ENZYME INHIBITORY PROPERTIES OF ZERUMBONE

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Zerumbone (ZER) is a well-known sesquiterpene compound can be set up in the rhizomes of *Zingiber zerumbet* (Smith), having anti-cancer, anti-inflammatory and anti-hyperglycemic effects. This study aimed to investigate the inhibitory potential of ZER against drug-target enzymes involved in human pathologies, namely, obesity (Pancreatic lipase) and idiopathic intracranial hypertension (carbonic anhydrase). Result: ZER inhibits pancreatic lipase and carbonic anhydrase at percentages of 52.5 and 71.3% with 184.1 ± 5 and 69.3 ± 0.43 $\mu\text{g/mL}$ IC_{50} values, respectively. Consequently, ZER has an excellent inhibitory action against pancreatic lipase and carbonic anhydrase, making it interesting anti-obesity drug candidate and avert patho-physiological-related carbonic anhydrase.

Keywords: Pancreatic lipase, carbonic anhydrase, *zingiber zerumbet*.

INTRODUCTION

Enzymes play very important role to biocatalyst the metabolism and have been interact with various substances taken in the body. Especially some enzymes are known as drug-target such as pancreatic lipase and carbonic anhydrase (Aslan *et al.*, 2019).

Obesity is a state in which there is excessive accumulation of fats in the body due to less energy expenditure as compared to high energy intake. It has increased in almost all countries and record approximately 1.9 billion people overweight throughout the world (Wang *et al.*, 2020). Obesity is associated with serious fitness harms like metabolic, hormonal, and cardiac ailments (Jaradat *et al.*, 2017). Recently, pancreatic lipase inhibitors have gained much attention as an ideal mechanism for treating obesity due to the fact that pancreatic lipase is responsible for breaking down fats for further absorption. Since, pancreatic lipase will be inhibited by compounds therefore dietary fats will not be broken down and will not be absorbed by the intestine. Due to this fact, many researchers are interested on searching potent natural compounds which possess anti-pancreatic lipase activity (Ong *et al.*, 2016).

Carbonic anhydrases (CA) is responsible for many physiological processes, including respiration, carbon dioxide and bicarbonate transport, pH and CO_2 balance in the lungs and metabolizing tissues (Kaya *et al.*, 2019). Carbonic anhydrases are necessary also involved in the biological/patho-physiological processes, such as tumorigenicity, gluconeogenesis, neurological disorders, and obesity, as well as gastric and duodenal ulcers (Ashiq *et al.*, 2017). Therefore, there is need of potent compounds from natural sources which

could act as safe CA inhibitors and consider as first line treatments for these diseases.

Plant-derived bioactive compounds are well flourished for their therapeutic actions in the prevention and treatment of various diseases. Zerumbone is a major compound derived from the rhizomes of *Zingiber zerumbet* (Smith) that has been traditionally used for the treatment of diarrhea, stomach ulcer, headache and asthma (Albaayit *et al.*, 2021a; Attyah and Ismail, 2013). Several pharmacological properties of ZER have been reported including antinociceptive, antimicrobial, immunomodulation effect, anti-inflammatory and anticancer towards several cancer cell lines (Albaayit *et al.*, 2020a; Albaayit and Maharjan, 2018). However, no scientific evidence toward enzyme inhibition against pancreatic lipase and carbonic anhydrase has been reported during our literature survey. Thus, this study was carried to evaluate the pancreatic lipase and carbonic anhydrase inhibition potential of ZER which could lead to control the effects of obesity, glaucoma cancer, and neurological disorders.

MATERIALS AND METHODS

The tested plant material having crystals of creamy white colored was obtained from Professor Dr. Rasedee Abdullah, Universiti Putra Malaysia (UPM). The crystallization of the plant material was done three times to attain unadulterated crystals of zerumbone and retained at 4°C till further usage.

***In vitro* Pancreatic Lipase inhibition:** The lipase inhibition assay was carried out spectrophotometrically in 96-well plate using Tris-HCl buffer as described by Ong *et al.* (2016). The reaction mixture contained various concentrations of ZER (0.2-0.025 mg/mL), PPL (200 units/mL) and *p*-nitrophenyl

butyrate (pNPB) substrate solution (10 mM in acetonitrile). After incubation at 37°C for 5 min, the absorbance of the released PPL was measured at 410 nm ELISA plate reader (Spectra Max M2, Molecular Devices, CA, USA). The percentage inhibition of enzyme had been used to analysis data from which IC₅₀ values were calculated.

% inhibitory activity of enzyme

$$= 100 - \frac{\text{O.D of enzyme with inhibitor}}{\text{O.D of enzyme without inhibitor}} \times 100$$

Carbonic anhydrase inhibition assay: Reaction mixture of 200 µL included 20 µL of ZER (0.1-0.0125 mg/mL) in DMSO, 140 µL of the HEPES–tris buffer (20 mM at pH 7.4), 20 µL of enzyme CA (0.1mg/mL) prepared in buffer, and 20 µL of a solution of 4-nitrophenyl acetate, incubated for fifteen minutes in a 96-well broad-plate. 20 µL (0.7 mM) of 4-nitrophenyl acetate (4-NPA) substrate was added, and change in absorbance was observed for thirty minutes min by steady breaks of one min, by means of microplate readers (Tecan, California, USA) (Kaya *et al.*, 2019). The enzyme inhibition was expressed as IC₅₀ (concentration of ZER that reduces the enzyme activity by 50%) value.

RESULT AND DISCUSSION

Since ancient time, mankind had been using different plants to treat various diseases; thus, collected knowledge has advanced the ethnomedicine area due to which many researchers are doing research in this field to find good candidate drugs (Albaayit, 2021; Albaayit *et al.*, 2021b; Albaayit *et al.*, 2021c; Albaayit *et al.*, 2020b; Al-Bahrani *et al.*, 2020; Albaayit *et al.*, 2019; Al-Ani *et al.*, 2018). Zerumbone is the most active compound from edible ginger *Zingiber zerumbet* has therapeutic efficacy against various types of diseases (Albaayit and Maharjan, 2018; Albaayit *et al.*, 2021d). As a development to that reviews, interestingly, the current review expect to investigate on the inhibition impacts of ZER against pancreatic lipase and carbonic anhydrase that may be considered as a potential therapeutic to prevent obesity, cancer, and neurodegenerative diseases.

Recently, anti-obesity researchers are trying to focus on the natural sources, particularly those that named conventional restorative and palatable sorts for the treatment of stoutness and for controlling of overweight because of their wellbeing issue. The significance of pancreatic lipase inhibition assay has increased because of their possible adequacy of regular items as against stoutness therapy. Pancreatic lipase assumes a key part in dietary triacylglycerol assimilation, hydrolyzing triacylglycerol to 2-monoacylglycerol and unsaturated fats. It is notable that dietary fat isn't straightforwardly consumed from the digestive tract except if it has been exposed to the activity of pancreatic lipase (Ado *et al.*, 2013).

Indeed, carbonic anhydrase considers ubiquitous in a variety of tissues, being involved in the reversible hydration of carbon dioxide to bicarbonate with the release of protons

(Sahin *et al.*, 2011). Therefore, imbalances levels of this enzyme have been associated with many diseases such as obesity, cancer, epilepsy, glaucoma, gastroduodenal ulcers, and neurodegenerative (Iqbal *et al.*, 2015).

From the outcome of present study, it is concluded that ZER showed enzyme inhibitory activity of 52.5 and 71.3% at high concentration, with IC₅₀ value of 184.1±5 and 69.3±0.43 µg/mL against pancreatic lipase and carbonic anhydrase, respectively (fig.1). The finding is in agreement with previous study by Arya *et al.* (2021), who reported that sesquiterpene has potential inhibitors of enzymes by binding to the substrate target of enzyme, preventing the hydrolysis of corresponding substrate.

Figure 1. Enzyme inhibition percentage of pancreatic lipase (A) and Carbonic anhydrase (B) after treating with zerumbone.

Conclusion: Zerumbone showed inhibition against pancreatic lipase and carbonic anhydrase enzyme, thus, in terms of safety and efficacy, this compound possess capacity to be used for the treatment of obesity, cancer, and neurodegenerative diseases.

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