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RESEARCH ARTICLE

Antimalarial and Cytotoxic Activities of *Cratoxylum sumatranum* (Jack) Bl. Twigs Dichloromethane Extract and Its Phytochemical Profiling by LC-MS/MS

Maylisa Natalia Corry[1], Hilkatul Ilmi[2,3], Lidya Tumewu[3], Hanifah Khairun Nisa[3], Firman Wicaksana[1], Irfan Rayi Pamungkas[1,3], Supriatno Salam[4,5], Hadi Kuncoro[4], Suciati[6], Che Puteh Osman[7,8], Achmad Fuad Hafid[3,6] and Aty Widyawaruyanti[3,6,*]

[1]*Master Program of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia*
[2]*Doctoral Program of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia*
[3]*Center for Natural Product Medicine Research and Development, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia*
[4]*Faculty of Pharmacy, Universitas Mulawarman, Samarinda, Indonesia*
[5]*Department of Chemistry, Faculty of Science and Mathematics, Sultan Idris Education Universiti, Kota Tanjong Malim, Perak, Malaysia*
[6]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia*
[7]*Faculty Sains Gunaan, Universiti Teknologi MARA, Selangor, Malaysia*
[8]*Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA, Cawangan Selangor, Kampus Puncak Alam, Selangor, Malaysia*

([*]Corresponding author's e-mail: aty-w@ff.unair.ac.id)

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Abstract

The genus *Cratoxylum* has been traditionally used to treat malaria. Several active antimalarial compounds were isolated from *C. mangiayi*, *C. cochinchinense*, and *C. glaucum*. Further exploration of another species within this genus, aparticularly *C. sumatranum*, is warranted based on a chemotaxonomic approach. This research aims to evaluate the *in vitro* inhibitory activity of *C. sumatranum* twig extract against *Plasmodium falciparum* strains 3D7 and Dd2, assess its cytotoxicity, and analyze metabolites responsible for antimalarial activity. The antimalarial activity was evaluated against *P. falciparum* strains 3D7 (chloroquine-sensitive) and Dd2 (chloroquine-resistant) using the lactate dehydrogenase (LDH) assay, and cytotoxicity was assessed on BHK-21 cells using the Resazurin assay. In addition, metabolite profiling of the twig extract was performed using LC-MS/MS to identify potential bioactive compounds. The results demonstrated that the dichloromethane extract exhibited high antimalarial activity against *P. falciparum* strains 3D7 and Dd2 with IC_{50} values of 0.28 ± 0.55 and 0.66 ± 0.02 µg/mL, respectively, and was considered non-toxic with a CC_{50} value of 38.11 ± 0.13 µg/mL. The phytochemical profiling by LC-MS/MS revealed that the extract contains compounds including xanthones, flavonoids, anthraquinones, terpenoids, coumarins, chalcones, phenylpropanoids, lignans, and amino acids with potential antimalarial properties. These findings highlight *C. sumatranum* as a promising candidate for developing new antimalarial agents.

Keywords: *Cratoxylum sumatranum*, Antimalarial, Cytotoxic, Phytochemical profile

Introduction

Malaria remains a major global health concern due to its high morbidity and mortality, particularly in subtropical and tropical regions. This burden necessitates sustained strategic interventions. The World Health Organization (WHO) 2023 report highlights the magnitude of the global malaria problem,

with 263 million cases recorded in 83 endemic countries and a death toll of around 608,000 [1]. In Indonesia, malaria cases exceeded 369,000, with notable increases in regions such as Gorontalo, East Nusa Tenggara, and Papua [2]. *Plasmodium falciparum* and *Plasmodium vivax* are the 2 main *Plasmodium* species that cause malaria in humans. Compared to other species, these parasites have the highest incidence of infection, as well as the highest rates of complications and mortality. Alarmingly, resistance to most available antimalarial agents, including artemisinin and its derivatives, has been confirmed in both species. The emergence of artemisinin resistance, initially documented in Cambodia in 2007 [3], and its subsequent rapid spread across Southeast Asia, has jeopardized the progress achieved in malaria control programs. Consequently, the emergence of new antimalarial drug candidates, particularly from herbal medicines, has become imperative.

The genus *Cratoxylum* has a history of traditional use for malaria treatment [4]. Several active antimalarial compounds have been isolated from *C. mangiayi*, *C. cochinchinense,* and *C. glaucum* [5,6]. A chemotaxonomic approach highlights the need to explore other species within this genus, including *C. sumatranum*. This study evaluated the *in vitro* inhibitory activity of *C. sumatranum* twig extract against *P. falciparum* strains 3D7 and Dd2, followed by assessing its cytotoxicity and analyzing metabolite profiles to identify compounds responsible for antimalarial activity. *C. sumatranum* (Jack) Bl., commonly known as Geronggang, is endemic to Kalimantan and West Sumatra, is a member of the Hypericaceae family, and has traditional medicinal value for treating various conditions, including fever, stomach ailments, dysentery, and burns [4,7,8]. Previous studies have also reported the presence of such metabolites in *C. sumatranum* extracts. *C. sumatranum* stem barks, twigs, and roots harbor diverse bioactive compounds, including xanthones, flavonoids, anthraquinones, and benzophenones [9,10]. The dichloromethane extract from *C. sumatranum* stem bark has successfully isolated caged xanthones, including Cochinchinone D and Cochinchinoxanthone, which exhibited IC_{50} values of 4.79 and 4.41 mM, respectively [9,10]. Dichloromethane, a solvent with intermediate polarity, effectively extracts semi-polar compounds such as xanthones, flavonoids, and anthraquinones, many of which are known for their antimalarial properties. In this study, the dichloromethane extract of *C. sumatranum* twigs (Cs-T-D) was found to contain several xanthones, including Gerontoxanthone I, Macluraxanthone, and γ-Mangostin. These compounds may contribute individually or synergistically to the extract's potent *in vitro* antimalarial activity against *P. falciparum* strains 3D7 and Dd2 [10,11]. Based on these results, our findings support the potential of *C. sumatranum* twigs as a promising source of antimalarial agents.

Materials and methods

Plant material

The twigs of *C. sumatranum* were collected from the Environmental and Forestry Instrument Standard Implementation Center, Samboja, Balikpapan, East Kalimantan, Indonesia. A licensed botanist at Purwodadi Botanical Garden Growth Conservation Center, East Java, Indonesia (1048/IPH.06/HM/IX/2019) confirmed the identity of the plant material, where voucher specimens (collection number: Cs-T-D) were deposited at the Institute of Tropical Disease, UNAIR.

Extraction of plant material

Cratoxylum sumatranum twigs were dried at room temperature (around 27 °C) without exposure to direct sunlight for 14 days and were mechanically powdered. A maceration process was conducted using successive extractions on 1 kg of the dried powder with 3 L of dichloromethane solvent. The extract underwent filtration, concentration using a rotary evaporator, and subsequent drying in an oven maintained at 40 °C to yield a dry extract. This process produced a single extract called *Cratoxylum sumatranum*-twigs dichloromethane extract (Cs-T-D). The extract was stored in glass bottles and kept at 4 °C in the refrigerator for future analysis.

P. falciparum culture

Plasmodium falciparum strains 3D7 and Dd2, obtained from Eijkman Institute for Molecular Biology, Jakarta and Department of Biomedical Chemistry, The University of Tokyo, Bunkyo-ku, Japan (resistant to chloroquine, pyrimethamine, and mefloquine), maintained at NPMRD-ITD Universitas Airlangga, was

cultured *in vitro* following a previously described method [10]. Parasite cultures were maintained in human O-type red blood cells at 37 °C in a controlled atmosphere (5% O_2, 5% CO_2 and 90% N_2) using RPMI-1640 medium (Gibco, Thermo Fisher Scientific, USA) containing 50 µg/mL hypoxanthine, 25 mM HEPES, 2 g/L $NaHCO_3$, 0.3 g/L L-glutamine, 11 mM glucose, 25 mM $NaHCO_3$, 2.5 µg/mL gentamicin, and 0.5% (w/v) Albumax II (SIGMA-Aldrich). Parasite cultures were synchronized to the ring stage using sorbitol treatment. The level of parasitemia was quantified by microscopic examination, counting the number of infected erythrocytes in a sample of 1,000 total erythrocytes.

Antimalarial activity test by LDH assay

The lactate dehydrogenase (LDH) assay evaluated the Cs-T-D extract's antimalarial properties against *P. falciparum* strains 3D7 and Dd2. The extract was dissolved in DMSO at a 5 µg/mL concentration and tested for initial screening. Extracts exhibiting > 50% inhibition of parasite growth were selected for IC_{50} determination. Eight 2-fold serial dilutions of these extracts and the standard drug chloroquine diphosphate were prepared and added to 96-well plates, with concentrations of 50 to 0.01 µg/mL for the extracts and 10 to 0.001 µg/mL for chloroquine diphosphate. Assays were performed in triplicate. Subsequently, 100 µL of Following the addition of parasitized red blood cells to each well, the plates were incubated at 37 °C under 5% oxygen, 5% carbon dioxide, and 90% nitrogen for 72 h. Following incubation, plates were harvested and stored at −30 °C. The LDH activity was then measured by adding 90 µL of prepared substrate to each well, incubating on a flatbed shaker at 650 rpm at room temperature for 30 min in the dark. Absorbance was read at 650 nm using a multiscan sky-high microplate spectrophotometer (Thermo Fisher Scientific). IC_{50} values were calculated using non-linear regression curve analysis with GraphPad PRISM 7.0 software (GraphPad Co., Ltd., San Diego, CA, USA).

Cytotoxicity test by resazurin assay

The method used to measure the cytotoxicity of the samples was the resazurin-based cell viability assay [12] on BHK-21 normal cell lines. Cells were cultured in D-MEM (High Glucose) enhanced with L-Glutamine, Phenol Red, $NaHCO_3$, 10% FBS, and 1% Penicillin-Streptomycin. For the assay, BHK-21 cells were seeded in 96-well plates at densities of 1×10^4 cells/well and exposed to various concentrations (100, 50, 25, 12.5 and 6.25 µg/mL in DMEM) of the extracts and fractions. After a 44-hour incubation period at 37 °C under 5% CO_2, 10 µL of a 0.5 mM resazurin solution was added to each well. Following a 4-hour reduction period, fluorescence was quantified using a Nivo plate reader (PerkinElmer) with wavelengths set at 530 and 595 nm, respectively. Cytotoxic concentrations (CC_{50}) were analyzed using non-linear regression curve analysis in GraphPad PRISM 7.0 software (GraphPad Co. Ltd., San Diego, CA, USA).

Sample preparation for LC-MS/MS analysis

The dichloromethane extract from *C. sumatranum* twigs (20 mg) was accurately weighed and dissolved in a solvent mixture of acetonitrile and ultra-pure water (98:2, v/v%). Before analysis, the solution was filtered through a 0.45 µm PTFE membrane. The TMstrata® C18-E column (Phenomenex) was preconditioned with ultra-pure water and acetonitrile (3 mL each), followed by an additional conditioning step using 3 mL of acetonitrile and ultra-pure water (98:2, v/v%). The prepared sample (20 mg/mL) was loaded onto the column, after which 2 mL of the same solvent mixture was added. The collected eluate was subsequently concentrated using a rotary evaporator at 40 °C, yielding 20 mg of purified twig extract.

Further analysis of the pre-treated *C. sumatranum* twig sample was conducted using an Orbitrap mass spectrometer. Separation was performed chromatographically using an Accucore™ Vanquish™ C18 column (100×2.1 mm, 1.5 µm, Thermo Scientific, Lithuania), where both solvents were supplemented with 0.1% formic acid. The elution gradient was set as follows with a mobile phase comprising ultra-pure water (solvent A) and acetonitrile (solvent B): 0 - 35 min, from 40% to 100% B; 20 - 25 min, held at 100% B; 25 - 29 min, from 100% to 40% B; and 29 - 35 min, maintained at 40% B. The analysis was run in positive ion mode (1 µL injection, 1,000 ppm, 0.2 mL/min flow rate) using the established UHPLC gradient. The mass spectrometric analysis was conducted at resolutions of 60,000 and 15,000 in full scan and MS/MS modes, respectively. Electro-spray ionization (ESI) was utilized as the ion source, operating at a spray voltage of 3,500

V. Additional parameters included an ion transfer tube temperature of 300 °C, sheath gas at 35 arb, auxiliary gas at 7 arb, vaporizer temperature at 275 °C, and RF lens setting at 60%. The scan range spanned from *m/z* 100 to 1,000. Thermo Scientific Xcalibur software version 4.2.47 was employed to acquire and process data.

Database export

The LOTUS: Natural Products Online (https://lotus.naturalproducts.net) database retrieved metabolite data associated with the genus *Cratoxylum* and the Hypericaceae family. The retrieved data were exported as an Excel (.xls) formatted file containing essential details such as chemical names, synonyms, molecular formulas, accurate masses, and SMILES representations. The SMILES data underwent manual curation to remove inconsistencies before being saved as a comma-separated values (CSV) file. The processed file was subsequently analyzed using DataWarrior software to verify and refine the structural representations of the identified metabolites [13].

Analysis of MS data using MZmine

Thermo raw data from the mass spectrometer were initially converted into mzML format using MSConvert from ProteoWizard. The resulting mzML files were subsequently imported into MZmine 3 for further processing. Before data analysis, the project was saved in the MZmine-specific format. The crude chromatogram was visualized using blank and crude sample data to assess the noise level before proceeding with mass spectrometry (MS) detection for $[M+H]^+$ (MS^1) and product ions *m/z* (MS^2) levels. A centroid mass detector was employed with polarity set to positive mode. The noise thresholds were configured at 2.0E6 for MS^1 and 0.0E0 for MS^2. Following this, chromatogram construction and resolution steps were performed, applying an isotope filter, alignment, and gap-filling procedures. The final processed dataset was exported as an mgf file for further structural elucidation using Sirius 5.8.3 software [13].

Phytochemical analysis

An academic account for Sirius was initially registered to facilitate the dereplication process. The procedure commenced by importing the mgf file into Sirius software, ensuring a stable connection to the web server. Custom databases were generated by incorporating curated SMILES representations of metabolites derived from Hypericaceae into the software. Before computation, all detected features were assigned the adduct type $[M+H]^+$. The Sirius analysis was initiated by activating the corresponding function, with molecular formula constraints limited to hydrogen (H), carbon (C), nitrogen (N), and oxygen (O). The mass spectrometry instrument settings were configured for an Orbitrap system with an MS^2 mass accuracy threshold of 15 ppm. The MS/MS isotope scoring was set to "SCORE".

Annotation was performed utilizing all available databases integrated within Sirius, including Biocyc, CHEBI, COCONUT, HMDB, KEGG, KEGG Mine, KNApSAcK, MeSH, NORMAN, Natural Products, PubChem, PubMed, YMDB, YMDB Mine, ZINC bio, SuperNatural, alongside the 2 custom-developed databases. Following this, the Zodiac function was activated, which was succeeded by fingerprint prediction, structure database search, and compound classification using CANOPUS. The computation process was finalized by selecting the compute function, allowing the system to complete the data analysis.

Results and discussion

The genus *Cratoxylum* (Hypericaceae) is known for its diverse pharmacological properties, including antimalarial potential. Phytochemical studies on *Cratoxylum* species have identified bioactive compounds such as xanthones, flavonoids, and anthraquinones, many exhibiting antimalarial activity [5,7,9,14]. This ethnopharmacological evidence highlights the promise of *C. sumatranum* as a source for antimalarial drug discovery, warranting further bioassay-guided research to validate its efficacy and identify active constituents.

Percentage yield of dichloromethane extract of *C. sumatranum* twigs (Cs-T-D)

The dichloromethane extract of *C. sumatranum* twigs (Cs-T-D) was produced with a dark green sticky solid with 14.73 g for a 1.473% (w/w) yield. In this study, dichloromethane was chosen as the extraction solvent for *C. sumatranum* based on previous studies on other *Cratoxylum* species, where dichloromethane

extracts showed significant antimalarial activity compared to extracts obtained with more polar (methanol extract) and non-polar (hexane extract) solvents [6,15]. Given the structural similarity of secondary metabolites across the genus, dichloromethane is considered a suitable solvent for selectively extracting bioactive compounds.

Antimalarial activity and cytotoxicity of the dichloromethane extract of *C. sumatranum* twigs

Dichloromethane extract of *C. sumatranum* twigs showed significant antimalarial potential against *Plasmodium falciparum* sensitive to chloroquine (3D7 strain) and resistant to chloroquine (Dd2 strain) with IC_{50} values of 0.28 ± 0.55 and 0.66 ± 0.02 µg/mL, respectively. Although the antimalarial activity against sensitive strains is higher than against resistant strains, the extract is still classified as very active against both test strains, based on the classification established in previous literature [12,14]. Statistical analysis revealed a significant difference in the extract efficacy between the 2 strains, with greater potency observed against 3D7 than Dd2 ($p < 0.05$). Nonetheless, the extract maintained a very active classification against both strains. Compared to the standard chloroquine diphosphate, the extract demonstrated significantly lower antimalarial activity in both strains ($p < 0.05$). Despite this, its strong inhibitory profile supports its potential as a promising natural antimalarial candidate.

Furthermore, an *in vitro* toxicity evaluation on BHK-21 cells using the Resazurin assay showed that the dichloromethane extract was non-toxic according to previously defined criteria, with a CC_{50} value exceeding 100 µg/mL [16]. The data is presented in **Table 1**. The potent antimalarial activity, coupled with the favorable safety profile of this multi-component extract from *C. sumatranum* twigs, highlights the potential for synergistic effects among its constituents. Subsequent isolation and identification of individual compounds could lead to the discovery of novel, effective, and safe antimalarial agents with activity comparable to chloroquine diphosphate.

Evaluating the selectivity index (SI) is essential in herbal drug research to determine the feasibility of further investigation. To determine the selectivity index (SI), the toxic concentration is divided by the effective bioactive concentration. An ideal drug exhibits high toxicity and low active concentrations [17]. A selectivity index of SI ≥ 2 is recommended as the acceptance criterion for selecting bioactive samples for further study [18]. **Table 1** indicates good selectivity for the extract, which showed selectivity towards malaria parasites, as its selectivity indices were more significant than 2.

Table 1 Antimalarial activity (IC_{50}), Cytotoxicity (CC_{50}), and SI of dichloromethane extract from *Cratoxylum sumatranum* twigs.

Sample	IC50 (µg/mL)*		CC50 (µg/mL)*	Selectivity Index (SI)	
	3D7	Dd2		3D7	Dd2
Dichloromethane extract (Cs-T-D)	0.28 ± 0.55	0.66 ± 0.02	38.11 ± 0.13	> 100	57.74
Chloroquine diphosphate	0.006 ± 0.10	0.015 ± 0.05	90.52 ± 0.24	> 100	> 100

Cs: *Cratoxylum sumatranum*, T: Twigs, D: Dichloromethane extract. Chloroquine diphosphate is the standard drug used in this study. *Mean ± SD of 3 replicates. Selectivity Index (SI) was calculated as: $SI = CC_{50}/IC_{50}$

Phytochemical analysis

Cratoxylum is widely recognized for its rich content of bioactive secondary metabolites with antimalarial potential. In this study, LC-MS/MS analysis of the dichloromethane extract from *C. sumatranum* twigs identified 41 compounds, including xanthones (17), flavonoids (10), terpenoids (4), coumarins (2), chalcones (2), anthraquinones (2), phenylpropanoids (2), lignans (1), and amino acids (1). The data is presented in **Figure 2**. The Total Ion Chromatogram (TIC) provided a comprehensive profile of these chemical constituents (**Figure 1**). **Table 2** lists selected metabolites based on abundance, structural uniqueness, and reported bioactivity. Compound

identification was achieved through MS/MS fragmentation pattern analysis and comparison with curated databases, including LOTUS, PubChem, and COCONUT. The complete compound list is available in Supplementary (**Table S1**)

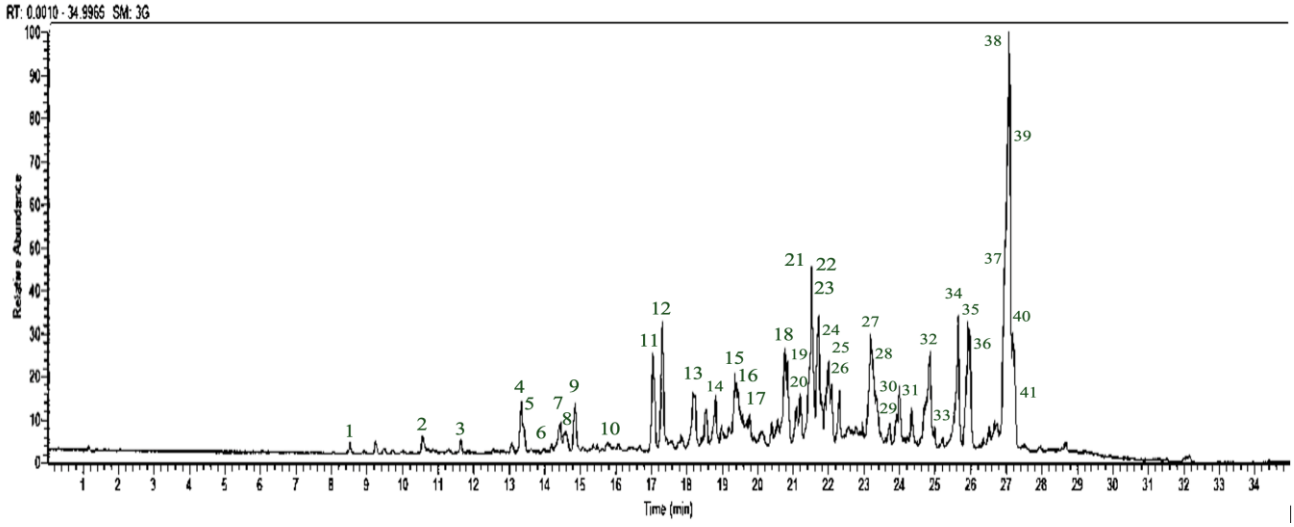

Figure 1 Total Ion Chromatography (TIC) of the dichloromethane extract from *C. sumatranum* twigs.

Among the identified metabolites (**Table 2**), several compounds have previously been isolated from other plant species and reported to exhibit antiplasmodial activity. Notably, xanthones such as Gerontoxanthone I from *C. mangiayi*, Macluraxanthone from *C. cochinchinense*, γ-Mangostin from *Garcinia mangostana,* and Trapezifolixanthone from *Chrysochlamys tenuis* demonstrated significant *in vitro* inhibitory activity against *Plasmodium falciparum*, with IC_{50} values ranging from 2.52 to 15.9 µM [5,19,20]. These compounds are known to affect parasite survival by inhibiting enzymes in the parasite's food vacuole, such as plasmepsin-II, falcipain-3, and M1-alanyl aminopeptidase, as well as by disrupting heme polymerization [10,19]. Chalcone compounds such as Pinocembrin chalcone have also exhibited activity against *Plasmodium berghei* [20]. In addition, the terpenoid Caryophyllene, isolated from *Copaifera reticulata*, effectively inhibited *P. falciparum* W2 and 3D7 strains with IC_{50} values of 1.66 and 2.54 µg/mL, respectively [21]. Furthermore, the anthraquinone Vismione B demonstrated strong antimalarial activity, with an IC_{50} value of 0.66 µg/mL [5]. According to Widyawaruyanti *et al.* [22], the flavonoid group, particularly prenylated flavones and chalcones, has exhibited strong antimalarial activity. Bilia *et al.* [23] stated that flavonoids exert their mechanism of action by inhibiting nutrient transport and blocking the degradation and detoxification of hemoglobin in *Plasmodium*. Flavonoids inhibit the influx of L-glutamine and Myo-inositol into *P. falciparum*-infected erythrocytes [24]. The presence of these compounds in the dichloromethane extract of *C. sumatranum* twig is believed to play a major role in the extract's strong antimalarial activity, as indicated by IC_{50} values of 0.28 µg/mL against the 3D7 strain and 0.66 µg/mL against the Dd2 strain of *Plasmodium falciparum.*

Table 2 Representative metabolites with reported antimalarial activity identified in the dichloromethane extract of *C. sumatranum* twigs.

Compound numbers*	RT (min)	Compound name	Class of compound	Molecular formula	$[M+H]^+$
8	14.56	Pinosembrin chalcone	Chalcone	$C_{15}H_{12}O_4$	257.0807
11	17.06	Gerontoxanthone I	Xanthone	$C_{23}H_{24}O_6$	397.1646

Compound numbers*	RT (min)	Compound name	Class of compound	Molecular formula	$[M+H]^+$
13	18.33	Vismione B	Anthraquinone	$C_{21}H_{22}O_5$	355.1538
18	20.77	γ-Mangostin	Xanthone	$C_{23}H_{24}O_6$	397.1641
29	23.47	Caryophyllene	Terpenoid	$C_{15}H_{24}$	205.1950
33	25.20	Macluraxanthone	Xanthone	$C_{23}H_{22}O_6$	395.1490
36	26.00	Trapezifolixanthone	Xanthone	$C_{23}H_{22}O_5$	379.1537

*Compound numbers correspond to **Figure 1**

Importantly, several other metabolites detected in the extract remain biologically uncharacterized. These include various xanthones and flavonoids with unknown bioactivity, as well as minor classes such as phenylpropanoids, lignans, and amino acid derivatives. Although not directly linked to antimalarial effects, these compounds may play auxiliary roles by enhancing pharmacokinetic properties, providing antioxidant or anti-inflammatory benefits, or modulating host-parasite interactions [25,26]. Their presence may contribute to the overall efficacy and safety profile of the extract through indirect or synergistic mechanisms [27,28]. The alignment between the phytochemical content and antimalarial activity observed *in vitro* highlights the value of further bioassay-guided fractionation. Future studies focusing on the isolation, structural elucidation, and biological evaluation of these uncharacterized compounds are warranted to uncover their roles and potential as novel therapeutic agents [29].

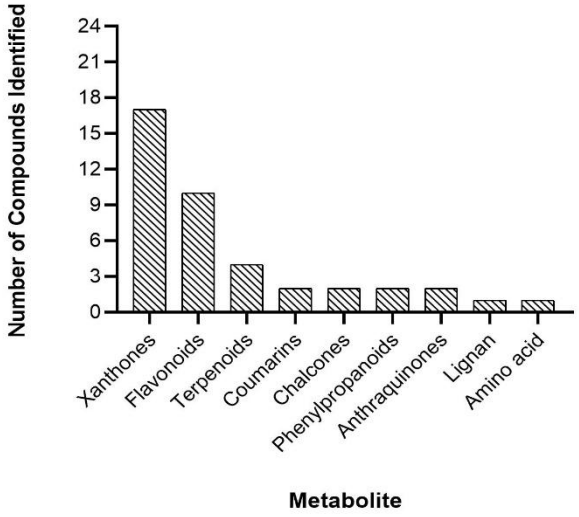

Figure 2 The metabolite classes in the dichloromethane extract of *C. sumatranum* twigs with potential antimalarial activity.

Conclusions

The dichloromethane extract of *Cratoxylum sumatranum* twigs displayed antimalarial activity against *Plasmodium falciparum* strains 3D7 and Dd2, with minimal cytotoxicity against BHK-21 cells. LC-MS/MS profiling revealed a diverse array of metabolites, predominantly xanthones and flavonoids, many of which have known or potential antimalarial properties. These results suggest that the observed bioactivity is likely due to the combined effects of multiple constituents. This study highlights the potential of *C. sumatranum* as a promising source of antimalarial agents. Future research should focus on bioassay-guided isolation of individual active compounds, evaluation of their mechanisms of action, and *in vivo* efficacy studies

to validate their potential as lead candidates in the development of antimalarial drugs.

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Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tools (e.g., ChatGPT by OpenAI) in the preparation of this manuscript, specifically for language editing and grammar correction. No content generation or data interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this work.

CRediT Author Statement

Maylisa Natalia Corry: Investigation, Writing-original manuscript, Visualization.

Hilkatul Ilmi: Methodology, Formal analysis, Writing-review & editing manuscript

Lidya Tumewu: Data curation and Writing-review & editing manuscript

Hanifah Khairun Nisa: Methodology and Data curation

Firman Wicaksana: Data Curation and Writing-original manuscript

Irfan Rayi Pamungkas: Data Curation and Writing-original manuscript

Supriatno Salam: Resources and Validation

Hadi Kuncoro: Resources and Validation

Suciati: Conceptualization, Methodology, Validation, Writing - Review & Editing, Supervision

Che Puteh Osman: Methodology, Validation, and Writing - Review & Editing manuscript

Achmad Fuad Hafid: Conceptualization, Validation, and Supervision

Aty Widyawaruyanti: Conceptualization, Methodology, Validation, Writing - Review & Editing, Supervision, Project Administration, Funding Acquisition.

References

[1] World Health Organization. *World malaria report 2021.* World Health Organization, Geneva, Switzerland, 2021.

[2] P Li. Annual report 2022. *AIMS Energy* 2023; **11(1)**, 135-139.

[3] N Mishra, RS Bharti, P Mallick, OP Singh, B Srivastava, R Rana, S Phookan, HP Gupta, P Ringwald and N Valecha. Emerging polymorphisms in falciparum Kelch 13 gene in Northeastern region of India. *Malaria Journal* 2016; **15(1)**, 4-9.

[4] CY Bok, J Low, E Kat, D Augundhooa, H Ariffin, YB Mok, KQ Lim, S Le Chew, S Salvamani, KE Loh, CF Loke, B Gunasekaran and SA Tan. Comprehensive review of *Cratoxylum* genus: Ethnomedical uses, phytochemistry, and pharmacological properties. *Pertanika Tropical Agricultural Science* 2023; **46(1)**, 213-241.

[5] S Laphookhieo, W Maneerat and S Koysomboon. Antimalarial and cytotoxic phenolic compounds from *Cratoxylum maingayi* and *Cratoxylum cochinchinense*. *Molecules* 2009; **14(4)**, 1389-1395.

[6] S Suryanto, L Tumewu, H Ilmi, AF Hafid, S Suciati and A Widyawaruyanti. Antimalarial activity of Cratoxyarborenone E, a prenylated xanthone, isolated from the leaves of *Cratoxylum glaucum* Korth. *Pharmacia* 2024; **71(1)**, 1-7.

[7] MLG Dapar. *Cratoxylum sumatranum (Jack) Blume Hypericaceae. In*: FM Franco (Ed.). Ethnobotany of the mountain regions of Southeast Asia. Springer, Cham, Switzerlands, 2021, p. 333-337.

[8] A Arnida, ER Sahi and S Sutomo. Aktivitas antiplasmodium *in vitro* dan identifikasi golongan senyawa dari ekstrak etanol batang manuran (*Coptosapelta tomentosa* Valeton ex K.Heyne) asal kalimantan Selatan (*in Indonesian*). *Jurnal Ilmiah Ibnu Sina* 2017; **2(2)**, 270-278.

[9] C Tantapakul, W Maneerat, T Sripisut, T Ritthiwigrom, RJ Andersen, P Cheng, S Cheenpracha, A Raksat and S Laphookhieo. New benzophenones and xanthones from *Cratoxylum sumatranum* ssp. *neriifolium* and their antibacterial and antioxidant activities. *Journal of Agricultural and Food Chemistry* 2916; **64(46)**, 8755-8762.

[10] F Wicaksana, FY Wardana, H Ilmi, L Tumewu, T Widiandani, AF Hafid and A Widyawaruyanti. Antimalarial activity of caged xanthone isolated compounds from *Cratoxylum sumatranum* stem bark: *In vitro* and *in silico* approaches. *Journal of Advanced Pharmaceutical Technology & Research* 2024; **15(4)**, 352-358.

[11] L Tumewu, FY Wardana, H Ilmi, AA Permanasari, AF Hafid and A Widyawaruyanti. *Cratoxylum sumatranum* stem bark exhibited antimalarial activity by Lactate Dehydrogenase (LDH) assay. *Journal of Basic and Clinical Physiology and Pharmacology* 2021; **32(4)**, 817-822.

[12] MF Dolabela, SG Oliveira, JM Nascimento, JM Peres, H Wagner, MM Póvoa and AB de Oliveira. *In vitro* antiplasmodial activity of extract and constituents from *Esenbeckia febrifuga*, a plant traditionally used to treat malaria in the Brazilian Amazon. *Phytomedicine* 2008; **15(5)**, 367-372.

[13] HA Abd Karim, NE Rasol, NH Ismail, C Wiart and CP Osman. Metabolites annotation of dichloromethane extract of *Kibatalia maingayi* woods using orbitrap high-resolution mass spectrometry. *Malaysian Journal of Chemistry* 2022; **24(4)**, 161-172.

[14] S Laphookhieo, JK Syers, R Kiattansakul and K Chantrapromma. Cytotoxic and antimalarial xanthones. *Chemical and Pharmaceutical Bulletin* 2006; **54**, 745-747.

[15] DK Sari, G Jeelani, H Ilmi, L Tumewu, R Wahyuni, A Widyawaruyanti, T Nozaki and AF Hafid. Therapeutic potential of Indonesian plant extracts in combating malaria and protozoan neglected tropical disease. *BMC Complementary Medicine and Therapies* 2024; **24(1)**, 416.

[16] P García-Huertas, A Pabón, C Arias and S Blair. Evaluation of cytotoxic effect and genetic damage of standardized extracts of *Solanum nudum* with antiplasmodial activity. *Biomédica* 2013; **33(1)**, 78-87.

[17] G Indrayanto, GS Putra and F Suhud. Validation of *in-vitro* bioassay methods: Application in herbal drug research. *Profiles of Drug Substances Excipients and Related Methodology* 2021; **46**, 273-307.

[18] B Kwansa-Bentum, K Agyeman, J Larbi-Akor, C Anyigba and R Appiah-Opong. *In vitro* assessment of antiplasmodial activity and cytotoxicity of *Polyalthia longifolia* leaf extracts on *Plasmodium falciparum* strain NF54. *Malaria Research and Treatment* 2019; **2019(1)**, 6976298.

[19] M Zakiah, RA Syarif, M Mustofa, J Jumina, N Fatmasari and EN Sholikhah. *In vitro* antiplasmodial, heme polymerization, and cytotoxicity of hydroxyxanthone derivatives. *Journal of Tropical Medicine* 2021; **2021(1)**, 8866681.

[20] Y Melaku, M Solomon, R Eswaramoorthy, U Beifuss, V Ondrus and Y Mekonnen. Synthesis, antiplasmodial activity and *in silico* molecular docking study of pinocembrin and its analogs. *BMC Chemistry* 2022; **16(1)**, 36.

[21] GAG de Souza, NC da Silva, J de Souza, KRM de Oliveira, AL da Fonseca, LC Baratto, ECP de Oliveira, F de Pilla Varotti and WP Moraes. *In vitro* and *in vivo* antimalarial potential of oleoresin obtained from *Copaifera reticulata* Ducke (Fabaceae) in the Brazilian Amazon rainforest. *Phytomedicine* 2017; **24**, 111-118.

[22] A Widyawaruyanti and NC Zaini. Mekanisme dan aktivitas antimalaria dari senyawa flavonoid yang diisolasi dari cempedak (*Artocarpus Champeden*) (*in Indonesian*). *Jurnal Bina Praja* 2011; **13(2)**, 67-77.

[23] AR Bilia, P Melillo de Malgalhaes, MC Bergonzi and FF Vincieri. Simultaneous analysis of artemisinin and flavonoids of several extracts of *Artemisia annua* L. obtained from a commercial sample and a selected cultivar. *Phytomedicine* 2006; **13(7)**, 487-493.

[24] JB Lekana-Douki, JB Bongui, SLO Liabagui, SEZ Edou, R Zatra, U Bisvigou and P Druilhe. *In vitro* antiplasmodial activity and cytotoxicity of 9 plants traditionally used in Gabon. *Journal of Ethnopharmacology* 2011; **133(3)**, 1103-1108.

[25] CW Wright. Traditional antimalarials and the development of novel antimalarial drugs. *Journal of Ethnopharmacology* 2005; **100(1-2)**, 67-71.

[26] K Kaur, M Jain, T Kaur and R Jain. Antimalarials from nature. *Bioorganic & Medicinal Chemistry* 2009; **17(9)**, 3229-3256.

[27] B Gilbert and L Alves. Synergy in plant medicines. *Current Medicinal Chemistry* 2005; **10(1)**, 13-20.

[28] R Verpoorte, YH Choi and HK Kim. Ethnopharmacology and systems biology: A perfect holistic match. *Journal of Ethnopharmacology* 2005; **100(1-2)**, 53-56.

[29] AL Harvey. Natural products in drug discovery. *Drug Discovery Today* 2008; **13(19-20)**, 894-901.

Supplementary Materials

Table S1 Identified metabolites in the dichloromethane extract of *C. sumatranum* twigs

No.	RT (min)	Compound Name	Class of Compound	Molecular Formula	$[M+H]^+$	Product ions *m/z*	Mass Error (ppm)	Compound Sources
1.	8.52	7-hydroxy-2,6-dimethylchromen-4-one	Coumarin	$C_{11}H_{10}O_3$	191.0702	176.0471, 163.0757, 151.0390, 115.0541	0.37	PubChem
2.	10.57	1,3,8-trihydroxy-2,4-dimethoxy-xanthen-9-one	Xanthone	$C_{15}H_{12}O_7$	305.0653	275.0182, 261.0385, 188.0449	0.92	COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, Natural Products, PubChem, PubMed
3.	11.64	Laurentixanthone B	Xanthone	$C_{17}H_{16}O_7$	333.0967	301.0339, 275.0544, 239.0324	0.54	COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, HMDB, KNApSAcK, MeSH, Natural Products, PubChem, PubChem class-bio and metabolites, PubMed, SuperNatural, ZINC bio
4.	13.35	2,3-dihydroxy-1,6,7-trimethoxyxanthone	Xanthone	$C_{16}H_{14}O_7$	319.0812	275.0546, 259.0594, 203.0338, 184.0517	0.09	COCONUT, LOTUS: Hypericaceae, Natural Products, PubChem, PubMed, SuperNatural
5.	13.40	Buchanaxanthone	Xanthone	$C_{14}H_{10}O_5$	259.0598	227.0336, 207.0285, 160.0517, 137.0231, 118.0414	1.16	COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, KEGG Mine, KNApSAcK, Natural Products, PubChem, PubMed, SuperNatural, Training Set, ZINC bio, additional
6.	13.98	3,5-dihydroxy-1,2-dimethoxy-9h-xanthen-9-one	Xanthone	$C_{15}H_{12}O_6$	289.0703	274.0467, 259.0230, 229.0484, 218.0579, 203.0348	1.26	COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, KNApSAcK, Natural Products, SuperNatural
7.	14.44	3,6-dihydroxy-1,5,7-trimethoxyxanthone	Xanthone	$C_{16}H_{14}O_7$	319.0810	271.0231, 261.0387, 230.0572	0.72	COCONUT, LOTUS: Hypericaceae, Natural Products, PubChem, PubMed, SuperNatural
8.	14.56	Pinosembrin chalcone	Chalcone	$C_{15}H_{12}O_4$	257.0807	239.0701, 229.0858, 211.0753, 171.0802	0.53	Biocyc, CHEBI, COCONUT, HMDB, KEGG, KNApSAcK, MeSH, Natural Products Plantcyc, PubChem, PubChem class-bio and metabolites, PubMed, SuperNatural, ZINC bio
9.	14.85	3,8-dihydroxy-1,2-dimethoxy-xanthen-9-one	Xanthone	$C_{15}H_{12}O_6$	289.0706	273.0397, 259.0231, 229.0490, 171.0438	0.22	COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, KNApSAcK, Natural Products, PubChem, SuperNatural
10.	15.78	8-hydroxy-3-(4-hydroxy-3-methoxy-phenyl)-2-(hydroxymethyl)-10-methoxy-2,3-dihydro-[1,4]dioxino[2,3-c]xanthen-7-one	Xanthone	$C_{24}H_{20}O_9$	453.1182	435.1066, 329.0641, 275.0551, 256.0368, 162.0675, 147.0442,	-0.42	COCONUT, LOTUS: *Cratoxylum,* LOTUS: Hypericaceae, Natural Products, PubChem

No.	RT (min)	Compound Name	Class of Compound	Molecular Formula	$[M+H]^+$	Product ions *m/z*	Mass Error (ppm)	Compound Sources
						137.0593, 109.1010		
11.	17.06	Gerontoxanthone I	Xanthone	$C_{23}H_{24}O_6$	397.1646	369.1687, 351.1586, 329.1017, 295.0959, 243.1372, 201.0909, 153.0180	-0.09	COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, KNApSAcK, Natural Products, PubChem, PubMed, SuperNatural
12.	17.32	2-(3-hydroxy-4-methoxy-phenyl)-5,7-dimethoxy-8-(3-methylbut-2-enyl) chroman-4-one	Flavonoid	$C_{23}H_{26}O_6$	399.1802	381.1698, 339.1219, 303.1226, 261.0754, 217.0857, 153.0181	0.04	COCONUT, MeSH, Natural, Products, PubChem, PubMed, SuperNatural
13.	18.33	Vismione B	Anthraquinone	$C_{21}H_{22}O_5$	355.1538	322.1193, 294.1261, 262.1000	0.56	COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, KNApSAcK, MeSH, Natural, Products, PubChem, PubMed, SuperNatural
14.	18.81	1-(3,3-dimethylbutyl)-3,6,8-trihydroxy-2-methoxy-7-(3-methylbut-2-en-1-yl)-9H-xanthen-9-one	Xanthone	$C_{25}H_{30}O_6$	427.2114	409.2017, 377.1750, 343.1541, 321.1123, 274.1194, 257.0806	0.27	COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, Natural Products, PubChem, SuperNatural
15.	19.36	6-[2-(4-benzyltetralin-1-yl)acetyl]-5-hydroxy-7-methyl-4-[3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]oxy-naphthalene-2-carboxylic acid	Flavonoid	$C_{37}H_{38}O_{10}$	643.2536	625.2426, 569.1799, 533.1796, 355.1538, 327.1592, 295.0955, 269.0802	0.27	COCONUT, SuperNatural
16.	19.45	Isocalycopterone	Flavonoid	$C_{36}H_{36}O_{10}$	629.2383	611.2274, 597.2123, 523.1412, 355.1543, 337.1437	-0.28	COCONUT, KNApSAcK, Natural Products, PubChem, PubMed, SuperNatural
17.	19.77	Osajetin, dimethyl ether	Coumarin	$C_{26}H_{30}O_5$	423.2166	367.1543, 355.1532, 339.1210, 313.1062, 301.1067, 289.1061, 259.0957, 135.1163	0.00	PubChem
18.	20.77	γ-Mangostin	Xanthone	$C_{23}H_{24}O_6$	397.1641	341.1019, 285.0391, 273.0383	1.17	CHEBI, COCONUT, LOTUS: *Cratoxylum*, HMDB, LOTUS: Hypericaceae, KEGG Mine, KNApSAcK, MeSH, Natural Products, PubChem, PubChem class-bio and metabolites, PubChem class-safety and toxic, PubMed, SuperNatural, Training Set, ZINC bio additional

No.	RT (min)	Compound Name	Class of Compound	Molecular Formula	$[M+H]^+$	Product ions *m/z*	Mass Error (ppm)	Compound Sources
19.	21.08	9-hydroxy-5,10-dimethoxy-11-(3-methoxy-3-methyl-butyl)-2,2-dimethyl-3,4-dihydropyrano[2,3-a]xanthen-12-one	Xanthone	$C_{26}H_{32}O_7$	457.2220	301.1068, 284.1040, 259.0959	0.17	COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, Natural Products, PubChem, SuperNatural
20.	21.13	*N*-[(3,5-di*tert*-butyl-4-hydroxyphenyl) methyl]acetamide	Flavonoid	$C_{17}H_{27}NO_2$	278.2113	219.1740, 163.1113	0.56	PubChem
21.	21.49	8,9*a*-dihydroxy-6-methoxy-3*a*-(4-methoxyphenyl)-3-[2-(2,4,5-trimethoxyphenyl) vinyl]-2,3-dihydrofuro[3,2-b]chromen-9-one	Flavonoid	$C_{30}H_{30}O_{10}$	551.1906	477.1180, 343.1165, 287.0536, 167.0702	1.04	COCONUT, KNApSAcK, MeSH, NORMAN, Natural Products, PubChem
22.	21.53	[4-[[4-[6-(ethenoxymethoxy) hexoxy]phenoxy]methoxy]-2-methoxyphenyl] 6-prop-2-enoyloxynaphthalene-2-carboxylate	Phenylpropanoid	$C_{37}H_{38}O_{10}$	643.2534	551.1691, 355.1535, 295.1325, 253.0860, 241.0854, 229.0855	0.56	PubChem
23.	21.72	[9-hydroxy-1,5-dimethyl-5-(4-methylpent-3-enyl)-6,15-dioxatetracyclo[9.3.1.0^{4,13} .0^{7,12}]pentadeca-7,9,11-trien-8-yl]-phenyl-methanone	Terpenoid	$C_{28}H_{32}O_4$	433.2373	351.1593, 323.1278, 297.1118, 243.0644, 203.1789, 165.0178, 109.1007	0.08	COCONUT, Natural Products, PubChem, PubMed
24.	21.93	[6-(3,7-dimethylocta-2,6-dienyl)-3,5,7-trihydroxy-2,2-dimethyl-chroman-8-yl]-phenyl-methanone	Flavonoid	$C_{28}H_{34}O_5$	451.2480	283.0965, 243.0647, 165.0179, 109.1007	-0.22	COCONUT, LOTUS: Hypericaceae, KEGG Mine, KNApSAcK, Natural Products, PubChem, SuperNatural
25.	22.00	3,6,8-trihydroxy-1,1,7-tris(3-methylbut-2-enyl)-4*a*,9*a*-dihydroxanthene-2,9-dione	Xanthone	$C_{28}H_{34}O_6$	467.2427	259.0591, 243.0642, 231.0648, 205.0495, 165.0177, 109.1008	0.25	COCONUT
26.	22.08	[9-hydroxy-1,5-dimethyl-5-(4-methylpent-3-enyl)-6,15-dioxatetracyclo[9.3.1.0^{4,13} .0^{7,12}]pentadeca-7,9,11-trien-8-yl]-phenyl-methanone	Terpenoid	$C_{28}H_{32}O_4$	433.2372	309.1124, 283.0961, 243.0646, 231.0652, 203.1792, 165.0178, 135.1164, 109.1007	0.31	COCONUT, Natural Products, PubChem, PubMed
27.	23.17	5,9,10-trihydroxy-2,2-dimethyl-12-(3-methylbut-2-enyl)pyrano[3,2-b]xanthen-6-one	Xanthone	$C_{23}H_{22}O_6$	395.1489	339.0859, 321.0755, 293.0800	0.04	COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, KNApSAcK, Natural Products, PubChem, SuperNatural
28.	23.37	1,3,7-trihydroxy-2,4-diisoprenylxanthone	Xanthone	$C_{23}H_{24}O_5$	381.1697	325.1071, 269.0440	-0.13	CHEBI, COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, KNApSAcK, Natural Products, PubChem, PubMed, SuperNatural
29.	23.47	Caryophyllene	Terpenoid	$C_{15}H_{24}$	205.1950	163.1477, 137.1321, 109.1007	0.38	Biocyc, CHEBI, COCONUT, LOTUS: *Cratoxylum*, HMDB, LOTUS: Hypericaceae, KEGG, KNApSAcK, MeSH, NORMAN, Natural Products,

No.	RT (min)	Compound Name	Class of Compound	Molecular Formula	$[M+H]^+$	Product ions *m/z*	Mass Error (ppm)	Compound Sources
								Plantcyc, PubChem, PubChem class-bio and metabolites, PubChem class-food, PubChem class-safety and toxic, PubMed, SuperNatural, ZINC bio
30.	23.97	Phenyl-[3,5,7-trihydroxy-2-methyl-6-(3-methylbut-2-enyl)-2-(4-methylpent-3-enyl)chroman-8-yl]methanone	Flavonoid	$C_{28}H_{34}O_5$	451.2475	433.2377, 323.1278, 313.1057, 273.0749, 243.0648, 231.0637	0.89	COCONUT, LOTUS: Hypericaceae, Natural Products, PubChem, SuperNatural
31.	24.00	6-[2-(4-benzyltetralin-1-yl)acetyl]-5-hydroxy-7-methyl-4-[3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]oxy-naphthalene-2-carboxylic acid	Flavonoid	$C_{37}H_{38}O_{10}$	643.2536	625.2429, 592.2089, 520.1519, 495.1429, 355.1543, 313.1436, 301.1068, 282.0883, 269.0806	0.27	COCONUT, SuperNatural
32.	24.85	Ethyl 2-[4-methoxy-3,5-bis(phenylmethoxy)benzoyl]-4-oxo-4-(3,4,5-trimethoxyphenyl)butanoate	Phenylpropanoid	$C_{37}H_{38}O_{10}$	643.253	625.2416, 582.1871, 356.1614, 309.1482, 271.0958, 259.0953, 241.0848	1.20	PubChem
33.	25.20	Macluraxanthone	Xanthone	$C_{23}H_{22}O_6$	395.1490	380.1253, 353.1013, 297.0754	-0.22	CHEBI, COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, KEGG, KNApSAcK, MeSH, Natural Products, PubChem, PubMed, SuperNatural, Training Set, ZINC bio additional
34.	25.64	9-ethenyl-6-hydroxy-2-[(1*S*)-1-hydroxy-3-methylbutyl]-1-methoxy-8-methyl-10*H*-benzo[b][1,5]benzodioxocin-12-one	Lignan	$C_{23}H_{26}O_6$	399.1801	381.1695, 337.1432, 279.1016, 243.0647, 167.0701, 111.0801	0.29	PubChem
35.	25.92	(1*R*,3*aS*,5*aR*,5*bR*,7*aS*,11*aS*,11*bR*,13*aR*,13*bR*)-5*a*,5*b*,8,8,11*a*-pentamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7*a*,11,11*b*,12,13,13*a*,13*b*-tetradecahydrocyclopenta[a]chrysene-3*a*-carboxylic acid	Terpenoid	$C_{30}H_{46}O_2$	439.3570	393.3514, 301.2161, 259.1689, 219.1380, 173.1323, 137.1322	0.13	PubChem
36.	26.00	Trapezifolixanthone	Xanthone	$C_{23}H_{22}O_5$	379.1537	323.0911, 305.0804	0.79	COCONUT, LOTUS: *Cratoxylum*, LOTUS: Hypericaceae, KEGG Mine, KNApSAcK, MeSH, Natural Products, PubChem, PubMed, SuperNatural
37.	26.96	2-(2,2-dimethylchroman-6-yl)-5,7-dihydroxy-8-(3-methylbut-2-enyl) chroman-4-one	Flavonoid	$C_{25}H_{28}O_5$	409.2006	391.1540, 283.0948, 243.0648, 231.0654, 165.0182, 105.0333	0.86	COCONUT, KNApSAcK, Natural Products, PubChem

No.	RT (min)	Compound Name	Class of Compound	Molecular Formula	$[M+H]^+$	Product ions *m/z*	Mass Error (ppm)	Compound Sources
38.	27.05	butyl 2-fluoro-4-[6-(4-methylidenecyclohexanecarbonyl) oxyhexoxy] benzoate	Amino Acid	$C_{25}H_{35}FO_5$	437.2574	340.1078, 243.0645, 185.0336, 165.0177	3.29	PubChem
39.	27.13	2-hydroxy-3-[(2*E*)-6-hydroxy-3,7-dimethylocta-2,6-dienyl]-4-methoxy-6-(2-phenylethyl) benzoic acid	Flavonoid	$C_{26}H_{32}O_5$	425.2319	301.1065, 283.0961, 259.0959	0.83	PubChem
40.	27.17	7-[(2E)-3,7-dimethylocta-2,6-dienyl]-3,8,9-trihydroxy-6-methoxy-3-methyl-2,4-dihydroanthracen-1-one	Anthraquinone	$C_{26}H_{32}O_5$	425.2319	301.1065, 283.0961, 259.0959	0.83	COCONUT, LOTUS: *Cratoxylum,* LOTUS: Hypericaceae, Natural Products, PubChem, SuperNatural
41.	27.25	(*E*)-1-[2-hydroxy-3-[(1*S*,2*R*)-2-hydroxycyclohexyl]-4,6-dimethoxyphenyl]-3-(2-hydroxyphenyl) prop-2-en-1-one	Chalcone	$C_{23}H_{26}O_6$	399.1796	381.1695, 301.1066, 283.0960, 259.0958	1.54	PubChem