (n=3).

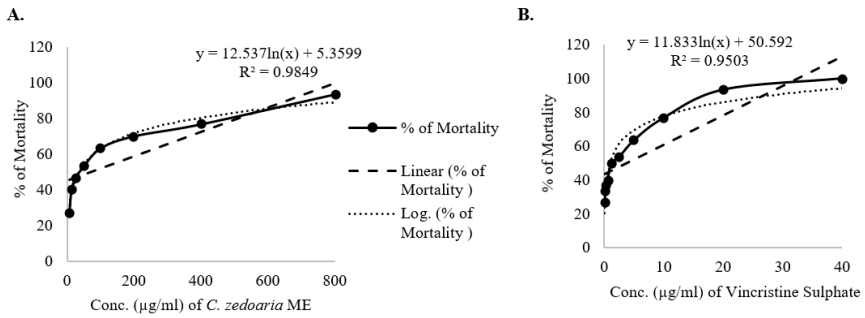

Figure 2. Brine Shrimp Lethality (LC_{50}) Bioassay of (A) *C. zedoaria* ME & (B) Vincristine Sulphate

4. Discussion

Plants are among the most significant areas to explore for uncovering and developing safe and potential drug candidates. The probable hepatoprotective benefits of the plant-based extract are commonly investigated using the PCM intoxication model (Kumar *et al.*, 2004). Elevated levels of hepatic enzymes (ALT, ALP, AST), and TB indicate cellular leakage in addition to collapse of the functional integrity of the hepatic cell membranes (Poole & Leslie, 1989). Lee (2009) states that N-acetyl P-bezoquinoneimine, a hazardous PCM byproduct, can bind covalently to protein sulfhydryl groups, resulting in lipid peroxidation and hepatocyte necrosis. Figure 1 demonstrates that elevated blood levels of TB and hepatic enzymes were significantly counteracted ($p<0.001$) by *C. zedoaria* rhizome ME in a dose-dependent manner. Based on a currently published study, its EE possesses a protective ability against CuSO4

pentahydrate-induced hepatocyte injury at 1000 mg/kg BW dose (Sari, *et al*., 2022). Likewise, Sumarheni *et al*., (2023) demonstrated that at doses of 350 & 525 mg/kg, the EE effectively reduced ($p < 0.05$) AST and ALT levels in doxorubicin-induced hepatotoxicity. Regarding these enzyme reduction rates, the lower dose of ME (300 mg/kg) proved more effective in this study than the EE of Sumarheni *et al*. (2023). Prasad *et al*. (2015) also claimed the hepatoprotective benefit of the ME through enzyme lowering response ($p < 0.05$). However, at nearly similar doses, the AST and ALT enzyme-lowering ability of the ME in the current investigation was greater than in the aforementioned study. Nevertheless, very few studies have been published on the hepatoprotective potential of the ME, and to the best of our knowledge, no work on hepatoprotective activity has been reported in Bangladesh so far. Previously reported phytochemical screening studies on its ME have confirmed the presence of flavonoids, tannins, saponins, sterols, and more (Sumathi *et al*., 2013; Setyani *et al*., 2020). Among them, flavonoids, tannins, and saponins have been reported to possess profound antioxidant and anti-inflammatory properties (Sandhar *et al*., 2011; Pithayanukul *et al*., 2009; Elekofehinti *et al*., 2012). According to Mujeeb *et al*. (2009), the antioxidant properties of this rhizome could facilitate the protective action of hepatocytes. By interacting with the endoplasmic reticulum's activated radicals, it may halt the peroxidative destruction of membrane lipids which may be connected to its ability to decrease enzyme levels. Considering these facts, it is reasonable to hypothesize that the hepatoprotective ability was a result of the bio-constituents' synergistic action. Another report on the possible mechanism claimed that the extract successfully corrected PCM-induced hepatotoxicity owing to its ability to inhibit cytochrome P450 and/or stimulation of the PCM Glucuronidation (Porchezhian & Ansari, 2005). Han *et al*. (2006), on the other hand, claimed that PPA (peroxisome proliferators activated) receptor activation is a prerequisite for all cellular pathways linked to hepatoprotective activity in PCM-induced hepatotoxicity. Flavonoids and saponins found abundantly in this herb have been reported to demonstrate the capacity to activate this receptor (Manautou *et al*., 1996; Liang *et al*., 2001). However, more research is required before we can draw any firm conclusions about the precise mechanism(s).

Researchers have been looking for natural sources of antibacterial to combat the challenges imposed by antibiotic resistance. This investigation to explore the antibacterial potential of *C. zedoaria* was intrigued by the herb's extensive traditional applications in infectious diseases. The outcomes of this study demonstrated that the extract appreciably hindered the growth of our selected microbial strains (Table 2) at least at 600 µg dose level. However, the maximum inhibition (a ZOI of 25.25 ± 0.854 mm) was observed against *P. aeruginosa* at 800 µg dose. Based on the CLSI (2023) guidelines and ZOI values obtained for the reference standard antibiotics, it can be concluded that an 800 µg dose of the ME has a prominent antibacterial effect ($p<0.01$). Compared to this investigation, Chachad *et al*. (2016)'s antibacterial activity test on EE (400 µg) showed lower efficacy against gram-negative microbes (*E. coli* & *P. aeruginosa*). Moreover, Islam *et al*. (2017)'s assessment also indicated a lower potency of EE (800 µg) against both gram-positive (*S. aureus*) and gram-negative (*E. coli* & *P. aeruginosa*) species. Therefore, it can be recommended that, at similar doses, the ME has superior dose-dependent inhibitory potential than EE against the selected microbes, except *K. pneumoniae*. Some published studies also have claimed that ME can suppress these bacteria (Banisalam *et al*., 2011; Silalahi, 2020) however the data is very limited and discrete. The compounds responsible for the antibacterial effect are still unknown; so, further works are warranted using suitable chromatographic techniques, to isolate and identify the active constituents.

The cytotoxic action of *C. zedoaria* was observed to be lower than the standard vincristine sulfate (Table 3, Table 4, and Figure 2). Nonetheless, the *in vitro* cytotoxicity shown by the ME can be taken as a preliminary indicator of potential *in vivo* antitumor activity. According to a report by Gharge *et al*. (2021), the crude ME of the plant expressed cytotoxicity against numerous cancer cell lines. Moreover, this extract contains a broad spectrum of phytomolecules (alkaloids, carbohydrates, flavonoids, tannins, polyphenols, saponins, terpenoids, and amino acids) that may demonstrate nonspecific cell destruction. Animal models might be used to validate the chemotherapeutic ability of specific compounds through cell-specific toxicity. Thus, scrutinizing the *in vivo* anticancer properties of its specific phytoconstituents may be a topic of interest in the future.

5. Conclusion

The study findings demonstrate that the ME of *C. zedoaria* rhizome exhibits promising dose-dependent hepatoprotective ability against PCM-induced liver cirrhosis. Moreover, it showed notable antibacterial properties against some perilous bacterial strains, and so could be potentially useful to treat numerous infectious disorders caused by resistant microbes. Besides, it possesses mild cytotoxicity as well which may serve as a prelude for subsequent pharmacological and phytochemical research to detect possible correlations between this plant's bioactivity and brine shrimp mortality. Therefore it is recommended to study in detail and explore the nature and extent of bioactive principles in the plant extract.

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Appendix

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BW	Body weight
CLSI	Clinical and laboratory standards institute
C. zedoaria	*Curcuma zedoaria*
DMSO	Dimethylsulfoxide
EE	Ethanolic extract
ICDDRB	International centre for diarrheal disease research, Bangladesh
LC_{50}	Lethal concentration 50
ME	Methanolic extract
Na-CMC	Sodium carboxymethyl cellulose
PCM	Paracetamol
SEM	Standard error of mean
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
TB	Total bilirubin
ZOI	Zone of inhibition