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Cytotoxicity of the rhizome of medicinal plants

Shakhawoat Hossain, Golam Kader, Farjana Nikkon, Tanzima Yeasmin*

Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi-6205, Bangladesh

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ABSTRACT

Objective: To investigate the cytotoxicity of the crude ethanol extract of the rhizome of *Zingiber zerumbet* (*Z. zerumbet*) (L) Smith. and *Curcuma zedoaria* (*C. zedoaria*) Rosc. against *Artemia salina* Leach. **Methods:** Fresh rhizomes of *Z. zerumbet* (L) Smith. and *C. zedoaria* Rosc. were extracted separately in cold with ethanol (2.5 L) and after concentration a brownish syrupy suspension of ethanol extracts of *Z. zerumbet* (L) Smith. and *C. zedoaria* Rosc. was obtained. The cytotoxic effect of the crude ethanol extracts of both plants was determined by brine shrimp lethality bioassay. **Results:** Crude ethanol extracts of the rhizome of *Z. zerumbet* (L) Smith. showed the highest cytotoxicity (LC_{50} was 1.24 μg/mL) against brine shrimp nauplii as compared with *C. zedoaria* Rosc. (LC_{50} was 33.593 μg/mL) after 24 h of exposure. **Conclusions:** It can be concluded that the rhizome of *Z. zerumbet* (L) Smith. and *C. zedoaria* Rosc. can be used as a source of cytotoxic agent.

1. Introduction

Natural substances serve as the sources of most drugs and medicinal agents. Several of these substances are believed to have potential value as cancer chemopreventive or therapeutic agents[1]. Moreover, from the ancient time, many plants are used as folk medicines to treat infectious diseases such as urinary tract infections, diarrhea, cutaneous abscesses, bronchitis and parasitic diseases[2-5]. Researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infections[6,7]. Hence, investigation of the chemical compounds within medicinal plants has become desirable. Therefore, it is essential to establish the scientific basis for their therapeutic actions as these may serve as the source for the development of effective drugs[8].

Zingiber zerumbet (*Z. zerumbet*) (L) Smith. and *Curcuma zedoaria* (*C. zedoaria*) Rosc. are perennial rhizomatous herb that belongs to the Zingiberaceae family. *Z. zerumbet* (L) Smith. is commonly known as the pinecone or shampoo ginger and also known by various names for example, "Bon adha" (Bangladesh), "Ghatian" and "Yaiimu" (India), "Lempoyang" (Malaysia and Indonesia), "Awapuhi" (Hawaii), "Zurunbah" (Arab), "Hong qiu jiang"(China) and "Haeo dam" or "Hiao dam" (Northern Thailand)[3,4,6,9], whereas *C. zedoaria* Rosc. is known as white turmeric, zedoaria or gajutsu, but in Bangladesh it is known as "Shoti"[10,11]. The plants are indigenous to Bangladesh, Sri Lanka and India, and are also cultivated in China, Japan, Brazil, Nepal, Malaysia and Thailand[12,13]. The rhizomes of *Z. zerumbet* (L) Smith. are used in the traditional medicine as a cure for swelling, loss of appetite, lumbago, diabetes, inflammation, chest pain, rheumatic pains, bronchitis, dyspepsia and sore throat[3,4,6,14]. The rhizome of *C. zedoaria* Rosc. has been used for stomachic, emmenagogic, antiallergant, carminative, expectorant activities and for treatment of vomiting, menstrual haematometra[15]. The juice of the boiled rhizomes of both plants has also been used in indigenous medicine for worm infestation in children[16,17]. From the pharmacological point of view, *Z. zerumbet* has been reported to have antimicrobial[8], anti-inflammatory[4], antinociceptive[18], antiallergic[19], antioxidant[20], antiplatelet aggregation[21], anthelmintic[22] and antihypertensive activities[23]. The rhizome of *C. zedoaria* Rosc. has been reported to have antimicrobial[24], antinociceptive[25], hepatoprotective[26] and antioxidant[2] activities. The present study was undertaken to investigate the cytotoxic effect of *Z. zerumbet* (L) Smith. and *C. zedoaria* Rosc. against *Artemia salina* (*A. salina*) Leach (brine shrimp).

*Corresponding author: Tanzima Yeasmin, Professor, Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi-6205, Bangladesh.
Tel: +88-0721-750041/Ext: 4109
Fax: +88-0721-750064
E-mail: yeasmin_bio@yahoo.com
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2. Materials and methods

2.1. Plants materials

Fresh rhizomes of *Z. zerumbet* (L) Smith. and *C. zedoaria* Rosc. were collected from the hilly areas of Chittagong, Bangladesh and authenticated by a taxonomist, Dr. Mohammed Yusuf, BCSIR Laboratory, Chittagong, Bangladesh where the voucher specimens (No: 1061 for *Z. zerumbet* and 93613 for *C. zedoaria*) of these collections were preserved.

2.2. Extraction of the rhizome of the plants

Fresh rhizomes of *Z. zerumbet* (L) Smith. and *C. zedoaria* Rosc. were washed, sun dried for 7 days and finally autoclaved in an electric oven below 60 ℃ for 23 h and ground to fine powders using a blender. Powdered plant materials of *Z. zerumbet* (L) Smith. and *C. zedoaria* Rosc. (800 g of each) were extracted separately in cold with ethanol (4 L) in an aspirator bottle for a week and then filtered. The filtrates were then concentrated by using a rotary evaporator at 40 ℃ under reduced pressure to afford a brownish syrupy suspension of ethanol extracts of *Z. zerumbet* (L) Smith. (15.0 g) and *C. zedoaria* Rosc. (16.5 g). Crude ethanol extracts of both plants were stored at 4 ℃ for determination of cytotoxicity.

2.3. Test organism

A. salina Leach (brine shrimp eggs) was collected from pet shops and used as test organism. The hatching tray *i.e.* a rectangular dish (22 cm × 32 cm) was half-filled with filtered brine solution and eggs of brine shrimps (50 mg) were sprinkled in it and incubated at 37 ℃ for 24 h under illumination. After the incubation period, brine shrimps were hatched and the plants extracts were applied to test the cytotoxic effect.

2.4. Brine shirmp cytotoxicity assay

The cytotoxicity of crude ethanol extracts of the rhizome of *Z. zerumbet* (L) Smith. and *C. zedoaria* Rosc. was determined by the procedure described by Meyer *et al*[27]. Test plant extracts (3 mg of each) were dissolved in 0.6 mL of pure dimethyl sulfoxide (DMSO) to get stock solutions of 5 mg/mL. Each of the stock solution (10, 20, 40 and 80 μL) was transferred to different vials and 5 mL of sea water (38 g sea salt/L distilled water) was added to each vials. The final concentrations of each vials were 10, 20, 40 and 80 μg/mL, respectively. Ampicillin trihydrate was used as positive control and DMSO used as negative control. After hatching and maturation of *A. salina* Leach, 10 larvae were placed in each vials using a Pastuer pipette and incubated at (25-27) ℃ for 24 h under illumination.

2.5. LC_{50} determination

After 24 h of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial was counted and noted. From this data, the percentage of mortality of the nauplii was calculated for each concentration and LC_{50} values with 95% confidence limits were determined using Probit analysis[27].

3. Results

The toxicity of ethanol extracts of the rhizome of *Z. zerumbet* (L) Smith. and *C. zedoaria* Rosc. was observed against brine shrimp nauplii and all the samples showed significant toxicity (Table 1). Between the tested samples, crude ethanol extracts of the rhizome of *Z. zerumbet* (L) Smith. showed the highest toxicity and the LC_{50} value was 1.240 μg/mL after 24 h of exposure, and the crude ethanol extract of the rhizome of *C. zedoaria* Rosc. showed moderate activity with LC_{50} value of 33.593 μg/mL. Results were compared with standard amphicillin trihydrate whose LC_{50} value was 16.067 μg/mL (Table 1).

Table 1
Cytotoxic activity of tested plants extract[a].

Tested materials	LC_{50} (24 h) (μg/mL)	95% *CI*
Z. zerumbet (L) Smith (rhizome)	1.240	13.998-21.946
C. zedoaria Rosc. (rhizome)	33.593	26.692-42.278
Ampicillin trihydrate[b]	16.067	7.157-36.068

[a]: All determinations were done in triplicate.
[b]: Reference standard.

4. Discussion

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxicity and anti-tumor properties[28]. Presently there is an increasing interest world wide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases[13]. The plants belonging to Zingiberaceae family have been received much attention for producing many complex compounds that are useful in food as herbs and spices, flavoring and seasoning, and in the cosmetics and medicinal industries as a pharmacological agents[2].

Cytotoxicity of the ethanol extracts of rhizome of *Z. zerumbet* (L) Smith. and *C. zedoaria* Rosc. against brine shrimp nauplii was evaluated and the extracts were found to be biologically active. However, more research should be directed towards the isolation of bioactive compounds from these plants and further toxicological studies (acute, sub-acute and chronic toxicity) on mice and different cancer cell

lines are needed in order to establish it as medicine.

In conclusion, the rhizomes of *Z. zerumbet* (L) Smith. and *C. zedoaria* Rosc. are cytotoxic to *A. salina* Leach and can be used as a source of cytotoxic agent.

Conflict of interest statement

We declare that we have no conflict of interest.

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