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Research Article

Chemical Compound Profile of Bajakah Kalalawit (*Uncaria gambir* Roxb) Stem Extract Using Liquid Chromatography High-Resolution Mass Spectrometry

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Abstract

Uncaria gambir Roxb., commonly known as Bajakah Kalalawit, is a plant endemic to Kalimantan, Indonesia, with potential medicinal properties. While previous studies have investigated the phytochemical composition of U. *gambir* leaves, limited information exists regarding the constituents of its stem extract. This study aimed to comprehensively characterize the chemical composition of *U. gambir* stem extract using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS). Ethanol (96%) was employed as the solvent for maceration extraction (1 : 5, plant material : solvent ratio) for three days. Subsequently, the dried extract was subjected to LC-HRMS analysis. Compounds with an Area Under the Curve (AUC) value greater than or equal to 1% were considered as major constituents. The results revealed the presence of 18 distinct chemical compounds in the *U. gambir* stem extract, providing valuable insights into its phytochemical profile and laying the foundation for further investigations into its potential pharmacological activities.

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INTRODUCTION

The utilization of plants for medicinal purposes has a long-standing tradition within Indonesian communities. Empirical evidence suggests a wide array of plant species possess therapeutic properties against various ailments[1]. This medicinal significance of plants is largely attributed to their diverse repertoire of bioactive compounds[2]. These compounds, often complex molecular entities capable of forming and breaking down into simpler substances, can be broadly categorized as active or inactive. The active, or bioactive, chemical constituents found in the plant and animal kingdoms are frequently harnessed as medicines. Their therapeutic effects are mediated through metabolic interactions, enabling the prevention or treatment of various physiological dysfunctions in other organisms[3], contrasting with inactive compounds that lack such curative potential at the atomic level[4]. Indonesia's rich biodiversity harbors numerous indigenous plants containing potent bioactive compounds, exemplified by *Uncaria gambir* Roxb. or Bajakah Kalalawit, a term used by the Dayak tribe in Kalimantan.

Uncaria gambir has been the subject of prior investigations[5,6]. Phytochemical screening of the stem ethanol extract of *U. gambir* revealed the presence of various secondary metabolites, including alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and glycosides. Notably, *U. gambir* is recognized as a flavonoid-rich plant, primarily containing polyphenolic compounds, with catechins being a prominent active constituent[7]. Traditionally, communities in Kalimantan have empirically employed *U. gambir* to treat a spectrum of conditions, including cancer, tumors, wounds, diabetes, premature aging, and other health issues[7,8]. Research has further indicated the presence of tannins, terpenoids, flavonoids, alkaloids,

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and saponins in *U. gambir* roots, alongside demonstrable antioxidant properties. Quantitative analysis has reported a flavonoid content of 3.6 ± 0.086% w/w in the ethanol extract of *U. gambir* stems from Loksado District, South Kalimantan[9]. Despite these preliminary findings, comprehensive research on the chemical constituents of *U. gambir* stems remains limited. Specifically, there has been a notable absence of in-depth studies employing advanced analytical techniques such as liquid chromatography-high resolution mass spectrometry (LC-HRMS) for precise compound identification. Therefore, this study is crucial to accurately identify the active compounds present in *U. gambir* stem extract that may possess significant therapeutic potential. This research aims to strengthen the scientific understanding of the traditional medicinal uses of this plant and pave the way for its potential application in the pharmaceutical field. Consequently, the primary objective of this study was to analyze and identify the chemical compound profile of a 96% ethanol extract of *U. gambir* stems using LC-HRMS, thereby obtaining accurate and detailed information regarding its bioactive constituents.

MATERIALS AND METHODS

Materials

The plant material utilized in this study consisted of *U. gambir* stem simplicia powder, which was authenticated at the Biology Laboratory of Universitas Lambung Mangkurat (certificate number 076/UN8.1.2.3.2/PG/Lab.PMIPA/Bio/2023). The solvents employed for extraction and analysis included 96% ethanol and methanol (analytical grade). For the LC-HRMS analysis, formic acid and acetonitrile (HPLC grade) were utilized as mobile phase modifiers. The chromatographic separation was performed using a Vanquish™ UHPLC Binary Pump system (Thermo Fisher Scientific). Mass spectrometric detection was carried out using a Q Exactive™ Hybrid Quadrupole-Orbitrap™ High-Resolution Mass Spectrometer (Thermo Fisher Scientific). Compound separation was achieved using a Phenyl-Hexyl column (100 mm × 2.1 mm ID × 2.6 µm, Thermo Fisher Scientific). Data processing and compound identification were performed using Compound Discoverer™ 3.2 software (Thermo Fisher Scientific).

Methods

Plant extraction

The stems of *U. gambir* were subjected to maceration using 96% ethanol as the solvent, employing a plant material to solvent ratio of 1 : 5 (w/v). The maceration process was conducted for a duration of 72 hours (3 days) at room temperature under intermittent shaking to facilitate efficient solute extraction. Following maceration, the resulting extract was separated from the plant residue by filtration. The collected filtrate was then concentrated under reduced pressure using a rotary evaporator maintained at a temperature of 55°C and a rotational speed of 60 rpm until the volume was reduced to approximately one-third of the initial volume. The concentrated extract was subsequently dried to a powdered form using a water bath set at a controlled temperature of 55°C to minimize thermal degradation of bioactive compounds. The obtained dry extract was carefully transferred to a sterile glass bottle, sealed tightly, and stored at 4°C under dark conditions to preserve its integrity and stability for subsequent experimental analyses.

LC-HRMS analysis

Sample preparation involved accurately weighing 5-10 mg of each sample, which was subsequently dissolved in 1 mL of methanol (MS grade) within a 1.5 mL vial. To ensure complete dissolution and remove particulate matter, the resulting sample solution was sonicated for 30 minutes and then filtered through a 0.22 µm PTFE syringe filter. The HRMS analysis was performed using a mobile phase system consisting of 0.1% formic acid in water (mobile phase A, MS grade) and 0.1% formic acid in acetonitrile (mobile phase B, MS grade), delivered at a constant flow rate of 0.3 mL/minute. The chromatographic separation was achieved using a gradient elution program: initial conditions of 5% mobile phase B were linearly increased to 90% over 16 minutes, maintained at 90% for 4 minutes, and then returned to 5% over 5 minutes, followed by a 5-minute re-equilibration period at the initial conditions, resulting in a total run time of 25 minutes. The column temperature was maintained at 40°C, and the injection volume was 3 µL. The HRMS data acquisition was conducted in full MS/data-dependent MS^2 (dd-MS^2) mode with positive ionization, utilizing XCalibur 4.4 software for instrument control and data processing. Ion source parameters were optimized as follows: nitrogen was employed as the sheath gas (32 AU), auxiliary gas (8 AU), and sweep gas (4 AU); the spray voltage was set to 3.30 kV; the capillary

temperature was 320°C; and the auxiliary gas heater temperature was 30°C. Mass spectra were acquired over a mass-to-charge ratio (m/z) range of 66.7 to 1000, with a resolution of 70,000 for full MS scans and 17,500 for dd-MS^2 scans.

Data analysis

Raw sample data and corresponding solvent blank data were processed using Compound Discoverer™ 3.2. Compound identification was performed through an automated workflow employing spectral library and database search tools, including mzCloud™ and ChemSpider™. The resulting compound annotations were subsequently filtered based on stringent criteria: unambiguous identification of compound names and structures; mass accuracy within a ±5 ppm range; and the presence of preferred fragment ions in the MS^2 spectra.

RESULTS AND DISCUSSION

LC-HRMS analysis

The chromatogram illustrating the separation of these identified compounds is presented in **Figure 1**. The LC-HRMS analysis of the 96% ethanol extract revealed the presence of 18 compounds with relative abundance, as indicated by AUC ≥1% (**Table I** and **Figure 2**). Data processing, including background subtraction using solvent blanks, was performed with Compound Discoverer™ 3.2. Compound identification was achieved through automated database searching and spectral matching using mzCloud™ and Chemspider™, with a mass accuracy tolerance set between -5 and 5 ppm. Only compounds with unambiguous names and well-defined MS^2 fragmentation patterns were included in the final list. This analysis identified 18 prominent compounds in the 96% ethanol extract of *U. gambir* stem (AUC ≥1%), including: the triterpenoids asiatic acid, 3-oxoglycyrrhetic acid, tomentosic acid, and 3-hydroxy-11-ursen-28,13-olide; lipid metabolites 9-oxoODE, oleamide, α-eleostearic acid, ethyl palmitoleate, goshuyic acid, and stearidonic acid; the glycosides bis (4-ethyl benzylidene) sorbitol, quinovin, and sweroside; the phenols 4-octylphenol and dimetofrine; the carbonyl compound asarylaldehyde; the coumarin scopoletin; and the steroid ester bolmantalate.

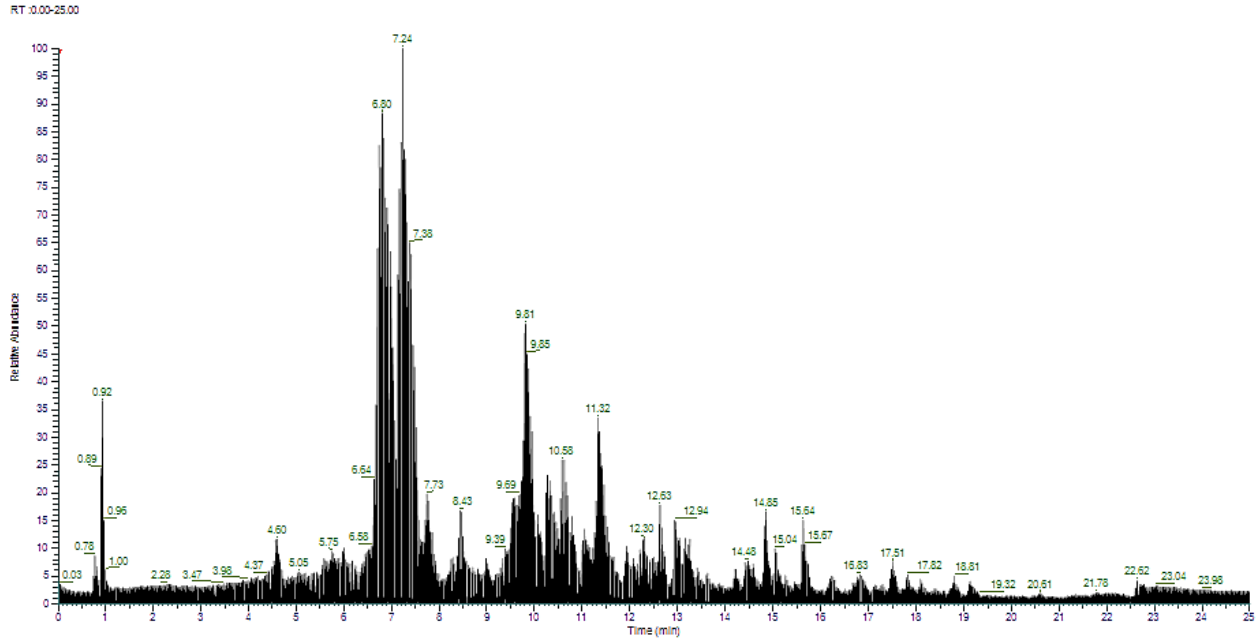

Figure 1. Chromatogram of 96% ethanol extract of *U. gambir* stems.

Biological activity of compounds from U. gambir stem profiling

Phytochemical analysis of the 96% ethanol extract from *U. gambir* stems revealed the presence of several health-related chemical compounds. Existing literature suggests that the identified 18 compounds within this extract possess a range of beneficial bioactivities, potentially contributing to the observed effects. Further investigation into the specific roles and synergistic interactions of these constituents is warranted to fully elucidate the extract's therapeutic potential.

Table I. Compounds identified in 96% ethanol extract of *U. gambir* stems.

No	Chemical compounds	Formula	MW	RT (minute)	Area (max)	AUC (>1%)
1	3-hydroxy-11-ursen-28,13-olide	$C_{30}H_{46}O_3$	454.34	13.26	814356972.4	1.98
2	3-oxoglycyrrhetic acid	$C_{30}H_{44}O_4$	468.32	10.32	3085830541	14.96
3	4-octylphenol	$C_{14}H_{22}O$	206.17	13.22	806311254.1	2.57
4	9-oxoODE	$C_{18}H_{30}O_3$	294.22	13.01	3028367379	5.20
5	α-eleostearic acid	$C_{18}H_{30}O_2$	278.22	12.67	2146338370	3.68
6	Asarylaldehyde	$C_{10}H_{12}O_4$	196.07	4.61	1506062636	2.58
7	Asiatic acid	$C_{30}H_{48}O_5$	488.35	11.41	3373840874	5.79
8	Bis(4-ethylbenzylidene)sorbitol	$C_{24}H_{30}O_6$	414.20	11.59	763228240.5	1.35
9	Bolmantalate	$C_{29}H_{40}O_3$	436.30	10.68	1163261593	2.31
10	Dimetofrine	$C_{11}H_{17}NO_4$	227.12	6.02	609098415.8	1.77
11	Ethyl palmitoleate	$C_{18}H_{34}O_2$	282.26	15.68	2031131756	3.50
12	Goshuyic acid	$C_{14}H_{24}O_2$	224.18	11.97	795650097.6	1.36
13	Oleamide	$C_{18}H_{35}NO$	281.27	14.88	2400825877	4.12
14	Scopoletin	$C_{10}H_8O_4$	192.04	5.79	744196096.9	1.68
15	Stearidonic acid	$C_{18}H_{28}O_2$	276.21	12.25	712121475.8	1.34
16	Sweroside	$C_{16}H_{22}O_9$	358.13	4.61	891465575	1.53
17	Tomentosic acid	$C_{30}H_{48}O_6$	504.34	9.91	1367063207	3.91
18	Quinovin	$C_{36}H_{56}O_9$	632.39	10.36	1971080166	3.78
					Total AUC (%)	**63.41**

Asiatic acid

The multifaceted therapeutic potential of asiatic acid is increasingly supported by scientific literature. Several studies have elucidated its capacity to mitigate oxidative stress and inflammation, attributed to its inherent anti-inflammatory and antioxidant properties[10,11]. Furthermore, asiatic acid has demonstrated promising neuroprotective effects, including the reduction of cognitive impairments, enhancement of antioxidant enzyme activity, and promotion of mitochondrial function, particularly in the context of neurodegenerative conditions such as Alzheimer's disease[12]. In the realm of metabolic disorders, research suggests that asiatic acid can improve β-cell insulin secretory function by preserving β-cell maturity, potentially through modulation of the TNF-α/MFN2 signaling pathway, thereby highlighting its therapeutic relevance in the management of type 2 diabetes[13]. Beyond its antioxidant and metabolic effects, asiatic acid has also exhibited inhibitory activity against prostate cancer metastasis by modulating key molecular mechanisms associated with cell migration and invasion[14].

3-oxoglycyrrhetic acid

The literature reviewed indicates that 3-oxoglycyrrhetic acid and its derivatives possess significant antiproliferative properties across various cancer cell lines. Mechanistically, these compounds have been shown to induce apoptosis through both intrinsic and extrinsic signaling pathways[15]. Furthermore, evidence suggests that these derivatives exhibit anti-angiogenic activity by inhibiting cell migration and angiogenesis-dependent motility in diverse cell types[16]. Notably, structural modifications of glycyrrhizic acid have yielded cytotoxic derivatives with promising antiproliferative activity against tumor cells, highlighting their potential as novel anticancer agents[17].

9-oxoODE

Previous investigations have elucidated the cytotoxic effects of 9-oxo-(10E,12E)-octadecadienoic acid or 9-oxoODE and its derivatives on human ovarian cancer cells, with the underlying mechanism involving the regulation of mitochondrial function, ultimately leading to cell death[18]. Furthermore, 9-oxoODE has demonstrated significant antioxidant properties and exhibits the potential to modulate inflammatory responses. This anti-inflammatory activity is likely mediated through the suppression of pro-inflammatory cytokine and nitric oxide production by macrophages[19].

Oleamide

Evidence suggests that oleamide can stimulate the NOD-like receptor pyrin domain-containing 3 (NLRP3) inflammasome, subsequently promoting M1 macrophage polarization and enhancing the production of interleukin-1 beta (IL-1β)[20]. This pro-inflammatory modulation may hold therapeutic promise in the context of NLRP3-related inflammatory disorders. Furthermore, oleamide has demonstrated potential anticancer properties through the blockade of high-pressure sodium and potassium channels, resulting in antiproliferative and anti-invasive effects on various cancer cell lines, and even exhibiting the capacity to potentiate the efficacy of conventional chemotherapy regimens[21]. Beyond its immunomodulatory

and anticancer activities, oleamide has also been implicated in the maintenance of skeletal muscle health. Studies indicate that oleamide can rescue muscle atrophy by restoring autophagy flux and activating the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathway, suggesting its potential to preserve muscle mass and function[22].

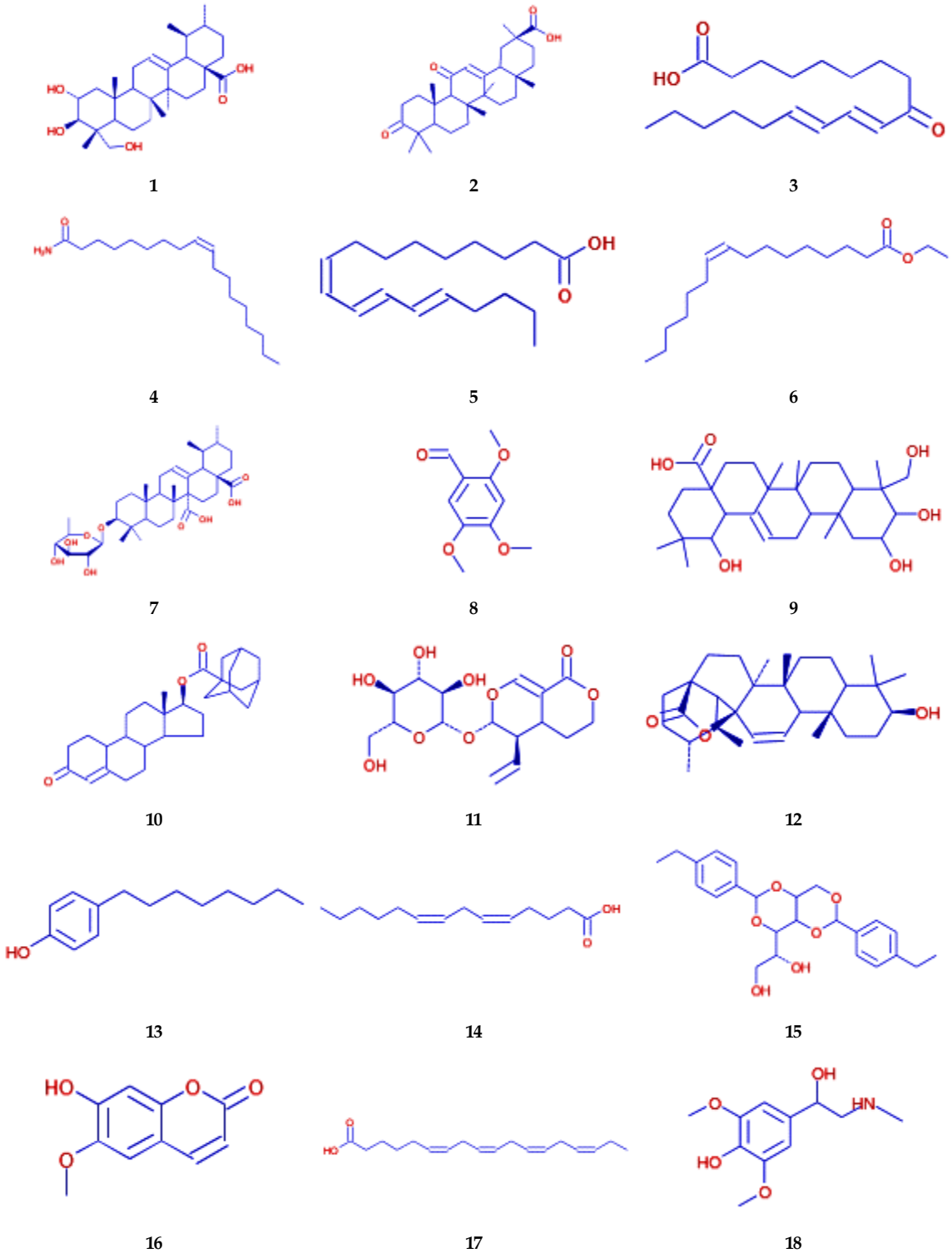

Figure 2. Chemical compound structure; (**1**) asiatic acid, (**2**) 3-oxoglycyrrhetic acid, (**3**) 9-oxoODE, (**4**) oleamide, (**5**) α-eleostearic acid, (**6**) ethyl palmitoleate, (**7**) quinovin, (**8**) asarylaldehyde, (**9**) tomentosic acid, (**10**) bolmantalate, (**11**) sweroside, (**12**) 3-hydroxy-11-ursen-28,13-olide, (**13**) 4-octylphenol, (**14**) goshuyic acid, (**15**) bis(4-ethylbenzylidene)sorbitol, (**16**) scopoletin, (**17**) stearidonic acid, and (**18**) dimetofrine.

α-eleostearic acid

Existing research has established the potent anticancer properties of α-eleostearic acid (α-ESA), particularly its significant activity against breast cancer cell lines. Mechanistically, α-ESA exerts its effects by impeding crucial cellular processes such as cell division and triggering apoptosis through the modulation of various molecular pathways. These multifaceted actions on the cell cycle phase, as elucidated in previous studies[23,24], underscore the potential of α-ESA as a promising therapeutic agent in the context of breast cancer treatment.

Ethyl palmitoleate

In vitro studies of ethyl palmitoleate have demonstrated its protective role against Zika virus-induced cellular damage, where palmitoleate shielded placental trophoblasts from apoptosis and endoplasmic reticulum (ER) stress, suggesting a potential mechanism for preventing fetal complications[25]. Beyond its antiviral properties, palmitoleate has been implicated in the modulation of metabolic pathways. Research indicates its capacity to attenuate inflammation, insulin resistance, and hepatic steatosis in the context of a high-fat diet, potentially mediated through the activation of peroxisome proliferator-activated receptor alpha (PPAR-α)[26]. Furthermore, in the context of obesity, palmitoleic acid and its derivative, palmitoleylethanolamide, have shown promise in ameliorating metabolic imbalances, exhibiting efficacy in reducing weight gain, hepatic steatosis, inflammation, and dyslipidemia in models of diet-induced obesity[23]. Notably, endogenously produced palmitoleate via de novo lipogenesis has been associated with beneficial lipidomic remodeling, potentially conferring protection against atherosclerosis and metabolic diseases by preventing ER stress and inflammation in macrophages[24].

Quinovine

Quinovine has demonstrated significant antiviral activity against Tobacco Necrosis Virus (TNV), alongside notable inhibitory effects on the growth of pathogenic bacteria, while exhibiting a comparatively modest antifungal activity[27]. Furthermore, quinovine has emerged as a promising antiviral agent with reported efficacy against the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)[28]. This aligns with the observed properties of structurally related compounds such as quinacrine, which has shown potential in neurodegenerative disorders by dissociating amyloid-β (Aβ) plaques in the brain. This action is associated with a reduction in neuroinflammation and an improvement in synaptic function, ultimately mitigating synaptic dysfunction characteristic of Alzheimer's disease[29].

Asarylaldehyde

Asarylaldehyde exhibits a significant inhibitory effect on platelet aggregation induced by both arachidonic acid and collagen. This observed anti-aggregatory activity strongly suggests the potential of asarylaldehyde as a therapeutic agent in the prevention of thrombotic events and the management of conditions associated with aberrant blood clot formation[30].

Tomentosic acid

In oncology, studies have demonstrated tomentosic acid anti-tumorigenic properties in Burkitt lymphoma cells, characterized by the induction of cell cycle arrest and apoptosis, downregulation of immune system-related pathways, and upregulation of proapoptotic genes[31]. Furthermore, tomentosic acid has anti-cancer effects in gastric cancer cell lines by inhibiting proliferation, induction of programmed cell death, and reduction of key inflammatory markers[32]. Beyond cancer, tomentosic acids have shown promise in neuroprotection, as evidenced by their ability to attenuate inflammation and oxidative stress, leading to improved motor function in a murine model of Parkinson's disease[33]. In human keratinocytes, tomentose derivatives have displayed significant antioxidant activity, mediated via the Nrf2 pathway, resulting in reduced reactive oxygen species (ROS) levels and the activation of MAPK signaling while concurrently inhibiting aryl hydrocarbon receptor (aHR) signaling, thereby offering protection against environmental pollutants[34]. Additionally, tomentosic acid have demonstrated anti-inflammatory properties by effectively suppressing NF-κB activation and MAPK phosphorylation in macrophages, suggesting their therapeutic potential in managing inflammatory disorders[35].

Bolmantalate

Bolmantalate, a boron-containing compound, emerges as a crucial micronutrient involved in a spectrum of biological processes. Consistent with prior research[36,37] indicate that boron supplementation exerts a modulatory influence on the metabolism of essential substances, including calcium, magnesium, and amino acids. Furthermore, the established anti-

inflammatory and antioxidant properties of boron compounds underscore their potential therapeutic relevance. Notably, evidence from both animal and human studies[38] suggests a positive association between boron intake and improved metabolic status, glucose homeostasis, and the maintenance of cell membrane integrity. The multifaceted impact of boron on enzymatic activity, mineral homeostasis, and energy metabolism[39] further highlights its significance in optimizing organismal function and in the context of addressing bone structural disorders.

Sweroside

Research indicates that sweroside exerts hepatoprotective effects by mitigating hepatic steatosis through the AMPK/mTOR signaling pathway[40]. Furthermore, its capacity to modulate the cholinergic system and enhance brain antioxidant activity suggests a promising role in improving cognitive function and reducing anxiety[41]. Beyond these effects, sweroside has been shown to promote osteoblast differentiation and bone formation via the BMP2/CBFA1 pathway, indicating its potential in osteoporosis treatment[42]. Finally, its anti-inflammatory properties, mediated by the inhibition of the NF-κB signaling pathway, offer protection against inflammation and acute lung injury[43], while cardioprotective effects are observed through the Keap1/Nrf2 pathway by reducing oxidative stress and pyroptosis in cardiac ischemia-reperfusion injury[44].

3-hydroxy-11-ursen-28,13-olide

3-hydroxy-11-ursen-28,13-olide, commonly known as ursolic acid, has demonstrated pro-longevity effects in *Caenorhabditis elegans* by significantly increasing its maximum lifespan. This lifespan extension mirrors the effects observed with dietary restriction, a well-established longevity-promoting intervention, and is mechanistically linked to the upregulation of downstream target genes such as *daf-9* and *gcs-1*[45].

4-octylphenol

4-octylphenol (4-OP) and its derivative, 4-octyl itaconate (4-OI), present a complex profile of health effects. Preclinical studies have indicated that 4-OI exhibits cardioprotective properties by mitigating myocardial injury through the reduction of oxidative stress, inflammation, and apoptosis[46]. Conversely, 4-OP exposure has been linked to reproductive and developmental toxicity in animal models, demonstrating estrogenic activity and adversely affecting spermatogenesis and reproductive outcomes[47]. Given its potential to disrupt both brain development and reproductive health, 4-OP remains a significant environmental and health concern.

Goshuyic acid

Goshuyic acid, commonly known as rosmarinic acid (RA), is a naturally occurring phenolic compound recognized for its diverse array of health-promoting properties. Scientific investigations have demonstrated that RA exhibits significant antioxidant and anti-inflammatory activities, alongside antimicrobial, immunomodulatory, anti-diabetic, and hepato-renal protective effects[48]. Pharmacokinetic studies in humans have further elucidated RA's potential therapeutic applications in managing metabolic syndrome, enhancing cognitive function, and addressing various dermatological, allergic, and osteoarthritic conditions[49]. Notably, research has also revealed RA's neuroprotective capacity, specifically its ability to shield neural cells from iron-induced damage by preventing mitochondrial dysfunction and oxidative stress, key pathogenic mechanisms in neurodegenerative disorders[50].

Bis(4-ethylbenzylidene) sorbitol

Bis(4-ethylbenzylidene)sorbitol, a natural product ligand identified in this study, exhibited the most potent antibacterial activity among the tested compounds, suggesting its potential as an alternative active pharmaceutical ingredient to combat bacterial infections[51].

Scopoletin

Scopoletin, a naturally occurring coumarin, demonstrates significant therapeutic potential across multiple disease areas. Research indicates its independent ability to reverse insulin resistance and improve β-cell function, positioning it as a promising candidate for antidiabetic drug development[52], while also exhibiting anticancer effects through modulation of signaling pathways, induction of apoptosis, and cell cycle arrest[53,54], suggesting its utility in chemoprevention and chemotherapy. Furthermore, its diverse pharmacological profile, encompassing anti-inflammatory, anti-hypertensive, anti-hyperuricemic, cardioprotective, and hepatoprotective properties[55], suggests its broader applicability in managing various chronic diseases with a potentially favorable safety profile.

Stearidonic acid

Stearidonic acid (SDA), a plant-derived omega-3 polyunsaturated fatty acid, has garnered significant scientific attention for its promising therapeutic effects on human health. Research indicates that SDA consumption is associated with a reduced risk of several chronic diseases, including cancer, obesity, inflammation, and cardiovascular disease[56]. Notably, the human body efficiently converts SDA to eicosapentaenoic acid (EPA), positioning it as a valuable plant-based alternative to fish oils for mitigating the risk of Alzheimer's Disease and protecting hippocampal neurons from amyloid-beta-induced neurotoxicity[57]. Moreover, studies have revealed that SDA-rich oils can confer protection against neurological and cardiovascular health deterioration, improve overall fatty acid profiles, modulate the expression of genes involved in oxidative stress and inflammation, and lower lipid levels, thus counteracting the detrimental effects of a high-calorie diet[58,59]. Mechanistically, SDA has been shown to minimize nitric oxide production, contributing to its anti-inflammatory properties, and to inactivate key inflammatory signaling pathways such as NFκB and MAPK, suggesting its potential to dampen inflammatory responses[60].

Dimetofrine

Dimetofrine, also recognized as tetrandrine, exhibits significant therapeutic potential as evidenced by prior research. Studies have demonstrated tetrandrine's efficacy in mitigating silicosis by effectively inhibiting key inflammatory and fibrosis pathways[61]. Furthermore, tetrandrine has shown promising anticancer effects in triple-negative breast cancer by targeting the epithelial-to-mesenchymal transition and cancer stemness, ultimately leading to the inhibition of tumor growth and metastasis[62].

Uses of U. gambir

Uncaria gambir has a long-standing history of use for treating a diverse range of ailments, including diarrhea, swollen gums, sore throat, dysentery, obesity, and arteriosclerosis[63]. Its applications extend across various traditional medicinal systems[64-67], and recent scientific investigations[9] have begun to elucidate the bioactive compounds responsible for its therapeutic potential. Traditionally, the roots and stems of *U. gambir* are the primary plant parts utilized. These are processed to yield extracts that form the basis of various medicinal preparations. While some studies[68,69] suggest potential benefits from the leaves and flowers, the roots and stems remain the dominant components in traditional medicinal applications[5,6,9,65,67,70], reflecting a historical preference likely based on observed efficacy or ease of access and preparation.

The traditional applications of *U. gambir* are supported by a growing body of scientific evidence demonstrating its diverse pharmacological activities. These include its use as an agent to enhance stamina and body energy, believed to combat fatigue and reduced vitality[5], as well as its anti-inflammatory[71-73], anti-cancer[70,74], antioxidant[9,65,67,75-78], anti-hyperglycemic[78-82], and anti-hyperuricemic[83,84] properties. These effects are attributed to the presence of various bioactive compounds identified within *U. gambir*, notably flavonoids, alkaloids, tannins, and saponins. The identification of these phytochemicals provides a scientific rationale for the traditional use of *U. gambir*, as these compounds are known to exert a range of pharmacological effects that align with its historical applications[9,64].

CONCLUSION

This study successfully identified the major chemical constituents present in the ethanol extract of *U. gambir* stem using LC-HRMS analysis. The comprehensive metabolomic profiling revealed that 18 distinct compounds constituted 96% of the extract's composition, each exhibiting an AUC value of ≥1%. These identified compounds, in descending order of their relative abundance, are: asiatic acid, 3-oxoglycyrrhetic acid, 9-oxoODE, oleamide, α-eleostearic acid, ethyl palmitoleate, quinovin, asarylaldehyde, tomentosic acid, bolmantalate, sweroside, 3-hydroxy-11-ursen-28,13-olide, 4-octylphenol, goshuyic acid, bis(4-ethyl benzylidene) sorbitol, scopoletin, stearidonic acid, and dimetofrine. The detailed elucidation of these bioactive compounds provides a crucial foundation for future research aimed at investigating the specific pharmacological activities of *U. gambir* and potentially isolating key constituents responsible for its traditional medicinal uses.

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AUTHORS' CONTRIBUTION

Conceptualization: Ainun Fawaid, Nurkhasanah Mahfudh
Data curation: Ainun Fawaid, Nurkhasanah Mahfudh
Formal analysis: Ainun Fawaid, Nurkhasanah Mahfudh
Funding acquisition: Nurkhasanah Mahfudh
Investigation: Ainun Fawaid, Nurkhasanah Mahfudh
Methodology: Nurkhasanah Mahfudh
Project administration: Nurkhasanah Mahfudh
Resources: Nurkhasanah Mahfudh
Software: -
Supervision: Nurkhasanah Mahfudh
Validation: Nurkhasanah Mahfudh
Visualization: Nurkhasanah Mahfudh
Writing - original draft: Ainun Fawaid, Nurkhasanah Mahfudh
Writing - review & editing: Nurkhasanah Mahfudh

DATA AVAILABILITY

The structural data analyzed in this study are publicly available and can be accessed through the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB).

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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