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Small bodies, big potential: Comprehensive nutritional and bioactivity evaluation of five edible insects from Indonesia

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ABSTRACT

Edible insects are increasingly valued as sustainable functional food sources due to their rich nutritional content, including proteins, essential fatty acids, vitamins, and minerals. This study investigated five insect species/forms traditionally consumed in Indonesia—*Phyllophaga helleri* (Puthul), *Valanga nigricornis* (*Belalang kayu*), *Gryllus bimaculatus* (Jangkrik), and both the adult (*Ulat jati*) and cocoon (*Entung jati*) forms of *Hyblaea puera*. Methanol and aqueous extracts were evaluated for their inhibitory activities against α-glucosidase, α-amylase, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE). Antioxidant and antimicrobial properties were assessed alongside volatile, amino acid, and elemental profiles using GC-MS, LC-MS, and ICP-MS. Volatile analyses revealed limonene (53.9–72.2%) as the dominant aroma compound in *Entung jati*, *Ulat jati*, *Belalang kayu*, and Puthul. Fatty acid profiles were rich in oleic acid (40.8–71.3%), especially in *Entung jati* and *Ulat jati*. Alanine and tyrosine were the most abundant amino acids, reaching up to 1945.36 and 4744.03 nmol/mL, respectively. Essential elements (K, Ca, Fe, Cu, Zn, Se) were abundant, while toxic elements (As, Cd, Hg, Pb) were below international safety limits. *Entung jati* methanol extract showed the strongest α-glucosidase inhibition (85.09%), exceeding acarbose (77.61%). Jangkrik exhibited the highest α-amylase inhibition (29.45%), and *Ulat jati* aqueous extract had the strongest antioxidant activity. Some methanol extracts also showed moderate antimicrobial effects (MIC = 625–1250 μg/mL). *P. helleri* extract showed cytotoxicity at high doses, but genotoxicity was not observed. These findings support the nutritional and therapeutic potential of Indonesian edible insects.

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1. Introduction

Although entomophagy is not widely embraced in Western societies, it is a common dietary practice in many regions globally, with over 2 billion people consuming insects as part of their traditional diets. More than 1900 edible insect species have been identified, offering significant nutritional benefits. On a dry weight basis, insects typically contain 40-60% protein, 12-35% fat, 5-15% fiber, and 3-10% ash, with an energy value ranging between 409 and 509 kcal/100 g-comparable to or exceeding that of conventional meats. Protein quality, digestibility, and a well-balanced amino acid profile make many insect species promising alternative protein sources. Insect fats, particularly rich in unsaturated fatty acids such as oleic, linoleic, and α-linolenic acids, also contribute to cardiovascular and metabolic health. Some species, like the black soldier fly, are notable for their high lauric acid content. Fatty acid profiles, along with cholesterol content (ranging from 7 to 100 mg/100 g), are largely influenced by diet. Beyond their nutritional value, insects have a long-standing place in traditional medicine across various cultures. Ancient records, including the Egyptian Ebers Papyrus, document medicinal uses of insects and arthropods. Traditional remedies include fly larvae for wound healing and beetle oils for rheumatism. Insects have been employed to manage over 350 ailments, ranging from digestive and respiratory disorders to endocrine and nervous system conditions. Despite this rich ethnomedical history, modern research has largely focused on insects as food, while their therapeutic applications remain underexplored. Emerging studies highlight insect-derived compounds-such as chitin, unsaturated fats, and bioactive peptides-for their potential in managing conditions like type 2 diabetes, immune dysfunction, and inflammation. With increasing global interest in sustainable nutrition and functional foods, edible insects offer a unique intersection of dietary value and medicinal potential (Bernard and Womeni, 2017; Kim et al., 2019; Lenaerts et al., 2019; Rumpold and Schlüter, 2013; Verheyen et al., 2018, 2021).

Diabetes mellitus (DM) is a growing global health issue, associated with serious complications such as cardiovascular disease, kidney damage, neuropathy, and retinopathy. Heart failure is also a common early manifestation in type 2 diabetes, with high mortality risk in both T1DM and T2DM patients (Tomic et al., 2022). In 2019, 463 million adults worldwide were living with diabetes-a number projected to reach 700 million by 2045. That year, diabetes caused an estimated 4.2 million deaths. The highest crude prevalence was in North America and the Caribbean (13.3%), while the Middle East and North Africa had the highest age-adjusted rates (12.8%). Global health spending on diabetes reached $760.3 billion in 2019. Once seen as a disease of wealthy nations, diabetes is now rising rapidly in developing countries, especially in urban areas (Verheyen et al., 2021).

Alzheimer's disease (AD) is the most common neurodegenerative cause of dementia in the elderly. Amyloid-β (Aβ) peptides are central to its pathogenesis. Current treatments, including acetylcholinesterase inhibitors and NMDA antagonists, offer limited relief and have side effects. Thus, there is growing interest in natural compounds targeting Aβ with better safety and efficacy (Zhang et al., 2021).

Epidemiological and biological studies indicate a strong association between type 2 diabetes mellitus (T2DM) and AD. Individuals with diabetes are at higher risk for cognitive decline, affecting memory, attention, processing speed, and executive functions. Structural brain changes such as cortical atrophy and reduced cerebral blood flow have been observed. Recent research highlights insulin resistance and disrupted brain insulin signaling as potential mechanisms linking T2DM and AD. Both hyperglycemia and recurrent hypoglycemia contribute to cognitive impairment, possibly through oxidative stress or neuronal damage. A longitudinal study showed a 65% higher risk of developing AD in diabetic individuals. Another study reported that up to 80% of AD patients had diabetes or glucose intolerance (Akter et al., 2011; Barbagallo and Dominguez, 2014). Malnutrition remains a major public health problem in many regions of the world, particularly in developing countries, where inadequate intake of high-quality protein is a key contributing factor. Previous studies have reported that childhood malnutrition continues to affect a considerable proportion of infants, leading to impaired growth, weakened immune function, and increased mortality risk (Black et al., 2013). In response to the growing need for sustainable and nutrient-dense food sources, edible insects have been proposed as a viable alternative protein source due to their high nutritional value and low environmental footprint (Huis et al., 2013).

Traditional Chinese medicine uses various animal-derived substances to support cognitive health in aging. These include *Mylabris phalerata* (blister beetle), *Scolopendra subspinipes mutilans* (centipede), and *Pheretima aspergillum* (earthworm), traditionally used to enhance brain function and manage dementia. Some contain compounds with potential metal-binding or neuroprotective properties, though mechanisms remain unclear. AD, the most common cause of dementia, involves oxidative stress, inflammation, metal ion imbalance, and cholinergic dysfunction. Current treatments focus on increasing acetylcholine or reducing glutamate toxicity, but their effects are limited. Natural antioxidants are being investigated for their ability to neutralize reactive oxygen species (ROS), which contribute to AD pathology (Ren et al., 2006).

Insects are increasingly recognized as a sustainable food source that can help address global food security. While one-third of the world's population-mainly in Asia, Africa, Australia, and Latin America-traditionally consumes insects (Aydoğan, 2021; Durst, 2010), insects and their products also serve various ethnoentomological roles, including use in traditional medicine, adhesives, toys, fishing bait, and folklore (Kuntadi et al., 2018; Raheem et al., 2019). Ethnoentomology refers to the cultural use of insects, while entomophagy specifically relates to their consumption as food. Currently, insect consumption is practiced in at least 113 countries, with over 2100 edible species recorded globally (Jongema, 2017; Mitsuhashi, 2016).

While insect-based foods offer nutritional benefits, some species may pose health risks. For example, consumption of *Anaphe venata* caterpillars in Africa has been linked to thiamine deficiency due to high thiaminase content, which can cause seasonal ataxia. Proper cooking can reduce this risk (Nishimune et al., 2000).

Peptide-based drugs like insulin remain central to diabetes management, enhancing glucose uptake in muscles and suppressing glucagon release from pancreatic alpha cells (Li et al., 2022).

Indonesia, known for its ethnic diversity, has a long history of entomophagy in several regions (Bodenheimer, 1951; Kuntadi et al., 2018). In Papua, locals consume up to 100 insect species, while in Java, *Hyblaea puera* pupae are collected during outbreaks. However, in some areas like Datengan, this tradition is fading (Afrianto et al., 2023). Insects are consumed in various forms-eggs, larvae, pupae, and adults-either raw or prepared by boiling, roasting, or frying. Preferences vary by region, ethnicity, taste, religious beliefs, and availability.

Evidence supports that edible insects provide high-quality proteins, essential fatty acids, vitamins and minerals (Jankowski et al., 2025) while also carrying potential hazards such as allergens and chemical contaminants (ANSES, 2015).

Despite Indonesia being an ethnoentomophagous country, in general, very little research has been conducted on the consumption of insects (Kuntadi et al., 2018; Lukiwati, 2010). As part of this study, according to anthropoentomophagic literature, we find that species of the order Coleoptera are popular among Indonesian people for consumption purposes. Table 1 revealed that 117 species of insects are taken as food by different tribes of Indonesia. Depending on the species, generally larva and adults, but sometimes all, developmental stages are consumed. Among these edible insect species, consumed by rural and urban people in Indonesia, was highest for Coleoptera accounting for about 22%, followed by Orthoptera (21%), Hymenoptera (17%), Odonata (10%), Lepidoptera (9%), Hemiptera (8%), Isoptera (7%), Mantodea (3%), and the least are Psocodea (1%) and Ephemeroptera (1%), Phasmatodea (1%) (Fig. 1).

A list of edible insects in Indonesia, distribution by species, common English name, local name, stage of consumption and references are given in Table 1. Accordingly, frying, roasting, boiling, steaming and drying are common traditional methods. Insects are consumed at different life stages, such as eggs, larvae, pupae and adults.

According to a literature written by (Gahukar, 2011), *Trigona biroi* is misspelled, corrected as *Tetragonula biroi* in this study. Similarly, *Erionota thrax* misspelled, corrected as *Erionata thrax*; *Megapis dorsata* misspelled, corrected as *Apis dorsata*. There is no taxonomic information on *Tramea limhata euryala*, *Dundubia* sp., *Trigona* sp. which are given in (van der Meer Mohr, 1965), hence, they was not added to the list.

This study investigates the composition of toxic metal(oid) contents of four of the commonly eaten edible insects. There was no comprehensive report identified related to edible insects in Indonesia. Hence, an update of the list of edible insects, an assessment of the diversity of commonly eaten insects and their taxonomic analysis has been carried out. Additionally, this study investigated the cholinesterase inhibitory effects on acetylcholinesterase and butyrylcholinesterase enzymes, as well as the inhibitory activities against α-amylase and α-glucosidase enzymes. The antimicrobial, antioxidant, and genotoxic activities of aqueous and methanol extracts from five edible insects were also evaluated. The samples were subjected to quantitative analysis of 43 amino acids using LC-MS/MS, and their volatile composition was analyzed using GC-MS. To date, most studies on entomophagy have focused primarily on nutritional composition, consumer perception, and sustainability aspects, while relatively few have examined the biochemical and pharmacological potential of edible insects in a mechanistic context. Recent reviews emphasize the importance of integrating compositional, functional, and molecular-level analyses to better understand the health-promoting potential of insect-derived compounds (Jankowski et al., 2025; Pali-Schöll et al., 2019; Lange and Nakamura, 2021). In contrast to previous studies, the present work integrates comprehensive chemical profiling (GC–MS, LC–MS/MS, and ICP–MS) with in vitro enzymatic inhibition assays and biological activity evaluations to provide an in-depth characterization of edible insects traditionally consumed in Indonesia. This multidisciplinary approach enables a detailed assessment of their nutritional composition, bioactive compound profiles, and functional properties, including antioxidant, antidiabetic, antimicrobial, and enzyme inhibitory activities.

Rather than relying solely on compositional data, this study links chemical constituents with observed biological effects, thereby offering insight into the potential mechanisms underlying the antidiabetic and neuroprotective activities of these insects. By combining ethnobiological knowledge with modern analytical and bioactivity-based methodologies, this work contributes to bridging the gap between traditional consumption practices and scientific validation of edible insects as functional and health-promoting food sources.

2. Materials and methods

2.1. Study area

The fieldwork was performed from March to September 2022 in Indonesia. Indonesia has the fourth largest population in the world, it is a huge country, with more than 18,000 islands. It is the largest archipelago in the world that lies across the Equator. Also, it is a tropical country and has the third largest forest area in the world, so it is home to a great diversity of insects, many of them are edible (Fig. 2).

2.2. Collection and preparation of insect samples

Insect specimens were collected with the assistance of local residents in Indonesia, primarily during early morning hours when insect activity was optimal. Collection methods included handpicking and the use of entomological nets, particularly for capturing flying species during the morning and late afternoon. The selection of target insects was informed through direct communication with international undergraduate and graduate students at Atatürk University who traditionally consume these species (Fig. 3). The collected insects were identified by Prof. Dr. Ümit İncekara and Assoc. Prof. Dr. Zeynep Aydoğan. Representative samples are preserved in alcohol for reference purposes and stored in the laboratory collection at Atatürk University.

In total, five insect samples were obtained, representing four species, including two developmental forms of *Hyblaea puera*:

Table 1
Taxonomy of common edible insects in Indonesia. Each data indicates the scientific name, order, family, English and local name, edible life stage, eating preference according to the world peer-reviewed literature.

Order	Family	Species	English and Vernacular Name	Edible Life Stage	Eating Preference	Country	References
Coleoptera	Cerambycidae	*Batocera* spp. Castelnau, 1840	Ngatit, Buang Gavor	Adult	Toasted or grilled	Papua New Guinea, Indonesia, Malaysia Sabah	(Chung et al., 2003; Sabri et al., 2023)
		Batocera gigas Drapiez, 1819	mokah	Larva		Indonesia	Jongema (2017)
		Batocera rubus (Linnaeus, 1758)		Larva		Indonesia, India, Laos, Sri Lanka	Jongema (2017)
		Batocera tigris Voet, 1778		Larva		Indonesia	Jongema (2017)
		Batocera wallacei Thomson, 1858	Wallace's long-horn beetle	Adult		Indonesia	(Jongema, 2017; Ramandey and Mastrigt, 2010)
		Rosenbergia mandibularis Ritsema, 1881	Juwar	Adult		Indonesia	Ramandey and Mastrigt (2010)
		Macrotoma sp. Audinet-Serville, 1832		Adult		Indonesia, Malaysia Sabah	Mitsuhashi (2016)
		Osphryon sp. Pascoe, 1869				Indonesia	Mitsuhashi (2016)
	Dryophthoridae	*Protocerius* sp. Schoenherr, 1838	Giant weevil	Adult Larva	Adults are roasted, larvae are stir-fried, boiled, or cooked with rice.	Indonesia, Malaysia	(Pemberton, 1995; Sabri et al., 2023)
	Curculionidae	*Rhynchophorus bilineatus* (Montrouzier, 1857)	Sago beetle/grubs/ worms	Larva Adult	Raw or roasted	Indonesia	Ramandey and Mastrigt (2010)
		Rhynchophorus ferrugineus (Olivier, 1790)	Red stripe weevil, red palm weevil, ulat sagu, butod, wutod, tobindok, sungut, wot kinangan, sungkut oelar ratan, gendon, sabeta, oelar sagoe, or olakna mandjalin	Larva Adult	Eaten raw or cook in porridge with thin slices of ginger or stir-fried with shallots and soy sauce. Sometimes, grilled or toasted.	Thailand, Indonesia, Malaysia, Philippines, Vietnam, Sri Lanka, Laos	(Lukiwati, 2010; Pemberton, 1995; Sabri et al., 2023)
		Rhynchophorus richteri Wattanapongsiri, 1966	Singgabit			Indonesia	Jongema (2017)
		Rhynchophorus palmarum (Linnaeus, 1758)	cucarrón, cigarrón, casanga, suri or cocotero chontacuro, Mojojoy,	Larva		Indonesia, Ecuador, South America, Colombia	DeFoliart (1999)
		Behrensiellus glabradus Lona, 1937	Gulangge jangge			Indonesia	Ramandey and Mastrigt (2010)
	Dytiscidae	*Cybister* sp. Curtis, 1827	tangkub, ulod sek			Indonesia	Sabri et al. (2023)
		Cybister guerini (Aubé, 1838)		Adult		Indonesia, China, Japan	(Jongema, 2017; Siddiqui et al., 2023)
		Cybister tripunctatus (Olivier, 1795)	Diving beetle	Larva Adult		Thailand, Indonesia, India	(Jongema, 2017; van der Meer Mohr, 1965)

(*continued on next page*)

Table 1 (*continued*)

Order	Family	Species	English and Vernacular Name	Edible Life Stage	Eating Preference	Country	References
	Lucanidae	*Odontolabis celebensis* Leuthner 1885	Stag beetle, tangkayabang, buang lilot	Adult		Malaysia, Sabah, Indonesia, Kalimantan	(Jongema, 2017; Sabri et al., 2023)
	Scarabaeidae	*Chalcosoma moellenkampi* Kolbe, 1900	Moellenkampi beetle	Larva Adult		Indonesia, Malaysia, Sabah	(Chung, 2010; Pemberton, 1995)
		Chalcosoma atlas (Linnaeus, 1758)	Atlas beetle, Kumbang tanduk, golihot, rangoi, buang unga	Larva		Indonesia, Malaysia, Sabah	(Chung et al., 2003; Lukiwati, 2010)
		Exopholis hypoleuca (Wiedemann, 1819) syn. *Melolontha hypoleuca* Wiedemann, 1819		Adult		Indonesia, Malaysia Sabah	(Jongema, 2017; Mitsuhashi, 2016)
		Lepidiota stigma Fabricius, 1798	Sugarcane white grub, buang mapoh	Larva Adult		Thailand, Indonesia India, Sri Lanka	(Chung, 2010; Mitsuhashi, 2016; Pemberton, 1995; Sabri et al., 2023)
		Leucopholis rorida (Fabricius, 1801)	White grub	Adult		Indonesia	Jongema (2017)
		Psilopholis vestita (Sharp, 1881)				Indonesia	Jongema (2017)
		Xylotrupes gideon (Linnaeus, 1767)	Brown rhinoceros beetle	Larva Adult		Thailand, Myanmar, India, Sabah, Malaysia, Indonesia	Ramandey and Mastrigt (2010)
		Phyllophaga helleri (Hope, 1837) Syn. *Holotrichia helleri* Brenske, 1894	Puthul			Indonesia	Jongema (2017)
	Tenebrionidae	*Tenebrio molitor* Linnaeus, 1758	Yellow mealworm	Larva		Indonesia	(Adámková et al., 2017; Kuntadi et al., 2018)
		Zophobas morio (Fabricius, 1776)	Giant mealworm			Indonesia	(Adámková et al., 2017; Kuntadi et al., 2018)
Hemiptera	Alydidae	*Leptocorisa acuta* (Thunberg, 1783)	Paddy bug	Adult		Indonesia, India	Jongema (2017)
		Leptocorisa oratoria (Fabricius, 1764)	Rice bug, rice ear bug, pesisang	Adult	Raw	Indonesia, Malaysia, Sabah	(Chung, 2010; Pemberton, 1995)
	Belostomatidae	*Sphaerodema rusticum* Distant, 1906		Adult		Indonesia, Thailand	(Jongema, 2017; van der Meer Mohr, 1965)
	Nepidae	*Laccotrephes robustus* Stal, 1871	Water scorpion			Indonesia	(van der Meer Mohr, 1965)
	Pentatomidae	*Nezara viridula* (Linnaeus, 1758)	southern green stink bug (USA), southern green shield bug (UK), green vegetable bug (Australia, New Zealand), babukulit, tangkayomot, kaber bilat (Indonesia)	Adult Larva	raw	Indonesia, India, Malaysia, Sabah, Kalimantan	(Pemberton, 1995; Ramandey and Mastrigt, 2010; Sabri et al., 2023)
		Cosmopsaltria aurata Duffels 1983		Adult		Indonesia	Dossey et al. (2016)
		Cosmopsaltria giganta occidentalis Duffels 1983		Adult		Indonesia	Dossey et al. (2016)

(*continued on next page*)

Table 1 (*continued*)

Order	Family	Species	English and Vernacular Name	Edible Life Stage	Eating Preference	Country	References
		Cosmopsaltria papuensis Duffels, 1983		Adult		Indonesia	Dossey et al. (2016)
		Cosmopsaltria signata Duffels 1983		Adult		Indonesia	Dossey et al. (2016)
		Cosmopsaltria waine Duffels, 1983	Cicadas	Adult	Roasted or Stir-Fried	Indonesia	Gahukar (2011)
		Dundubia spp. Amyot and Audinet-Serville, 1843	Light green cicadas, tavir, sebeper	Adult	Roasted or Stir-Fried	Indonesia, Malaysia	(Gahukar, 2011; Sabri et al., 2023)
		Megapomponia merula (*Pomponia merula*) (Distant, 1905)	Riang-riang, Pagam, Sebeper, Cicada	Adult	Stir-Fried or Deep-Fried	Indonesia, Malaysia	(Chung, 2010; Pemberton, 1995; Sabri et al., 2023)
		Orientopsaltria spp. Kato, 1944		Adult	Roasted or Stir-Fried	Indonesia	Gahukar (2011)
		Platylomia sp. Stål, 1870		Adult		Indonesia, Thailand, Malaysia	(Jongema, 2017; van der Meer Mohr, 1965)
Hymenoptera	Apidae	*Apis cerana* Fabricius 1793	Indian honey bee	Larva Pupa		Indonesia, Thailand Philippines, India	(Chung, 2010; Pemberton, 1995)
		Apis (Apis) cerana javana Enderlein 1906				Indonesia	(van der Meer Mohr, 1965)
		Apis dorsata Fabricius, 1793	giant honey bee, rock bee	Larva Pupa		Indonesia, Philippines, Thailand, India, Nepal	(Chung, 2010; Pemberton, 1995)
		Apis mellifera Linnaeus, 1758		Larva Pupa		Indonesia	Afrianto et al. (2023)
		Apis florea Fabricius, 1787		Larva Pupa		Indonesia, Thailand Philippines, Malaysia, India, Nepal, Sabah	(Dossey et al., 2016; Gahukar, 2011)
		Tetragonula biroi (Friese, 1898)		Larva Pupa	boiled with porridge or rice, stir-fried or drunk together with honey	Indonesia	Gahukar (2011)
		Bombus sp.				Indonesia	(Jongema, 2017; van der Meer Mohr, 1965)
		Nannotrigona minuta (Lepeletier, 1836)		Larva Pupa		Indonesia	Jongema (2017)
		Melipona minuta Lepeletier de Saint Fargeau, 1836 (incertae sedis)		Larva Pupa		Indonesia	(Jongema, 2017; Mitsuhashi, 2016)
		Melipona vidua Lepeletier de Saint Fargeau 1836		Larva Pupa		Indonesia	(Jongema, 2017; Mitsuhashi, 2016)
		Homotrigona (*Tetrigona*) *vidua* (Lepeletier de Saint Fargeau)	stingless bee	Larva Pupa		Indonesia	Jongema (2017)
		Xylocopa aestuans (Linnaeus, 1758)	carpenter bee	Adult Larva Pupa		Thailand, Indonesia, Malaysia, Sabah	Jongema (2017)
		Xylocopa latipes (Drury, 1773)	broad-handed carpenter bee, tawon endas	Larva Pupa	fried with butter or onion and salt	Indonesia	Lukiwati (2010)

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Table 1 (*continued*)

Order	Family	Species	English and Vernacular Name	Edible Life Stage	Eating Preference	Country	References
	Formicidae	*Dinomyrmex gigas* (Latreille, 1802) Syn. *Camponotus gigas*	Giant rainforest ant, carpenter ant	Pupa Adult Larva	Used as a flavouring agent	Malaysia, Indonesia Brunei	Sabri et al. (2023)
		Crematogaster vandermeermohri Menozzi, 1930	acrobat ant	Larva Pupa		Indonesia	(van der Meer Mohr, 1965)
		Oecophylla smaragdina Fabricius, 1775	Kerengga, laga, haga, tontoh, tandarapas	Egg Larva Pupae	mixed with chili and salt or stir-fried with onions	Indonesia, Malaysia	Sabri et al. (2023)
	Vespidae	*Provespa anomala* (Saussure, 1854)	nihgt wasp	Larva		Indonesia, Kalimantan	(Chung, 2010; Pemberton, 1995)
		Provespa nocturna Vecht, 1935				Indonesia	(van der Meer Mohr, 1965)
		Vespa analis Fabricius, 1775	Golden wasp Kogata-suzumebachi	Larva Pupa		China, Japan, Taiwan, Indonesia	Mitsuhashi (2016)
		Vespa ducalis Smith, 1852	Small yellow jacket	Larva Pupa Adult		China, Indonesia	Mitsuhashi (2016)
		Vespa multimaculata Perez-Santaescolastica et al., 2022				Indonesia	Jongema (2017)
		Vespa tropica Linnaeus, 1758	greater banded hornet	Larva Pupa Adult		Thailand, Indonesia, Malaysia, Sabah, China India, Laos	(Jongema, 2017; van der Meer Mohr, 1965)
		Vespa luctuosa Saussure, 1854				Indonesia	(van der Meer Mohr, 1965)
		Vespa velutina (Lepeletier, 1836)		Larva Pupa		Indonesia, China, Taiwan, Thailand	Mitsuhashi (2016)
		Ropalidia sp. Guérin-Méneville 1831			raw or boiled with rice	Indonesia, Malaysia	Pemberton (1995)
Isoptera	Termitidae	*Macrotermes* sp. Holmgren, 1910		Adult		Indonesia	Jongema (2017)
		Macrotermes gilvus (Hagen, 1858)	tanai, kelkatu laron		Raw, stir-fried without oil, or cooked	Indonesia, Malaysia	(Afrianto et al., 2023; Sabri et al., 2023)
		Odontotermes sp. Holmgren, 1912				Indonesia, India, Laos	(van der Meer Mohr, 1965)
		Odontotermes javanicus Holmgren, 1912				Indonesia	Afrianto et al. (2023)
		Termes atrox Smeathman 1781		Adult	Roasted with flour or baked as a cake	Indonesia	(Jongema, 2017; Mitsuhashi, 2016)
		Termes fatalis Linnaeus, 1758		Adult	Roasted with flour or baked as a cake	Indonesia	Mitsuhashi (2016)
		Termes mordax Smeathman, 1781		Adult		Indonesia	(Jongema, 2017; Mitsuhashi, 2016)
		Termes destructor De Geer, 1778		Adult	Roasted with flour or baked as a cake	Indonesia, Guyana	(Jongema, 2017; Mitsuhashi, 2016)
Lepidoptera	Bombycidae	*Bombyx mori* (Linnaeus, 1758)	silkworm	Larva Pupa		Thailand, China, Korea, Indonesia, Vietnam,	Kuntadi et al. (2018)

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Table 1 (*continued*)

Order	Family	Species	English and Vernacular Name	Edible Life Stage	Eating Preference	Country	References
						Myanmar, India, Japan	
	Hesperiidae	*Erionata thrax* (Linnaeus, 1767)	banana skipper	Larva Pupa		Indonesia, Malaysia, Sabah, Thailand	(van der Meer Mohr, 1965)
	Hyblaeidae	*Hyblaea puera* (Cramer, 1777)	Moth cocoon, teak defoliator, *Ulat jati*, entung jati, entong, oengker	Larva Pupa	The cocoon is boiled until dry and stirred.	Indonesia	(Lukiwati, 2010; Sabri et al., 2023)
	Papilionidae	*Papilio lagleizei* Depuiset, 1877		Larva		Indonesia	Ramandey and Mastrigt (2010)
	Pieridae	*Catopsilia* sp. Hübner (1819)				Indonesia	(van der Meer Mohr, 1965)
		Eurema sp. Hübner (1819)				Indonesia	(van der Meer Mohr, 1965)
	Saturniidae	*Cricula trifenestrata* Helfer, 1837		Pupa		Indonesia	Jongema (2017)
		Syntherata apicalis Bouvier, 1928		Larva		Indonesia	Ramandey and Mastrigt (2010)
	Uraniidae	*Nyctalemon patroclus goldiei*		Larva		Indonesia, Papua	(Durst, 2010)
	Sphingidae	*Daphnis hypothous* (Cramer, 1780)				Indonesia	Mitsuhashi (2016)
		Theretra nessus (Drury, 1773)	Kiiro-suzume	Adult		Indonesia, China	Mitsuhashi (2016)
		Acherontia lachesis (Fabricius, 1798)	dunkala	Larva		Indonesia	(Ramandey and Mastrigt, 2010; van der Meer Mohr, 1965)
		Acherontia styx Westwood, 1847		Larva		Indonesia, Japan	(van der Meer Mohr, 1965)
		Agrius convolvuli (Linnaeus, 1758) Syn. *Herse convolvuli*		Larva		Botswana, Indonesia, South Africa, Zambia, Japan	(van der Meer Mohr, 1965)
Odonata	Aeshnidae	*Anax guttatus* (Burmeister, 1839)	hairy emperor	Adult		Indonesia, Thailand Laos	(van der Meer Mohr, 1965)
		Tetracanthagyna spp. Selys, 1883	Siferla	Adult Nymph	Wings removed, then lightly salted and boiled.	Indonesia	Sabri et al. (2023)
	Libellulidae	*Cratilla lineata assidua* Lieftinck, 1953		Adult		Indonesia	(Jongema, 2017; Mitsuhashi, 2016)
		Crocothemis sp.	river skimmers	Adult Nymph Larva		Indonesia, Vietnam, Laos, Myanmar, Thaliand	(Jongema, 2017; Mitsuhashi, 2016)
		Crocothemis servilia (Drury, 1773)		Adult Nymph		Indonesia, India	Jongema (2017)
		Neurothemis ramburii (Kaup in Brauer, 1866)				Indonesia	Mitsuhashi (2016)
		Orthetrum glaucum (Brauer, 1865)	kuyu, siferla	Adult		Indonesia	Sabri et al. (2023)
		Orthetrum sabina (Drury, 1770)				Indonesia	Mitsuhashi (2016)
		Pantala flavescens (Fabricius, 1798)	globe skimmer, globe wanderer, wandering glider, capung ciwet	Adult		Indonesia, China	(Lukiwati, 2010; van der Meer Mohr, 1965)

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Table 1 (*continued*)

Order	Family	Species	English and Vernacular Name	Edible Life Stage	Eating Preference	Country	References
		Potamarcha obscura (Ramandey and Mastrigt, 2010)		Adult		Indonesia	Jongema (2017)
		Tramea transmarina euryale Selys, 1878				Indonesia	Jongema (2017)
		Tramea limbata (Desjardins, 1832)				Indonesia	(van der Meer Mohr, 1965)
		Trithemis aurora (Burmeister, 1839)		Adult		Indonesia	Jongema (2017)
Orthoptera	Acrididae	*Acrida* spp. Linnaeus, 1758				Indonesia, Malaysia	Sabri et al. (2023)
		Choroedocus spp. Bolívar, 1914				Indonesia	Sabri et al. (2023)
		Cyrtacanthacris tatarica (Linnaeus, 1758) syn Acridium aeruginosum	black spotted, walang, belalang, djankirk, gansir, walang gapoek and belalang gambar	Adult		Thailand, Indonesia	(DeFoliart, 1999; Jongema, 2017)
		Gastrimargus marmoratus (Thunberg, 1815)				Indonesia	(Jongema, 2017; van der Meer Mohr, 1965)
		Melanoplus cinereus Scudder, 1878	jangkrik	Adult	Fried Roasted	Indonesia	Adi et al. (2020)
		Oxya chinensis (Thunberg, 1815)				Indonesia	(Jongema, 2017; van der Meer Mohr, 1965)
		Patanga succincta (Johannson, 1763) syn. *Nomadacris succincta*	Bombay locust, paddy locust, belalang patanga	Adult		Indonesia, Thailand, Philippines	(Kuntadi et al., 2018; Lukiwati, 2010)
		Phlaeoba antennata Brunner von Wattenwyl, 1893		Adult		Indonesia, India	(Jongema, 2017; van der Meer Mohr, 1965)
		Stenocatantops splendens (Thunberg, 1815)				Indonesia	(Jongema, 2017; Sabri et al., 2023)
		Valanga nigricornis (Burmeister, 1838)	Wooden grasshopper, Javanese grasshopper, Javan locust, Javan bird locus, *Belalang kayu*, belalang kunyit, kato mapoh		After removing legs and wings, salted, boiled and simmered or deep-fried, stir-fried, roasted. Marinated in palm sugar and spices before frying, also served with thiwul.	Indonesia, Malaysia, Sabah, Thailand	(Jongema, 2017; Kuntadi et al., 2018; Lukiwati, 2010; Sabri et al., 2023)
		Xenocatantops humilis (Serville, 1838)				Indonesia	(Durst, 2010)
	Gryllidae	*Acheta domesticus* (Linnaeus, 1758)	House cricket	Adult	Dry roasting, deep- fried	Indonesia	Adámková et al. (2017)
		Tarbinskiellus portentosus (Lichtenstein, 1796) Syn. *Brachytrypes portentosus* (Lichtenstein, 1796)	Gangsir			Thailand, India, Myanmar, Indonesia, Vietnam	(van der Meer Mohr, 1965)
		Gryllus assimilis (Fabricius, 1775)				Indonesia	Adámková et al. (2017)
		Gryllus bimaculatus De Geer, 1773	Two spotted cricket, Jangkrik	Nymph Adult		Indonesia	Magara et al. (2021)

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Table 1 (*continued*)

Order	Family	Species	English and Vernacular Name	Edible Life Stage	Eating Preference	Country	References
		Gymnogryllus elegans syn. *Gymnogryllus leucostictus* (Burmeister, 1838)				Indonesia	(van der Meer Mohr, 1965)
		Gryllus mitratus Burmeister, 1838 Syn. *Teleogryllus mitratus* (Burmeister, 1838)				Indonesia, Thailand, Laos	(van der Meer Mohr, 1965)
		Nisitrus vittatus (Haan, 1842)	Gentirik		Frying without oil	Indonesia, Malaysia	(Sabri et al., 2023; Sabri et al., 2023)
	Gryllotalpidae	*Gryllotalpa* sp.	Mole crique			Indonesia, Philippines, India	(DeFoliart, 1999; Jongema, 2017)
		Gryllotalpa africana (Pali-Schöll et al., 2019)	African mole cricket	Nymph Adult		Indonesia, Thailand, Philippines, Vietnam, India, Laos	(Jongema, 2017; van der Meer Mohr, 1965)
	Tettigoniidae	*Mecopoda elongata* (Linnaeus, 1758)	Wisin, kato buyot	Adult	After removing legs and wings, salted, boiled and simmered or deep-fried, stir-fried, roasted.	Indonesia, Malaysia Thailand, Laos, India	(Sabri et al., 2023; van der Meer Mohr, 1965)
	Pyrgomorphidae	*Atractomorpha crenulata* Fabricius, 1793	Tobacco grasshopper, buntak	Adult	After removing legs and wings, salted, boiled and simmered or deep-fried, stir-fried, roasted.	Indonesia	Sabri et al. (2023)
		Atractomorpha psittacina Haan, 1842	Pointed-nose grasshopper, tombuzungus, kato tachong	Adult	After removing legs and wings, salted, boiled and simmered or deep-fried, stir-fried, roasted.	Indonesia, Malaysia	Sabri et al. (2023)
Mantodea	Mantidae	*Hierodula venosa* Olivier, 1792		Larva		Indonesia	Jongema (2017)
		Hierodula vitrea (Stoll, 1813)				Indonesia	(van der Meer Mohr, 1965)
		Tenodera aridifolia (Stoll, 1813)				Indonesia	(van der Meer Mohr, 1965)
		Tenodera sinensis (Saussure, 1871)	Praying mantis, kato gadang	Adult	Grilled on an open fire	Indonesia, Thaliand	Sabri et al. (2023)
Psocodea	Pediculidae	*Pediculus humanus* Linnaeus, 1758		Adult		Indonesia	Jongema (2017)
Ephemeroptera	Ephemerellidae	*Ephemeroptera* sp.	laron	Adult		Indonesia, Vietnam, Kalimantan	Jongema (2017)
Phasmatodea	Heteropterygidae	*Haaniella grayii* (Westwood, 1859)		Egg		Indonesia	Gahukar (2011)

1. *Phyllophaga helleri* (puthul)
2. *Valanga nigricornis* (belalang kayu)
3. *Gryllus bimaculatus* (jangkrik)

4-a) *Hyblaea puera* (ulat jati – caterpillar form)
4-b) *Hyblaea puera* (entung jati – cocoon form)

After collection, insects were initially sun-dried and stored in pre-cleaned glass jars. Samples were transported to the laboratory at Atatürk University, Türkiye, for identification and further analysis. In the laboratory, all specimens were oven-dried at 40 °C for 15 h to ensure moisture removal without degrading sensitive compounds. The dried insects were then finely powdered to facilitate extraction and analytical procedures (Fig. 4).

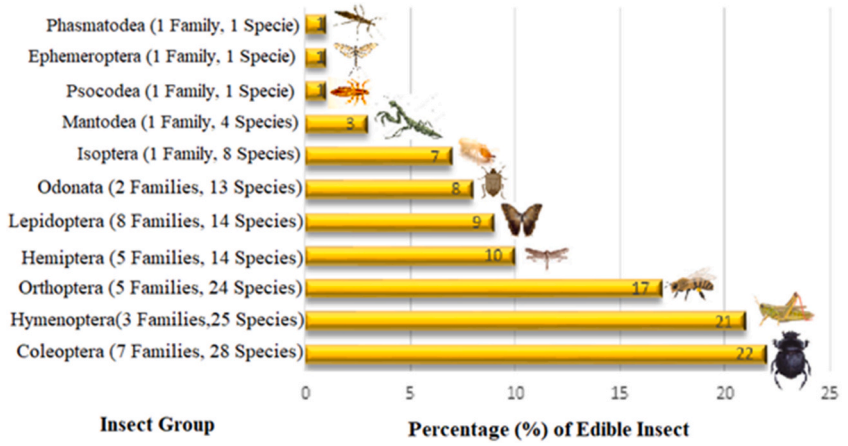


Fig. 1. Percentage of most commonly consumed insects by order, in Indonesia.



Fig. 2. Map of Indonesia.

2.3. Preparation of methanol and water extracts from insects

Insect samples were first dried and finely ground to a uniform powder. Precisely weighed portions (5–10 g) of the powdered material were extracted separately with methanol (100 mL) and distilled water (50 mL). For the methanol extraction, samples were macerated at room temperature for 72 h (24 h × 3 days) with occasional shaking, using an appropriate solvent-to-sample ratio. The mixture was filtered every 24 h, and the combined filtrates were concentrated under reduced pressure using a rotary evaporator until complete solvent removal. For the aqueous extraction, distilled water was added at the defined ratio, and the mixture was extracted under the same conditions. After filtration, the aqueous extracts were either freeze-dried or concentrated under vacuum.

To ensure methodological transparency, the concentrations and extraction yields were determined and reported in Table 2. The methanolic extracts ranged from 0.7769 to 1.846 g, corresponding to yields of 28.30–58.30%, while the aqueous extracts ranged from 69.8 to 544.5 mg, corresponding to yields of 4.50–27.45%. All extracts were stored in airtight containers at appropriate temperatures until further analysis.

2.4. Headspace-SPME

The manual SPME device (Supelco, Bellafonte, PA, USA) with a fibre precoated of a 65 μm thick layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB-blue) was used for extraction of the edible insect volatiles. The vial containing the dried insect (0.5 g) was sealed with parafilm. The fiber was pushed through the film layer for exposure to the headspace of the extract for 15 min at 50 °C. The



Fig. 3. The lady collecting insect samples for this study eats some of the insects she collects.



Fig. 4. Powdering the collected insects after drying.

Table 2
Powdered, extract amounts and yield of samples.

Samples	Puthul	*Belalang kayu*	Jangkrik	*Ulat jati*	*Entung jati*
Powdered (g)	1.9840	2.8102	1.5531	3.6243	3.9876
Methanol (g)	1.1566	1.1846	0.7769	1.5210	1.1285
Aqueous (mg)	544.5	148.1	69.80	366.6	462.5
Samples	**Yield (%)**				
Methanol	58.30	42.15	50.02	41.97	28.30
Aqueous	27.45	5.27	4.50	10.12	11.60

fiber was then inserted immediately into the injection port of the GC-MS for the desorption of the adsorbed volatile compounds for analysis.

2.5. Analysis of volatile compounds

The volatiles were analyzed by GC-MS using a Hewlett Packard GCD system. An HP-Innowax FSC column (60 m × 0.25 mm inner diameter, with 0.25 mm film thickness) was used with helium as carrier gas (1 ml/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, then kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min, at splitless mode. The injector temperature was at 250 °C. EI-mass spectra were recorded at 70 eV. Mass range was from 35 to 425 *m/z*.

2.6. Identification of components

Identification of the components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder Software 4.0), and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils (Hochmuth, D. H., 2008; McLafferty, 1989).

2.7. Chemical compositions of fatty acids

Fatty oils were obtained from the dried insect samples (0.5 g) by Soxhlet apparatus with *n*-hexane. Analysis was performed after the methylation process (Azcan et al., 2004).

2.8. Amino acid measurement analysis by LC-MS/MS

In Eppendorf tubes, 50 μL of samples was placed for both experimental and control groups. By adding 50 μl of internal standard, vortexing was performed for 10 s. To each tube, 700 μL of amino acid solvent solution (mobile phase A, mobile phase B, V:V, 1:4) was added. After vortexing each sample for 1 min, it was centrifuged at 4 °C for 8 min. A 0.45 μm filter was used for the separation and filtration of the supernatant. The LC-MS/MS system (Agilent 6460 Triple Quadrupole, USA) was used for measuring the samples (Atila et al., 2021).

2.9. Elemental analysis

Elemental analysis was conducted to determine both nutritionally essential and potentially toxic elements in insect species traditionally consumed as food. The examined elements were classified into two categories:

- *Nutritional elements*: Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca), Manganese (Mn), Iron (Fe), Zinc (Zn), Cobalt (Co), Strontium (Sr), Rubidium (Rb), and Selenium (Se)
- *Potentially toxic elements*: Aluminum (Al), Scandium (Sc), Chromium (Cr), Arsenic (As), Cesium (Cs), Barium (Ba), Lanthanum (La), Cerium (Ce), Samarium (Sm), and Uranium (U)

All insect samples were oven-dried at 40 °C for 15 h to remove moisture content. The dried specimens were ground into fine powders, and 1.0 g of each sample (on a dry weight basis) was accurately weighed for elemental analysis. The measurements were carried out using an inductively coupled plasma mass spectrometry (ICP-MS) instrument at the Eastern Anatolian High Technology Application and Research Center (DAYTAM), Atatürk University. Analytical results were expressed on a dry weight basis and compared with maximum permissible limits set by international regulatory authorities, including the Environmental Protection Agency (EPA) and the World Health Organization (WHO), to assess the nutritional value and safety of insect consumption.

2.10. Biological activity studies

2.10.1. α-Glucosidase enzyme inhibition assay

The determination of α-glucosidase inhibitory activity was conducted with some modifications to the method described by Bachhawat et al. (2011). In a 96-well microplate, 50 μL of phosphate buffer (50 mM, pH 6.9), 10 μL of α-glucosidase enzyme (1 Unit/mL), and 20 μL of insect extracts (concentration range: 1-5000 μg/mL) was mixed and incubated at 37 °C for 5 min. Then, 20 μL of 3 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) was added as a substrate, and the mixture was incubated at 37 °C for 30 min. The reaction was stopped by adding 50 μL of 0.1 M sodium carbonate. All solutions was prepared in the buffer system. Acarbose was used as a positive control. The amount of yellow-colored p-nitrophenol (pNP) formed was determined at 405 nm. Each assay was repeated three times. The results was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

2.10.2. α-Amylase enzyme inhibition assay

The determination of α-amylase inhibitory activity was carried out according to Nampoothiri et al. (2011). In a microplate, equal volumes (100 µL) of sample (concentration range: 1-5000 µg/mL) and a 1% starch solution in 20 mM sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) was incubated at 25 °C for 10 min. Then, 100 µL of porcine pancreatic α-amylase (0.5 mg/mL) was added to each well, and the samples was further incubated at 25 °C for 10 min. The reaction was stopped by adding 200 µL of dinitrosalicylic acid (DNS) color reagent, and the tubes was incubated at 100 °C for 5 min. After cooling the samples to room temperature, 50 µL was transferred from each tube to a 96-well microplate. The reaction mixture in each well was diluted by adding 200 µL of distilled water, and the absorbance was measured at 540 nm. The results was compared with the control. Acarbose was used as a positive control. Each assay was repeated three times. The results was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

2.10.3. Acetylcholinesterase and butyrylcholinesterase enzyme inhibition assays

The assays for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme activity was conducted with minor modifications based on the method described by Ingkaninan et al. (2000). In a 96-well plate, 125 µL of 5,5′-dithiobis-(2-nitrobenzoic acid) (3 mM, DTNB, Ellman's Reagent), 25 µL of substrate (15 mM, acetylthiocholine iodide for AChE and butyrylthiocholine iodide for BChE), 50 µL of Tris-HCl buffer (50 mM, pH 8), and 25 µL of the sample was mixed. Finally, 25 µL of the respective enzyme (AChE or BChE) was added to the mixture, and the AChE enzyme inhibition assay was incubated for 10 min, while the BChE enzyme inhibition assay was incubated for 15 min. At the end, the absorbance was measured spectrophotometrically at 405 nm. Donepezil was used as a positive control. Each assay was repeated three times. The percentage of inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

2.10.4. Antioxidant activity assay

2.10.4.1. DPPH radical scavenging capacity assay. The DPPH radical scavenging capacities of the extracts obtained from insects were tested using a modified method based on the one developed by Blois (1958). To create the free radical, a 1 mM $DPPH^{\bullet}$ solution was prepared. As reference standards, *α*-tocopherol and trolox were employed. The antioxidant capacity of the standards were tested against $DPPH^{\bullet}$ solution in the concentration range of 1-150 µg/mL. Considering the percent inhibition values of the standards, the dose range of the samples to be studied was decided. The extracts were diluted appropriately within the concentration range of 10-150 µg/mL. The antioxidant capacity of the samples was evaluated by observing their reaction with the $DPPH^{\bullet}$ solution. In the experiment, the control group consisted of ethanol and $DPPH^{\bullet}$ solution, all measurements were recorded at 517 nm against the ethanol blank. To ensure accuracy and reliability, each measurement was repeated three times.

The percentage of inhibition was calculated using the formula provided below:

$$DPPH^{\bullet} \text{ scavenging capacity } (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

A: Absorbance value at 517 nm.

2.10.4.2. ABTS radical scavenging capacity assay. The ABTS cation radical scavenging capacity was determined according to the method developed by Re et al. (1999) and modified by our team. To prepare the radical solution, a 2 mM ABTS solution was initially prepared. Then, a 2.45 mM potassium persulfate solution was added to the ABTS solution in a 1:1 ratio to generate $ABTS^{\bullet+}$. As reference standards, *α*-tocopherol and trolox were employed. The antioxidant capacity of the standards were tested against $ABTS^{\bullet+}$ solution in the concentration range of 1-150 µg/ml. Considering the percent inhibition values of the standards, the dose range of the samples to be studied was decided. Serial dilutions of the extracts was prepared at different concentrations (10-150 µg/mL) to obtain stock solutions. The antioxidant capacity of the samples was evaluated by observing their reaction with the $ABTS^{\bullet+}$ solution. In the experiment, the control group consisted of phosphate buffer (pH 7.4) and $ABTS^{\bullet+}$ solution, all measurements were recorded at 734 nm against the buffer blank. To ensure accuracy and reliability, each measurement was repeated three times.

The percentage of ABTS cation radical scavenging capacity for the extracts was calculated using the following equation:

$$ABTS^{\bullet+} \text{ scavenging capacity } (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

A: Absorbance value at 734 nm.

2.10.5. Antimicrobial activity assay

The antimicrobial effects of the extracts obtained from insects was evaluated against Gram-negative *Escherichia coli* ATCC 8739, Gram-positive *Staphylococcus aureus* ATCC 6538, and *Candida albicans* ATCC 10231 strains.

Moxifloxacin, and fluconazole (0.125-128 μg/mL) were used as standard antimicrobial agents. The concentration of *Belalang kayu* extract (39.06-1250 μg/mL), the concentration of Puthul extract (78.12-2500 μg/mL), and concentration of Jangkrik (19.53-625 μg/mL), and the other extracts were 312.5-5000 μg/mL used in activity.

The microdilution technique recommended by the Clinical and Laboratory Standards Institute (CLSI, 2008, 2006) for aerobic microorganisms (M100-S16) and for fungi (M27-A2) were modified and employed for evaluation. The standard culture strains to be used in antimicrobial efficacy tests was stored at −85 °C until use. To revive the cultures, they were streaked onto petri dishes containing Mueller Hinton Agar (MHA), Potato Dextrose Agar (PDA), or Sabouraud Dextrose Agar (SDA) and incubated at 37 °C for 24 h. After incubation, single colonies grown on the culture media was transferred to tubes containing Mueller Hinton Broth (MHB) (or RPMI medium for *Candida species*) and incubated again at 37 °C for 24 h. Following the 18-24-h incubation, the cultures grown in liquid medium was adjusted to a turbidity corresponding to McFarland No: 0.5 (approximately 108 cfu/mL for bacteria or 106 cfu/mL for yeast cultures) using a turbidometer. Microplate petri dishes with 96 "U"-shaped wells (Brand) were used for the experiment. After these procedures, the lids of the microplate petri dishes were closed, and they were incubated at 35-37 °C for 16-20 h. At the end of the incubation period, 15 μL of 0.5 mg/mL resazurin was added to the plates to determine the presence of microbial growth in the wells. Subsequently, the plates were further incubated at 37 °C for 3 h for color development. The MIC was defined as the lowest concentration of extracts that prevented a color change from blue to pinks of resazurin.

2.10.6. Human fibroblast cell culture

The human fibroblast cell line (HDFa, ATCC® PCS-201-012™) was cultured in DMEM medium containing 1% penicillin/streptomycin antibiotic mixture, 10% FBS, 5% CO_2, and at a temperature of 37 °C until reaching confluency. Extracted insect samples were tested on the fibroblast cell line grown in 48-well cell culture plates for toxicological studies. The experiments were conducted in three replicates, including a fibroblast cell culture with the application of a different insect isolate, and a negative control without any treatment. Fibroblast cell culture is a reliable model for genotoxicity and cytotoxicity analysis due to its characteristics of being a healthy human cell type and adhering to the cell culture surface. Isolate samples were applied to the cell cultures within the minimum dose range (1.5-200 μg/mL) typically used in cytotoxic and genotoxicity studies.

2.10.7. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is a colorimetric technique used to measure the viability percentage in an in vitro environment. Cells were seeded onto a 48-well plate at a density of 10a (a = 5) cells per well in 100 μL of culture medium, along with the treated isolates and untreated controls. The cells were incubated at 37 °C in a CO_2 incubator for 24 h, after which 10 μL of MTT mixture was added to each well. The plate was gently shaken for 1 min on a circular shaker and then incubated at 37 °C in a CO_2 incubator for 3-4 h. At the end of the incubation, dark crystals of formazan was visible at the bottom of the wells. The culture medium was carefully aspirated from each well without disturbing the cell layer. Alternatively, the plate can be centrifuged at 400×*g* for 10 min to pellet non-adherent cells before aspirating. To solubilize the formazan crystals, 100 μL of dimethyl sulfoxide (DMSO) was added to each well. The absorbance of each sample was measured at 570 nm using a microplate reader.

2.10.8. Nuclear structure analysis with hoechst 33258 fluorescent staining

Hoechst 33258 staining was used to detect mutated nuclei. Different concentrations of insect isolates were applied to the fibroblast cell culture, and a cell culture without any isolate application was served as the negative control. The cells was incubated for 24 h to analyze nuclear morphology. Subsequently, the cells was fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4 °C for 30 min. After washing the cells with PBS, the nuclear DNA was incubated with 1 μM Hoechst 33258 fluorescent dye at room temperature for 5 min. The cells was observed and photographed under a fluorescence microscope (Leica® DM IL LED) at 20X magnification.

2.10.9. Chromosomal aberration test

The chromosomal aberration test was performed with minor modifications based on a previous report (Türkez et al., 2010). Preheated Karyotyping Medium B (6 mL) was added to the cell culture tubes. Then, 0.5 mL of whole blood was added to the culture medium. Edible insect extracts was added to the tubes at a final concentration of IC_{50}. The tubes was inverted and placed in a 37 °C culture incubator. After 70 h and 15 min from the start of the culture, colchicine solution (10 μm/mL) was added in a volume of 0.1 mL. At the end of 72 h, the tubes were vortexed several times and centrifuged at 900 rpm for 10 min. The supernatant was discarded, and 8 mL of hypotonic solution was added dropwise to the tubes. After incubating the samples for 15-20 min, they were centrifuged again at 900 rpm for 10 min. Then, 5 mL of fixative was added dropwise onto the pellet, and all samples was centrifuged at 900 rpm for 5 min. The fixative treatment steps were repeated three times. After discarding the final supernatant, the pellets was resuspended by vortexing. The resuspended pellets was dropped onto pre-labeled slides, which have been washed and stored at 4 °C, using a Pasteur pipette. The slides were left to dry for 3 days and then stained with Giemsa stain for 15 min. After the staining process, the slides were left to completely dry for 2-3 days. Subsequently, thirty metaphase plates were examined for each test group, and the presence of gaps or breaks in chromosome chromatin level will be evaluated to calculate the average chromosomal aberration value for each group.

2.10.10. Apoptosis-necrosis test

In the cell cultures where experimental procedures was performed, cells was detached from the culture plate surface by adding trypsin at room temperature for 5 min. The cells was collected by centrifugation at 5x104 cells and resuspended in 500 μL of 1X binding buffer. Subsequently, 5 μL of Annexin V-FITC and 5 μL of propidium iodide (PI, 50 μg/mL) was added to the cultures, and they were

incubated in the dark for 5 min. Then, the cultures was analyzed using a flow cytometry device with a dual filter set for FITC and rhodamine.

2.10.11. Statistical analysis

The group analyses was performed using the One-way ANOVA procedure. The toxic effects of the isolates on the cells was compared against the control group using Dunnett's test and Tukey's multiple comparison analysis. The significance level was set at 5% ($p < 0.05$). The calculations was conducted using GraphPad Prism version 7.0 software. All experiments were performed in triplicate. Statistical significance was assessed using the Kruskal-Wallis test. The obtained results were analyzed using SPSS software (IBM SPSS Statistics 20, IBM Corporation, Armonk, NY, USA) with a significance level of $P = 0.05$. The mean and standard deviation (STD) of percent inhibition and IC_{50} values for the samples are reported in the results section.

Table 3
The volatile composition of the edible insects from Indonesia.

Component	RRI	Puthul %	*Belalang kayu* %	Jangkrik %	*Ulat jati* %	*Entung jati* %	Odor description
Myrcene	1174	2.1	1.8	-	-	-	earthy, fruity, and clove-like
Heptanal	1194	-	1.4	-	-	-	Fruity
Limonene	1203	**68.1**	**66.7**	**72.2**	**53.9**	27.6	citrus type odor
n-Butyl-*n*-butyrate	1230	1.2	2.4	-	0.7	-	sweet fruity
Ethyl hexanoate	1239	2.3	1.5	-	1.7	-	Fruity
γ-Terpinene	1255	1.0	<0.1	-	-	-	herbaceous-citrusy
Styrene	1272	2.6	13.9	3.2	1.3	-	pungent, gassy; slightly fresh-aromatic
p-Cymene	1280	4.2	2.6	3.8	1.8	-	woody and terpy-like
2,5-Dimethyl pyrazine	1332	-	-	-	-	3.1	chocolate type odor
2,6-Dimethyl pyrazine	1337	-	-	-	-	0.3	chocolate type odor
2,3-Dimethyl pyrazine	1358	-	-	-	-	1.1	chocolate type odor
1-Hexanol	1360	0.2	-	-	0.4	-	freshly mown grass
Methyl octanoate	1399	0.6	-	-	0.9	-	waxy type odor
Nonanal	1400	0.4	0.3	-	0.4	-	rose-orange odor
Tetradecane	1400	-	-	-	0.5	-	gasoline-like to odorless
Trimethyl pyrazine	1409	-	-	-	-	12.1	nutty type odor
Butyl hexanoate	1423	0.6	-	-	2.7	-	Fruity
Hexyl butyrate	1424	0.5	-	-	2.7	-	sweet, slightly waxy, fruity
Ethyl octanoate	1444	0.6	-	-	2.9	-	strong odor of fruit and flowers
3-Methylbutyl hexanoate (=*Isoamyl hexanoate*)	1468	-	-	-	0.4	-	Fruity
Acetic acid	1475	-	-	-	-	3.8	strong vinegar-like odor
Tetramethyl pyrazine	1482	-	-	-	-	**32.8**	musty, fermented, coffee odor
2,3,5-Trimethyl-6-ethyl-pyrazine	1492	-	-	-	-	0.5	roasted nut, baked potato odor
Methyl nonanoate	1500	-	-	-	-	-	Fruity
3,5-Octadien-2-one	1520	-	1.3	-	9.2	-	fruity fatty mushroom
Methyl 3-methylthio propionate	1541	-	-	-	-	-	sulfurous type odor
Benzaldehyde	1541	0.7	0.9	1.3	0.7	-	bitter almond odor
Linalool	1553	0.2	-	-	0.3	-	floral, spicy wood
(*E,E*)-3,5-Octadien-2-one	1573	-	0.4	-	5.1	-	fruity green grassy
Butanedioic acid dimethly ester (=*Dimethyl succinate*)	1580	1.9	0.3	1.7	-	-	pleasant, ethereal, winey odor
Hexyl hexanoate	1617	-	-	-	1.7	-	green type odor
γ-Butyrolactone	1651	-	0.8	-	-	-	sweet, pleasant, and creamy
Isovaleric acid	1685	-	-	-	-	2.4	cheesy type odor
6-*oxo*-Isophorone	1704	-	-	-	0.8	3.4	
Valencene	1740	-	-	-	2.1	-	sweet, fresh citrus, grapefruit, woody, orange odor
(*E,E*)-Farnesene	1758	-	-	-	1.4	-	green apple odor
Naphthalene	1763	0.4	0.3	0.1	-	-	Mothballs
Acetamide[a]	1769	0.3	1.9	4.1	-	-	odorless mouse-like with impurities
Dihydrooxophorone	1793	-	-	-	2.1	10.2	Saffron
δ-Valerolactone[a]	1838	-	0.8	-	-	-	floral, honey, citrus
Pyrrolidone[a]	2061	-	-	3.8	-	-	faint and amine-like odor
2-Piperidinone (=*Valerolactam*)[a]	2162	-	-	2.7	-	-	ammoniacal, pepper-like
Total		**87.9**	**97.3**	**92.9**	**93.7**	**97.3**	

RRI Relative retention indices calculated against *n*-alkanes.
% calculated from total ion chromatogram (TIC).
[a] From Wiley; tentative identification.

3. Results

3.1. Extraction

Table 2 presents data on the powdered sample weights, extract amounts, and extraction yields of five edible insect species: Puthul, *Belalang kayu*, Jangkrik, *Ulat jati*, and *Entung jati*. Among the samples, *Entung jati* had the highest initial dry weight (3.9876 g), followed by *Ulat jati* (3.6243 g), *Belalang kayu* (2.8102 g), Puthul (1.9840 g), and Jangkrik (1.5531 g). In terms of methanol extraction, the highest amount was obtained from *Ulat jati* (1.5210 g), while Jangkrik yielded the lowest (0.7769 g). For aqueous extracts, Puthul provided the highest amount (544.5 mg), and Jangkrik again had the lowest (69.80 mg).

Extraction yields with methanol were significantly higher than those with water. Puthul showed the highest methanol extract yield at 58.30%, followed by Jangkrik (50.02%), *Belalang kayu* (42.15%), *Ulat jati* (41.97%), and *Entung jati* (28.30%). Aqueous extraction yields were relatively low across all species, with Puthul again yielding the most (27.45%) and Jangkrik the least (4.50%). These results indicate that methanol was a more effective solvent than water for extracting compounds from all insect samples. Notably, Puthul consistently produced the highest extract amounts and yields in both extraction methods, suggesting it may be particularly rich in solvent-soluble bioactive compounds.

3.2. The volatiles and fatty oils extraction and analysis

The compositions of the volatiles and fatty oils derived from various edible insects are detailed in Tables 3 and 4, respectively, showcasing the major constituents and their respective proportions.

3.2.1. Volatile components

Table 3 presents the volatile compound profiles of five edible insect species traditionally consumed in Indonesia: *Puthul*, *Belalang kayu*, *Jangkrik*, *Ulat jati*, and *Entung jati*. A total of 52 volatile compounds were identified across the samples, revealing substantial variation in both composition and concentration, indicating species-specific aroma characteristics and rich chemical diversity. Among these, limonene, a monoterpene with a distinct citrus aroma, was the most dominant compound in four of the five species. It accounted for 68.1% of the total volatiles in *Entung jati*, 66.7% in *Ulat jati*, 72.2% in *Belalang kayu*, and 53.9% in *Puthul*. This high limonene content contributed to the strong citrus-type aroma associated with these samples. In contrast, the volatile profile of *Jangkrik* was dominated by tetramethyl pyrazine (32.8%), limonene (27.6%), and trimethyl pyrazine (12.1%), creating a savory, roasted, and nutty scent profile often linked to Maillard reaction products. Additional compounds contributed to the sensory complexity of each species. Myrcene, with its earthy and fruity aroma, was present in all samples, especially *Belalang kayu* and *Jangkrik*. Smaller amounts of ethyl acetate, ethyl butyrate, and linalool-compounds with fruity and floral characteristics-were identified in *Puthul* and *Ulat jati*, enhancing their pleasant aromatic qualities. Compounds such as acetic acid (3.8% in *Ulat jati*) and 3,5-octadien-2-one (9.2% in *Puthul*) contributed vinegar-like and green-grassy notes, respectively. Trace amounts of additional volatiles, including naphthalene, hexyl butyrate, and acetatede, appeared sporadically across the samples, subtly enriching the overall aroma matrices. Total identified volatile contents, based on total ion chromatogram (TIC), were high in all species: *Puthul* (87.9%), *Belalang kayu* (97.3%), *Jangkrik* (92.9%), *Ulat jati* (93.7%), and *Entung jati* (97.3%), indicating strong analytical coverage. Overall, the volatile profiles highlight the unique organoleptic potential of each species. *Ulat jati*, *Entung jati*, *Belalang kayu*, and *Puthul* featured citrus-dominant aromas, while *Jangkrik* offered a distinct savory-nutty profile. These differences underscore the potential of edible insects not only as nutritional resources but also as novel aromatic components in food applications and product development.

3.2.2. Fatty oils

Table 4 summarizes the fatty acid compositions of five Indonesian edible insect samples: *Puthul*, *Belalang kayu*, *Jangkrik*, *Ulat jati*, and *Entung jati*. The results reveal substantial variation in fatty acid profiles among the species, reflecting their distinct biological and

Table 4
Fatty acid compositions of the insect samples from Indonesia.

Fatty acids	Puthul %	*Belalang kayu* %	Jangkrik %	*Ulat jati* %	*Entung jati* %
Myristic (14:0)	0.1	0.7	-	0.1	<0.1
Pentadecanoic (15:0)	-	-	-	0.3	<0.1
Palmitic (16:0)	36.1	37.5	-	34.3	29.5
Palmitoleic (16:1)	0.5	0.2	9.7	0.6	0.6
Margaric (17:0)	-	0.2	-	-	1.2
Stearic (18:0)	6.7	5.6	2.5	6.2	6.3
Oleic (18:1)	40.8	41.8	71.3	18.6	25.9
Elaidic (18:1)	1.0	0.9	1.3	0.4	0.5
Linoleic (18:2)	13.6	9.3	11.1	5.7	5.6
Linolenoic (18:3)	0.7	1.7	2.8	29.5	28.6
Arachidic (20:0)	<0.1	0.5	1.2	0.4	0.5
Total	**99.5**	**98.4**	**99.9**	**96.1**	**98.7**

% calculated from FID data.

ecological attributes. Oleic acid (C18:1), a monounsaturated fatty acid associated with cardiovascular benefits, was the most dominant fatty acid across all samples. *Jangkrik* exhibited the highest oleic acid content (71.3%), followed by *Belalang kayu* (41.8%) and *Puthul* (40.8%), suggesting that these insects could serve as valuable sources of heart-healthy fats. In contrast, *Ulat jati* and *Entung jati* contained lower levels of oleic acid, with 18.6% and 25.9%, respectively.

Palmitic acid (C16:0), a saturated fatty acid commonly found in edible oils, was also notably present in all samples, ranging from 29.5% in *Entung jati* to 37.5% in *Belalang kayu*. Stearic acid (C18:0), another saturated fatty acid, was consistently detected across all species, contributing between 2.5% (*Jangkrik*) and 6.7% (*Puthul*). Among the monounsaturated and polyunsaturated fatty acids, palmitoleic acid (C16:1) and linolenic acid (C18:3) were particularly abundant in *Jangkrik* and *Ulat jati*. Notably, *Jangkrik* contained 9.7% palmitoleic acid, while *Ulat jati* and *Entung jati* demonstrated substantial levels of alpha-linolenic acid (29.5% and 28.6%, respectively), an essential omega-3 fatty acid, indicating their potential as functional food sources.

Linoleic acid (C18:2), an essential omega-6 fatty acid, was also present in all insect oils, with the highest concentrations observed in *Puthul* (13.6%) and *Jangkrik* (11.1%). Minor fatty acids such as myristic, margaric, pentadecanoic, arachidic, and elaidic acids appeared in trace amounts (typically $<0.1\%$), while the total fatty acid content of the samples ranged from 96.1% in *Ulat jati* to 99.9% in *Jangkrik*, based on FID data.

In terms of overall composition, *Entung jati* fatty oil comprised nine compounds, accounting for 99.5% of the total oil, with oleic acid (40.8%) and palmitic acid (36.1%) as the dominant constituents. *Ulat jati* contained ten compounds making up 98.4% of the oil, primarily oleic acid (41.8%) and palmitic acid (37.5%). The fatty oil of *Belalang kayu*, composed of seven compounds, represented 99.9% of the oil, with oleic acid being the principal component (71.3%). *Puthul* oil included ten fatty acids, amounting to 96.1% of the total, with palmitic acid (34.3%) and linolenic acid (29.5%) as the major contributors. In *Jangkrik*, eleven fatty acids were identified, comprising 98.7% of the oil, with high levels of palmitic acid (29.5%), linolenic acid (28.6%), and oleic acid (25.9%).

Collectively, these findings underscore the nutritional diversity of edible insect oils, emphasizing their potential as sustainable sources of beneficial fatty acids, particularly omega-3 and omega-9, for human health.

3.3. Quantitative analysis of amino acids

The amino acid profiles of five edible insect species traditionally consumed in Indonesia-*Entung jati*, *Ulat jati*, *Belalang kayu*, *Puthul*, and *Jangkrik*-were analyzed using both methanol and aqueous extracts. Quantitative analysis was performed using LC-MS/MS to assess the levels of 43 amino acids, of which 34 were identified across the samples. The results revealed distinct differences in amino acid composition based on species and extraction solvent, emphasizing species-specific biochemical diversity and the efficiency of solvent systems.

Overall, methanol extracts yielded higher concentrations and a greater diversity of amino acids than aqueous extracts. Notably, *Ulat jati* methanol extract exhibited the highest concentration of alanine (1945.36 nmol/mL), while the aqueous extract of the same species had the highest tyrosine content (4744.03 nmol/mL). Similarly, *Belalang kayu* methanol extract recorded the highest glutamic acid concentration (1520.70 nmol/mL). *Puthul* aqueous extract was rich in arginine (850.95 nmol/mL), further highlighting the nutritional potential of these insects.

Across the samples, glutamic acid, alanine, arginine, asparagine, and proline were consistently found in high amounts. *Ulat jati* extracts, both methanol and aqueous, were especially rich in branched-chain and aromatic amino acids such as valine, leucine, isoleucine, phenylalanine, and tyrosine, indicating a high-quality protein profile. *Jangkrik* methanol extract also contained significant levels of beta-alanine and tryptophan, amino acids associated with cognitive and metabolic benefits (Table 5).

In addition to these abundant amino acids, several conditionally essential and functional amino acids such as sarcosine, citrulline, ornithine, and hydroxylysine were detected in select species. For instance, *Entung jati* methanol extract had a notably high citrulline level (465.65 nmol/mL), while *Puthul* methanol extract exhibited elevated sarcosine (33.67 nmol/mL). Some amino acids, such as cysteine, glutamine, and 5-hydroxylysine, were either absent or present only in trace amounts, depending on the insect species and extraction method.

These results demonstrate that Indonesian edible insects are rich in essential and bioactive amino acids, positioning them as valuable alternatives to conventional protein sources. Their amino acid profiles support their potential role in addressing protein malnutrition and enhancing functional food formulations. Methanol extraction, in particular, proved more effective in yielding a broader and higher concentration of amino acids, making it the preferred method for comprehensive amino acid recovery from insect materials.

3.4. ICP-MS

Today, many scientific literature has shown that insects are a good source of essential nutrients such as fatty acids, protein, vitamins and minerals. This paper studies the toxic element level of selected insects, commonly consumed by Indonesian. In addition, many species of insects are eaten as part of Indonesian food culture. There are knowledge gaps and very little published information on edible insects in Indonesia. Therefore, the contemporary list of edible beetles of Indonesia is revised according to the world peer-reviewed literature. Information about the insects studied and their use in Indonesia is given below.

Phyllophaga helleri, local name is “Puthul” in Indonesia. Commonly known as may beetles, June bugs, and July beetles. It is a species of beetles in the family Scarabaeidae (Coleoptera). They are devastating to plant life. In total, 55 species of scarabaeids are consumed worldwide (Siddiqui et al., 2023). Due to their high peptide, fat and ash content, scarabaeids are used in various types of food such as soup, cereal snacks, bread, meat emulsions.

Table 5
Amino acid compositions of the insect samples from Indonesia.

Amino Acids (nmol/mL)	*Entung jati*		*Ulat jati*		*Belalang kayu*		Puthul		Jangkrik	
	MeOH	Aqueous	MeOH	Aqueous	MeOH	Aqueous	MeOH	Aqueous	MeOH	Aqueous
Taurine	302.2027	82.4221	0.0000	0.0000	526.5468	69.0750	479.7533	320.1510	10.1551	0.0000
Tryptophan	76.0255	29.7087	24.2982	41.3368	26.2747	0.0000	0.0000	0.0000	20.4741	0.0000
Phenylalanine	44.8713	0.0000	43.3044	0.0000	4.6420	0.0000	0.0000	0.0000	0.0000	0.0000
Tyrosine	181.5301	82.3987	2008.2174	4744.0343	137.9532	0.0000	123.2589	68.8663	54.8035	56.1443
Leucine	430.9708	49.2989	142.7989	28.5299	132.3320	30.8494	111.8029	65.5126	39.3346	53.7968
Isoleucine	296.5706	60.1132	180.9466	62.7911	117.9611	48.9715	79.0226	52.2825	74.3100	67.5612
Methionine	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3-Aminoisobutyric acid	33.7916	0.6436	51.2438	2.9581	14.8077	0.0000	8.4240	3.3040	0.0000	33.2384
gamma-Aminobutyric acid	37.5137	1.6905	35.3859	8.4697	13.4459	0.0000	10.2860	2.2418	0.0000	24.1370
2-Aminoadipic acid	0.0000	0.0000	0.0000	0.0000	27.0075	4.4797	13.7226	27.6288	0.0000	0.0000
Norvaline	28.9568	0.0000	11.1855	0.0019	5.1202	0.0000	2.1257	0.0000	0.7754	0.0000
Valine	601.0553	0.0000	257.2538	8.6527	119.2330	0.0000	54.9618	0.0000	19.2118	2.0363
Glutamic acid	881.7907	209.5375	331.0971	183.5148	1520.6978	187.4122	230.9075	220.4548	63.1439	78.6307
Ethanolamine	477.8282	162.8738	84.3538	66.3210	55.6499	39.6731	58.3782	54.7833	37.9412	43.4506
Beta-Alanine	209.2503	11.5944	103.4782	34.9033	3.6032	0.0000	26.0466	0.0000	0.0000	0.0000
Aspartic acid	56.7269	45.2176	36.1288	26.1010	105.0238	0.0000	28.1001	26.9200	0.0000	8.5097
2-Aminobutyric acid	56.0791	29.7902	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	48.3279	42.0777
Threonine	200.3668	1.1926	206.2214	54.3616	77.0178	0.0000	16.5663	0.0000	0.0000	0.0000
Serine	518.8113	112.5954	410.2589	135.5682	74.7005	0.0000	7.8854	0.0000	0.0000	0.0000
Alanine	1945.3602	241.4003	1884.1449	483.4378	523.3053	156.3659	447.4044	241.5979	445.1257	420.9041
Glycin	441.5437	246.9519	180.2781	452.0783	736.5017	0.0000	0.0000	0.0000	0.0000	246.5378
trans-4-Hydroxyproline	48.3132	21.2917	23.4338	22.3228	29.9798	20.5594	22.2333	19.9772	19.6595	21.1843
Asparagine	39.1882	79.9137	41.3099	73.3378	140.1584	0.0000	0.0000	0.0000	0.0000	0.0000
Proline	840.5056	1.0662	576.5685	40.6686	167.2017	0.0000	353.2060	6.2950	0.0000	0.0000
Glutamine	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Lysine	0.0000	0.0000	0.8071	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Sarcosine	50.5982	41.1141	44.7158	39.8499	47.4971	33.6667	39.9421	38.1515	0.0000	36.1445
Homocitrulline	0.0000	38.7997	0.0000	0.0000	0.0000	0.0000	0.0000	46.8627	0.0000	0.0000
Citrulline	465.6514	101.2600	0.0000	0.0000	0.0000	78.6797	0.0000	0.0000	0.0000	0.0000
Ortho-Phosphoserine	0.0000	0.0000	0.0000	0.0000	62.9616	0.0000	0.0000	0.0000	0.0000	0.0000
Ortho-Phosphorylethanolamin	155.4518	71.6149	19.8592	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Homocystine	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cystine	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Argininosuccinic acid	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Arginine	13.8762	26.8582	94.1730	450.1094	339.5033	60.2656	104.9254	850.9475	10.3454	41.7735
Cystathionine	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Histidine	0.0000	28.8834	0.0000	16.4160	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Ornithine	164.9118	428.7361	0.0000	0.0000	24.2482	1.2237	36.2027	4.7109	0.0000	27.8174
5-Hydroxylysine	0.0000	5.2620	0.0000	5.0826	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Carnosine	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1-Methylhistidine	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3-Methylhistidine	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Anserine	7.7446	3.9792	4.3185	5.1721	2.5040	3.0329	5.7654	7.9597	3.3242	6.6551

Table 6
Element composition of the edible insect species commonly eaten in Indonesia.

No	Element group	Elements	Sample Name					Recommended Daily Intake	Source
			Phyllophaga helleri (Puthul)	*Valanga nigricornis* (*Belalang kayu*)	*Gryllus bimaculatus* (Jangkrik)	*Hyblaea puera* (Adult) (*Ulat jati*)	*Hyblaea puera* (Cocoon) (*Entung jati*)		
1.	**Major elements at g/kg**	Na	<0.000	7423.804	78809.906	10423.847	8977.967	≤2300 mg	WHO, 2012
2.	**Concentrations**	Mg	2437.361	3514.491	6818.665	3564.706	10145.030	310–420 mg	IOM, 2001
3.		Al	3243.813	3583.037	4045.046	7225.301	2446.485	1–2 mg (tolerable limit)	WHO, 2007
4.	**Trace elements at mg/kg**	K	9374.4	5321.8	5195.2	11338.3	24901.8	4700 mg	WHO, 2003
5.	**Concentrations**	Ca	139.30	44.89	96.39	246.6	356.6	1000–1300 mg	IOM, 2002
6.		Fe	54.83	59.20	191.35	547.9	253.4	8–18 mg	IOM, 2001
7.		Rb	23.239	41.891	29.695	58.861	6.583	No data available	-
8.		Sr	12.665	63.569	35.286	131.851	175.624	No data available	-
9.		Cu	17.13	53.11	13.59	8.036	21.73	0.9 mg	IOM, 2001
10.		Zn	178.29	120.14	88.006	72.2	202.36	8–11 mg	IOM, 2001
11.		Co	7.747	<0.000	<0.000	9.411	<0.000	5–8 μg	WHO, 1996
12.		Cd	0.016	0.025	0.009	0.019	0.016	≤0.007 mg	WHO, 2001
13.		Hg	0.003	0.002	0.004	0.005	0.004	≤0.004 mg	USEPA and U.S. E.P.A., 2000
14.		Pb	0.081	0.171	0.429	0.176	0.065	≤0.2–2 mg (PTWI)	WHO, 2001
15.	**Ultratrace elements at μg/kg**	Se	0.0069	0.27	0.56	0.107	0.28	0.055 mg	IOM, 2000
16.	**Concentrations**	As	0.020	0.033	2.092	0.102	0.034	0.01–0.1 mg (tolerable limit)	WHO, 2001
17.		Sc	<0.000	<0.000	<0.000	<0.000	<0.000	No data available	-
18.		Cr	<0.000	<0.000	18.572	16.782	100.888	0.025–0.035 mg	IOM, 2001
19.		Mn	<0.000	<0.000	<0.000	67.101	44.012	1.8–2.3 mg	IOM, 2001
20.		Cs	<0.000	<0.000	<0.000	<0.000	<0.000	No data available	-
21.		Ba	<0.000	27.446	<0.000	11.538	22.757	1–2 mg	WHO, 2001
22.		La	<0.000	19.013	0.009	8.646	19.995	No data available	-
23.		Ce	<0.000	37.141	<0.000	22.596	19.942	No data available	-
24.		Sm	<0.000	7.585	2.953	1.418	7.251	No data available	-
25.		U	1.070	1.134	<0.000	<0.000	<0.000	0.6 μg/kg/day	WHO, 2001

PTWI: Provisional Tolerable Weekly Intake. IOM: Institute of Medicine (part of the U.S. National Academies). USEPA: United States Environmental Protection Agency. WHO: World Health Organization. Some rare earth elements (e.g., Sm, Ce, La, Sc) and elements like rubidium and cesium have no defined Recommended Daily Intake (RDI), as they are not considered essential or there is insufficient data regarding their biological roles in humans. The IUPAC Compendium of Chemical Terminology (2nd edition) defines a trace element as one with an average concentration of less than approximately 100 parts per million (ppm) or 100 μg per gram (μg/g). However, with the continuous evolution of analytical technologies, our ability to detect and quantify elements at increasingly lower concentrations has significantly improved. As a result, this traditional threshold for "trace" elements no longer reflects the current sensitivity of modern instrumentation. To capture these lower concentration ranges, the term ultra-trace analysis has been introduced. Although a universally accepted definition is lacking, ultra-trace analysis typically refers to the quantification of elements present at mass fractions below 10^{-6} to 10^{-8} g/g—that is, concentrations under 1 ppm to 10 parts per billion (ppb) (Brown and Milton, 2005).

Valanga nigricornis, local name is "belalang kayu" in Indonesia. Commonly known as wooden grasshopper, Javanese grasshopper, Javan locust or Javan bird locust. It is a species of grasshopper in the family Acrididae and is native to southern Thailand, Malaysia, Indonesia and the Philippines. From Java (East and Central) to Sumatra and Kalimantan, it is commonly eaten by rural communities. Fried and smoked grasshoppers are a local culinary speciality sold by the people of Mount Kidul, Yogyakarta province. They are marinated in palm sugar and then fried. Also served with Thiwul (Sabri et al., 2023).

Gryllus bimaculatus, commonly known as "Jangkrik" in Indonesia. It is a species of cricket in the family Gryllidae (Orthoptera). It is rich in glutamic acid, lysine, linoleic acid, oleic acid and palmitic acid, low in polyunsaturated fatty acids (PUFA), high in calcium and iron (Magara et al., 2021).

Hyblaea puera the catepillar and cocoon form are commonly known in Indonesia as "ulat jati" and "entung jati" respectively. It is the sole genus of Hyblaeidae (Lepidoptera). The teak moth, or teak caterpillar, is commonly eaten in Indonesia in the regions where it is found. Consumers are usually caught for consumption at the cocoon stage because of the ease of collection from the ground. The cocoons are fried in coconut or palm oil, seasoned with salt, and it is believed that the consumption of teak caterpillar cocoons increases vitality (Lukiwati, 2010). However, the adult form is also a food for the local people. It provides an essential, easily accessible source of food, particularly for the villagers, who are generally farmers.

Most of the research on edible insects has focused on the nutritional value and less attention given to elements especially toxic elements. Therefore, the present paper is intended to analyses toxic metal(oid) concentrations of these edible insects, which are mostly consumed in Indonesia. Table 6 shows the content of toxic elements in four species of edible insects from orders Coleoptera, Orthoptera and Lepidoptera. The content of As, Cd, Hg, Pb in wild *Phyllophaga helleri*, *Valanga nigricornis*, *Gryllus bimaculatus*, *Hyblaea puera* were below the recommended limits, which make these insects safe for consumption (Balali-Mood et al., 2021; USEPA and U.S.E.P.A., 2000; WHO, 2001). Also, the results obtained showed that larva and pupa form of *Hyblaea puera* have different level of elements.

Even when the toxic element concentrations are all below the recommended limit, it is important to note that potential risks from the toxic elements may still exist in edible insects due to bioaccumulation and biomagnification.

Lead (Pb) is a potentially toxic and non-essential to plants, animals and humans. Increased levels of Pb in industrial activities such as mining, agrochemicals, manufacturing of batteries, gasoline, paints, etc., can lead to Pb contamination in the environment and thus contamination of the food chain. Studies have shown that 10 μg/dl Pb exposure leads to neurological and cognitive dysfunction, hepatic and renal dysfunction etc., (Aydoğan, 2021; Kumar et al., 2020). The maximum permitted Pb levels in food by WHO, USEPA and EC (European Community) are 2, 0.5 and 0.2 μg/g respectively (USEPA and U.S.E.P.A., 2000; WHO, 2001). With the exception of *Gryllus bimaculatus* (0.429 ppm), Pb concentrations in this study were lower than the permitted levels.

Cadmium (Cd) has a long biological half life (10-30 years) and is genotoxic and carcinogenic to humans. Similarly, increased industrial and anthropogenic activity increases Cd levels in the environment such as Ni-Cd batteries, agriculture, alloy and glass production, etc. The safe limit is 7 μg cadmium/week/kg body weight, or 0.4-0.5 mg/week or 25 μg cadmium/kg body weight per month (WHO, 2001). Cd concentrations in this study were lower than the safe limits.

Arsenic (As) has no known biological function in humans and is considered a non-essential toxic element. The acute and chronic toxicity of arsenic causes damage to many tissues and organs in the body. Arsenic and its compounds have been used in industrial, medical, veterinary and agricultural applications. The Environment Protection Agency and World Health Organisation recommended limit for arsenic from 50 ppb to 10 ppb and 10 μg l^{-1} (USEPA and U.S.E.P.A., 2000; WHO, 2001). With the exception of *Gryllus bimaculatus* (2.092 ppm) and *Hyblaea puera* (adult) (0.102 ppm), As concentrations in this study were below the safe limits. Agricultural pesticides may be responsible for this.

Like As, Cd, and Pb, Mercury (Hg) has a variety of adverse effects on living organisms and concentrates, possibly magnified up the food web. It causes liver damage, cardiac disease, neurodevelopmental impairments, loss of memory etc. The dominant sources of anthropogenic mercury emissions are coal-fired power plants, dental amalgam, gold mining, pesticides, etc. Studies show that the half-life of mercury in the whole body is about 40 days, while in the brain it can be up to 20 years (Rice et al., 2014). Acceptable daily intake is 0.71 μg/kg/bwt (WHO, 2001). Hg concentrations in this study were below the safe limits.

Heavy metals pose a serious threat when they exceed permissible levels in the body. It can be concluded that the non-essential elemental composition of edible insects depends largely on the environment in which they live and diet (Table 6).

The table presents a detailed analysis of the elemental composition of five edible insect species commonly consumed in Indonesia: *Phyllophaga helleri* (Puthul), *Valanga nigricornis* (*Belalang kayu*), *Gryllus bimaculatus* (Jangkrik), and both the adult and cocoon forms of *Hyblaea puera* (*Ulat jati* and *Entung jati*). The elements are grouped into major, trace, and ultratrace categories, and their concentrations are compared against established recommended daily intake (RDI) values from WHO, USEPA, and IOM.

Among the major elements, sodium (Na) shows the highest level in *Gryllus bimaculatus* (78809.906 mg/kg), while it is undetectable in *Phyllophaga helleri*. Although sodium is an essential electrolyte, its intake should not exceed 2300 mg per day. Magnesium (Mg), another vital macroelement, is particularly abundant in *Hyblaea puera (Cocoon)*, with 10145.030 mg/kg, significantly higher than the recommended 310–420 mg. Aluminum (Al), though not essential for human nutrition, appears in high concentrations in all species, most notably in *Hyblaea puera (Adult)* (7225.301 mg/kg). Potassium (K), essential for nerve and muscle function, is present in extremely high amounts across all samples, especially in *Hyblaea puera (Cocoon)* (24901.8 mg/kg), which far exceeds the recommended 4700 mg/day. Calcium (Ca) content is also highest in the cocoon form of *Hyblaea puera* (356.6 mg/kg), suggesting its potential as a supplemental calcium source.

In terms of trace elements, iron (Fe) content is remarkably high in *Hyblaea puera (Adult)* (547.9 mg/kg), making it a strong candidate for addressing iron deficiency, with a daily requirement of 8–18 mg. Copper (Cu) is most concentrated in *Valanga nigricornis* (53.11 mg/kg), and zinc (Zn) is highest in *Hyblaea puera (Cocoon)* (202.36 mg/kg); both values surpass the daily needs of 0.9 mg and 8–11 mg, respectively. Selenium (Se) levels also exceed the RDI of 0.055 mg, particularly in *Gryllus bimaculatus* (0.56 mg/kg). Arsenic

(As), lead (Pb), cadmium (Cd), and mercury (Hg) are present in low amounts, and while they do not exceed tolerable intake limits, their detection still necessitates attention due to potential toxicity. Notably, cobalt (Co) in *Gryllus bimaculatus* (9.411 mg/kg) exceeds the 5–8 μg recommended intake, indicating possible bioaccumulation.

Several ultratrace elements were also detected. Chromium (Cr), important for glucose metabolism, is most concentrated in *Hyblaea puera (Cocoon)* (100.888 μg/kg), aligning with the RDI of 0.025–0.035 mg. Manganese (Mn) levels reach 67.101 μg/kg in *Hyblaea puera (Adult)*, fitting within the recommended daily range of 1.8–2.3 mg. Other elements such as scandium (Sc), cesium (Cs), lanthanum (La), cerium (Ce), and samarium (Sm) lack established RDI values and are generally not considered essential. Uranium (U) is present in all samples in very small amounts, with the highest detected in *Valanga nigricornis* (1.134 μg/kg), but still below the WHO tolerable threshold of 0.6 μg/kg/day when considered in realistic consumption levels.

In summary, these edible insect species exhibit high nutritional potential, particularly *Hyblaea puera*, due to their rich profiles of essential elements like iron, calcium, magnesium, zinc, and potassium. However, the concurrent presence of non-essential and toxic elements warrants careful monitoring and further safety evaluations to ensure that their consumption remains within safe limits.

3.5. α-Glucosidase and α-amylase inhibition assays

The results of α-glucosidase and α-amylase is presented in Table 7 inhibition. When compared to acarbose, the most effective extract in terms of α-glucosidase inhibition is the *Entung jati* (*Hyblaea puera*, cocoon) MeOH extract. The *Entung jati* MeOH extract provides 85.09% inhibition, whereas acarbose only achieves 77.61% inhibition. The other extracts, on the other hand, were ineffective in α-glucosidase inhibition. The most effective extracts for α-amylase inhibition were Jangkrik MeOH (29.45%) and Puthul Aqueous (24.44%) extracts, while acarbose achieves 62.74% inhibition. Based on our knowledge, as far as the literature indicates, these are the initial studies conducted on α-glucosidase and α-amylase inhibition within these species except Jangkrik (*Gryllus bimaculatus*).

3.6. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition assays

The results of acetylcholinesterase and butyrylcholinesterase inhibition is presented in Table 7 inhibition. In the acetylcholinesterase inhibition assay, standard drug donepezil demonstrated an effect of 93.03%, whereas all the extracts prepared from insects are ineffective. When compared to donepezil, the extracts that exhibit significant effects on butyrylcholinesterase inhibition, along with their respective percentages, are as follows: 6.67% for *Entung jati* MeOH, 14.31% for *Ulat jati* MeOH, and 12.79% for Puthul MeOH. It's important to note that all the other extracts, including the aqueous extracts, do not show significant effects on butyrylcholinesterase inhibition. Donepezil, on the other hand, demonstrated a very high inhibition of 100.00%. To the best of our knowledge and as per the available literature, these represent the first investigations conducted on anticholinesterase inhibition within these species, with the exception of Jangkrik (*Gryllus bimaculatus*).

Table 7
The results of α-glucosidase, α-amylase, acetylcholinesterase, and butyrylcholinesterase inhibition.

Samples	α-Glucosidase Inhibition (%) (5000 μg/mL) (mean ± std)	α-Amylase Inhibition (%) (5000 μg/mL) (mean ± std)	Acetylcholinesterase Inhibition (%) (5 μg/mL) (mean ± std)	Butyrylcholinesterase Inhibition (%) (500 μg/mL) (mean ± std)
Entung jati MeOH	**85.09 ± 2.12**	15.78 ± 3.77	ND	**6.67 ± 5.66**
Entung jati Aqueous	ND	ND	ND	0.37 ± 0.48
Ulat jati MeOH	ND	18.11 ± 6.41	ND	**14.31 ± 5.97**
Ulat jati Aqueous	ND	ND	ND	ND
Belalang kayu MeOH	ND	15.77 ± 4.03	ND	ND
Belalang kayu Aqueous	ND	18.78 ± 3.63	ND	ND
Puthul MeOH	ND	ND	ND	**12.79 ± 6.08**
Puthul Aqueous	ND	**24.44 ± 4.16**	ND	0.59 ± 2.41
Jangkrik MeOH	ND	**29.45 ± 2.99**	ND	ND
Jangkrik Aqueous	ND	0.45 ± 5.86	ND	ND
Acarbose	**77.61 ± 4.70**	**62.74 ± 1.85**	-	-
Donepezil	-	-	**93.03 ± 0.22**	**100.00 ± 0.49**

ND: Not determined.

3.7. Antioxidant activity assay

Antioxidant activity of extracts from the insects species were presented in Table 8. In DPPH radical scavenging tests, when the % inhibition values of the standards [α-tocopherol (TC), troloks (TR)] and extracts were compared at a concentration of 100 μg/mL, it was determined that especially water extract of *Entung jati* had a higher % inhibition value on DPPH$^{\bullet}$ [(TR) 93.0 > (TC) 88.2 > (*Entung jati*) 9.0 > (Jangkrik) 8.6 > (*Ulat jati*) 5.3 > (*Belalang kayu*) 1.7 > (Puthul) 1.0%; 100 μg/ml % inhibition for water extracts of insects species], [(TR) 93.0 > (TC) 88.2 > (Jangkrik) 6.9 > (*Belalang kayu*) 6.3 > (*Ulat jati*) 4.2 > (*Entung jati*) 3.8 > (Puthul) 1.6%; 100 μg/mL % inhibition for methanol extracts of insects species]. When the % inhibition values of standards and extracts in ABTS cation radical scavenging tests were compared at 100 μg/mL concentration, it was seen that the *Ulat jati* water extract came to the fore in terms of antioxidant effect. In this context, the results were found to be compatible with DPPH radical scavenging capacity tests [(TR) 99.2 > (TC) 89.7 > (*Ulat jati*) 19.0 > (*Entung jati*) 16.4 > (Jangkrik) 13.8 > (Puthul) 9.5 > (*Belalang kayu*) 7.8%; 100 μg/ml % inhibition for water extracts of insects species], [(TR) 99.2 > (TC) 89.7 > (*Ulat jati*) 14.5 > (*Entung jati*) 11.7 > (*Belalang kayu*) 7.9 > (Jangkrik) 6.8 > (Puthul) 2.8%; 100 μg/ml % inhibition for methanol extracts of insects species]. The antioxidant capacities of the extracts were found to be average compared to the standards.

3.8. Antimicrobial activity

Antimicrobial activity results is given in Tables 9 and 10. The water exracts of *Entung jati*, *Ulat jati*, *Belalang kayu*, Puthul, and Jangkrik was not found effective tested concentrations against all microorganisms. The MIC values were found to be greater than the upper concentration value at which they were tested.

The methanol extracts of insects were found more effective against *C. albicans*. Especially, *Belalang kayu* and Jangkrik were found as MIC = 156.25, and MIC = 78.12 μg/mL, respectively. The MIC values of *Belalang kayu*, Puthul and Jangkrik exratcs were effective at 312.5 μg/mL for *S. aureus* and *E. coli.* The methanol extracts of *Entung jati* and *Ulat jati* were showed as MIC = 625-1250 μg/mL for all microorganisms.

3.9. Cytotoxicity and genotoxicity analyses

Samples were applied to human fibroblast (HDFa) and lymphocyte cell cultures for 24 h to investigate their possible cytotoxic and genotoxic properties. According to the cell viability analyses of the extracts on the HDFa cell line *P. helleri* methanol isolate cause a significant cytotoxicity up between concentration of 1000 μg/mL to 125 μg/mL. On the other hand, *P. helleri* extracts did not show any cyotoxicity up to 1000 μg/mL concentration. Also, *V. nigricomis* extracts exhbited similar cytotoxicity properties on the cell culture. Only water extracts of *V. nigricomis* showed a significant cytotoxic feature on the HDFa cell culutre in higher concentrations (from 250 μg/mL to 1000 μg/mL). Besides, both water and methanol extracts of *G. bimaculatus*, *H. puera* Ca. and *H. puera* Co. showed cyotoxic properties on the cell culture in a some extend. In these isolates, 125 μg/mL concentration of water extracts was found to exhibit a significant cytotoxicity bu, only 1000 μg/mL concentration of methanol extract showed cytotoxic properties on the HDFa cell cultures (Fig. 5).

Moreover, Hoechst 33258 flourescein staining technique was used to investigate possible genotoxic properties of the isolates for 24 h of applications. Results put forth that there was no significant tpxicological effects of the extract applications compared to the

Table 8
The results of antioxidant activity assay.

DPPH$^{\bullet}$ Scavenging Activity of Standards and Methanol Extracts (% Inhibition of 100 μg/mL± standard deviation)		
α-Tocopherol	88.264 ± 0.0007	
Trolox	93.018 ± 0.0003	
Extracts	**Methanol**	**Water**
Entung jati	3.876 ± 0.0011	9.058 ± 0.0022
Ulat jati	4.239 ± 0.0026	5.354 ± 0.0052
Belalang kayu	6.380 ± 0.0076	1.731 ± 0.0089
Puthul	1.619 ± 0.0036	1.063 ± 0.0127
Jangkrik	6.921 ± 0.0041	8.641 ± 0.0011
ABTS$^{\bullet+}$ Scavenging Activity of Standards and Methanol Extracts (% Inhibition of 100 μg/mL ± standard deviation)		
α-Tocopherol	89.707 ± 0.0013	
Trolox	99.258 ± 0.0009	
Extracts	**Methanol**	**Water**
Entung jati	11.773 ± 0.0026	16.457 ± 0.0167
Ulat jati	14.582 ± 0.0068	19.096 ± 0.0047
Belalang kayu	7.978 ± 0.0086	7.885 ± 0.0048
Puthul	2.838 ± 0.0022	9.575 ± 0.0042
Jangkrik	6.819 ± 0.0092	13.834 ± 0.0046

Table 9
Minimum inhibitory concentrations (MIC), μg/mL.

Aqueous extracts	*Escherichia coli* ATCC 8739	*S. aureus* ATCC 6538	*C. albicans* ATCC 10231
Entung jati	>2500	>2500	>5000
Ulat jati	>2500	>2500	>5000
Belalang kayu	>0.625	>0.625	>1250
Puthul	>1250	>1250	>2500
Jangkrik	>312.5	>312.5	>1250
Moxifloxacin	0.125>	0.125>	-
Fluconazole	-	-	0.125>

Table 10
Minimum inhibitory concentrations (MIC), μg/mL.

Methanol extracts	*Escherichia coli* ATCC 8739	*S. aureus* ATCC 6538	*C. albicans* ATCC 10231
Entung jati	625	625	625
Ulat jati	1250	1250	625
Belalang kayu	312.5	312.5	156.25
Puthul	312.5	312.5	312.5
Jangkrik	312.5	312.5	78.12
Moxifloxacin	0.125>	0.125>	-
Fluconazole	-	-	0.125>

negative control (non-treated HDFa cell culture). On the other hand, there were some differences that were observed in nuclear abnormality numbers after *P. helleri* methanol, *G. bimaculatus* dH_2O, *G. bimaculatus* methanol and *H. puera* Ca. dH_2O isolates applications. In the negative control group, nuclear abnormality number were observed as 0.004 ± 0.002/1000 cell count and the mentione group has 0.005 ± 0.003/1000, 0.006 ± 0.003/1000, 0.007 ± 0.003/1000, 0.006 ± 0.003/1000 cell count in aspect of nuclear abnormality, respectively. Also, it was observed that positive control group (0.001 M concentration of Cadmium acetate application) have significantly higher nuclear abnormality numbers with 0.017 ± 0.006/1000 cell count compared to the experimental groups (Fig. 6 and Table 11).

Furtheremore, cytotoxic effects of insect extracts were analyzed on the lymphocyte cell culture for 24 h of applications by using MTT cell viability assay. It was observed that *P. helleri* water and methanol extract exhibited a significant cytotoxic effect on the lymphocyte cell culture at higher dose intervals between 1000 μg/mL and 250 μg/mL concentrations. Also, *V. nigricomis* extracts showed a lower cytotoxic property on the cell culture and only 1000 μg/ml concentration of water and methanol extract exhibit a significant cytotoxicity on the lymphocyte cell culture. In a same manner, *G. bimaculatus*, *H. puera* Ca and *H. puera* Co extract showed cytoxicity only at 1000 μg/mL concentration. Furtheremore, *H. puera* Ca and *H. puera* Co water extract did not exhibit any toxicological feature up to 1000 μg/mL concentration on the lymphocyte cell culture for 24 h of application (Fig. 7).

For further genotoxicity analyses, chromosomal abberation tests were performed for the insect isolates on the human lymphocyte cell culture for 24 h of applications. As a result, there were no significant genotoxicolog properties observed after all of the extract application. But, there were some negligible changes were observed in chromosomes in respect to structures. *P. helleri* water, *P. helleri* methanol, *G. bimaculatus* water and *H. puera* Co. methanol extract application was observed to change chromosomal aberration numbers as 1.2 ± 0.06, 0.98 ± 0.04, 1.1 ± 0.05 and 0.99 ± 0.04, respectively. On the other hand, these numbers were insignificant when compared to the negative control (0.8 ± 0.04) and the positive control (3.62 ± 0.10) in aspect of genotoxicological outcome (Fig. 8 and Table 12).

The examination of cytotoxic properties in isolates derived from edible insects is a captivating and emerging field, largely driven by the growing emphasis on insects as a sustainable protein alternative (Fasolin et al., 2019; Henchion et al., 2017; Pan et al., 2022). Our investigations have been primarily centered on assessing the prospective health implications of these insect-derived isolates, with a specific emphasis on their cytotoxic capabilities in relation to human cell lines. In the resent study, insect isolates were tested on the HDFa and human lymphocyte cell cultures for 24 h of application to investigate potential cytotoxicity properties. Results put forth that *P. helleri* methanol and *V. nigricomis* methanol extracts did not show any cytotoxicity on HDFa cell line up to 1000 μg/mL. Also, *H. puera* ca. and *H. puera* co. water extracts did not exhibit significant cytotoxicity on the human lymphocyte cell cultures up to 1000 μg/mL. Moreover, other isolates showed variable cytotoxic effects on both human cell cultures but, the concentration levels were observed to be high dose levels such as *V. nigricomis* and *G. bimaculatus* extracts did not change cell viability ratios up to 500 μg/mL concentration on the human lymphocyte cell cultures. On the other hand, the examination of insect species as viable food sources remains notably limited.

4. Discussion

In this study, all insect samples were analyzed after controlled drying, as also documented in the photographic records. Drying is a commonly applied preservation method in traditional Indonesian practices and is known to reduce moisture content, limit microbial degradation, and improve sample stability without causing extensive degradation of bioactive compounds when applied at moderate

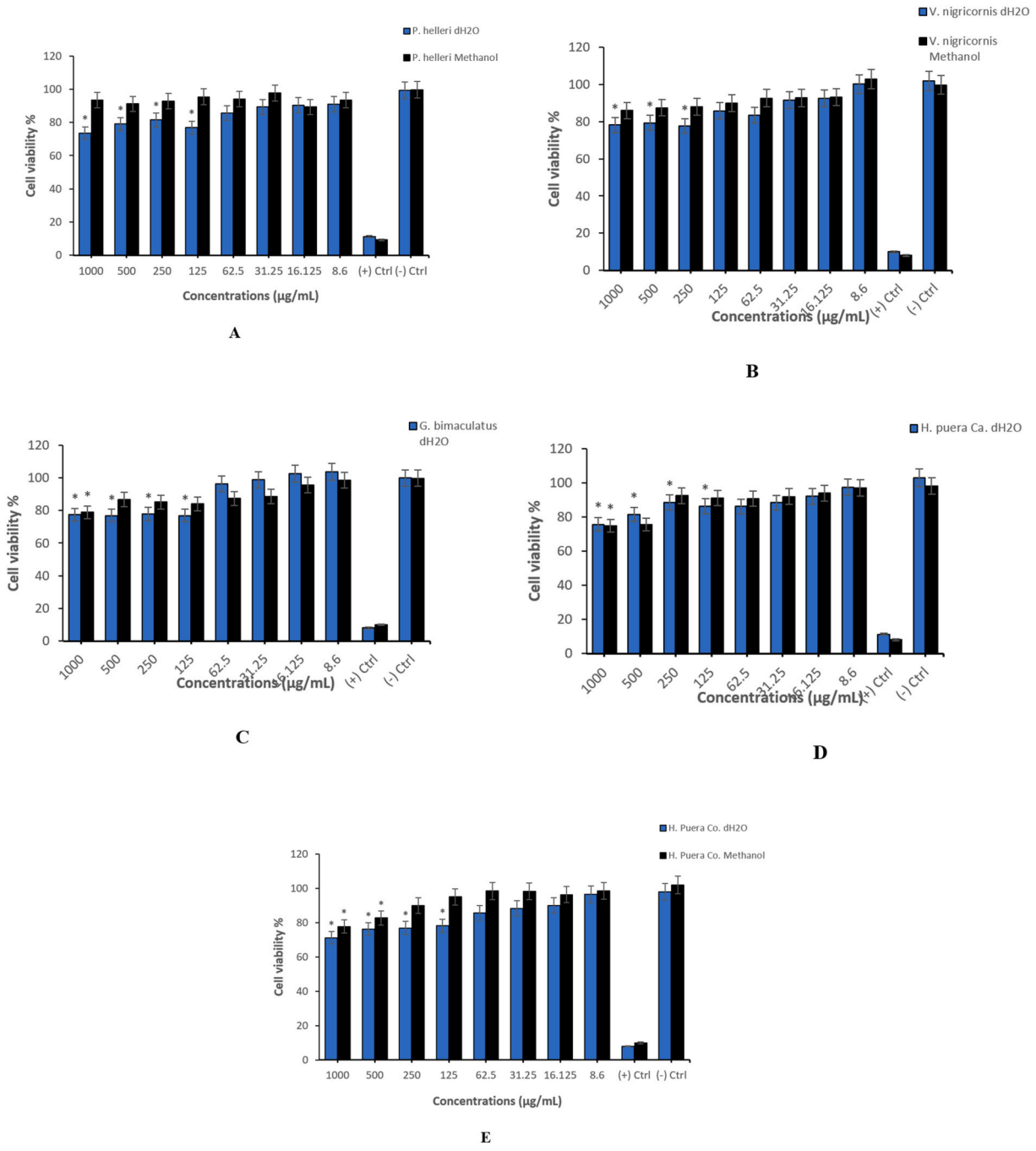


Fig. 5. Cytotoxic effects of insect extracts (water and methanol) on the fibroblast (HDFa) cell culture for 24 h of applications. **A-** Puthul (*P. helleri*), **B-** *Belalang kayu* (*V. nigricrnis*), **C-** Jangkrik (*G. bimaculatus*), **D-** *Ulat jati* (*H. puera*) (caterpillar) and **E-** *Entung jati* (*H. puera*) (cocoon). Symbol (*) represent statistically significant difference compared to the negative control ((−) Ctrl).

temperatures. Previous studies have shown that low-temperature drying preserves phenolic compounds, amino acids, and antioxidant capacity more effectively than high-temperature cooking methods such as frying or roasting (Rumpold and Schlüter, 2013; Orkusz, 2021). Therefore, the present results reflect the intrinsic bioactive potential of dried insect materials, rather than that of cooked products. Nevertheless, it is well established that traditional cooking methods may further modify bioactive profiles through thermal degradation or transformation of metabolites (Zielińska et al., 2015). Future studies comparing dried and cooked forms are needed to better understand the impact of culinary processing on functional properties.

The extraction protocol was limited to methanol and distilled water. It is acknowledged that the use of additional solvents with

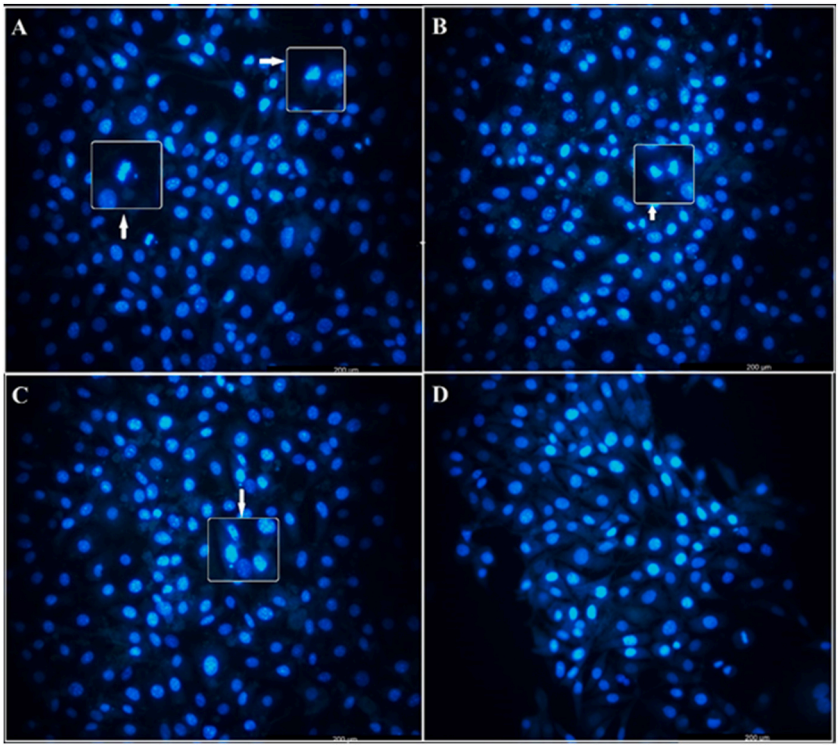


Fig. 6. Hoechst 33258 staining analyses for the nuclear abnormality investigations. Sample images for **A-** Puthul (*P. helleri),* **B-** *Belalang kayu* (*V. nigricrnis*), **C-** Jangkrik (*G. bimaculatus*) and **D-** Negative control (non-treated HDFa cell culture). White arrows indicate nuclear abnormalities.

Table 11
Genotoxicity properties of insect extracts on the human fibroblast (HDFa) cells in respect to nuclear abnormalities (MN: Micro Nucleus, Notch and Lobbed nucleus) by using Hoechst 33258 staining.

Groups	Concentrations (μg/mL)	Scored Cell Number	MN	Notch	Lobb	Total NAi + SD
Puthul	1000	1000	2	1	0	0.003 ± 0.001
Aqueous	500	1000	2	0	0	0.002 ± 0.001
Puthul	1000	1000	4	1	0	0.005 ± 0.002
Methanol	500	1000	1	0	0	0.001 ± 0.001
Belalang kayu Aqueous	1000	1000	3	0	1	0.004 ± 0.002
	500	1000	1	1	0	0.002 ± 0.001
Belalang kayu Methanol	1000	1000	2	1	0	0.003 ± 0.001
	500	1000	1	0	1	0.002 ± 0.001
Jangkrik	1000	1000	5	1	0	0.006 ± 0.003
Aqueous	500	1000	2	1	0	0.003 ± 0.001
Jangkrik	1000	1000	3	2	2	0.007 ± 0.003
Methanol	500	1000	0	2	0	0.002 ± 0.001
Ulat jati	1000	1000	4	2	0	0.006 ± 0.003
Ca. Aqueous	500	1000	0	1	1	0.002 ± 0.001
Ulat jati	1000	1000	2	1	1	0.004 ± 0.002
Ca. Methanol	500	1000	1	0	0	0.001 ± 0.001
Ulat jati	1000	1000	3	1	1	0.005 ± 0.002
Co. Aqueous	500	1000	2	1	0	0.003 ± 0.001
Ulat jati	1000	1000	0	1	2	0.003 ± 0.001
Co. Methanol	500	1000	0	1	1	0.002 ± 0.001
(+) Ctrl (Cadmium acetate 0.001 M)		1000	8	5	4	0.017 ± 0.006
(−) Ctrl		1000	2	1	1	0.004 ± 0.002

different polarities may allow the recovery of a broader range of compounds. However, the selection of methanol and water was based on their complementary polarity characteristics and their widespread use as reference solvents in bioactivity-oriented extraction studies. Together, these solvents are known to cover a broad polarity range and are effective for extracting polar and moderately polar constituents, which are commonly associated with nutritional and biological activity (Snyder, 1978; Dai and Mumper, 2010).

Methanol and water were specifically chosen as extraction solvents because they are standard, widely accepted media for broad-

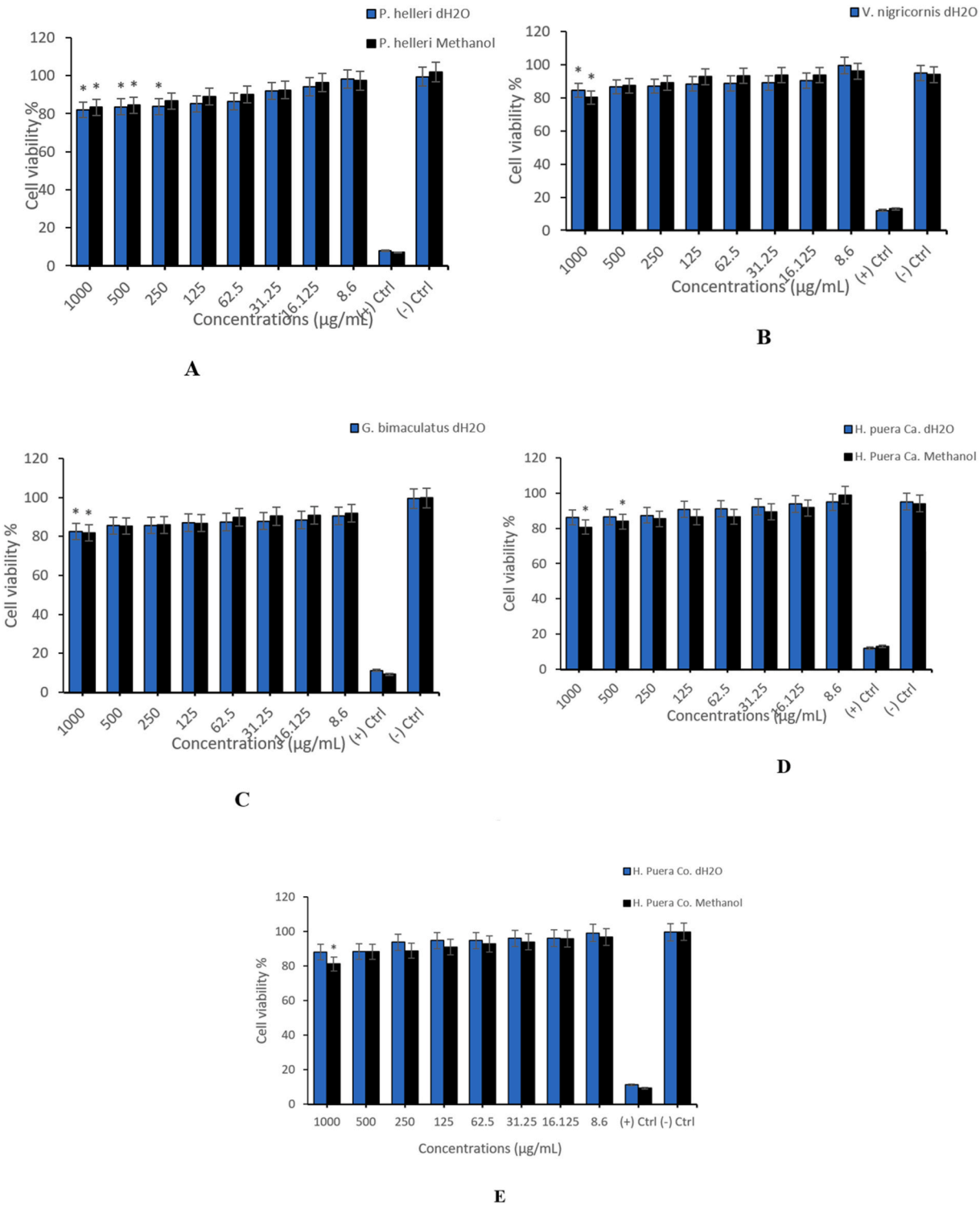


Fig. 7. Cytotoxic effects of insect extracts (water and methanol) on the lymphocyte cell culture for 24 h of applications. **A-** Puthul (*P. helleri*), **B-** *Belalang kayu* (*V. nigricrnis*), **C-** Jangkrik (*G. bimaculatus*), **D-** *Ulat jati* (*H. puera*) (caterpillar) and **E-** *Entung jati* (*H. puera*) (cocoon). Symbol (*) represent statistically significant difference compared to the negative control ((−) Ctrl).

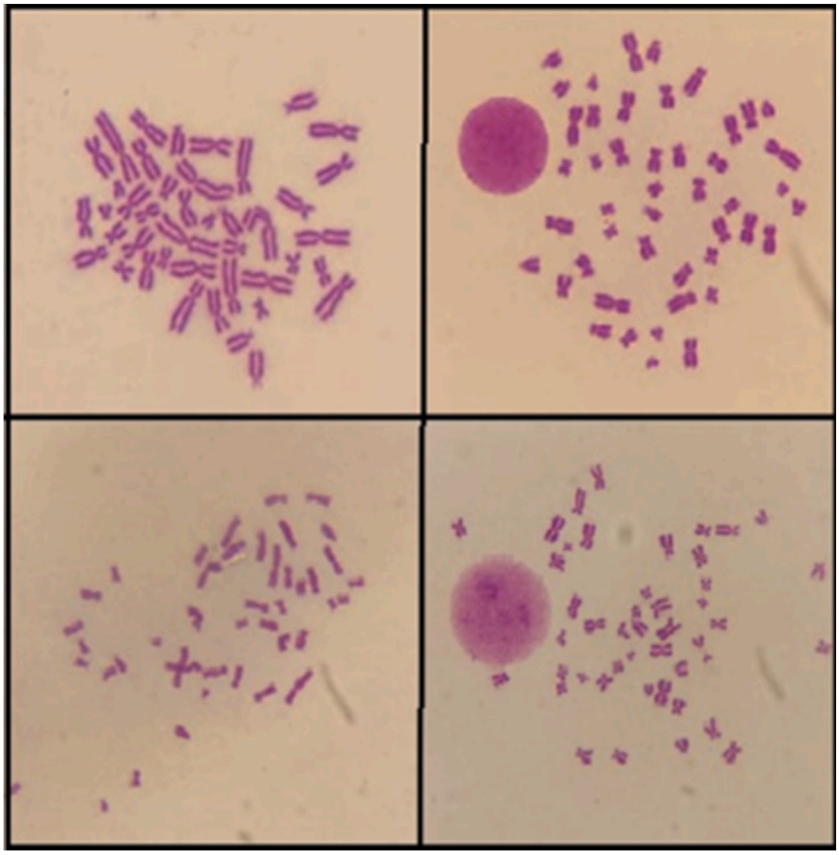


Fig. 8. Sample images of metaphase plates evaluated in the chromosomal aberration test.

Table 12
Frequency of chromosomal aberrations observed in human lymphocyte cell treated with 1000 μg/mL of insect extracts for 72 h (ChG: Chromosomal gaps, ChB: Chromosomal breaks, Ch'G: Chromatin gaps, Ch'B: Chromatin breaks, Others: including tetrad chromosome, ring chromosome, fused chromosome. SD: Standard deviation.)

Groups	Methapahase Plaque	ChG	ChB	Ch'G	Ch'B	Others	Mean CAi + SD
Puthul Aqueous	30	9	3	6	0	18	1.2 ± 0.06
Puthul Methanol	30	9	0	3	3	15	0.98 ± 0.04
Belalang kayu Aqueous	30	6	0	0	9	12	0.9 ± 0.04
Belalang kayu Methanol	30	6	3	0	0	15	0.8 ± 0.04
Jangkrik Aqueous	30	6	0	0	3	24	1.1 ± 0.05
Jangkrik Methanol	30	6	6	0	0	0	0.4 ± 0.02
Ulat jati Ca. Aqueous	30	6	0	0	0	18	0.8 ± 0.04
Ulat jati Ca. Methanol	30	12	0	0	0	12	0.8 ± 0.04
Ulat jati Co. Aqueous	30	12	6	0	6	0	0.8 ± 0.04
Ulat jati Co. Methanol	30	24	0	6	0	0	0.99 ± 0.04
(+) Ctrl (Cadmium acetate 0.001 M)	30	18	0	6	6	18	3.62 ± 0.10
(−) Ctrl	30	6	0	0	6	12	0.8 ± 0.04

spectrum phytochemical extraction and provide a useful comparative baseline for biological studies. Water is highly polar and efficiently solubilizes very polar compounds such as sugars, amino acids, some tannins, and glycosides, while methanol has moderate polarity that allows it to extract a broader range of secondary metabolites including phenolics, flavonoids, alkaloids, and terpenoids that are often associated with bioactivity in antimicrobial and antioxidant assays. Methanol also generally yields a higher total

extractable phytochemical content than water in many plant and organism studies, making it a common choice in initial screenings of biological activity. In antimicrobial studies, differences in solvent polarity have been shown to influence both the yield and strength of bioactive constituents. For example, methanol extracts often exhibit stronger antibacterial effects and higher recovery of phenolic compounds than aqueous extracts in comparative assays, depending on the test organism and matrix. However, it is also well documented that other solvents with different polarities—such as ethanol, ethyl acetate, acetone, chloroform, or even non-polar solvents like hexane—can extract distinct classes of compounds that may exhibit more potent antimicrobial activity than those obtained with water or methanol alone. The solvent polarity strongly affects the solubility of secondary metabolites, and some highly lipophilic antimicrobial agents (such as certain terpenoids, fatty acids, or sterols) are better recovered with less polar solvents. Studies comparing multiple extraction solvents have shown that solvent choice can significantly change both the chemical profile and the biological activity of crude extracts (Borges et al., 2020; Mhamdi et al., 2025; Lee et al., 2024). Therefore, while methanol and water were appropriate for generating representative extracts for broad screening and comparative purposes, further work using a broader solvent system or sequential polarity extraction might reveal more potent antimicrobial constituents that are not efficiently recovered by methanol or water alone.

In the present study, the amount of insect material available was limited, and expanding the extraction protocol to multiple solvent systems would have required substantially higher sample quantities, potentially compromising sample comparability. Therefore, while acknowledging this methodological limitation, the selected solvent system was considered sufficient to obtain a representative extract profile within the scope and objectives of the study.

In the investigation of volatile compounds in edible insects, an extensive examination unveiled a comprehensive categorization of the 406 identified compounds. These substances were grouped into distinct chemical families, encompassing straight-chain hydrocarbons, aromatic and cyclic hydrocarbons, aldehydes, ketones, esters, alcohols, carboxylic acids, pyrazines, nitrogenous compounds, sulfur compounds, phenols, terpenes, and furans. Some aroma compounds such as 3-methylbutanoic acid, 2-methylbutanoic acid and 2,3-butanediol, 2,4-nonadienal, hexanoic and nonanoic acid, (E,E) 1-hexanol, 1-heptanol and 1-octen-3-ol, 2,4-butanedione, 2-heptanone and 3-octen-2-one, ocimene, diacetyl, dimethylsulphide, nonanal and 2- and 3-methylbutanal, 3-sulfanylhexyl acetate and 3-sulfanyl-1-hexanol were analyzed in some edible insects (Perez-Santaescolastica et al., 2022). Volatile compounds identified in *Allomyrina dichotoma* included 2-methylbutanol, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine (M. H. Lee et al., 2022). Research has demonstrated that the oils from the desert locust *Schistocerca gregaria* and the African bush-cricket *Ruspolia differens* exhibit a higher composition of omega-3 fatty acids, flavonoids, and vitamin E compared to olive and sesame oils (Cheseto et al., 2020). This is the first study of Puthul, *Belalang kayu*, Jangkrik, *Ulat jati*, and *Entung jati* volatile components.

In *Acheta domesticus*, the primary fatty acids reported are linoleic acid, oleic acid, palmitic acid, and stearic acid. Lauric acid was predominantly found in *Hermetia illucens*. Palmitic acid, oleic acid, and linoleic acid were identified as the predominant fatty acids in *Tenebrio molitor* and *Alphitobius diaperinus* (Riekkinen et al., 2022). During their larval/nymphal stage, house crickets (*Acheta domesticus*), lesser mealworms (*Alphitobius diaperinus*), and black soldier flies (*Hermetia illucens*) were given diets enriched with 1%, 2%, or 4% flaxseed oil, in addition to a control diet. Fatty acid compositions of both the diets and the insects were analyzed using GC-MS. All three species exhibited distinct fatty acid profiles across the four diets, yet responded similarly to the supplementation of flaxseed oil. With each percent increase in the flaxseed oil content in the diet, the α-linolenic acid content in the insects rose by 2.3%-2.7% (Oonincx et al., 2020). This is the first study of Puthul, *Belalang kayu*, Jangkrik, *Ulat jati*, and *Entung jati* volatile fatty acid components.

Initial trials suggested that islet β-cells metabolized l-alanine significantly, yet insulin secretion remained largely unaltered by the amino acid. Subsequent, more detailed investigations revealed that l-alanine serves as a potent trigger for insulin secretion when coupled with glucose in normal rodent islets and β-cell lines. Furthermore, the metabolic pathways of l-alanine, alongside its augmentation of glucose metabolism, play a role in the stimulatory impact of this amino acid on in vitro insulin secretion (Brennan et al., 2002). A collection of 72 compounds associated with N-[4-(benzyloxy)benzoyl]alanine (I) was synthesized, formulated, and evaluated for their inhibitory effects on α-glucosidase. Among these, four compounds exhibited robust inhibitory activity, six displayed moderate inhibition, and 16 were characterized as weak inhibitors. Notably, N-[4-(benzyloxy)benzoyl]serine emerged as a potent α-glucosidase inhibitor, achieving 100% inhibition at a concentration of 1 μM (Kundu et al., 2002). The potential antidiabetic impact of L-alanine was assessed in alloxan-induced diabetic rats. Thirty-five male albino Wistar rats were categorically assigned into five groups. The results revealed that the administration of 300 mg/kg L-alanine led to a noteworthy reduction ($p < 0.05$) in both weight and blood glucose levels. L-alanine exhibited a restorative effect on tissue antioxidants, as well as kidney and liver functions, evidenced by improvements in crucial parameters. Histopathological examinations underscored the regenerative potential of L-alanine on the islets of Langerhans. These findings collectively imply that L-alanine possesses a mitigating effect on alloxan-induced diabetes (Dandare et al., 2021). Repetitive amino acid sequences within proteins are commonly observed, and the glycine-serine-alanine repeat is a motif with a general tendency to form β-sheet aggregates, prevalent in key pathological factors associated with various neurodegenerative diseases. The effects of specific glycine-serine-alanine repeat peptides (SNPs), particularly SNP-9, on Alzheimer's disease (AD)-like abnormalities were assessed in transgenic mice, *Caenorhabditis elegans*, and in rat and cell models. The entry of SNPs into the brain, SNP activity in neuronal cells, and peptide penetration into cells were scrutinized both in vivo and in vitro. Utilizing cell-free systems and the yeast two-hybrid system, potential targets of SNP-9 were explored, and interactions of these targets with SNP-9 were corroborated in cell-based systems (Yao et al., 2023). To investigate protein metabolism, plasma levels of 22 amino acids were analyzed using high-pressure chromatography in elderly patients with late-onset Alzheimer's disease (AD) at both early and late stages of progression. Disruptions in protein metabolism were evident in 16 untreated patients compared to 7 age-matched, healthy controls. Elevated fasting levels of plasma cystine and reduced fasting levels of plasma tyrosine and plasma tryptophan were noted. The ratio of fasting levels of plasma tryptophan to the sum of plasma phenylalanine, tyrosine, leucine, isoleucine, and valine showed a significant

decrease. While the ratio of fasting levels of plasma tyrosine to the sum of tryptophan, phenylalanine, leucine, isoleucine, and valine was low, it did not reach statistical significance compared to controls. Plasma levels of taurine, threonine, serine, asparagine + glutamate, glutamine, glycine, alanine, citrulline, alpha-aminobutyrate, valine, methionine, isoleucine, leucine, phenylalanine, ornithine, lysine, 1-M-histidine, histidine, arginine, and proline remained within normal fasting ranges. Given that tryptophan and tyrosine serve as precursors for dopamine/norepinephrine and serotonin, respectively, the observed deficiencies in these amino acids suggest a potential reduction in monoaminergic activities in the central nervous system (CNS) during the early stages of late-onset AD. These findings prompt consideration for a trial involving precursor loading to address tryptophan and tyrosine deficiencies, potentially enhancing mental capacity and performance. The observations in mild to early moderate stages of late-onset AD align with previous reports (Forssell et al., 1989). It was observed that the amount of alanine and tyrosine were high in the extracts showing high activity.

The present study revealed distinct differences in the nutritional and bioactive profiles of *Hyblaea puera* between its adult and cocoon forms, reflecting developmental stage–specific metabolic and physiological adaptations. The cocoon form (*Entung jati*) exhibited a richer biochemical composition, with higher relative levels of essential fatty acids—particularly oleic and linolenic acids—and elevated concentrations of key amino acids such as alanine and tyrosine. This enhanced nutrient density aligns with the functional role of the pupal stage as a metabolic reservoir that supports metamorphosis and tissue differentiation (Rumpold and Schlüter, 2013; Huis et al., 2013).

In contrast, the adult form (*Ulat jati*) displayed comparatively lower levels of these constituents, which may reflect a shift in metabolic priorities toward locomotion, reproduction, and energy utilization rather than nutrient storage. Comparable developmental differences have been observed in other insect species, where larvae or pupae often contain higher lipid and protein reserves than their adult counterparts due to the energetic demands of metamorphosis (Zielińska et al., 2015; Payne et al., 2016).

Functionally, the cocoon form demonstrated stronger bioactivity in several assays, including α-glucosidase inhibition and antioxidant capacity. These activities are likely associated with its elevated levels of phenolic compounds and amino acids, both of which have been reported to contribute to enzyme inhibition and redox modulation (Tundis et al., 2010; Oboh et al., 2014). In contrast, the adult form showed relatively lower biological activities, consistent with its reduced content of these bioactive constituents.

Elemental analysis further indicated that the cocoon stage contained higher concentrations of nutritionally significant minerals such as potassium, calcium, zinc, and iron, which are involved in metabolic regulation, enzymatic processes, and antioxidant defense mechanisms. Similar nutrient enrichment during immature developmental stages has been documented in other edible insects, emphasizing the potential nutritional advantage of these stages (Bukkens, 1997; Payne et al., 2016).

Overall, these findings suggest that the cocoon form of *H. puera* may offer superior nutritional and functional benefits compared to the adult form. This developmental distinction highlights the importance of life stage consideration in evaluating edible insects for functional food and nutraceutical applications. The observed biochemical variation reinforces the potential of *H. puera*, particularly its cocoon form, as a sustainable and value-added food resource with promising health-promoting properties.

In a study, the *α*-glucosidase enzyme inhibition effect of *G. bimaculatus* samples collected on different days during the fermentation process was evaluated. It was observed that the activity increased during the fermentation process; however, no comparison was made with a standard drug (J.-S. Lee et al., 2022). In another study, the functionality of *G. bimaculatus* fermented by *Bacillus* and *Lactobacillus* strains was investigated. *G. bimaculatus* underwent fermentation by the following strains: *B. amyloliquefaciens*, *L. plantarum*, *B. amyloliquefaciens*, and *L. plantarum*. Remarkably, *L. plantarum* exhibited complete inhibition of *α*-glucosidase (>96%). With an increase in the concentration of *L. plantarum*, the *α*-amylase inhibition activity exhibited a corresponding rise, ultimately reaching 68.48%. Conversely, the other fermented *G. bimaculatus* products showed no significant effect on the inhibition of *α*-amylase. It's important to note that no comparison with a standard drug was conducted in this study (Jang and Kim, 2021).

The marked difference observed between α-glucosidase and α-amylase inhibitory activities can be explained by the distinct structural and functional characteristics of these enzymes. Although both enzymes play essential roles in carbohydrate digestion, they differ significantly in substrate specificity, catalytic mechanisms, and accessibility of their active sites. α-Amylase initiates starch digestion by cleaving internal α-1,4-glycosidic bonds, whereas α-glucosidase acts at the intestinal brush border to hydrolyze terminal glucose residues, facilitating glucose absorption. Consequently, compounds capable of inhibiting α-glucosidase do not necessarily exhibit comparable inhibitory activity against α-amylase.

Natural bioactive compounds, particularly phenolics, flavonoids, and small polar metabolites, have been reported to interact more efficiently with α-glucosidase due to favorable hydrogen bonding and π–π interactions within its active site. In contrast, the deeper and more sterically constrained active site of α-amylase limits the binding efficiency of many naturally occurring inhibitors. This structural selectivity explains why plant- and insect-derived extracts frequently show stronger α-glucosidase inhibition than α-amylase inhibition, as also reported in previous studies (Tundis et al., 2010; Tadera et al., 2006; Kim et al., 2005).

Importantly, selective inhibition of α-glucosidase without strong suppression of α-amylase is considered pharmacologically advantageous. Excessive α-amylase inhibition is associated with undesirable gastrointestinal side effects, such as bloating and diarrhea, due to excessive fermentation of undigested carbohydrates in the colon. Therefore, extracts that preferentially inhibit α-glucosidase while exerting minimal effects on α-amylase are regarded as more suitable for managing postprandial hyperglycemia (Bischoff, 1994).

In the present study, the stronger α-glucosidase inhibition observed in selected extracts may be attributed to their rich composition of phenolic compounds, amino acids, and other low-molecular-weight bioactives, which are known to modulate carbohydrate-hydrolyzing enzymes. Conversely, the comparatively weaker α-amylase inhibition suggests a selective and potentially safer inhibitory profile. This selective enzyme modulation highlights the therapeutic relevance of the studied insect-derived extracts as promising functional ingredients for glycemic control.

In a study, to assess the biological activities associated with Alzheimer's disease, cricket (*G. bimaculatus*) specimens were subjected to ethanol extraction and subsequently examined for acetylcholinesterase inhibition. However, the study did not reveal any inhibitory

effect on acetylcholinesterase enzyme function as in our study (Boonsin et al., 2018).

In a study, the antioxidant activity of *Gryllus bimaculatus* was tested on diabetic mice. In hepatocellular examinations, it was determined that oxidative damage indicators decreased significantly (Ahn et al., 2020). In another study investigating the antioxidant activity of *G bimaculatus*, which is known to be rich in unsaturated fatty acids and protein, it was observed that fermentation increased the antioxidant effect (Jang and Kim, 2021). There are not many studies in the literature about the antioxidant activity of these edible insect species. Therefore, our study is important in terms of revealing the biological activities of these species.

As it stands, only a minor fraction, less than 34 species spanning the orders of Lepidoptera, Coleoptera, Orthoptera, Hemiptera, Hymenoptera, Blattodea, Diptera, and Amorphosceloidea, have undergone rigorous toxicological evaluation. Notwithstanding, the heterogeneity of these studies poses significant challenges in comparing and synthesizing results due to variations in methodology and endpoints. However, despite these constraints, the emerging consensus from existing data underscores the general safety of these insects for consumption (Baiano, 2020; Gao et al., 2018; Gnana Moorthy Eswaran et al., 2023).

Micronucleus and chromosomal aberration tests are two commonly used assays for evaluating the genotoxic potential of various substances, including potential food sources. These tests serve as an integral part of toxicology studies, assessing the potential of a substance to cause genetic damage that could lead to conditions like cancer (Memiş et al., 2013; Noh et al., 2018; Turkez et al., 2014). In our study, the genotoxicity of the insect isolates was evaluated using the Hoechst 33258 fluorescent staining technique over a 24-h period for micronucleus assessments. The results from these investigations revealed no significant toxicological effects from the extract applications in comparison to the negative control (non-treated HDFa cell culture), suggesting that the isolates were generally non-genotoxic under these conditions. However, certain variations were noticed in the number of nuclear abnormalities post-application of specific isolates, namely *P. helleri* methanol, *G. bimaculatus* dH_2O, *G. bimaculatus* methanol, and *H. puera* Ca. dH_2O. The observed nuclear abnormalities in the negative control group stood at 0.004 ± 0.002 per 1000 cell count. The aforementioned isolates exhibited slightly higher nuclear abnormality counts of $0.005 \pm 0.003/1000$, $0.006 \pm 0.003/1000$, $0.007 \pm 0.003/1000$, and $0.006 \pm 0.003/1000$ cells respectively, which points towards a minor genotoxic effect. Notably, the positive control group, which received a 0.001 M concentration of Cadmium acetate, exhibited significantly elevated nuclear abnormality numbers ($0.017 \pm 0.006/1000$ cell count) compared to the experimental groups. This comparison further underscores the relative genotoxic safety of the insect isolates in the study. Further investigations were performed to evaluate the genotoxicity of the insect isolates through chromosomal aberration tests on human lymphocyte cell cultures over a 24-h application period. The results showed no significant genotoxic effects after the application of the extracts, further substantiating the safety of these isolates. In a summary, while minimal chromosomal alterations were observed following the application of the extracts, the changes were relatively insignificant and did not indicate substantial genotoxic effects. These findings suggest the relative genotoxic safety of the insect isolates, although continued investigation and monitoring are warranted to confirm these preliminary results. The findings from our study align with previous research in the field, further reinforcing the consensus on the safety profile of these insect isolates. Previous investigations into the cytotoxicity and genotoxicity of various edible insects and their extracts have similarly reported a generally low toxicological impact, especially at the concentrations typically consumed (Belluco et al., 2013; Borges et al., 2022; Jatoi and Sahito, 2023).

The cytotoxic effects observed in *Phyllophaga helleri* are most likely associated with a combination of phenolic compounds, lipid-derived metabolites, and oxidative stress–related mechanisms, rather than the action of a single toxic compound. Phenolic compounds are well known to exhibit dual biological behavior, acting as antioxidants at low concentrations while exerting pro-oxidant and cytotoxic effects at higher doses through mitochondrial dysfunction and oxidative stress induction (Galati and O'Brien, 2004; Dai and Mumper, 2010).

In addition, lipid-derived compounds—particularly unsaturated fatty acids and their oxidation products—have been reported to disrupt membrane integrity and induce apoptosis in mammalian cells. Lipid peroxidation products such as aldehydes and hydroperoxides may interfere with cellular redox balance and contribute to cytotoxic outcomes under in vitro conditions (Ayala et al., 2014; Catalá, 2009). These mechanisms may partially explain the concentration-dependent cytotoxic effects observed for *P. helleri* extracts in the present study.

Furthermore, trace elements detected in *P. helleri*, such as iron and aluminum, may indirectly enhance oxidative stress through Fenton-type reactions, thereby amplifying cellular damage when present alongside redox-active metabolites (Valko et al., 2005). However, as these elements were detected below internationally accepted toxic thresholds, their contribution is likely synergistic rather than primary.

Importantly, despite the observed cytotoxicity at higher concentrations, no significant genotoxic effects were detected, indicating that the bioactive constituents exert reversible or non–DNA-damaging effects. This finding is consistent with previous reports demonstrating that insect-derived extracts often display moderate cytotoxicity without genotoxic risk (Belluco et al., 2013; Borges et al., 2022).

5. Conclusion

This comprehensive investigation highlights the remarkable nutritional and bioactive potential of five traditionally consumed Indonesian edible insect species. The methanol and aqueous extracts of *Phyllophaga helleri*, *Valanga nigricornis*, *Gryllus bimaculatus*, and both adult and cocoon forms of *Hyblaea puera* demonstrated diverse and species-specific phytochemical profiles. Notably, *Hyblaea puera* (*Entung jati*) emerged as a promising candidate due to its high α-glucosidase inhibitory activity, rich limonene and oleic acid content, and favorable amino acid composition, including elevated levels of alanine and tyrosine. Elemental analysis revealed that all studied insects are valuable sources of essential minerals such as potassium, calcium, zinc, iron, and copper, while toxic elements (As, Cd, Hg, Pb) remained within internationally accepted safety limits. Although cytotoxic effects were observed at high extract

concentrations in certain cell lines, genotoxicity assessments confirmed a generally safe profile under tested conditions. Furthermore, selected extracts exhibited antioxidant and antimicrobial activities, reinforcing their potential as functional food ingredients with added therapeutic value. The variation in chemical and biological activities among species also underscores the importance of species selection and extraction methods in maximizing functional benefits. In summary, the findings support the incorporation of these edible insect species, particularly *Hyblaea puera*, into novel food systems aimed at improving nutritional quality and promoting sustainable protein alternatives. These insights contribute valuable data to the expanding field of entomophagy and encourage further research into optimizing their safety, functionality, and acceptance in modern diets.

CRediT authorship contribution statement

Hafize Yuca: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Zeynep Aydoğan:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Özge Çağlar Yıldırım:** Methodology, Investigation, Data curation, Conceptualization. **Bilge Aydın:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mehmet Enes Arslan:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alptuğ Atila:** Methodology, Investigation, Data curation, Conceptualization. **Gamze Göger:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Betül Demirci:** Methodology, Formal analysis, Data curation, Conceptualization. **Najeebullah Mohammadi:** Resources, Formal analysis, Data curation. **Artina Prastiwi:** Resources, Formal analysis, Conceptualization. **Ahmet Taşer:** Investigation, Formal analysis, Data curation, Conceptualization. **Ümit İncekara:** Writing – original draft, Supervision, Funding acquisition, Data curation, Conceptualization. **Songül Karakaya:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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