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Research article

Gas chromatography-mass spectrometry profiling, bioactive compounds and cytotoxicity of edible-cricket extracts as inhibitors for α-glucosidase, α-amylase, tyrosinase, and acetylcholinesterase

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Abstract

Importance of the work: In Thailand, consumption is increasing of edible crickets as a future prospective food, due to their various nutrient components and high level of proteins.
Objectives: The crude extracts of several edible cricket insect species were investigated for their levels of enzyme inhibition activity regarding diabetes, Alzheimer's disease and melanogenesis.
Materials & Methods: Several bioactive compounds, including alkaloids, saponins, steroids, triterpenoids, tannins, phenolics, and flavonoids, were investigated in the cricket extracts using the solvent extraction method and subsequently analyzed using gas chromatography-mass spectrometry (GC-MS) profiling and their inhibitory levels regarding α-glucosidase, α-amylase, tyrosinase and acetylcholinesterase.
Results: The total phenolic and flavonoids in the methanol extracts had greater inhibition levels than the other extracts. α-Glucosidase and acetylcholinesterase activities were inhibited by the cricket extracts in dichloromethane, similar to standard drugs. The crude extracts of the short-tailed cricket had the same anti-tyrosinase ability as kojic acid. These extracts showed no cytotoxicity in mouse subcutaneous connective tissue and human keratinocyte immortal cells. The GC-MS analysis confirmed that the inhibitory activity levels of the unsaturated and saturated fatty acids were the major components in the cricket extracts.
Main finding: Cricket extracts from the domestic house cricket, field cricket and short-tailed cricket, displayed diverse potential as enzyme inhibitors for α-glucosidase, α-amylase, tyrosinase and acetylcholinesterase, supporting the future use of crickets as functional food ingredients.

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Introduction

Edible insects have been classified as a prospective food and key solution for food insecurity by the Food and Agricultural Organization (Zielińska et al., 2015). Compared to traditional livestock, numerous insects contain a variety of nutrient compositions, including fatty acids, vitamins, minerals, amino acids and high levels of proteins (Pyo et al., 2020). In addition, insect production releases less CO_2 and greenhouse gases than other animal sources due to the insects having a higher conversion rate of feed to body mass (Nino et al., 2021a). Various countries in Africa, Asia and Latin America have widespread consumption of over 2,100 insect species (Kulma et al., 2020). In Thailand, there has been a substantial increase in the consumption of edible crickets, such as *Acheta domesticus* (house cricket), *Gryllus bimaculatus* (field cricket), *Brachytrupes portentosus* (short-tailed cricket) and *Gryllotalpa africana* (mole cricket) (Bolat et al., 2021). Therefore, the proximate compositions of nutrients, minerals, and vitamins of edible crickets were examined. Other researchers have reported that the house cricket had a greater protein level (71.7±0.5% dry weight) than other crickets (Udomsil et al., 2019), while field crickets had the highest fatty acid content (23.4±0.1% dry weight), while the highest amount of carbohydrates was found in mole crickets (47.2±0.3% dry weight) (Raksakantong et al., 2010; Musundire et al., 2016). The major mineral components of house crickets and field crickets were phosphorus (52.4−53.2%) and potassium (22.7−24.4%) (Udomsil et al., 2019). Recently, biological activity has been reported regarding insect protein hydrolysates, including antioxidant, antimicrobial, α-glucosidase inhibitory and angiotensin-converting enzyme inhibitory activity (da Silva Lucas et al., 2020).

Among many metabolic illnesses, diabetes and Alzheimer's disease have risen globally (Makki and Rahman, 2023). Diabetes mellitus (DM) is a chronic endocrine metabolic condition caused by defective insulin production by pancreatic cells that can be divided into type 1 diabetes mellitus (T1DM) or type 2 (T2DM) involving decreased sensitivity to pancreatic-cell-secreted insulin (Settu et al., 2021). The blood glucose level of T2DM involves the carbohydrate digestion process, controlled by α-amylase and α-glucosidase (Naveen and Baskaran, 2018; Gong et al., 2020;). α-Amylase is produced in human salivary glands and the intestinal lumen, hydrolyzing α-1,4-glycosidic bonds of carbohydrates. Then, α-glucosidase hydrolyzes the obtained oligosaccharides or disaccharides. Subsequently, potential α-amylase and α-glucosidase inhibitors have been discovered for use in anti-diabetic drugs (Alam et al., 2019). The protein hydrolysates from edible crickets have been reported to inhibit α-glucosidase activity (Yoon et al., 2019), while cricket glycosaminoglycan also displayed anti-diabetic activity (Ahn et al., 2020).

Alzheimer's Disease (AD) is the most prevalent kind of dementia, defined by memory loss and other intellectual skills severe enough to interfere with everyday living (Colović et al., 2013). Acetylcholine (Ach), as a neurotransmitter, plays an essential role in the acquisition and storage of transmitted memory through synaptic impulse transmission, with AD patients having inadequate Ach levels, regulated by acetylcholinesterase inhibitors (AChEIs) at the synapse that have been utilized as anti-AD drugs, such as galantamine, alkaloids, coumarins, terpenes, and polyphenols (Santos et al., 2018). Fatty acids have been reported as acetylcholinesterase inhibitors (Öztürk et al., 2014). Notably, unsaturated and saturated fatty acids have been identified in several edible cricket species (Raksakantong et al., 2010). The current work carried out further investigation of the anti-acetylcholinesterase activity of cricket extracts.

In addition, melanin pigment is synthesized from melanogenesis, which plays an important role in skin photocarcinogenesis (Di Petrillo et al., 2016). Tyrosinase (EC 1.14.18.1), as the key enzyme in melanin synthesis, catalyzes the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA, monophenolase activity) and the oxidation of L-DOPA to dopaquinone (diphenolate activity) (Muddathir et al., 2017). Overproduction of melanin can promote hyperpigmentation effects or various human skin disorders, such as freckles, ephelides, melasma and malignant melanomas (Cui et al., 2018). Consequently, tyrosinase inhibitors from natural sources have been developed to prevent pigmentation disorders and widely applied in cosmetic whitening agents, since commercial tyrosinase inhibitors, including arbutin and kojic acid, have many limitations, such as excessive cytotoxicity, inadequate penetrating power, limited activity and low stability (Chang et al., 2013). Notably, anti-tyrosinase activity has been reported from the consumption of edible insects or their extracts, including mealworms, (Hae et al., 2019). In addition, mericin and oil extracted from native silkworms (*Bombyx mori*) exhibited tyrosinase inhibition; however, their half maximal inhibitory concentration (IC_{50}) values were less than for kojic acid (Manosroi et al., 2010). The antioxidant activity of the ethanolic extract from edible insects has also been studied (Pyo et al., 2020).

Recently, the methanolic extracts of edible house cricket showed strong antioxidant activity (Nino et al., 2021a). The antioxidant activity may be related to anti-melanogenesis activity by inhibiting tyrosinase, tyrosinase-related protein-1 (TRP-1) and TRP-2 activities (Lee et al., 2019; Kim et al., 2023). Although edible crickets have been raised commercially for consumption in Thailand, the anti-diabetic, anti-melanogenesis and anti-Alzheimer activities from the crude extracts of diverse cricket species have not been fully investigated to date.

Therefore, the current work used solvent extraction to investigate the crude extracts of several edible cricket species—the domestic house cricket (*Acheta domesticus*), field cricket (*Gryllus bimaculatus*), short-tailed cricket (*Brachytrupes portentosus*) and mole cricket (*Gryllotalpa africana*). Several bioactive compounds of the crude extracts were evaluated to determine the secondary metabolites: alkaloids, saponins, triterpenoids, steroids and tannins. Gas chromatography-mass spectrometry (GC-MS) was used to undertake volatile chemical profiling of the cricket extracts. In addition, the crude extracts from all cricket species were investigated for their inhibitory activity levels regarding α-glucosidase, α-amylase, tyrosinase and acetylcholinesterase. Furthermore, the cytotoxicity of the cricket extracts for the effective enzyme inhibition was elucidated in human keratinocyte immoral cells (HaCat) and mouse subcutaneous connective tissue cells (L929).

Materials and Methods

Chemicals and equipment

All chemicals and reagents used were of analytical grade and purchased from commercial suppliers. The following items were obtained: α-glucosidase from *Saccharomyces cerevisiae*; α-amylase from *Aspergillus oryzae*; coupled enzyme (peroxide-glucose oxidase enzyme); tyrosinase from mushroom; acetylcholinesterase from *Electrophorus electricus* (electric eel, type VI); and *p*-nitrophenyl-α-glucosidase (4-*p*NPG), amylose from potatoes. The following chemicals were purchased from Sigma Aldrich (Saint Louis, MO, USA): acarbose, kojic acid, 3,4-dihydroxy-L-phenylalanine (L-DOPA), 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB), tacrine hydrochloride, acetylthiocholine iodide (ATC), sodium phosphate monobasic monohydrate ($NaH_2PO_4{\cdot}H_2O$), sodium phosphate dibasic (Na_2HPO_4), and Tris-HCl. Sodium carbonate (Na_2CO_3) was purchased from KemAus (Cherrybrook, NSW, Australia). Deionized water (R > 18 M Ω/cm[1]) was used in all experiments. Absorbance readings were measured on a Biochrom EZ Read 2000 microplate reader (Waterbeach Cambridge, United Kingdom).

Preparation of crude extracts from edible insects

Frozen cricket insects (house cricket, field cricket, short-tailed cricket and mole cricket) were purchased from the Talad Thai market, Pathum Thani province, Thailand (Fig. S1). The insect samples were kept at -20°C for 1 wk. Samples of short-tailed cricket (2 kg), mole cricket (2 kg), domestic house cricket (3 kg) and field cricket (3 kg) were dried in a hot-air oven at 60°C for 2 d and then ground in a mechanical blender to produce brown powders of short-tailed cricket (511.0 g, 26% dry weight), mole cricket (395.0 g, 19.8% dry weight), domestic house cricket (831.6 g, 28% dry weight) and field cricket (700.4 g, 24% dry weight), respectively. The crude extracts were prepared using the extraction process by increasing the polarity of the organic solvents, including hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and methanol (MeOH), in a 1:5 ratio of the powder to each organic solvent for 3 d at room temperature. The supernatant was passed through filter paper and then evaporated under reduced pressure to provide the crude extracts of each cricket species—short-tailed cricket: hexane (23.0 g, 4.5% dry weight), CH_2Cl_2 (34.9 g, 6.8% dry weight), EtOAc (14.0 g, 2.7% dry weight) and MeOH (24.4 g, 4.8% dry weight); mole cricket: hexane (63.9 g, 16.2% dry weight), CH_2Cl_2 (32.8 g, 8.3% dry weight), EtOAc (7.3 g, 1.8% dry weight) and MeOH (12.5 g, 3.2% dry weight); domestic house cricket: hexane (81.9 g, 9.8% dry weight), CH_2Cl_2 (49.7 g, 6.0% dry weight), EtOAc (13.1 g, 1.6% dry weight) and MeOH (34.4 g, 4.1% dry weight); field cricket: hexane (75.1 g, 10.7% dry weight), CH_2Cl_2 (60.2 g, 8.6% dry weight), EtOAc (19.8 g, 2.8% dry weight) and MeOH (32.6 g, 4.7% dry weight).

Bioactive compound analysis

The determination of bioactive compounds was performed using the method described by Chelladurai and Chinnachamy (2018).

Alkaloids were qualitatively analyzed using Mayer’s test. Briefly, the crude extract (10.0 mg) in 28% ammonia (NH_3) solution (1.5 mL) was mixed for 5 min and then, the solution was added with chloroform (5.0 mL). After mixing for 10 min, the reaction mixture was passed through filter paper

and evaporated using a water bath to obtain the crude extract. Mayer's reagent was prepared, consisting of: mercury(II) chloride ($HgCl_2$, 0.2716 g) and potassium iodide (KI, 1.0 g) in deionized water (20 mL). The resulting crude extract was added to Mayer's reagent (1.5 mL) to provide a white or cream-colored precipitate suggesting the presence of alkaloids.

Saponins were tested using the Forth method. Briefly, each crude extract (10.0 mg) in deionized water (5.0 mL) was heated in boiling water for 5 min, followed by passing through filter paper. The filtrate (2.0 mL) in deionized water (5.0 mL) was added to olive oil (3 drops) and shaken immediately. Emulsion and permanent bubbles appeared after incubation at room temperature for 30 min, suggesting the presence of saponins.

Triterpenoids were determined using the Liebermann Burchard method. Briefly, each cricket crude extract (5.0 mg) was dissolved in chloroform (2.0 mL). Acetic anhydride (1.0 mL) and then concentrated sulfuric acid (H_2SO_4, 1.0 mL) were added to the reaction solution. The formation of a purple-red solution indicated the presence of triterpenoids.

The Salkowaski reaction was utilized for the determination of steroids. Briefly, each solution of crude extract (10.0 mg) in chloroform (2.0 mL) was prepared in a test tube with vigorous shaking. After the addition of concentrated H_2SO_4 (2.0 mL) down the internal side of the test tube, red in the upper layer and yellow with green fluorescence in the acid layer suggested the presence of steroids.

Tannins were investigated based on colorimetric formation with Iron (III) chloride. Briefly, acetic anhydride (Ac_2O, 1.0 mL) was added to each solution of crude extract (3.0 mg/mL) in chloroform (1.0 mL). After the addition of concentrated H_2SO_4 (1.0 mL) down the internal side of the test tube, a green mixture suggested the presence of tannins.

Determination of total phenolic contents

The total phenolic contents of each cricket extract were elucidated using the Folin-Ciocalteu method (Masoongnoen, 2022). Each extracts (2.0 mg) was dissolved in methanol (2.0 mL). Then, 10% Folin-Ciocalteu reagent (50 µL) was added to the sample solution (150 µL) and incubated at room temperature under dark conditions for 5 min. Next, 5% sodium carbonate (Na_2CO_3, 50 µL) was added to the reaction solution. After incubating at room temperature under dark conditions for 60 min, the absorbance of the solution at 570 nm was measured using an ultraviolet-visible light (UV-Vis) spectrometer. The total phenolic contents of the crude extract were determined as milligrams of gallic acid equivalent (GAE) per gram of the crude extract from the standard calibration curve of gallic acid at concentrations of 0.4 µg/mL, 0.8 µg/mL, 1.0 µg/mL, 2.5 µg/mL, 10 µg/mL, 25 µg/mL and 40 µg/mL.

Determination of total flavonoid contents

The total flavonoid contents of each cricket extract were determined using the colorimetric detection of aluminum chloride (Sansenya et al., 2021). The crude extract (1.0 mg) was dissolved in methanol (1.0 mL). Then, 1.0 M sodium acetate (CH_3COONa, 50 µL) and 10% aluminum chloride ($AlCl_3$, 50 µL) were added to the sample solution (500 µL), which was added to methanol (400 µL). After incubation at room temperature under dark conditions for 45 min, the absorbance of the solution was measured at 415 nm using the UV-Vis spectrometer. The total flavonoid contents of the cricket extract were determined as milligrams of quercetin (QE) per gram of the crude extract from the standard calibration curve of quercetin at concentrations of 0 µg/mL, 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL.

Gas chromatography-mass spectroscopy analysis

The volatile compounds from each cricket crude extract were analyzed using GC-MS (Agilent 7890A GC-7000 Mass Triple Quad; Santa Clara, CA, United States). The crude extracts were dissolved in CH_2Cl_2 or MeOH and then passed through a regenerated cellulose membrane filter (0.22 µm). The solution (2 µL) of the crude extracts was injected into a DB-Wax (60 m × 0.25 mm i.d. × 0.25 µm film thickness) fused silica capillary column (J&W Scientific; Folsom, CA, USA). The injector port was set at 260°C and split mode was applied with a split ratio of 20:1. Helium gas was used as the carrier with a constant flow rate of 1.0 mL/min. The GC oven temperature regime was: commence at 70°C for 4 min; increase to 175°C at 13°C/min and hold for 27 min; and increase to 250°C at 3°C/min and hold for 60 min. The total run time was 124 min. The mass spectrometer was used in electron ionization mode, with the ion source temperature set at 250°C and the ionization energy set at 70 eV and in scan mode, with a scan range of 30–500 m/z. The Agilent MassHunter Qualitative Analysis B.04.00 software (https://agilent-masshunter-workstation-qualitative-analysis-b-04-00.updatestar.com/en) was used for data analysis. Identification of volatile compounds was performed by comparing their mass spectra with NIST mass spectral libraries (National Institute of Standards, 2011 version, https://chemdata.nist.gov/). The content of the volatile compound was calculated based on the peak area.

α-Glucosidase inhibition activity

The determination of α-glucosidase inhibition activity was carried out using the modified method of Nanok and Sansenya (2020). Briefly, *p*-nitrophenyl-α-glucosidase (4-*p*NPG) as the substrate and acarbose as the standard inhibitor were utilized in the α-glucosidase inhibition assays. Stock solutions of each crude extract were prepared in 5% (volume per volume; v/v) dimethyl sulfoxide/deionized water (DMSO/H_2O). The sample solutions (15 µL) and 4-*p*NPG (10 mM, 15 µL) were mixed in sodium phosphate buffer (20 mM, pH 6.8, 50 µL). The α-glucosidase solution (0.05 mg/mL, 20 µL) in sodium phosphate buffer (20 mM, pH 6.8) was added to the sample solution. After incubation at 37°C for 30 min, sodium carbonate (Na_2CO_3, 0.5 M, 100 µL) was added to the reaction solution to stop the enzymatic activity. The *p*-nitrophenol released from the α-glucosidase activity was measured at 405 nm using the a microplate reader. The α-glucosidase inhibition percentage was determined based on Equation 1:

$$\% \text{ Inhibition} = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100 \quad (1)$$

where $A_{control}$ is the absorbance of the enzyme activity and A_{sample} is the absorbance of the enzyme activity in the addition of the sample solution.

The IC_{50} value of acarbose was determined from the α-glucosidase inhibition percentage in the concentration range 0.02−0.3 mg/mL. The IC_{50} values of the crude extracts were evaluated from the α-glucosidase inhibition percentage in the concentration ranges of 0.01–8.0 mg/mL (domestic house cricket), 0.01−2.0 mg/mL (short-tailed cricket), 0.01−4.0 mg/mL (field cricket) and 0.02−30.0 mg/mL (mole cricket). The IC_{50} values of the triplicate experiment were calculated using the Grafit 5.0 computer software (Erithacus Software; Horley, UK).

α-Amylase inhibition activity

The determination of α-amylase inhibition activity was performed according to Nanok and Sansenya (2020). The α-amylase inhibition tests used acarbose as the standard inhibitor and amylose as the substrate. The stock solutions of the crude extract were prepared in 5% (v/v) DMSO/H_2O. The sample solutions were prepared by mixing the crude solution (10 µL) and amylose solution (0.5 mg/mL, 10 µL) in sodium phosphate buffer (20 mM, pH 6.8, 70 µL). The α-amylase solution (1.0 mg/mL, 10 µL) was added to the reaction solution. After incubation at 37°C for 60 min, the solution was soaked in boiling water for 5 min to stop the enzyme activity. Peroxidase-glucose oxidase assay was used to determine the released glucose molecules. The coupled enzyme (100 µL) and ABTS (1.0 mg/mL, 100 µL) were added to the reaction solution and then incubated at 37°C for 60 min. The absorption of the solution was measured at 405 nm by a microplate reader. The α-amylase inhibition percentage was determined using the same method as the α-glucosidase inhibition percentage. The IC_{50} value of acarbose was determined from the α-amylase inhibition percentage in the concentration range 0.02−0.3 mg/mL. The IC_{50} values of the crude extracts were evaluated from the α-amylase inhibition percentage in the concentration ranges of 0.04−14.0 mg/mL (domestic house cricket), 0.02-10.00 mg/mL (short-tailed cricket), 0.02-12.00 mg/mL (field cricket) and 0.01−40.0 mg/mL (mole cricket). The IC_{50} values of the triplicate experiment were calculated using the Grafit 5.0 computer software (Erithacus Software; Horley, UK).

Tyrosinase inhibition activity

The tyrosinase inhibition activity of the crude extracts was investigated using L-DOPA as the substrate and kojic acid as the standard inhibitor (El-hady et al., 2014). The stock solutions of each crude extract were prepared in 5% (v/v) DMSO/H_2O. The sample solutions were prepared by mixing the crude solution (10 µL) and tyrosinase solution (0.1 mg/mL, 10 µL) in sodium phosphate buffer (20 mM, pH 6.8, 70 µL). The reaction solution was pretreated at 37°C for 20 min. The L-DOPA solution (2.0 mM, 20 µL) in sodium phosphate buffer (20 mM, pH 6.8) was added to the reaction solution and then incubated at 37°C for 30 min. The absorption of the dopachrome formation was determined at 492 nm using the microplate reader. The tyrosinase inhibition percentage was calculated using the same method as the α-glucosidase inhibition percentage. The IC_{50} value of kojic acid was determined from the tyrosinase inhibition percentage in the concentration range 0.02−0.4 mg/mL. The IC_{50} values of the crude extracts were evaluated from the tyrosinase inhibition percentage in the concentration ranges of 0.01−8.0 mg/mL (domestic house cricket), 0.01−3.0 mg/mL (short-tailed cricket), 0.01−10.0 mg/mL (field cricket) and 0.02−40.0 mg/mL (mole cricket). The IC_{50} values of the triplicate experiment were calculated using the Grafit 5.0 computer software (Erithacus Software; Horley, UK).

Acetylcholinesterase inhibition activity

The acetylcholinesterase inhibition activity of the crude extracts was investigated using acetylthiocoline iodide (ATC) as the substrate and tacrine hydrochloride as the standard inhibitor (Ferreira et al., 2006). The stock solutions of each crude extract were prepared in 5% (v/v) DMSO/H_2O. The sample solutions were prepared from the crude solution (10 µL), ATC (5.0 mM, 5 µL) and H_2O (25 µL) in Tri-HCl buffer (0.1 M, pH 6.8, 50 µL). The acetylcholinesterase solution (0.01 mg/mL, 5 µL) and DTNB (1.0 mM, 10 µL) were added to the reaction solution. After incubation at 37°C for 20 min, the absorption at 415 nm was investigated using microplate reader. The acetylcholinesterase inhibition percentage was calculated using the same method as the α-glucosidase inhibition percentage. The IC_{50} value of tacrine hydrochloride was determined from the acetylcholinesterase inhibition percentage in the concentration range 0.02−0.4 mg/mL. The IC_{50} values of the crude extracts were evaluated from the acetylcholinesterase inhibition percentage in the concentration ranges of 0.01−16.0 mg/mL (domestic house cricket), 0.01-7.00 mg/mL (short-tailed cricket), 0.01-14.00 mg/mL (field cricket) and 0.01−30.0 mg/mL (mole cricket). The IC_{50} values of the triplicate experiment were calculated using the Grafit 5.0 computer software (Erithacus Software; Horley, UK).

Cytotoxicity

The cytotoxicity test of the crude extracts was determined based on a triplicate experiment using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Saidi et al., 2020). HaCat and mouse subcutaneous connective tissue cells (L929) were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 8% fetal bovine solution in a 95% humidified atmosphere of 5% CO_2 at 37°C. The cells (1.0×10^5 cells/mL) were seeded in a 96-well plate with incubation at 37°C for 24 hr. The medium was removed and then the sample in DMEM (100 µL) was added to the plate. After incubation at 37°C for 24 hr, the sample medium was removed. The cells were incubated with MTT (1.0 mg/mL, 100 µL) in a 95% humidified atmosphere of 5% CO_2 at 37°C for 4 hr. After removing the MTT solution, the cells were treated with formazan solution (100 µL) in 10% sodium dodecyl sulfate-polyacrylamide/DMSO (1:9, v/v) for 5 min. The absorption was measured at 570 nm using the microplate reader to determine the %cytotoxicity of the sample according to Equation 2:

$$\%\ \text{Cytotoxicity} = \left[\frac{A_c - A_s}{A_c}\right] \times 100 \quad (2)$$

where A_c is the absorbance of the solution of cells in DMEM medium and A_s is the absorbance of the solution of cells treated with the sample solution.

Statistical analysis

The IC_{50} values of α-glucosidase, α-amylase, tyrosinase and acetylcholinesterase inhibitory activity, as well as cytotoxicity test, were expressed as mean ± SD values based on triplicate determination. The SPSS Statistics 23 software (IBM Corp.; Armonk, NY, USA) was used for the statistical analysis that was based on one-way analysis of variance and Duncan's multiple range test. Values of $p < 0.05$ were considered to be statistically significant.

Results and Discussion

Bioactive compound analysis of the crude extracts from the cricket species

The crude extracts of all the cricket species were prepared based on solid-liquid extraction in several organic solvents (hexane, CH_2Cl_2, EtOAc, and MeOH) (Fig. S2). The results of the bioactive compound analyses of the cricket extracts are provided in Table S1. All the crude extract samples contained alkaloids, saponins and steroids, corresponding to Musundire et al. (2014). However, triterpenoids were found in all the mole cricket extracts, the domestic house cricket extracts in CH_2Cl_2, EtOAc and MeOH and in the field cricket extract in EtOAc, which were in agreement with reported triterpenoids in insects (Garg et al., 2020). Although edible insects contain high amounts of nutritional and protein contents, tannins as an anti-nutrient component have also been reported, including in the domestic house cricket (Shantibala et al., 2014; Botella-Martínez et al., 2021). In the current work, tannins were also present in the MeOH extracts of the domestic house cricket and short-tailed cricket. From the reported literature, bioactive compounds, including alkaloids, saponins, and steroids, are potent α-glucosidase inhibitors (Yin et al., 2014). In addition, steroids have displayed tyrosinase inhibition (Chang, 2009), while flavonoids, alkaloids, terpenoids and saponins have been reported as potential anti-acetylcholinesterase inhibitors in Alzheimer's disease

therapy (Konrath et al., 2013; Jiang et al., 2017). These results suggested that the cricket extracts were composed of alkaloids, saponins, and steroids, which could have inhibition potential for α-glucosidase, α-amylase, tyrosinase or acetylcholinesterase.

Furthermore, insects have been reported as the source of bioactive phenolic compounds, with biological activities, such as antioxidant, anti-inflammatory and anticancer (Nino et al., 2021b), while phenolic and flavonoid compounds have been reported as α-glucosidase, α-amylase, tyrosinase or acetylcholinesterase inhibitors (Yin et al., 2014; Muddathir et al., 2017; Hae et al., 2019; Abd El-Aziz et al., 2021). Therefore, the total phenolic and flavonoid contents of the cricket extracts were determined, with all samples having these contents, as shown in Table 1. However, the total phenolic and flavonoid contents of almost all the mole cricket extracts were less than those of the other cricket extracts. The methanol extracts of the crickets had higher phenolic and flavonoid contents than the other extracts. These results indicated that the cricket extracts had inhibitory activities for α-glucosidase, α-amylase, tyrosinase or acetylcholinesterase.

Gas chromatography-mass spectrometry analysis

The volatile chemical profiles of the cricket extracts except mole crickets were investigated based on GC-MS analysis (Table S2). Although, the crude extracts of the mole crickets had greater levels of α-amylase inhibition activity than the other cricket extracts; however, the crude extracts of mole crickets were not studied since they exhibited levels of biological inhibition activity that were less than the standard inhibition. The peak identification at the retention time of interest was performed using the NIST mass spectral library (https://chemdata.nist.gov/) and the relative quantity was calculated based on the percentage of the peak area (Figs. S3–S14). The chemical profiles of the cricket extracts included carboxylic acids, esters, amides, alcohols, hydrocarbons and phytosterols (Table 2). Carboxylic acids, consisting of tetradecanoic acid, *n*-hexanoic acid, *cis*-9-hexadecenoic acid, octadecanoic acid, *cis*-9-octadecenoic acid, (*Z,Z*)-9,12-octadecadienoic acid), amides (octadecanamide, (*Z*)-9-octadecenamide) and phytosterol (cholesterol) were found in all crude extracts of all cricket species.

Table 1 Total phenolic and total flavonoid contents of crude cricket extracts

Edible insect	Crude extract	Total phenolic (mg GAE/g dry weight)	Total flavonoid (µg QE/g dry weight)
Domestic house cricket	Hexane	0.39±0.05^{e}	213.71±8.82^{f}
	CH_2Cl_2	0.23± 0.01^{d}	22.27±9.14^{ab}
	EtOAc	0.15±0.01^{c}	32.26±6.81^{bc}
	MeOH	1.19±0.06^{h}	432.20±5.14^{i}
Short-tailed cricket	Hexane	0.39±0.04^{e}	44.63±7.35^{c}
	CH_2Cl_2	0.59±0.06^{f}	32.01±4.65^{bc}
	EtOAc	0.23±0.02^{d}	31.18±4.62^{bc}
	MeOH	1.58±0.08^{i}	282.49±24.25^{g}
Field cricket	Hexane	0.65±0.06^{f}	72.23±32.26^{de}
	CH_2Cl_2	0.59±0.03^{f}	91.37±1.79^{e}
	EtOAc	0.16±0.02^{cd}	65.10±2.21^{d}
	MeOH	1.01±0.05^{g}	345.16±11.45^{h}
Mole cricket	Hexane	ND	5.45±1.56^{a}
	CH_2Cl_2	0.02±0.00^{ab}	45.01±1.38^{c}
	EtOAc	0.01±0.00^{a}	7.77±0.34^{a}
	MeOH	0.08±0.00^{b}	82.33±0.91^{de}

GAE = gallic acid equivalents; QE = quercetin equivalents; ND = not detected.

Mean ± SD (n = 3) within each column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

Table 2 Gas chromatography-mass spectrometry profiles of crude extracts from cricket species

Serial Number	Compound	Retention time (min)	Molecular formula	Area (%)											
				Field cricket				Domestic house cricket				Short-tailed cricket			
				Hexane	CH_2Cl_2	EtOAc	MeOH	Hexane	CH_2Cl_2	EtOAc	MeOH	Hexane	CH_2Cl_2	EtOAc	MeOH
Carboxylic acid															
1	Acetic acid	13.392	$C_2H_4O_2$	-	-	0.20	0.48	-	-	0.51	2.24	-	-	-	0.73
2	Butanoic acid, 3-methyl-	14.761	$C_5H_{10}O_2$	-	-	0.09	-	-	-	-	-	-	-	-	-
3	Hexanoic acid	17.677	$C_6H_{12}O_2$	-	-	-	-	-	-	-	-	0.07	-	-	0.19
4	Octanoic acid	23.823	$C_8H_{16}O_2$	-	-	-	-	-	-	-	-	-	-	0.07	-
5	Tetradecanoic acid	59.103	$C_{14}H_{28}O_2$	0.31	0.30	0.37	0.29	0.36	0.36	0.38	0.25	0.71	0.63	0.81	0.54
6	16-Hydroxyhexadecanoic acid	61.503	$C_{16}H_{32}O_3$	-	-	-	-	-	-	-	-	-	-	0.42	-
7	Pentadecanoic acid	62.515	$C_{15}H_{30}O_2$	-	-	-	-	0.06	0.07	0.10	0.06	0.44	0.49	0.55	0.14
8	n-Hexadecanoic acid	65.739	$C_{16}H_{32}O_2$	17.82	17.51	19.62	14.80	18.76	27.59	21.93	23.10	23.24	21.50	28.49	16.68
9	cis-9-Hexadecenoic acid	66.795	$C_{16}H_{30}O_2$	1.37	2.02	1.11	1.31	1.01	1.36	1.25	0.60	1.12	1.01	1.13	0.85
10	Heptadecanoic acid	72.441	$C_{17}H_{34}O_2$	-	-	0.17	-	0.16	0.31	0.16	-	0.61	0.37	0.40	0.26
11	Octadecanoic acid	73.512	$C_{18}H_{36}O_2$	6.88	6.82	8.20	5.28	8.48	16.88	11.43	12.09	8.42	7.06	7.94	4.50
12	cis-9-Octadecenoic Acid	74.965	$C_{18}H_{34}O_2$	25.66	25.26	25.22	20.62	22.51	17.99	20.19	11.92	26.88	24.69	24.67	20.00
13	9,12-Octadecadienoic acid (Z,Z)-	77.609	$C_{18}H_{32}O_2$	27.32	28.11	25.06	24.11	34.0	26.04	30.06	20.09	18.07	17.80	15.60	15.65
14	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	81.36	$C_{18}H_{30}O_2$	0.42	0.49	1.09	0.43	0.41	0.29	0.51	-	2.24	1.31	1.24	1.03
15	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl	81.744	$C_{21}H_{36}O_4$	-	-	-	-	-	-	-	-	-	-	-	0.55
16	Eicosanoic acid	85.09	$C_{20}H_{40}O_2$	0.37	0.35	0.31	-	-	0.24	0.18	0.30	0.16	-	-	-
Ester															
17	Hexadecanoic acid, methyl ester	32.429	$C_{17}H_{34}O_2$	-	-	-	4.08	1.02	0.53	0.60	0.55	0.05	-	-	0.87
18	9-Hexadecenoic acid, methyl ester, (Z)-	34.289	$C_{17}H_{32}O_2$	-	-	-	0.23	-	-	-	-	-	-	-	-
19	Hexadecanoic acid, ethyl ester	35.182	$C_{18}H_{36}O_2$	-	-	0.29	0.37	-	-	0.30	-	-	-	-	-
20	1,2,3-Propanetriol, 1-acetate	36.932	$C_5H_{10}O_4$	-	-	0.09	-	-	-	0.39	0.44	-	-	-	-
21	Octadecanoic acid, methyl ester	47.652	$C_{19}H_{38}O_2$	-	-	-	1.53	0.35	0.20	0.19	0.23	-	-	-	0.27
22	9-Octadecenoic acid, methyl ester, (E)-	48.616	$C_{19}H_{36}O_2$	-	-	-	-	0.87	0.47	0.48	0.47	0.10	-	-	0.95
23	9-Octadecenoic acid, methyl ester, (Z)-	48.695	$C_{19}H_{36}O_2$	-	-	-	4.49	-	-	-	-	-	-	-	-
24	13-Octadecenoic acid, methyl ester	49.054	$C_{19}H_{36}O_2$	-	-	-	0.15	-	-	-	-	-	-	-	-
25	Octadecanoic acid, ethyl ester	49.603	$C_{20}H_{40}O_2$	-	-	0.11	0.12	-	-	0.09	-	-	-	-	-
26	9-Octadecenoic acid, ethyl ester, (Z)-	50.519	$C_{20}H_{38}O_2$	0.33	0.34	0.49	0.53	0.10	-	0.23	0.09	-	-	-	-
27	Methyl 9-*cis*,11-*trans*-octadecadienoate	50.969	$C_{19}H_{34}O_2$	-	-	-	-	-	-	-	-	0.06	-	-	-
28	9,12-Octadecadienoic acid, methyl ester, (Z,Z)-	51.071	$C_{19}H_{34}O_2$	-	0.09	-	5.64	1.35	0.72	0.75	0.80	-	-	-	0.74
29	9,12-Octadecadienoic acid, ethyl ester	52.667	$C_{20}H_{36}O_2$	0.36	0.30	0.43	0.52	0.08	-	0.29	0.11	-	-	-	-
30	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	53.974	$C_{19}H_{32}O_2$	-	-	-	0.20	-	-	-	-	-	-	-	-
31	DL-Proline, 5-oxo-, methyl ester	55.506	$C_6H_9NO_3$	-	-	-	-	-	-	-	0.10	-	-	-	-

Table 2 Continued

Serial Number	Compound	Retention time (min)	Molecular formula	Area (%)											
				Field cricket				Domestic house cricket				Short-tailed cricket			
				Hexane	CH_2Cl_2	EtOAc	MeOH	Hexane	CH_2Cl_2	EtOAc	MeOH	Hexane	CH_2Cl_2	EtOAc	MeOH
32	Hexanoic acid, 2-dimethylaminoethyl ester	56.559	$C_{10}H_{21}NO_2$	-	-	-	-	-	-	-	-	-	-	-	0.25
33	*i*-Propyl 12-methyltetradecanoate	61.472	$C_{18}H_{36}O_2$	-	-	-	-	-	-	-	-	-	0.10	-	-
34	Hexadecanoic acid, butyl ester	64.082	$C_{20}H_{40}O_2$	-	-	-	-	-	-	-	-	-	0.17	-	-
35	Octanoic acid, 2-dimethylaminoethyl ester	64.095	$C_{12}H_{25}NO_2$	-	-	-	-	-	-	-	-	-	-	-	0.43
36	Isopropyl palmitate	64.107	$C_{19}H_{38}O_2$	-	-	-	-	0.08	-	-	-	-	-	-	-
37	Glycerol 1-palmitate	64.108	$C_{19}H_{38}O_4$	-	-	-	-	-	-	0.13	-	0.15	-	0.40	-
38	9,12-Octadecadienoic acid (Z,Z)-, 1-methylethyl ester	70.433	$C_{21}H_{38}O_2$	-	0.07	-	-	-	0.05	-	-	-	-	-	-
39	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	72.428	$C_{21}H_{40}O_4$	-	-	-	-	-	-	-	-	0.09	0.13	0.43	-
40	Methyl 5,12-octadecadienoate	98.963	$C_{19}H_{34}O_2$	-	-	-	-	-	-	-	1.02	-	-	-	-
41	Butyl 9,12-octadecadienoate	99.054	$C_{22}H_{40}O_2$	1.10	0.87	0.65	0.57	0.48	0.27	0.57	-	0.96	1.03	0.91	1.50
42	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	106.571	$C_{21}H_{40}O_4$	-	-	-	-	-	-	-	-	-	-	2.82	5.80
Amide															
43	2-Pyrrolidinone	24.153	C_4H_7NO	-	-	-	-	-	-	-	0.08	-	-	-	-
44	Palmidrol	53.979	$C_{18}H_{37}NO_2$	-	-	-	-	-	-	-	-	0.09	0.08	0.05	-
45	Tetradecanamide	68.426	$C_{16}H_{29}NO$	-	0.43	0.30	0.30	0.12	0.13	0.18	0.44	0.22	0.42	0.34	0.62
46	cis-11-Eicosenamide	85.843	$C_{20}H_{39}NO$	-	-	-	-	-	-	-	0.15	-	-	-	-
47	Octadecanamide	91.843	$C_{18}H_{37}NO$	0.46	0.48	0.38	0.37	0.36	0.23	0.36	0.69	0.43	0.52	0.40	0.81
48	9-Octadecenamide, (Z)-	94.215	$C_{18}H_{35}NO$	11.94	11.96	11.09	9.17	6.56	4.04	6.75	16.32	11.76	15.12	10.13	21.76
Alcohol															
49	Glycerin	39.791	$C_3H_8O_3$	-	-	0.15	0.4	-	-	-	3.41	-	-	0.13	1.14
50	1-Dodecanol, 2-hexyl-	60.149	$C_{18}H_{38}O$	0.28	0.24	-	-	-	-	-	-	-	-	-	-
51	1-Heptacosanol	60.421	$C_{27}H_{56}O$	0.51	0.47	0.31	0.23	-	-	-	-	-	-	-	-
52	Eicosen-1-ol, cis-9-	67.998	$C_{20}H_{40}O$	1.15	-	0.72	0.52	-	-	-	-	-	-	-	-
Hydrocarbon															
53	Pentacosane	59.546	$C_{25}H_{52}$	0.55	0.51	0.32	0.16	-	0.05	-	-	-	-	-	-
54	Octacosane	62.769	$C_{28}H_{58}$	0.12	0.34	0.25	-	0.09	0.07	-	-	-	-	2.06	-
55	Triacontane	64.322	$C_{30}H_{62}$	-	0.10	-	-	-	-	0.17	-	0.54	-	0.36	-
56	Nonacosane	64.448	$C_{29}H_{60}$	-	-	-	-	0.40	0.29	-	-	-	-	-	-
57	1,21-Docosadiene	67.998	$C_{22}H_{42}$	-	1.12	-	-	-	-	-	-	-	-	-	-
58	Tetratriacontane	71.159	$C_{34}H_{70}$	-	0.09	-	-	0.61	0.33	0.19	-	2.84	2.68	-	0.98
59	Tetracontane	101.161	$C_{40}H_{82}$	-	-	-	-	0.62	0.30	-	-	-	-	-	-
Phytosteroid															
60	Ethyl iso-allocholate	72.456	$C_{26}H_{44}O_5$	-	-	-	-	0.06	-	-	-	-	-	-	-
61	Cholesterol	110.1	$C_{27}H_{46}O$	1.95	1.16	2.00	2.58	1.11	1.10	1.62	3.86	0.68	3.80	0.61	1.49

The major component in the chemical profiles of all crude extracts was carboxylic acids (61−91%), with unsaturated fatty acids (38−72%) in greater amounts than saturated fatty acids (22−38%), as shown in Table 3. Similarly, *Musca domestica* larvae had a high composition of unsaturated fatty acids (Anankware et al., 2021). Of the saturated fatty acids, the level of *n*-hexadecanoic acid (palmitic acid, 15−29%) was greater than for octadecanoic acid (stearic acid, 5–17%) and tetradecanoic acid (myristic acid, 0.3–0.8%), respectively. Of the unsaturated fatty acids, *cis*-9-octadecenoic acid (oleic acid) and (*Z*,*Z*)-9,12-octadecadienoic acid (linoleic acid) were predominantly detected in all cricket species, whereas *cis*-9-hexadecenoic acid (palmitoleic acid) and (*Z*,*Z*,*Z*)-9,12,15-octadecatrienoic acid (α-linolenic acid, omega-3) were detected at lower than 2.5% of the relative content. There were relatively high contents of linoleic acid (omega-6) in the field cricket and domestic house cricket that were higher than in the short-tailed cricket. Several edible insects (*R. phoenicis*, *Homorocoryphus nitidulus* and *Zonocerus variegates*) had high contents of polyunsaturated fatty acids, such as linoleic acid and α-linolenic acid (Anankware et al., 2021). In the current work, the monounsaturated fatty acids in the shorted-tailed cricket and field cricket were greater than in the domestic house cricket. Another study reported that the polyunsaturated fatty acid components, including omega-3 and omega-6, in the domestic house cricket were higher than in the field cricket and short-tailed cricket, respectively (Udomsil et al., 2019). Recently, the volatile profiles of fatty acids from the domestic house cricket and field cricket were investigated using GC-MS analysis, with the quantitative area analysis of the extracts from domestic house cricket identifying major fatty acids, such as linoleic acid, palmitic acid, oleic acid, stearic acid and linolenic acid (Spano et al., 2023). Furthermore, the extract from field crickets have been reported to contain the same main fatty acids as the domestic house cricket (Mlček et al., 2018).

The relative contents of ester derivatives (26 compounds) were in the range of 1.8−18.4%, as shown in Table 2. The high relative ester contents in the methanol extracts of the field cricket and short-tailed cricket were 18.4% and 10.8%, respectively. Butyl 9,12-octadecadienoate was found in all cricket extracts, except for the methanol extract of the domestic house cricket. The ethyl ester derivatives of (*Z*)-9-octadecenoic acid and 9,12-octadecadienoic acid were in the range of 0.3−0.5% for the field cricket extracts. Many methyl ester derivatives—hexadecanoic acid, methyl ester (0.6–1.0%), octadecanoic acid, methyl ester (0.2−0.4%), (*E*)-9-octadecenoic acid, methyl ester (0.5−0.9%), (*Z*,*Z*)-9,12-octadecadienoic acid and methyl ester (0.7−1.4%) were detected in all the crude extracts from the domestic house cricket. In addition, other amide derivatives, such as (*Z*)-9-octadecenamide (4.0−21.8%), were identified as the major component in all crude extracts and greater than octadecenamide (0.2−0.8%). The levels for both the alcohols and hydrocarbons were less than 3.4%. Pentosane, in the field cricket

Table 3 Fatty acid compositions of edible crickets

Fatty acid components		Field cricket				Domestic house cricket				Short-tailed cricket			
		H	D	E	M	H	D	E	M	H	D	E	M
Saturated fatty acids													
Myristic acid	C14:0	0.31	0.30	0.37	0.29	0.36	0.36	0.38	0.25	0.71	0.63	0.81	0.54
Pentadecylic acid	C15:0	-	-	-	-	0.06	0.07	0.10	0.06	0.44	0.49	0.55	0.14
Palmitic acid	C16:0	17.82	17.51	19.62	14.80	18.76	27.59	21.93	23.10	23.24	21.50	28.49	16.68
Margaric acid	C17:0	-	-	0.17	-	0.16	0.31	0.16	-	0.61	0.37	0.40	0.26
Stearic acid	C18:0	6.88	6.82	8.20	5.28	8.48	16.88	11.43	12.09	8.42	7.06	7.94	4.50
Total saturated fatty acids		25.01	24.63	28.36	20.37	27.82	45.21	34.00	35.5	33.42	30.05	38.19	22.12
Unsaturated fatty acids													
Palmitoleic acid	C16:1	1.37	2.02	1.11	1.31	1.01	1.36	1.25	0.60	1.12	1.01	1.13	0.85
Oleic acid	C18:1	25.66	25.26	25.22	20.62	22.51	17.99	20.19	11.92	26.88	24.69	24.67	20.00
Linoleic acid*	C18:2	27.32	28.11	25.06	24.11	34.0	26.04	30.06	20.09	18.07	17.80	15.60	15.65
α-Linolenic acid**	C18:3	0.42	0.49	1.09	0.43	0.41	0.29	0.51	-	2.24	1.31	1.24	1.03
Eicosanoic acid	C20:1	0.37	0.35	0.31	-	-	0.24	0.18	0.30	0.16	-	-	-
Monounsaturated fatty acids		27.40	27.63	26.64	21.93	23.52	19.59	21.62	12.82	28.16	25.70	25.80	20.85
Polyunsaturated fatty acids		27.74	28.60	26.15	24.54	34.41	26.33	30.57	20.09	20.31	19.11	16.84	16.68

H = hexane; D = dichloromethane; E = ethyl acetate; M = methanol; * = omega 6; ** = omega 3

extracts, has been reported as an insect pheromone (Mitaka et al., 2020). Cholesterol (0.6−3.9%) was identified in all the crude extracts from all cricket species, corresponding to a report on edible insects (Mudalungu et al., 2023). Hydrocarbons were found in the hexane and dichloromethane extracts from all crickets. Furthermore, the acetic acid methyl ester fraction of the Chinese and the hexane extract of the German mole cricket displayed linoleic acid methyl ester that was confirmed using GCMS and nuclear magnetic resonance spectroscopy (Zimmer et al., 2006).

α-Glucosidase and α-amylase inhibitory activity

The α-glucosidase and α-amylase inhibitory activity levels of the crude extracts from the edible cricket species are presented in Table 4. The crude extracts from the mole cricket were not effective at α-glucosidase inhibition. All the crude extracts from the short-tailed cricket, the CH_2Cl_2 extract from the domestic house cricket and the hexane, CH_2Cl_2 and MeOH extracts from the field cricket had the same potential α-glucosidase inhibition as the commercial drug acarbose. According to GC-MS analysis, these cricket extracts were mostly composed of diverse α-glucosidase inhibitors, including saturated fatty acids, unsaturated fatty acids and esters (Murugesu et al., 2018). The binding affinity of these inhibitors, such as palmitic acid, heptadecanoic acid, 1-monoplamitin and pentadecanoic acid, resulted in protein-ligand complex formation with α-glucosidase *via* hydrogen bonding and hydrophobic interactions (Murugesu et al., 2018). Other studies (Su et al., 2013; Teng and Chen, 2017) reported diverse fatty acids as having effective α-glucosidase inhibitory activity, including unsaturated fatty acids: palmitoleic acid (IC_{50} = 26.5 μM), oleic acid (IC_{50} = 64.2 μM), linoleic acid (IC_{50} = 73.8 μM) and α-linolenic acid (IC_{50} = 17.9 μM); and saturated fatty acids: myristic acid (IC_{50} = 1.22 mM), palmitic acid (IC_{50} = 21.3 μM) and stearic acid (IC_{50} = 22.2 μM). Almost all the extracts with potent anti-α-glucosidase activity contained more unsaturated fatty acids (37.5−55.9%) than saturated fatty acids (22.0−45.1%), as shown in Table 2. Considering efficient α-glucosidase inhibitory action of the unsaturated fatty acids, oleic acid was the major component in the short-tailed cricket extracts, while the maximum levels of linoleic acid were in both the field cricket and house domestic cricket extracts. Oleic acid and linoleic acid have been reported as competitive inhibitors (Su et al., 2013). Thus, almost all the crude extracts of the domestic house cricket showed no potential anti-α-glucosidase activity, since they contained less oleic acid than linoleic acid compared to the other cricket extracts.

Table 4 Inhibition potential of the cricket extracts for α-glucosidase, α-amylase, tyrosinase, and acetylcholinesterase (AChE)

Edible insect	Crude extract	IC_{50} (mg/mL)			
		α-glucosidase	α-amylase	tyrosinase	AChE
Domestic house cricket	Hexane	0.73±0.05^{d}	4.88±0.38^{ij}	0.14±0.02^{abc}	3.54±0.11^{c}
	CH_2Cl_2	0.07±0.01^{a}	4.97±0.19^{j}	0.29±0.04^{bcd}	0.38±0.04^{a}
	EtOAc	3.20±0.20^{f}	6.50±0.06^{k}	1.56±0.25^{g}	4.07±0.20^{d}
	MeOH	0.42±0.02^{c}	1.25±0.10^{cd}	0.44±0.01^{d}	3.59±0.09^{c}
Short-tailed cricket	Hexane	0.11±0.01^{ab}	4.34±0.07^{h}	0.33±0.05^{cd}	0.08±0.01^{a}
	CH_2Cl_2	0.06±0.00^{a}	2.84±0.06^{g}	0.08±0.00^{a}	0.07±0.01^{a}
	EtOAc	0.28±0.03^{abc}	2.09±0.11^{e}	0.07±0.00^{a}	1.75±0.05^{b}
	MeOH	0.26±0.01^{abc}	2.15±0.13^{ef}	0.05±0.01^{a}	2.14±0.09^{b}
Field cricket	Hexane	0.15±0.01^{ab}	2.38±0.06^{f}	0.65±0.07^{e}	1.76±0.21^{b}
	CH_2Cl_2	0.06±0.00^{a}	1.99±0.16^{e}	0.94±0.07^{f}	0.22±0.02^{a}
	EtOAc	0.37±0.03^{bc}	4.70±0.28^{i}	2.16±0.22^{h}	4.47±0.27^{e}
	MeOH	0.07±0.01^{a}	2.69±0.14^{g}	0.10±0.01^{ab}	6.30±0.34^{g}
Mole cricket	Hexane	3.59±0.30^{g}	0.95±0.01^{b}	2.45±0.17^{i}	8.51±0.42^{h}
	CH_2Cl_2	3.54±0.32^{g}	2.25±0.10^{ef}	1.38±0.18^{g}	3.26±0.40^{i}
	EtOAc	2.44±0.26^{e}	1.50±0.08^{d}	2.42±0.12^{i}	5.06±0.46^{f}
	MeOH	5.77±0.18^{h}	1.05±0.05^{bc}	0.90±0.14^{f}	6.15±0.24^{g}
Acarbose		0.12±0.00^{ab}	0.11±0.01^{a}	NM	NM
Kojic acid		NM	NM	0.11±0.01^{ab}	NM
Tacrine hydrochloride		NM	NM	NM	0.01±0.00^{a}

IC_{50} = half maximal inhibitory concentration; NM = not measured

Mean ± SD (n = 3) within each column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

Furthermore, several methyl ester derivatives that could act in α-glucosidase inhibition were found in the cricket extracts: hexadecanoic acid, methyl ester (methyl palmitate, IC_{50} = 51.8 µM); octadecanoic acid, methyl ester (methyl stearate, IC_{50} = 24.8 µM); (*Z*)-9-octadecenoic acid, methyl ester (methyl oleate, IC_{50} = 20.1 µM); (*Z,Z*)-9,12-octadecadienoic acid, methyl ester (methyl linoleate, IC_{50} = 47.5 µM); and (*Z,Z,Z*)-9,12,15-octadecatrienoic acid, methyl ester (methyl linolenate, IC_{50} = 46.7 µM), corresponding to other reports (Teng and Chen, 2017; Chelladurai and Chinnachamy, 2018). These esters were the highest (16.2%) in the MeOH extract from the field cricket. The hexane and EtOAc extracts from the short-tailed cricket had glycerol 1-palmitate as the glyceryl ester, which has been investigated as an α-glucosidase inhibitor (Nokhala et al., 2020).

The α-amylase inhibition of the crude extract of the mole cricket was greater than for the other cricket extracts. Nevertheless, these inhibition activity levels were less than for the standard drug acarbose. Diverse fatty acids, such as the α-amylase inhibitors have been reported in cricket extracts: tetradecanoic acid, palmitic acid (IC_{50} = 38.7 µg/mL); oleic acid (IC_{50} = 97.3 µg/mL); and linoleic acid (IC_{50} = 22.8 µg/mL) (Teng and Chen, 2017; Lakshmanasenthil et al., 2018). Tetradecanoic acid could form a tetradecanoic acid–α-amylase complex through hydrogen bonding at THR 314, ARG 346 and THR314 (Lakshmanasenthil et al., 2018). Cholesterol in the phytosterol class was identified in cricket extract, which can inhibit α-amylase *via* hydrogen bonding, Van der Waals force and electrostatic interaction based on investigation using molecular docking (Settu et al., 2021).

Tyrosinase inhibitory activity

The results of the analyses of anti-tyrosinase activity of the crude extracts from all crickets are shown in Table 4. Diverse crude extracts from the edible insects exhibited the same potential inhibition as the standard kojic acid, including from the short-tailed cricket (CH_2Cl_2, EtOAc and MeOH), domestic house cricket (hexane) and field cricket (MeOH). According to the GC-MS profiles, hexanoic acid was detected in the MeOH extract and octanoic acid was detected in the EtOAc extract of the short-tailed cricket, leading to effective anti-tyrosinase activity. Both hexanoic acid and octanoic acid were reported as a mixed-type inhibitor for tyrosinase *via* intermolecular hydrogen bonding, while pigmentation was monitored by fatty acid through the proteasome destruction of tyrosinase (Guo et al., 2010). Compared to the other cricket extracts, the short-tailed cricket extracts with the efficient anti-tyrosinase activity had a higher concentration of α-linolenic acid. The IC_{50} values of anti-tyrosinase activity of these extracts may depend on the fatty acid composition. Saturated fatty acids, including palmitic acid and stearic acid, exhibited no tyrosinase inhibitory activity, whereas unsaturated fatty acids, such as oleic acid, linoleic acid, and α-linolenic acid, have been reported as tyrosinase inhibitors by inducing tyrosinase degradation (Ando et al., 2004). Almost all the extracts from the domestic house cricket showed no tyrosinase inhibitory activity due to their high relative components of palmitic acid and stearic acid. From other studies, effective tyrosinase inhibitors, including heteroarylcoumarins (Pintus et al., 2017), benzylidene-linked thiohydantoin (Kim et al., 2014) and kojic acid-linked thio-quinazoline derivatives (Sepehri et al., 2021), also decreased the melanin contents relating to the anti-melanogenesis activity. Therefore, the cricket extracts could inhibit melanin synthesis.

Acetylcholinesterase inhibitory activity

The results of acetylcholinesterase inhibition of the crude extracts from the crickets are shown in Table 4. The CH_2Cl_2 extracts from the domestic house cricket, field cricket and short-tailed cricket, as well as the hexane extract from the shorted-tailed cricket, exhibited similar effective acetylcholinesterase inhibition to that of tacrine hydrochloride as the standard inhibitor, with significant levels (71.1−88.5%) of oleic acid, lineoic acid, palmitic acid and stearic acid in these extracts. Unsaturated fatty acids, including oleic acid (IC_{50} = 0.13±0.03 mg/mL) and linoleic acid (IC_{50} = 0.27±0.05 mg/mL), have been reported as acetylcholinesterase inhibitors (Öztürk et al., 2014). Of the saturated fatty acids, palmitic acid and stearic acid (IC_{50} > 4.0 mg/mL) have been reported to slightly inhibit acetylcholinesterase (Öztürk et al., 2014). However, compared to other crude extracts there was less effective inhibition for acetylcholinesterase, which was consistent with the results reported for polar cricket extract, which did not exhibit anti-acetylcholinesterase activity (Tel et al., 2010).

Cytotoxicity test

The cricket extracts for potential α-glucosidase and acetylcholinesterase inhibition were tested for cytotoxicity

activity against mouse subcutaneous connective tissue (L929) cells and the results are shown in Table 5. The crude extracts of the domestic house cricket (CH_2Cl_2), field cricket (CH_2Cl_2 and MeOH) and short-tailed cricket (hexane and CH_2Cl_2) showed slight cytotoxicity to L929 cells (less than 10%). The hexane extract of the short-tailed cricket produced the lowest cytotoxicity compared to the other extracts. The CH_2Cl_2 extract of the domestic house cricket had significant greater cytotoxicity than the other extracts. In addition, the crude extracts from the short-tailed cricket for tyrosinase inhibition were tested for cytotoxicity using HaCat cells. The CH_2Cl_2 and MeOH extracts produced slightly lower cytotoxicity than the EtOAc extract. These results suggested that the cricket extracts with IC_{50} values of α-glucosidase, acetylcholinesterase and tyrosinase inhibition produced no cytotoxicity in living cells. Hence, the utilization of cricket extracts as powerful inhibitors of glucosidase, acetylcholinesterase and tyrosinase has great promise in the field of bioactive chemical sourcing. These extracts may have potential in various applications, such as food production, pharmaceutical development, and cosmetics manufacturing.

Table 5 Cytotoxicity test of crude extracts from edible crickets

Cell type	Insect	Crude extract	Concentration (mg/mL)	Cytotoxicity (%)
L929	House cricket	CH_2Cl_2	0.07	7.7±0.1^{d}
	Field cricket	CH_2Cl_2	0.06	3.9±0.1^{b}
		MeOH	0.07	4.6±0.5^{c}
	Short-tailed cricket	Hexane	0.11	0.3±0.1^{a}
		CH_2Cl_2	0.06	4.0±0.2^{b}
HaCat	Short-tailed cricket	CH_2Cl_2	0.08	2.7±0.3*
		EtOAc	0.07	12.4±0.1**
		MeOH	0.05	2.8±0.3*

Mean ± SD (n = 3) within each column superscripted by different lowercase letters and symbols (*, **) are significantly ($p < 0.05$) different.

Conclusion

Extracts from several edible cricket insects in Thailand (*Acheta domesticus*, *Gryllus bimaculatus*, *Brachytrupes portentosus* and *Gryllotalpa Africana*) were successfully obtained using hexane (5−16% yield), CH_2Cl_2 (6−9% yield), EtOAc (2−3% yield) and MeOH (3−5% yield). The bioactive compound analysis identified alkaloids, saponins, steroids, phenolics and flavonoids in all crude extracts. The methanol extracts of the crickets had the highest phenolic and flavonoid contents. Furthermore, the chemical profiles based on GC-MS analysis of the cricket species identified carboxylic acids, esters, amides, alcohols, hydrocarbons and phytosterols. The unsaturated (37.5−71.8%) and saturated (22.1−38.2%) fatty acids were the major components. Notably, linoleic acid (omega-6) was identified in the field cricket and domestic house cricket extracts. The crude extracts from the domestic house cricket, short-tailed cricket and field cricket showed potential α-glucosidase and acetylcholinesterase inhibition. The crude extracts from the short-tailed cricket and field cricket showed anti-tyrosinase inhibition. However, none of the crude extracts from the cricket species showed any potential for α-amylase inhibition. From these results, the crude extract from these cricket species could be developed further for their biological activity as functional food ingredients, novel bio-resources, or for their whitening potential in cosmetics.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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