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Cytotoxicity of the rhizome of medicinal plants

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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 $1.24 \mu\text{g/mL}$) against brine shrimp nauplii as compared with *C. zedoaria* Rosc. (LC_{50} was $33.593 \mu\text{g/mL}$) 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).

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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 °C 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 °C 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 °C 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 °C 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 shrimp 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 μ L) 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 μ g/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) °C for 24 h under illumination.

2.5. LC₅₀ 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 LC₅₀ 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 LC₅₀ value was 1.240 μ g/mL after 24 h of exposure, and the crude ethanol extract of the rhizome of *C. zedoaria* Rosc. showed moderate activity with LC₅₀ value of 33.593 μ g/mL. Results were compared with standard ampicillin trihydrate whose LC₅₀ value was 16.067 μ g/mL (Table 1).

Table 1

Cytotoxic activity of tested plants extract^a.

Tested materials	LC ₅₀ (24 h) (μ g/mL)	95% CI
<i>Z. zerumbet</i> (L) Smith (rhizome)	1.240	13.998–21.946
<i>C. zedoaria</i> 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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References

- [1] Kinghorn AD, Farnsworth NR, Soejarto DD, Cordell GA, Pezzuto JM, Udeani GO, et al. Novel strategies for the discovery of plant-derived anticancer agents. *Pharm Biol* 2003; **41**: 53–67.
- [2] Chen IN, Chang CC, Ng CC, Wang CY, Shyu YT, Chang TL. Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. *Plant Foods Hum Nutr* 2008; **63**: 15–20.
- [3] Bhuiyan MNI, Chowdhury JU, Begum J. Chemical investigation of the leaf and rhizome essential oils of *Zingiber zerumbet* (L.) Smith from Bangladesh. *Bangladesh J Pharmacol* 2009; **4**(1): 9–12.
- [4] Zakaria ZA, Mohamad AS, Chear CT, Wong YY, Israf DA, Sulaiman MR. Antiinflammatory and antinociceptive activities of *Zingiber zerumbet* methanol extract in experimental model systems. *Med Princ Pract* 2010; **19**(4): 287–294.
- [5] Yob NJ, Jofrry SM, Affandi MMR, Teh LK, Salleh MZ, Zakaria ZA. *Zingiber zerumbet* (L.) Smith: a review of its ethnomedicinal, chemical, and pharmacological uses. *Evid Based Complement Alternat Med* 2011; **2011**: 543216.
- [6] Tushar, Basak S, Sarma GC, Rangan L. Ethnomedicinal uses of zingiberaceous plants of Northeast India. *J Ethnopharmacol* 2010; **132**(1): 286–296.
- [7] Philip K, Malek SNA, Sani W, Shin SK, Kumar S, Lai HK, et al. Antimicrobial activity of some medicinal plants from Malaysia. *Am J Appl Sci* 2009; **6**(8): 1613–1617.
- [8] Kader G, Nikkon F, Rashid MA, Yeasmin T. Antimicrobial activities of the rhizome extract of *Zingiber zerumbet* Linn. *Asian Pac J Trop Biomed* 2011; **1**(5): 409–412.
- [9] Zakaria ZA, Mohamad AS, Ahmad MS, Mokhtar AF, Israf DA, Lajis NH, et al. Preliminary analysis of the anti-inflammatory activity of essential oils of *Zingiber zerumbet*. *Biol Res Nurs* 2010.
- [10] Mia MMK, Kadir MF, Hossan MS, Rahmatullah M. Medicinal plants of the Garo tribe inhabiting the Madhupur forest region of Bangladesh. *Am Eurasian J Sustain Agric* 2009; **3**(2): 165–171.
- [11] Shahriar M. Antimicrobial activity of the rhizomes of *Curcuma zedoaria*. *J Bangladesh Acad Sci* 2010; **34**(2): 201–203.
- [12] Saadia MS, Halijah I. *Proceedings of the national convention on herbal medicine*. Kuala Lumpur: Forest Research Institute Malaysia; 1995, p. 205–207.
- [13] Lobo R, Prabhu KS, Shirwaikar A, Shirwaikar A. *Curcuma zedoaria* Rosc. (white turmeric): a review of its chemical, pharmacological and ethnomedicinal properties. *J Pharm Pharmacol* 2009; **61**: 13–21.
- [14] Sulaiman MR, Tengku Mohamad TAS, Shaik Mossadeq WM, Moin S, Yusof M, Mokhtar AF, et al. Antinociceptive activity of the essential oil of *Zingiber zerumbet*. *Planta Med* 2010; **76**(2): 107–112.
- [15] Khare CK. *Indian medicinal plants: an illustrated dictionary*. 1st ed. New Delhi: Springer Pvt Ltd; 2007.
- [16] Ruslay S, Abas F, Shaari K, Zainal Z, Maulidiani, Sirat H, et al. Characterization of the components present in the active fractions of health gingers (*Curcuma xanthorrhiza* and *Zingiber zerumbet*) by HPLC–DAD–ESIMS. *Food Chem* 2007; **104**(3): 1183–1191.
- [17] Nadkarni KM. *Indian materia medica*. 3rd ed. Mumbai: Popular Prakashan Private Limited; 1999.
- [18] Sulaiman MR, Perimal EK, Akhtar MN, Mohamad AS, Khalid MH, Tasrip NA, et al. Anti-inflammatory effect of zerumbone on acute and chronic inflammation models in mice. *Fitoterapia* 2010; **81**: 855–858.
- [19] Tewtrakul S, Subhadhirasakul S. Anti-allergic activity of some selected plants in the Zingiberaceae family. *J Ethnopharmacol* 2007; **109**(3): 535–538.
- [20] Stanly C, Bhatt A, Ali HMD, Keng CL, Lim BP. Evaluation of free radical scavenging activity and total phenolic content in the petiole-derived callus cultures of *Zingiber zerumbet* Smith. *J Med Plant Res* 2011; **5**(11): 2210–2217.
- [21] Jantan I, Raweh SM, Sirat HM, Jamil S, Mohd Yasin YH, Jalil J, et al. Inhibitory effect of compounds from Zingiberaceae species on human platelet aggregation. *Phytomedicine* 2008; **15**(4): 306–309.
- [22] Goswami S, Pandey A, Tripathi P, Singh A, Rai A. An *in vitro* evaluation of the anthelmintic activity of *Hedychium spichatum* rhizomes and *Zingiber zerumbet* rhizomes on the *Pheretima posthuma* model: a comparative study. *Pharmacognosy Res* 2011; **3**(2): 140–142.
- [23] Chaung HC, Ho CT, Huang TC. Anti-hypersensitive and anti-inflammatory activities of water extract of *Zingiber zerumbet* (L.) Smith. *Food Agric Immunol* 2008; **19**(2): 117–129.
- [24] Bugno A, Nicoletti MA, Almodóvar AAB, Pereira TC, Auricchio MT. Antimicrobial efficacy of *Curcuma zedoaria* extract as assessed by linear regression compared with commercial mouthrinses. *Braz J Microbiol* 2007; **38**: 440–445.
- [25] Pamplona CR, Souza MMD, Mda SM, Filho CV, Navarro D, Yunes RA, et al. Seasonal variation and analgesic properties of different parts from *Curcuma zedoaria* Roscoe (Zingiberaceae) grown in Brazil. *Z Naturforsch C* 2006; **61**: 6–10.
- [26] Kim DI, Lee TK, Jang TH, Kim CH. The inhibitory effect of a Korean herbal medicine, zedoariae rhizoma, on growth of cultured human hepatic myofibroblast cells. *Life Sci* 2005; **77**: 890–906.
- [27] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nicolas DE, McLaughlin JL. Shrimp: a convenient general bioassay for active plant constituents. *Planta Med* 1982; **45**: 31–34.
- [28] Krishnaraju AV, Rao–Tayi VN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Assessment of bioactivity of Indian medical plants using brine shrimp (*Artemia salina*) lethality assay. *Int J Appl Sci* 2005; **3**: 125–134.