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Toxicological evaluation and mycochemical characterisation of aqueous extract of *Schizophyllum commune*

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Abstract

Growing demand for *Schizophyllum commune* Fr. reflects its pharmaceutical and nutraceutical potential, although its safety profile remains underexplored despite long-standing traditional use. This study aimed to evaluate the in vitro cytotoxicity, in vivo acute and subacute oral toxicity, and mycochemical composition of the aqueous extract of *S. commune* (AESC). Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on human gastric adenocarcinoma (AGS) and human hepatocellular carcinoma (HepG2) cell lines. Acute toxicity was evaluated in Sprague-Dawley rats given a single oral dose of 5000 mg/kg bw, while subacute toxicity involved daily oral doses of 250, 500, and 1000 mg/kg bw for 28 days. Parameters including clinical signs, body weight, relative organ weight, food and water intake, and haematological, biochemical, and histopathological changes were monitored throughout the study. Mycochemical characterisation was performed using qualitative screening tests and liquid chromatography–quadrupole time-of-flight mass spectrometry (LC–QTOF–MS). The MTT assay indicated minimal cytotoxicity, with half-maximal inhibitory concentration (IC_{50}) values exceeding 1000 µg/mL for both AGS and HepG2 cells. No mortality or adverse signs occurred in the acute toxicity study, indicating a median lethal dose (LD_{50}) greater than 5000 mg/kg bw. Similarly, the subacute study showed no adverse effects at any tested dose in either sex, with a no-observed-adverse-effect level (NOAEL) greater than 1000 mg/kg bw. Qualitative screening revealed no detectable levels of major secondary metabolite classes, while LC–QTOF–MS profiling identified amino acids, fatty acids, and low-abundance phenolic compounds as the predominant constituents of AESC. These findings confirm a favourable safety profile of AESC, supporting further evaluation for gastro- and hepato-protective applications.

Keywords *Schizophyllum commune*, Aqueous extract, Cytotoxicity, Acute toxicity, Subacute toxicity, Mycochemical composition

1 Introduction

Despite the growing interest in natural products and edible mushrooms, the assumption that such materials are inherently safe is increasingly questioned. Adverse effects, including toxic reactions, allergic responses, and interactions with conventional drugs, have been reported for several medicinal plants and mushrooms [1–3]. Therefore, systematic toxicological evaluation is essential to establish safety margins, identify potential target organ toxicity, and support future pharmacological or nutraceutical development. Standard safety assessments typically involve in vitro cytotoxicity screening followed by in vivo acute and subacute toxicity studies to evaluate both short- and repeated-dose exposure effects [1]. *Schizophyllum commune* Fr. is a widely distributed basidiomycete mushroom found across tropical, subtropical, and temperate regions, commonly growing on decaying wood and forest substrates [4, 5]. It is particularly abundant in Southeast Asia, including Malaysia, where it is known locally as kodop in Sabah and remains the only wild edible mushroom widely sold in local markets [6, 7]. Owing to its availability and long-standing dietary use, *S. commune* has attracted increasing scientific interest for its bioactive and therapeutic potential. Mycochemical investigations have shown that *S. commune* contains diverse bioactive constituents, including polysaccharides such as schizophyllan and secondary metabolites such as flavonoids and sesquiterpenes, which are associated with anti-tumour, anti-inflammatory, and anti-hyperlipidaemic activities [8].

Previous in vitro studies on *S. commune* have reported generally low cytotoxicity, depending on the extraction solvent. Aqueous extracts tested at 250–10,000 µg/mL exhibited a 50% cytotoxic concentration (CC_{50}) greater than 10,000 µg/mL against Vero cells, indicating high safety [9]. Similarly, ethyl acetate extracts showed weak cytotoxicity at 31.25–500 µg/mL, with half-maximal inhibitory concentration (IC_{50}) values exceeding 500 µg/mL against several human cancer cell lines [10]. In contrast, chloroform extracts demonstrated higher cytotoxicity toward human hepatocellular carcinoma (HepG2) cells, suggesting that non-polar constituents may contribute to membrane disruption and oxidative stress [11, 12]. These solvent-dependent cytotoxicity profiles are consistent with the distribution of mycochemical classes in *S. commune*, where hydrophilic constituents are generally associated with lower cytotoxicity, while non-polar fractions may contain more biologically reactive compounds [13–15].

Although pharmacological studies on *S. commune* are increasing, in vivo toxicological data remain scarce. To date, only one study has evaluated the acute toxicity of *S. commune* mycelial liquid in mice, reporting no observable toxicity across multiple administration routes [16]. However, the safety of aqueous fruiting body extracts, particularly following repeated oral exposure, has not been adequately investigated. Therefore, the present study aims to address this gap by evaluating the toxicity of the aqueous extract of *S. commune* (AESC) through in vitro cytotoxicity assays and in vivo acute and subacute oral toxicity studies, followed by mycochemical characterisation, thereby providing a focused and comprehensive safety assessment.

2 Materials and methods

2.1 Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade unless otherwise stated. Ham's F-12 Nutrient Mixture, Eagle's Minimum Essential Medium (EMEM), fetal

bovine serum (FBS), penicillin–streptomycin solution, and phosphate-buffered saline (PBS) were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Trypsin–ethylenediaminetetraacetic acid (0.25% trypsin–EDTA) and 3-(4,5-dimethylthiazol-2-yl)–2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Reagents used for qualitative mycochemical screening included Liebermann–Burchard reagent, ferric chloride, Dragendorff's reagent, magnesium turnings, concentrated hydrochloric acid, and sodium hydroxide, all obtained from Merck (Darmstadt, Germany). Formic acid, ammonium formate, and acetonitrile (LC–MS grade) used for liquid chromatography–mass spectrometry (LC–MS) analysis were purchased from Merck (Darmstadt, Germany). All reagents and solvents were prepared using ultrapure water and handled according to the manufacturers' instructions.

2.2 Sample collection and preparation

Whole *S. commune* samples were collected from Kampong Apin-Apin, Keningau, Sabah, Malaysia (5.470395° N, 116.271670° E) in September 2023 under a sampling permit approved by the Sabah Biodiversity Council [Licence Ref. No.: JKM/MBS.1000-2/2 JLD.17 (114)]. Permission for collection of the mushroom specimens from the sampling site was obtained from the relevant governing authority under this permit. The samples were authenticated by Assoc. Prof. Dr. Jaya Seelan Sathiya Seelan, a mycologist at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia. A voucher specimen of the collected material was deposited in the BORNEENSIS Herbarium, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, under voucher number BORH(F)03738.

The samples were thoroughly washed with distilled water to remove dirt and debris and oven-dried at 50 °C, a temperature commonly employed for edible and medicinal mushrooms to achieve effective dehydration while minimising thermal degradation of thermolabile bioactive constituents such as polysaccharides, amino acids, and phenolic acids [17]. Drying was performed with periodic turning to ensure uniform moisture removal, after which the samples were ground into coarse powder using a milling machine.

2.3 Sample extraction

Aqueous extraction of the dried *S. commune* powder was performed following a modified protocol adapted from Yim et al. [18]. A total of 10 kg of dried and powdered fruiting bodies was extracted by soaking in 200 L of ultrapure water, corresponding to a solid-to-solvent ratio of 1:20 (w/v), in a water bath shaker maintained at 37 °C for 24 h. After extraction, the mixture was allowed to settle, and the aqueous supernatant was carefully decanted and sequentially filtered through a cloth filter, cotton wool, and Whatman No. 1 filter paper to remove particulate matter. The clarified filtrate (approximately 180 L) was transferred into suitable containers and stored at − 80 °C for at least 48 h prior to freeze-drying. Lyophilisation was performed using a freeze dryer to obtain the first batch of dried AESC. The residual solid material was re-extracted with a fresh 200 L volume of ultrapure water under the same conditions for 48 h, followed by identical filtration, freezing, and freeze-drying procedures to obtain the second batch of dried AESC. Both batches were pooled to give a total yield of 13.00 ± 1.00% (w/w) of lyophilised AESC,

which was weighed, sealed in airtight containers, and stored at − 20 °C for a period not exceeding 2 months until further analysis and use in subsequent experiments. Reconstitution for in vivo studies was performed immediately prior to administration to minimise potential degradation during storage.

Stability of AESC during frozen storage was inferred from consistent liquid chromatography–quadrupole time-of-flight mass spectrometry (LC–QTOF–MS) profiles obtained from aliquots analysed before and after the storage period, with no qualitative changes observed in the major detected constituents. For in vitro assays, the lyophilised AESC was reconstituted in sterile ultrapure water and diluted with culture medium, with corresponding ultrapure water controls included. For in vivo studies, AESC was reconstituted in distilled water prior to oral administration, and distilled water alone was used as the vehicle control.

2.4 In vitro cytotoxicity assay

2.4.1 Cell culture and maintenance

Human gastric adenocarcinoma (AGS) cells (iCell Bioscience, Shanghai, China) were cultured in Ham's F-12 Nutrient Mixture, whereas HepG2 cells (ATCC, Manassas, VA, USA) were cultured in EMEM. Both media were supplemented with 10% FBS and 1% penicillin–streptomycin. Cells were maintained at 37 °C in a humidified incubator with 5% carbon dioxide (CO_2) and subcultured at 70–80% confluence by washing with phosphate-buffered saline (PBS) followed by treatment with 0.25% trypsin–EDTA. All procedures were conducted aseptically in a Class II biosafety cabinet.

2.4.2 MTT cytotoxicity assay

Cytotoxicity of AESC was evaluated using the MTT assay as described by Gavanji et al. [19]. Cells were seeded in 96-well plates at a density of 1×10^4 cells/well in 100 µL complete medium and incubated for 24 h at 37 °C. Cells were then treated with AESC at concentrations ranging from 0.01 to 2.00 mg/mL and incubated for an additional 24 h, with ultrapure water serving as the vehicle control. Subsequently, 20 µL of MTT solution was added to each well and incubated for 4 h at 37 °C. The medium was removed, and 100 µL detergent reagent was added to dissolve the formazan crystals. Plates were incubated in the dark for 2 h, and absorbance was measured at 570 nm. Cell viability was calculated according to Eq. (1) [20]. The IC_{50} value was determined from the dose–response curve generated by plotting cell viability against sample concentration.

$$\text{Cell viability } (\%) = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100 \quad (1)$$

2.5 In vivo toxicity studies

2.5.1 Animal housing and husbandry

Male and female Sprague–Dawley rats (200–250 g, 8–10 weeks old) were obtained from Laboratory Animal Facility and Management (LAFAM), Faculty of Pharmacy, Universiti Teknologi MARA (UiTM). Rats were housed in individually ventilated cages with corncob bedding under controlled conditions (22 ± 3 °C, 70–80% humidity, 12 h light–dark cycle). Standard pellet food and water were provided *ad libitum*. Animal handling and experimental procedures adhered to UiTM guidelines for investigations involving experimental pain in conscious animals. All experiments were conducted between 0830 h and

1730 h to minimise environmental variation. The study protocol was approved by the UiTM Committee on Animal Research and Ethics (CARE), Department of Research and Innovation, UiTM, Selangor, Malaysia [Approval No.: UiTM CARE 435/2023].

2.5.2 Acute toxicity evaluation

Acute toxicity was evaluated according to the Organisation for Economic Co-operation and Development (OECD) Guideline 423 [21]. Adult female Sprague–Dawley rats (200–250 g) were fasted overnight with free access to water and weighed prior to dosing. Initially, a single oral dose of 5000 mg/kg bw AESC was administered to one nulliparous, non-pregnant rat. Upon survival, two additional rats received the same dose. If one rat died, another group of three rats received the same dose; if two or more rats died, the dose was reduced to 2000 mg/kg bw. The control group received distilled water. After dosing, food was withheld for 4 h, and rats were observed for clinical signs of toxicity, behavioural changes, mortality, and daily body weight throughout the 14-day period at 0 h, 0.5 h, 4 h, 24 h, and thereafter. Humane endpoints, including persistent loss of righting reflex, laboured breathing, severe lethargy, or unresponsiveness, were predefined for early euthanasia to minimise suffering. No animals reached the predefined humane endpoints, and all rats survived and completed the 14-day observation period without adverse clinical signs.

At the end of the study, rats were euthanised by gradual-fill CO_2 inhalation, and death was confirmed by cessation of respiration and absence of corneal and pedal reflexes before tissue collection. The heart, lungs, stomach, spleen, liver, and kidneys were then harvested. Relative organ weight (ROW) was calculated based on the final body weight using Eq. (2) [22] to assess potential organ-specific toxicity. Organs were preserved, sectioned, and stained with haematoxylin and eosin (H&E) for histopathological examination, which was evaluated by Assoc. Prof. Dr. Nornazirah Azizan, Head of the Department of Pathology and Microbiology, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, using a compound light microscope (Leica DM 5500B, Leica Microsystems, Wetzlar, Germany).

$$\text{ROW}\ (\%) = \frac{\text{Organ weight}\ (\text{g})}{\text{Body weight of rat on sacrifice day}\ (\text{g})} \times\ 100 \tag{2}$$

2.5.3 Subacute toxicity evaluation

Subacute toxicity was evaluated according to OECD Guideline 407 [23]. Adult Sprague–Dawley rats (200–250 g) were randomly assigned into four groups (five males and five females per group): one control group (distilled water) and three treatment groups, which received AESC at 250, 500, or 1000 mg/kg bw daily by oral gavage for 28 consecutive days. Animals were monitored weekly for body weight, food intake, and water intake, and observed daily for physical and behavioural changes. Humane endpoints were applied as in the acute study to ensure animal welfare. No animals exhibited clinical signs requiring early euthanasia, and all rats completed the 28-day treatment period as planned. At the end of the treatment period, animals were anaesthetised by gradual-fill CO_2 inhalation, death confirmed by absence of respiration and corneal reflex, and blood samples were collected via cardiac puncture for haematological and serum biochemical analyses.

For haematology, whole blood was collected into EDTA tubes (purple cap), gently inverted eight times to ensure mixing, and analysed fresh. Parameters included red blood cell count (RBC), haemoglobin concentration (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EON), platelet count (PLT), and plasma protein (PP). Haematological parameters (except PP) were measured using a Sysmex XP-300 automated analyser (Sysmex Corporation, Kobe, Japan), while PP was determined from plasma separated from EDTA-treated blood via microhaematocrit centrifugation using a VEE GEE STX-3 clinical refractometer (VEE GEE Scientific, Kirkland, WA, USA). For serum biochemistry, blood was collected into plain tubes (red cap), allowed to clot at room temperature for at least 30 min, and centrifuged at 5000 rpm for 10 min. The liver panel was assessed by measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), while the kidney panel was assessed by measuring serum creatinine, phosphate, and urea. Following blood collection, animals were euthanised by CO_2 inhalation, and the heart, lungs, stomach, spleen, liver, and kidneys were harvested. ROW was calculated based on the final body weight [Eq. (2)] [22]. The organs were preserved, sectioned, and stained with H&E for histopathological examination, which was evaluated by the same pathologist using a compound light microscope.

2.6 Mycochemical analysis

2.6.1 Mycochemical screening analysis

Qualitative mycochemical screening was conducted to determine the presence of steroids, flavonoids, tannins, triterpenes, alkaloids, and saponins. A total of 2 g of AESC was dissolved in 20 mL of distilled water and subjected to standard qualitative tests. The Liebermann–Burchard test was used to detect steroids and triterpenes [24], the Shinoda test for flavonoids [25], the ferric chloride test for tannins [26], Dragendorff's reagent for alkaloids [27], and the froth test for saponins [28]. The presence of each mycochemical class was indicated by characteristic colour changes, precipitate formation, or stable frothing.

2.6.2 Mycochemical profiling analysis

Mycochemical profiling of AESC was performed following the method described by Jan Jam et al. [29] and established LC–QTOF–MS protocols [30]. Analysis was conducted using an LC–QTOF–MS 6520 system (Agilent Technologies, Santa Clara, CA, USA), operated in both positive and negative electrospray ionisation (ESI) modes. For positive ionisation, the mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). For negative ionisation, mobile phase A comprised 0.1% ammonium formate in water, while mobile phase B was acetonitrile. Data acquisition was performed using Agilent MassHunter Workstation Acquisition software (Version B.02.01), with continuous internal calibration using reference masses at m/z 121.0529 and 922.0098 to ensure mass accuracy.

Raw data files (.d format) were processed using Agilent MassHunter Qualitative Analysis software. Molecular features were extracted using the molecular feature extractor algorithm with a mass accuracy tolerance of 15 ppm and retention time alignment of 0.2 min. Noise reduction was achieved by setting the absolute abundance height

threshold to 200 counts. Metabolite annotation was performed using the Find by Formula algorithm, restricting charge states to +1 in positive mode and −1 in negative mode, with an absolute height threshold of 100 counts. Protonated (H^+) and sodium-adduct (Na^+) ions were considered in positive mode, while deprotonated (H^-) ions were considered in negative mode. Putative metabolite identification was achieved by matching accurate mass, isotope pattern, and MS/MS fragmentation data against the METLIN database in accordance with established metabolomics identification criteria [31].

2.7 Statistical analysis

All data are expressed as mean ± standard deviation. Descriptive statistics and bar graphs were generated using Microsoft Excel (2019) with the Analysis ToolPak add-in enabled. Statistical analyses were performed using Microsoft Excel and cross-validated using GraphPad Prism (Version 10) to ensure analytical reliability. Data were assessed for normality and homogeneity of variances prior to inferential testing. For the cell toxicity study, differences among groups were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. For the acute and subacute toxicity studies, one-way ANOVA followed by Dunnett's post hoc test was applied to evaluate treatment-related effects on body weight, ROW, and haematological and biochemical parameters by comparison with the control group. In addition, two-way ANOVA was performed for the subacute toxicity study to assess the main effects of dose and sex, as well as their interaction. Differences were considered statistically significant at $p < 0.05$.

3 Results

3.1 Cytotoxic activity of AESC

3.1.1 Cell viability of AGS cells

The viability of AGS cells treated with AESC at different concentrations is shown in Fig. 1(a). Cell viability remained close to 100% at lower concentrations (0.01–0.13 mg/mL). A concentration-dependent reduction in viability was observed at higher concentrations, with significant ($p < 0.05$) decreases from 0.50 mg/mL onwards. At 2.00 mg/mL, viability decreased to 81.04% ± 1.11%. The IC_{50} value exceeded 1000 µg/mL, indicating that a 50% reduction in AGS cell viability was not achieved within the tested concentration range.

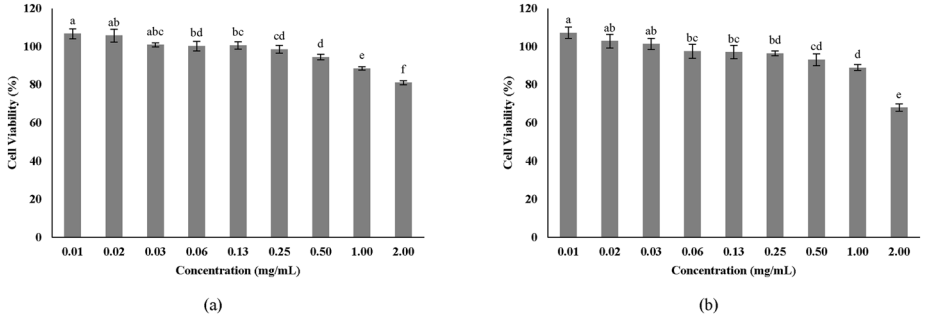

Fig. 1 Cell viability of (a) AGS and (b) HepG2 cell lines. Error bars represent the standard deviation of three independent replicates ($n = 3$). Different letters indicate significant differences ($p < 0.05$)

3.1.2 Cell viability of HepG2 cells

Figure 1(b) shows the viability of HepG2 cells following AESC treatment at different concentrations. A concentration-dependent reduction in viability was observed, with a significant ($p < 0.05$) decrease recorded at the highest concentration of 2.00 mg/mL (67.94% ± 1.88%). As the concentration decreased, cell viability increased, reaching a maximum at 0.01 mg/mL. At lower concentrations (0.01–0.03 mg/mL), viability remained close to or slightly above 100%. The IC_{50} value exceeded 1000 µg/mL, indicating that a 50% reduction in HepG2 cell viability was not observed within the tested concentration range.

3.2 Acute toxicity activity of AESC

3.2.1 Body weight changes

Figure 2 shows the body weight changes of control and AESC-treated groups (5000 mg/kg bw) over 14 days. Both groups exhibited steady increases in body weight. No significant differences ($p > 0.05$) were observed between groups, indicating comparable growth patterns throughout the acute toxicity study.

3.2.2 Relative organ weights

The ROW results of the heart, lungs, stomach, spleen, liver, and kidneys in control and AESC-treated groups (5000 mg/kg bw) are presented in Fig. 3. No significant differences ($p > 0.05$) were observed between groups, indicating that AESC did not affect organ weights at 5000 mg/kg bw.

3.2.3 Histopathological findings

Histopathological findings (Fig. 4) of the heart, lungs, stomach, spleen, liver, and kidneys showed no signs of inflammation or tissue damage in either control or AESC-treated groups (5000 mg/kg bw). Microscopic analysis revealed normal cellular architecture and an absence of inflammatory infiltration or necrosis in all examined tissues. These findings suggest that the median lethal dose (LD_{50}) of AESC exceeds 5000 mg/kg bw.

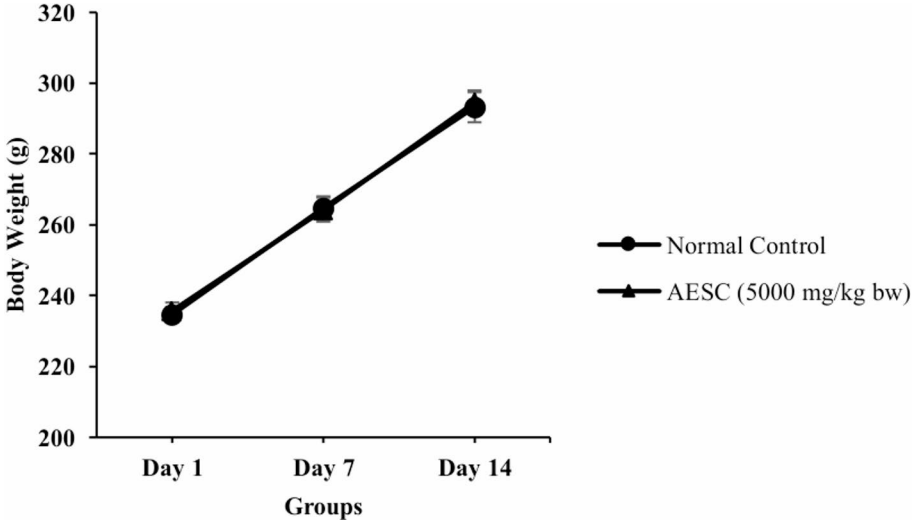

Fig. 2 Weekly body weight changes of control and AESC-treated groups during the 14-day acute toxicity study. Error bars represent the standard deviation of three independent replicates ($n = 3$). No significant differences were observed between the control and treatment groups ($p > 0.05$)

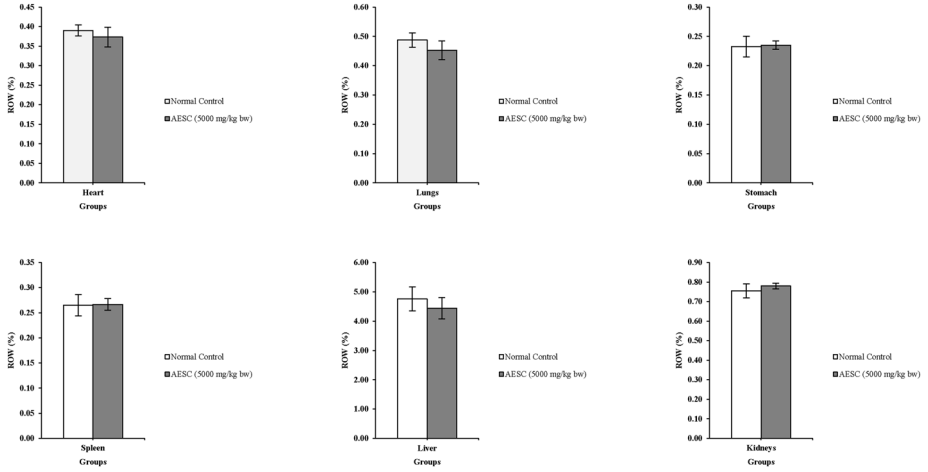

Fig. 3 ROWs of key organs in control and AESC-treated groups following the 14-day acute toxicity study. Error bars represent the standard deviation of three independent replicates ($n = 3$). No significant differences were observed between the control and treatment groups ($p > 0.05$)

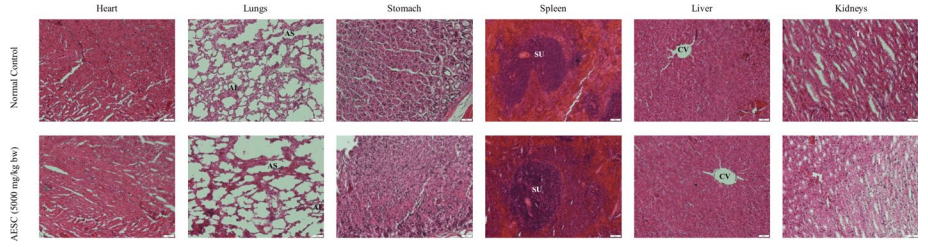

Fig. 4 Representative histological sections of key organs in control and AESC-treated groups (H&E staining, 10×). Control and AESC-treated groups show preserved tissue architecture with no evidence of inflammation, necrosis, or pathological alterations across all examined organs. AL, alveoli; AS, alveolar space; CV, central vein; SU, splenic unit; T, tubule

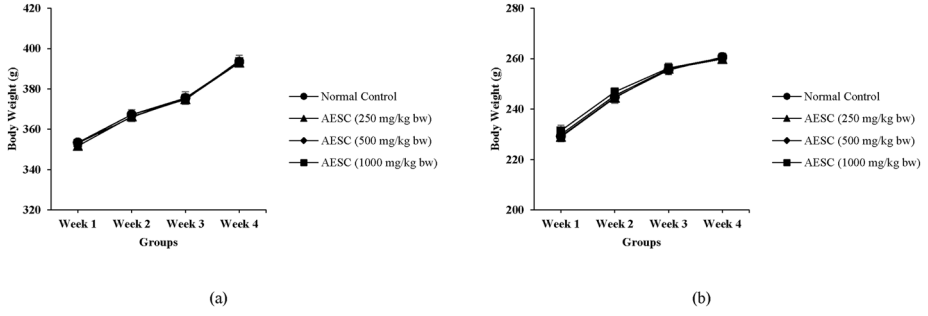

Fig. 5 Weekly body weight changes of (a) male and (b) female rats in control and AESC-treated groups during the 28-day subacute toxicity study. Error bars represent the standard deviation of five independent replicates ($n = 5$). No significant differences were observed between control and treatment groups or between sexes ($p > 0.05$)

3.3 Subacute toxicity activity of AESC

3.3.1 Body weight changes

Body weight changes in (a) male and (b) female rats during the 28-day subacute toxicity study are shown in Fig. 5. Control and AESC-treated groups (250, 500, and 1000 mg/kg bw) exhibited consistent and progressive weight gain throughout the study period in both sexes. No significant differences ($p > 0.05$) were observed between control and

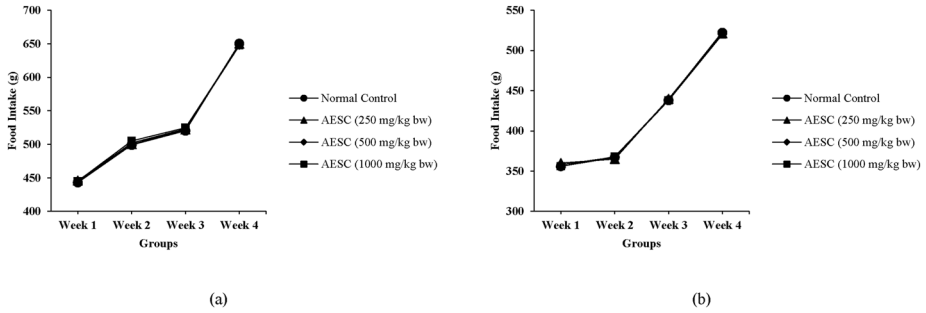

Fig. 6 Weekly food intake of (a) male and (b) female rats in control and AESC-treated groups during the 28-day subacute toxicity study

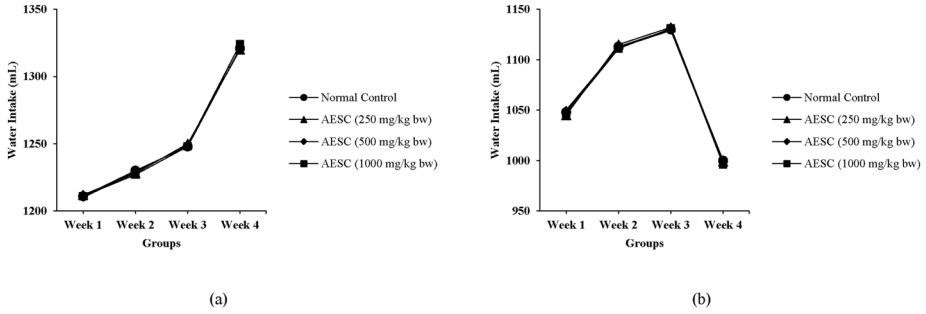

Fig. 7 Weekly water intake of (a) male and (b) female rats in control and AESC-treated groups during the 28-day subacute toxicity study

treated groups. Comparable body weight responses were observed between male and female rats, with no sex-dependent differences ($p > 0.05$) across dose levels.

3.3.2 Food intake

Figure 6 shows the food intake of (a) male and (b) female rats during the 28-day subacute toxicity study. Food intake remained stable and consistent across all groups. No differences were observed between control and AESC-treated groups, suggesting that AESC administration did not affect feeding behaviour.

3.3.3 Water intake

Water intake of (a) male and (b) female rats is illustrated in Fig. 7. Both control and AESC-treated groups maintained stable water intake throughout the study. No differences were observed between groups in either sex. However, female rats exhibited a slight decline in water intake from Week 3 to Week 4. Overall, AESC administration had no significant influence on drinking behaviour.

3.3.4 Relative organ weights

Figures 8 and 9 present ROW results for male and female rats, respectively, following the 28-day subacute toxicity study. Analysis included the heart, lungs, stomach, spleen, liver, and kidneys. No significant differences ($p > 0.05$) were observed between control and AESC-treated groups for either sex. ROWs were comparable between male and female rats, with no sex-dependent differences ($p > 0.05$) across dose levels. These findings indicate the absence of organ-specific toxicity following AESC administration.

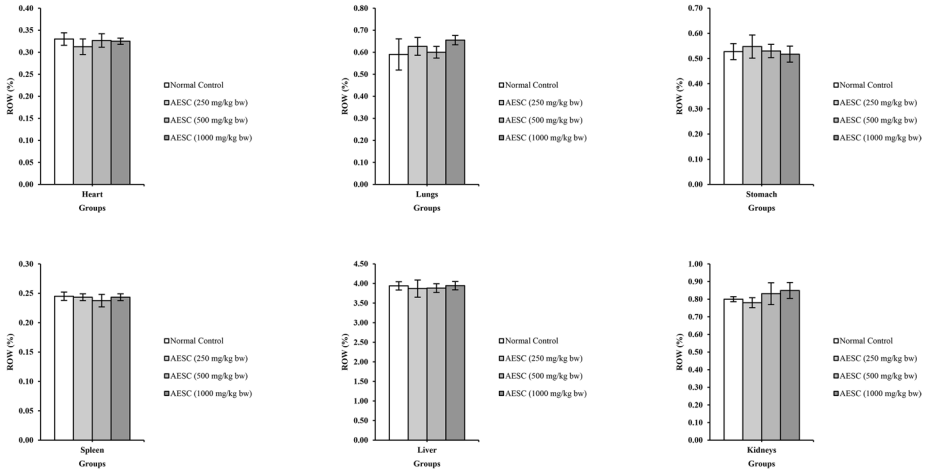

Fig. 8 ROWs of key organs of male rats in control and AESC-treated groups following the 28-day subacute toxicity study. Error bars represent the standard deviation of five independent replicates ($n = 5$). No significant differences were observed between control and treatment groups or between sexes ($p > 0.05$)

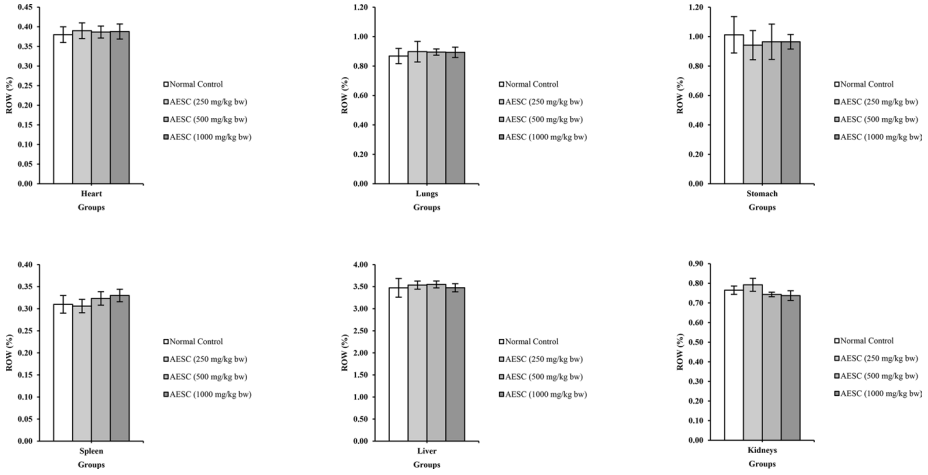

Fig. 9 ROWs of key organs of female rats in control and AESC-treated groups following the 28-day subacute toxicity study. Error bars represent the standard deviation of five independent replicates ($n = 5$). No significant differences were observed between control and treatment groups or between sexes ($p > 0.05$)

3.3.5 Haematological parameters

Table 1 (male rats) and Table 2 (female rats) present haematological parameters following subacute treatment. In male rats, no significant differences ($p > 0.05$) were observed between control and AESC-treated groups for RBC, HGB, PCV, MCV, MCHC, WBC, NEU, LYM, MON, EON, PLT, and PP. Female rats similarly showed no significant differences ($p > 0.05$). Additionally, comparison between male and female rats at equivalent dose levels revealed no significant differences ($p > 0.05$). AESC administration caused no notable haematological alterations in either sex during the subacute study.

3.3.6 Biochemical parameters

Biochemical parameters following subacute treatment in male and female rats are shown in Figs. 10 and 11, respectively. No significant differences ($p > 0.05$) were observed in hepatic enzymes (ALT, AST, and ALP) or renal markers (creatinine, phosphate, and urea) between control and AESC-treated groups of either sex. Comparable biochemical

Table 1 Haematological parameters of male rats in control and AESC-treated groups following the 28-day subacute toxicity study

Haemogram	Groups			
	Normal control	AESC (250 mg/kg bw)	AESC (500 mg/kg bw)	AESC (1000 mg/kg bw)
RBC ($\times 10^{12}$/L)	6.62 ± 1.33	6.43 ± 1.05	6.72 ± 1.27	5.93 ± 1.79
HGB (g/L)	117.00 ± 26.67	113.40 ± 16.94	115.20 ± 22.13	108.40 ± 34.36
PCV (L/L)	0.32 ± 0.04	0.35 ± 0.06	0.35 ± 0.06	0.31 ± 0.05
MCV (fL)	49.03 ± 5.92	54.27 ± 5.51	51.86 ± 3.39	53.17 ± 7.05
MCHC (g/L)	364.10 ± 52.99	327.95 ± 29.21	331.41 ± 23.42	348.65 ± 56.87
WBC ($\times 10^9$/L)	6.66 ± 1.81	5.73 ± 2.44	7.32 ± 6.08	11.85 ± 5.38
NEU ($\times 10^9$/L)	0.96 ± 0.87	0.43 ± 0.40	1.21 ± 1.82	1.89 ± 1.63
LYM ($\times 10^9$/L)	5.45 ± 2.18	5.08 ± 1.89	5.90 ± 4.03	9.47 ± 3.90
MON ($\times 10^9$/L)	0.17 ± 0.13	0.16 ± 0.11	0.17 ± 0.21	0.27 ± 0.15
EON ($\times 10^9$/L)	0.08 ± 0.06	0.06 ± 0.08	0.04 ± 0.08	0.22 ± 0.13
PLT ($\times 10^9$/L)	256.80 ± 126.39	352.00 ± 204.20	445.00 ± 287.07	662.00 ± 427.92
PP (g/L)	84.40 ± 5.55	76.00 ± 1.41	75.00 ± 5.29	78.80 ± 14.46

Error bars represent the standard deviation of five independent replicates ($n = 5$). No significant differences were observed between control and treatment groups or between sexes ($p > 0.05$)

Table 2 Haematological parameters of female rats in control and AESC-treated groups following the 28-day subacute toxicity study

Haemogram	Groups			
	Normal control	AESC (250 mg/kg bw)	AESC (500 mg/kg bw)	AESC (1000 mg/kg bw)
RBC ($\times 10^{12}$/L)	8.70 ± 0.52	8.21 ± 0.47	7.99 ± 0.36	8.72 ± 0.63
HGB (g/L)	162.50 ± 14.76	155.40 ± 6.58	154.60 ± 4.98	162.00 ± 6.75
PCV (L/L)	0.51 ± 0.05	0.48 ± 0.02	0.46 ± 0.05	0.47 ± 0.03
MCV (fL)	58.99 ± 2.26	58.10 ± 3.29	57.91 ± 4.75	55.36 ± 0.98
MCHC (g/L)	316.64 ± 10.66	326.48 ± 9.07	336.58 ± 36.97	339.64 ± 12.96
WBC ($\times 10^9$/L)	5.51 ± 3.19	6.50 ± 0.56	5.53 ± 1.32	6.56 ± 2.27
NEU ($\times 10^9$/L)	0.52 ± 0.22	0.32 ± 0.09	0.54 ± 0.53	0.77 ± 0.24
LYM ($\times 10^9$/L)	4.66 ± 2.90	5.85 ± 0.43	4.72 ± 1.53	5.56 ± 2.16
MON ($\times 10^9$/L)	0.27 ± 0.19	0.27 ± 0.13	0.22 ± 0.10	0.14 ± 0.09
EON ($\times 10^9$/L)	0.06 ± 0.06	0.05 ± 0.06	0.04 ± 0.07	0.10 ± 0.06
PLT ($\times 10^9$/L)	1038.80 ± 2.83	889.40 ± 90.53	873.00 ± 255.85	928.20 ± 153.48
PP (g/L)	84.00 ± 2.83	82.40 ± 4.98	84.00 ± 8.00	84.80 ± 4.15

Error bars represent the standard deviation of five independent replicates ($n = 5$). No significant differences were observed between control and treatment groups or between sexes ($p > 0.05$)

responses were observed between male and female rats across dose levels, with no sex-dependent differences ($p > 0.05$) detected. These results indicate no biochemical alterations associated with AESC administration, even at the highest dose (1000 mg/kg bw).

3.3.7 Histopathological findings

Histopathological examinations (Figs. 12 and 13) of the heart, lungs, stomach, spleen, liver, and kidneys from male and female rats revealed no evidence of toxicity following subacute AESC administration. Microscopic evaluation showed intact tissue architecture without signs of inflammation, necrosis, fibrosis, or cellular degeneration. All examined tissues appeared normal and comparable to those of control groups, indicating absence of morphological alterations due to AESC treatment.

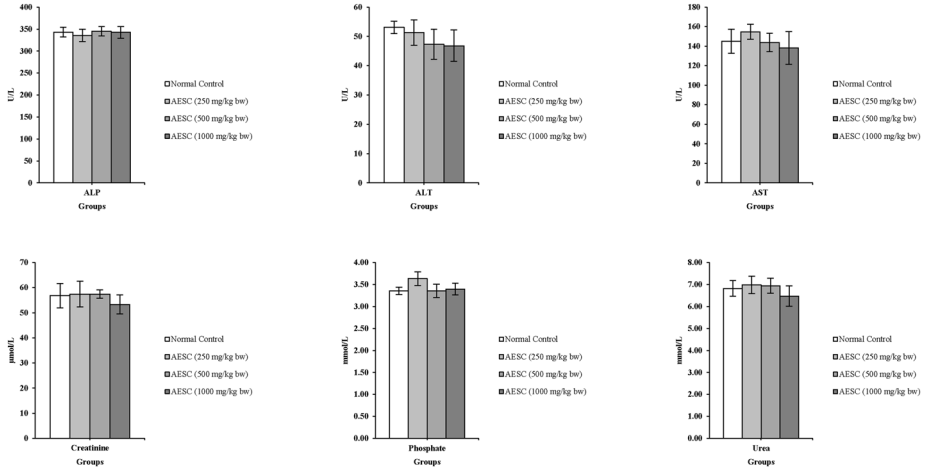

Fig. 10 Biochemical parameters of male rats in control and AESC-treated groups following the 28-day subacute toxicity study. Error bars represent the standard deviation of five independent replicates ($n = 5$). No significant differences were observed between control and treatment groups or between sexes ($p > 0.05$)

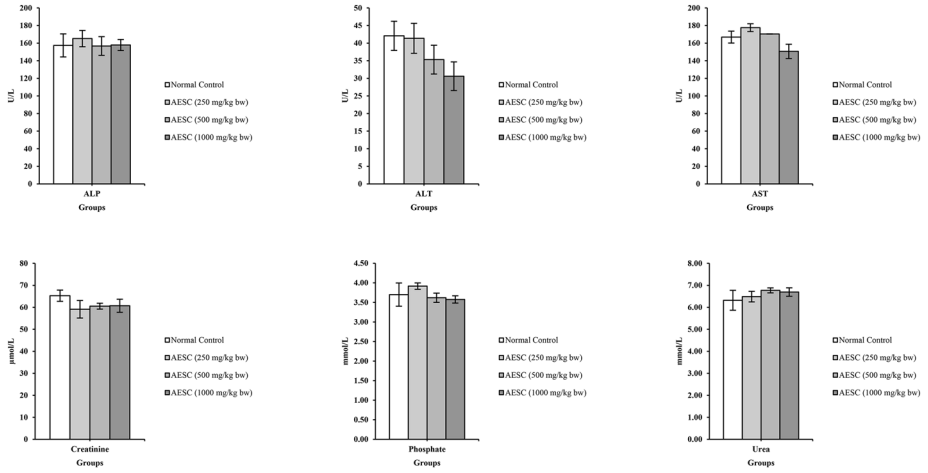

Fig. 11 Biochemical parameters of female rats in control and AESC-treated groups following the 28-day subacute toxicity study. Error bars represent the standard deviation of five independent replicates ($n = 5$). No significant differences were observed between control and treatment groups or between sexes ($p > 0.05$)

3.4 Mycochemical analysis of AESC

3.4.1 Qualitative screening

Qualitative mycochemical screening of AESC revealed the absence of all tested major classes of secondary metabolites, including steroids, flavonoids, tannins, triterpenes, alkaloids, and saponins, as shown in Table 3. These findings indicate that AESC did not contain detectable levels of these common mycochemical groups based on standard chemical screening tests.

3.4.2 Qualitative profiling

Based on Table S1 (Supplementary Material), a total of 68 compounds were detected in AESC under positive ionisation mode. As summarised in Table 4, purine was the most abundant compound (7.93%), followed by docosanedioic acid (1.10%) and trimethylolpropane triacrylate (0.80%). Only two compounds exceeded the 1% abundance threshold, while the remaining detected compounds were present at levels below 1%.

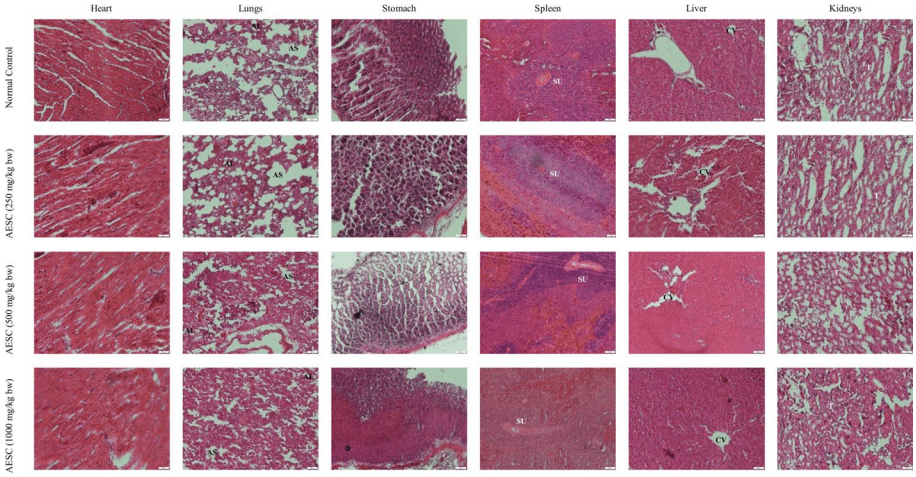

Fig. 12 Representative histological sections of key organs in control and AESC-treated groups in male rats (H&E staining, 10×). Control and AESC-treated groups (250, 500, and 1000 mg/kg bw) show preserved tissue architecture with no evidence of inflammation, necrosis, or pathological alterations across all examined organs. AL, alveoli; AS, alveolar space; CV, central vein; SU, splenic unit; T, tubule

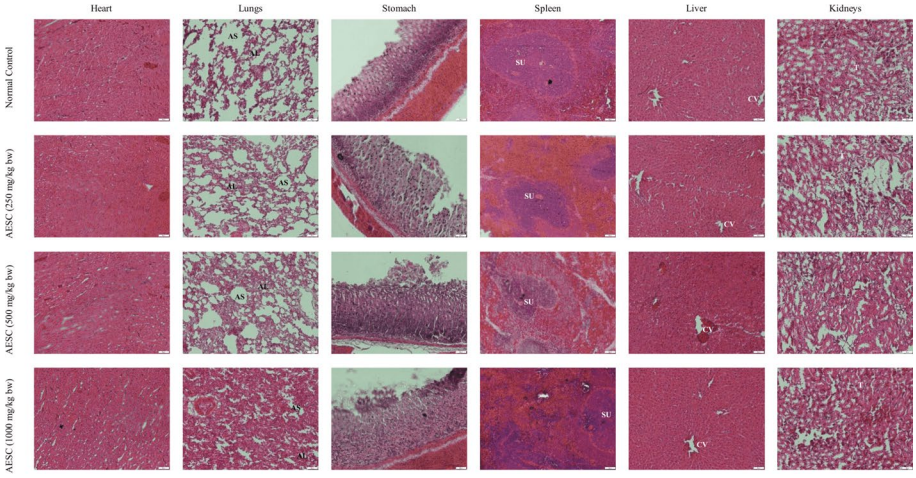

Fig. 13 Representative histological sections of key organs in control and AESC-treated groups in female rats (H&E staining, 10×). Control and AESC-treated groups (250, 500, and 1000 mg/kg bw) show preserved tissue architecture with no evidence of inflammation, necrosis, or pathological alterations across all examined organs. AL, alveoli; AS, alveolar space; CV, central vein; SU, splenic unit; T, tubule

Table 3 Qualitative mycochemical screening of AESC

Compound class	Score
Steroids	–
Flavonoids	–
Tannins	–
Triterpenes	–
Alkaloids	–
Saponins	–

(–): absent; 1+: negligible amount; 2+: small amount; 3+: abundant amount

Table 4 LC–MS profile of selected compounds in AESC under positive ionisation mode

Peak	RT	m/z	Mass (observed)	Mass (DB)	Score (DB)	Diff (DB, ppm)	Compound name	Compound class	Vol. (%)
45	1.124	121.0509	120.0437	120.0436	97.78	−0.75	Purine	Other	7.84
54	1.488	121.0509	120.0443	120.0436	59.29	−5.82	Purine	Other	0.09
79	4.879	297.1329	296.1256	296.1260	99.02	1.17	Trimethylolpropane triacrylate	Other	0.80
186	25.336	409.2728	370.3093	370.3083	91.75	−2.7	Docosanedioic acid	Fatty acid	1.10

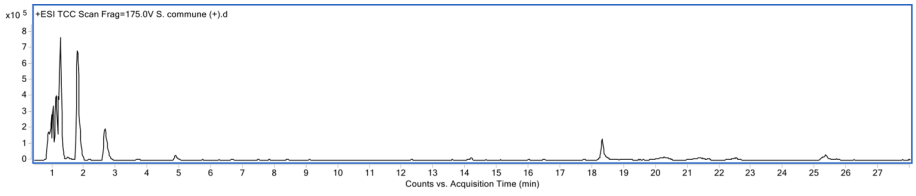

Fig. 14 Chromatogram of AESC based on positive ionisation

Approximately 84.03% of the total ion signal was classified as not detected. The corresponding chromatographic profile is presented in Fig. 14.

Under negative ionisation mode, a total of 189 compounds were detected in AESC in Table S2 (Supplementary Material). As shown in Table 5, aminocaproic acid exhibited the highest relative abundance (18.20%), followed by 2-hexyl-decanoic acid (13.41%), carboxymethyloxysuccinate (6.35%), L-glutamate (2.36%), and 4-methylaminobutyrate (2.12%). In total, 18 compounds exceeded the 1% threshold, while the majority were present at lower relative abundances. Approximately 11.96% of the total ion signal was categorised as not detected. The chromatographic profile is shown in Fig. 15.

4 Discussion

The MTT assay was conducted as a preliminary step to evaluate the cytotoxicity of AESC in AGS and HepG2 cell lines, primarily to determine safe concentrations for subsequent analyses, particularly for assessing endogenous antioxidant enzyme activities [32]. The assay measures cellular metabolic activity, providing direct evidence of cell viability and potential toxicity following treatment exposure. The findings demonstrated minimal cytotoxic effects, with cell viability ranging from 68% to 81% in both cell lines, reflecting low cytotoxicity towards these cancer-derived models. AGS and HepG2 cell lines served as relevant models for gastric and hepatic tissues, respectively, selected to establish non-toxic concentrations for antioxidant enzyme assays rather than to evaluate anticancer potency, as both are well characterised for antioxidant activity [33, 34]. The observed low toxicity may partly result from the high antioxidant content of AESC, which could mitigate oxidative stress and preserve cellular integrity by reducing non-specific oxidative damage [35]. These results are consistent with previous reports where extracts of *S. commune* demonstrated similarly low cytotoxicity and preserved cellular functionality [9, 10]. Selecting appropriate, non-toxic concentrations is crucial for subsequent antioxidant enzyme analyses, as excessive cytotoxicity could impair cellular metabolism and confound enzyme activity measurements [36]. Maintaining high cell viability ensures that observed antioxidant effects directly reflect AESC treatment rather than non-specific cellular injury or stress responses [33, 34, 37]. In line with standard practices

Table 5 LC–MS profile of selected compounds in AESC under negative ionisation mode

Peak	RT	m/z	Mass (observed)	Mass (DB)	Score (DB)	Diff (DB, ppm)	Compound name	Compound class	Vol. (%)
11	0.913	117.0194	118.0267	118.0266	87.36	−0.51	Methylmalonic acid	Fatty acid	2.02
17	0.928	191.0203	192.0276	192.0270	97.20	−3.18	Carboxymethyloxysuccinate	Fatty acid	6.35
23	0.930	146.0459	147.0532	147.0532	47.62	−0.12	L-Glutamate	Amino acid	2.36
24	0.931	132.0301	133.0371	133.0375	91.53	2.74	D-Aspartic acid	Amino acid	1.63
57	0.999	217.0123	172.0138	172.0137	81.01	−0.59	D-Glycerol 1-phosphate	Other	1.32
71	1.008	241.0933	182.0795	182.0790	46.67	−2.35	D-Sorbitol	Other	1.07
85	1.015	154.0618	155.069	155.0695	84.72	3.29	L-2-Amino-3-(1-pyrazolyl) propanoic acid	Amino acid	1.07
105	1.039	116.0717	117.0785	117.0790	74.50	4.37	4-Methylaminobutyrate	Amino acid	2.12
134	1.112	111.0198	112.0271	112.0273	87.26	1.16	Uracil	Other	1.30
137	1.119	135.0309	136.0381	136.0385	86.99	2.85	Hypoxanthine	Other	1.08
140	1.125	151.0262	152.0335	152.0334	99.74	−0.32	Xanthine	Other	2.10
148	1.170	130.0877	131.0948	131.0946	97.83	−1.63	Aminocaproic acid	Amino acid	18.20
165	1.301	131.0715	132.0788	132.0786	99.71	−0.96	5R-Hydroxy-hexanoic acid	Fatty acid	1.88
179	5.003	341.125	342.1321	342.1315	96.79	−1.88	4-Hydroxy-3-(2-hydroxyethyl)acetophenone 4-glucoside	Phenolic	1.91
180	5.003	331.0956	296.1261	296.1260	98.10	−0.43	Trimethylolpropane triacrylate	Other	1.22
236	22.136	255.2342	256.2413	256.2402	91.73	−4.23	2-Hexyl-decanoic acid	Fatty acid	13.41
270	23.564	367.3573	368.3645	368.3654	90.41	2.50	20-Tetracosene-1,18-diol	Fatty acid	1.02
277	24.45	283.2646	284.2717	284.2715	98.52	−0.54	14-Methylheptadecanoic acid	Fatty acid	1.84

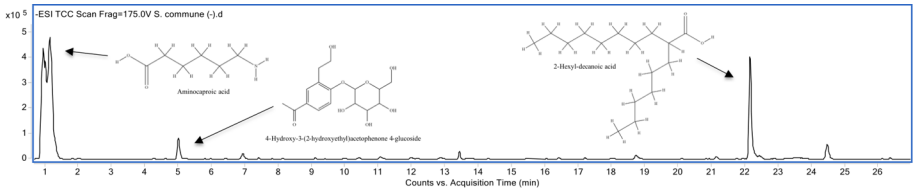

Fig. 15 Chromatogram of AESC based on negative ionisation

in antioxidant research, sub-toxic concentrations were selected to optimise biological responsiveness without compromising cellular integrity. Typically, biological effects are concentration-dependent, with increasing concentrations enhancing responses up to a safe threshold [38, 39]. Therefore, the observed high viability across both AGS and HepG2 cells confirms the suitability of the selected AESC concentrations, supporting their use in future studies examining cellular antioxidant defence mechanisms, including endogenous antioxidant enzymes and oxidative stress responses.

Animal models serve as essential tools for preclinical drug screening, providing critical data on safety, toxicity, and efficacy before human application [40]. Among laboratory animals, Sprague–Dawley rats are frequently employed due to their well-characterised

physiology, ease of handling, and high sensitivity to toxicological effects, making them an established model for systemic toxicity evaluation [41–43]. In this study, AESC administration at a single oral dose of 5000 mg/kg bw did not result in mortality or observable toxic signs during the 14-day observation period. Body weight increased steadily in both control and AESC-treated groups without significant differences, indicating no adverse impact on growth. ROW analysis showed no significant differences or organ enlargement in the heart, lungs, stomach, spleen, liver, or kidneys. These findings were corroborated by histopathological examination, which revealed no inflammation, necrosis, or tissue injury in either control or treated groups. Collectively, these results confirm that AESC is non-toxic at the administered dose of 5000 mg/kg bw. Consequently, the LD_{50} of AESC exceeds 5000 mg/kg bw, categorising it as non-toxic according to OECD Guideline 423 [21]. This classification aligns with the Globally Harmonised System (GHS), where substances with an LD_{50} greater than 5000 mg/kg bw are considered Category 5 or "unclassified" for acute toxicity. Comparable outcomes were reported for aqueous mushroom extracts of *Lentinus squarrosulus* and *Coriolus versicolor*, which exhibited no observable toxicity at similar dosages [44, 45]. The absence of toxicity may also be attributed to the water-based extraction process, which generally excludes harmful organic solvents and reduces potentially toxic constituents [46]. Furthermore, the antioxidant-rich composition of AESC could have contributed to reduced oxidative stress, thereby protecting organs and reinforcing the observed safety profile [47, 48]. Overall, these findings substantiate the favourable acute toxicity profile of AESC, supporting its further evaluation in subacute toxicity studies.

The subacute toxicity results indicate that administration of AESC at doses of 250, 500, and 1000 mg/kg bw for 28 days caused no significant toxicological effects in either male or female Sprague–Dawley rats. These findings were supported by normal body weight progression, consistent food and water intake, and stable haematological and biochemical parameters relative to control animals. These physiological and metabolic indicators are standard measures of systemic toxicity, and their stability implies that AESC did not adversely affect general health or metabolic functions [49, 50]. Responses to AESC were comparable between male and female rats at the same dose levels, with no evidence of sex-dependent differences across the assessed endpoints, supporting a consistent safety profile irrespective of sex. Nevertheless, a slight decrease in water intake observed in female rats between Week 3 and Week 4 may reflect natural fluctuations in voluntary consumption rather than treatment-related toxicity, as female rats often exhibit such variations without compromising overall health [51]. Histopathological analysis of vital organs, including the heart, lungs, stomach, spleen, liver, and kidneys, revealed no inflammation, necrosis, or structural abnormalities in any group, further confirming the absence of organ-specific toxicity. These results align with previous reports showing low toxicity of mushroom aqueous extracts from *Lignosus tigris* and *Ophiocordyceps sinensis* following similar exposure periods [52, 53]. The favourable safety profile observed in this study may be attributed to the water-based extraction, which typically removes harmful constituents, and to the antioxidant-rich composition of AESC, which likely protected organs from oxidative damage [46, 48]. The absence of dose-dependent toxicity supports the identification of a no-observed-adverse-effect level (NOAEL) greater than 1000 mg/kg bw. According to OECD Guideline 407, NOAEL represents the highest dose that causes no detectable clinical, biochemical, or histopathological changes [23,

54]. Establishing NOAEL is essential for guiding clinical dose selection and evaluating the potential severity of adverse effects [54]. Inclusion of both sexes further enhances the reliability and general applicability of these findings, aligning with current regulatory recommendations [23, 55, 56]. Taken together, these findings provide robust preclinical safety evidence supporting the continued development of AESC for pharmacological and therapeutic applications. The consistent tolerability observed across all tested doses suggests a broad safety margin, justifying future studies at these concentrations.

The absence of detectable levels of major mycochemical classes in AESC, including steroids, flavonoids, tannins, triterpenes, alkaloids, and saponins, as revealed by standard qualitative screening, is consistent with the LC–MS profiling results. Although LC–MS detected compounds structurally related to some of these classes, their signal intensities were below the detection limits of conventional qualitative tests, indicating genuinely low abundance rather than methodological failure. The use of water as the extraction solvent may further explain this profile, as it preferentially solubilises polar compounds while limiting extraction of less polar or structurally complex bioactives [57, 58]. LC–QTOF–MS analysis revealed distinct chemical profiles under positive and negative ionisation modes. Under positive ionisation, 68 compounds were detected, with purine representing the most abundant component (7.93%), followed by docosanedioic acid (1.10%) and trimethylolpropane triacrylate (0.80%). Only two compounds exceeded the 1% threshold, while most were present at trace levels. In contrast, negative ionisation mode detected a broader spectrum of compounds (189 in total), with aminocaproic acid (18.20%), 2-hexyl-decanoic acid (13.41%), carboxymethyloxysuccinate (6.35%), L-glutamate (2.36%), and 4-methylaminobutyrate (2.12%) showing the highest relative abundances. These differences reflect the fundamental characteristics of the two ionisation modes. Positive ionisation enhances detection of basic or proton-affinitive molecules, whereas negative ionisation favours acidic or electronegative compounds that readily form negatively charged ions [59]. Negative ionisation is generally less susceptible to matrix-induced suppression and provides improved sensitivity for polar metabolites, consistent with comparative evaluations of ionisation efficiency across compound classes [60, 61]. The predominance of detected metabolites under negative ionisation therefore suggests closer alignment between the chemical characteristics of AESC and this ionisation mode. A substantial fraction of the total ion signal was classified as not detected, accounting for 84.03% in positive mode and 11.96% in negative mode. This observation may be attributed to limited ionisation efficiency, suppression effects, or incomplete spectral coverage in the METLIN database, particularly for uncommon or mushroom-specific metabolites [62]. Nevertheless, the overall profile indicates that AESC predominantly contains polar, acidic, or electronegative constituents, notably amino acids and organic acid derivatives. Although several compound classes associated with biological activity were detected at low abundance, including steroids, flavonoids, terpenoids, alkaloids, saponins, and phenolics, most were present below 1%.

The toxicological outcomes of this study complement existing ethnomycological knowledge of *S. commune*, which has long been consumed by the Kadazan-Dusun community in Sabah, Malaysia [6, 7]. Ethnomycological evidence recognises this species for its cultural, nutritional, and potential medicinal value, with traditional harvesting guided by indigenous ecological knowledge and preparation commonly involving boiling or cooking in water-based dishes [6, 8, 63]. Water, widely recognised as a safe and

environmentally friendly polar solvent [57], has been consistently employed in ethnomycological practices, particularly in decoctions and infusions used to extract medicinal compounds from mushrooms [64]. This long-established practice supports the effective solubilisation of hydrophilic bioactive constituents such as polysaccharides, amino acids, and phenolic acids [13–15], and remains prevalent across various indigenous communities due to accessibility, non-toxic nature, and cultural familiarity [64]. Despite this long history of use, systematic toxicological evaluations of *S. commune* have been limited, emphasising the importance of the present scientific validation, as traditional consumption alone cannot substitute for empirical safety assessment [65]. Beyond its cultural context, *S. commune* continues to attract global attention for its bioactive composition, particularly amino acids, fatty acids, and phenolic compounds associated with antioxidant and anti-inflammatory activities [47]. In the present study, amino acids and fatty acids constituted the most abundant metabolite groups, with compounds such as aminocaproic acid, glutamate, and aspartate potentially contributing indirectly to gastro- and hepato-protective effects through support of glutathione synthesis, mucosal defence, and epithelial integrity [66, 67], while fatty acids such as 2-hexyl-decanoic acid have been reported to modulate inflammatory pathways and hepatic lipid metabolism [68, 69]. The phenolic compound 4-hydroxy-3-(2-hydroxyethyl)acetophenone 4-glucoside, although detected at low abundance, may further contribute to protective effects through established antioxidant and anti-inflammatory mechanisms [70–72]. The predominance of these hydrophilic metabolites reflects the use of water as the extraction solvent, which preferentially extracts polar constituents [15, 73], while the detection of some fatty acids may result from membrane disruption or matrix-assisted solubilisation during extraction [74, 75]. In summary, these findings highlight the convergence of indigenous knowledge and modern toxicological science, supporting biocultural conservation and responsible bioprospecting [76], while providing a scientific foundation for the development of *S. commune*-based functional foods or natural therapeutic products, albeit with further targeted studies required to characterise less polar bioactives [57, 58].

5 Conclusions

This study provides clear toxicological evidence supporting the safety of AESC based on combined in vitro and in vivo evaluations, together with complementary mycochemical characterisation. While AESC demonstrated acceptable cytocompatibility at lower concentrations, a reduction in cell viability was observed in HepG2 cells at the highest tested concentration, indicating concentration-dependent cytotoxicity. In contrast, in vivo acute and subacute oral toxicity studies showed no mortality, clinical toxicity, or organ damage, indicating a wide safety margin for oral exposure in both sexes. Mycochemical analysis revealed the absence of detectable levels of major secondary metabolite classes by qualitative screening, while LC–QTOF–MS profiling identified amino acids, fatty acids, and low-abundance phenolic compounds as the predominant constituents of AESC, consistent with extraction using water as a polar solvent. These findings are particularly relevant for public health, as they support the safety of traditional dietary consumption and local medicinal use of *S. commune* in Sabah when AESC is used within appropriate exposure limits. Importantly, the established safety profile and chemical composition suggest that beneficial biological effects observed at non-cytotoxic concentrations are unlikely to be confounded by nonspecific toxicity. Overall, the data provide

a scientific basis for the continued traditional use of *S. commune* and support its further development for gastro- and hepato-protective applications, with due consideration of dose-dependent effects.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s44187-026-00941-w.

Supplementary Material 1.

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Data availability
Data will be made available on request.

Declarations

Ethics approval and consent to participate
Wild fruiting bodies of *S. commune* used in this study were collected in accordance with local biodiversity regulations under a sampling permit issued by the Sabah Biodiversity Council [Licence Ref. No.: JKM/MBS.1000-2/2 JLD.17 (114)]. The mushroom specimen used in this study was taxonomically identified by Assoc. Prof. Dr. Jaya Seelan Sathiya Seelan, a mycologist at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, and a voucher specimen [BORH(F)03738] was deposited at BORNEENSIS Herbarium, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. All experimental procedures involving animals were conducted in accordance with institutional and international guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the UiTM CARE, Department of Research and Innovation, UiTM, Selangor, Malaysia (Approval No.: UiTM CARE 435/2023).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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