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| RESEARCH ARTICLE

GC-MS and LC-MS/MS Analysis of Secondary Metabolites in the Methanolic Extract of *Uncaria callophylla* Blume ex Korth. Stems

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| ABSTRACT

Uncaria callophylla Blume ex Korth. is a wild plant species belonging to the Rubiaceae family. These plants thrive in the peat swamp forest of Kalimantan, Indonesia. The stems of *Uncaria callophylla* have been used by the Dayak Ngaju tribe to treat various diseases and to restore stamina while they work in the forest. The present study was conducted to identify the secondary metabolites in the methanolic extract of *Uncaria callohylla* stems to increase the knowledge about this plant and its potential in medicinal applications and drug discovery. The analysis was conducted using GC-MS and LC-MS/MS. GC-MS analysis was performed with the Agilent Technologies 7890 Gas Chromatography equipped with an Auto Sampler and 5975 Mass Selective Detector. The LC-MS analysis was carried out in the C18 column of the Waters Acquity UPLC system. MS analysis was performed with an electrospray ionization source (ESI) in positive and negative ion modes, and the compounds were identified using a UNIFI data processor with a mass spectrum library of natural active substances from the Waters Traditional Medical Scientific Library database based on UPLC/QTof MSE Data Acquisition. GC-MS analysis revealed the presence of 24 peaks, and nine of them showed quality 90% or higher, namely n-Hexadecanoic acid (16.63%), 1,3,7-Trimethyl-3,7-Dihydro-1H-Purine-2,6-Dione (13.07%), Hexadecanoic Acid, Methyl Ester; 1,2-Benzenedicarboxylic Acid, Diethyl Ester; 9,12-Octadecadienoic Acid, Methyl Ester; Methyl 14-Methylheptadecanoate; (9E,12E)-9,12-Octadecadienoic Acid; Z, E-3,13-Octadecadien-1-o1; and 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexanethyl-, (all-E)-. LC-MS/MS analysis revealed the presence of five compounds, namely Arecatannin A1, Sweroside_2, Uncarine A, Epianhydrobelachinal, and Betulonic acid. All the secondary metabolites identified from GC-MS and LC-MS/MS analysis have biological activity, which indicates their medicinal potencies. From the result, it can be concluded that *Uncaria callophylla* stem methanolic extract contains various bioactive compounds justifies its traditional use, and therefore it can be developed further for new drug discovery.

| KEYWORDS

Uncaria callophylla, Rubiaceae, natural product, GC-MS, LC-MS/MS, biochemical compound.

| ARTICLE INFORMATION

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1. Introduction

Uncaria callophylla Blume ex Korth. is a wild plant species from the Rubiaceae family that thrives in the peat swamp forests of Central Kalimantan, Indonesia. The Dayak Ngaju tribe of Central Kalimantan often used the tea made from the stems of *Uncaria callophylla* as a tonic or stimulant as part of the remedy for various diseases. The water comes out from the cut stems, usually drunk, to increase or restore stamina while working in the forest. The dried powder of the stem is often used to treat scabs and wounds by sprinkling them on top of the wound, which has been practiced for hundreds of years (Almeida et al., 2022).

The genus Uncaria comprises 34 species, including 3 species in Africa and Madagascar, 2 species in tropical America, and 29 species in Asia and Australia. Many of these are usually used as traditional Chinese medicines (TCMs) to treat hypertension, fever, headache,

gastrointestinal illness, and fungal infection (Liang et al., 2020). Zhang et al. (2015) found that 19 species of the genus Uncaria are important folk medicines used to cure asthma, rheumatism, hyperpyrexia, hypertension, and headaches, in China, Malaysia, the Philippines, Africa, and Southeast America. More than 200 different substances, such as indole alkaloids, triterpenes, flavonoids, phenols, and phenylpropanoids, have been identified from Uncaria plants. Indole alkaloids, which are characteristic components, have been thought to be the primary effective component for treating hypertension, epilepsy, depression, Parkinson's disease, and Alzheimer's disease. In addition, pharmacokinetic and metabolism investigations reveal that indole alkaloids are likely to be absorbed, metabolized, and excreted at early time points. Many species from the genus *Uncaria* are important sources of natural medicines due to their rich bioactive compounds, especially alkaloids and triterpenes. Alkaloids, terpenes, quinovic acid glycosides, flavonoids and coumarins have been isolated from Uncaria. The potential for the development of leads from Uncaria continues to grow, particularly in the area of immunomodulatory, anti-inflammatory and vascular-related conditions (Heitzman et al., 2004; Zhang et al., 2015; Qin et al., 2021). Uncaria species that most studied are *Uncaria gambir*, *Uncaria nervosa, Uncaria longiflora, Uncaria tomentosa*, *Uncaria cordata, Uncaria macrophylla, Uncaria rhynchophylla, and Uncaria hirsuta* (Almeida et al., 2022; Munggari et al., 2022; Huang et al., 2021). *Uncaria callophylla* is one of the Uncaria species that is still under-researched.

In 1989 Chang et al. (1989) reported the cardiovascular effect of dihydrocorynantheine, an alkaloid isolated from *Uncaria callophylla*. Ahmad *et al.* (2011) reported that the stem extract of *Uncaria callohylla* contains very high levels of phenolics and flavonoids and has very strong antioxidant activity. However, it has never been analyzed more deeply what phytochemical compounds are present in the stem extract. Information about the compounds contained in a natural product is needed to give insights into the vital role of the natural product in drug development. In this study, the phytochemical screening using GC-MS and LC-MS/MS was performed to identify the phytoconstituents in the methanolic extract of *Uncaria callohylla* stems to increase the knowledge about this plant and its potential in medicinal applications and drug discovery.

2. Literature Review

2.1 Botanical aspects of Uncaria callophylla

Uncaria callophylla Blume ex Korth. is a flowering plant belongs to the Rubiacea family. Growing mainly in the humid tropical area, it is native to Indonesia, Malaya, New Guinea, the Philippines, Thailand, and Queensland. In Indonesia, this plant thrives, especially in Kalimantan, Sumatera, and Maluku (POWO, 2023). *U. callophylla* is a liana with vine stem diameters up to 14 cm. Stem vessels are very large and easily visible to the naked eye. Stem pitch square in transverse section. The leaf blades are about 8-11.5 x 3-5 cm, and the petioles are about 0.5-1 cm long. Domatia are tufts of hair surrounding or bordering a slit-like foveola or gash. The supporting leaves are found between pairs of leaflets. Stipules are about 5-9 mm long, blunt at the apex. Hooks or tendrils develop from short shoots in the leaf axils and resemble shepherd's crooks about 2-2.5 cm long. Flowers appear in the leaf axils in the form of a long tube with short lobes. Calyx is linear, with lobes up to 2.5 mm long, bent and persistent at the apex. Corolla lobes are finely pubescent. Fruits are longitudinally ribbed, borne in umbels of 50-60 fruits. Each fruit is clavate or spindle-shaped, measuring about 10-20 x 3-3.5 mm on a stalk about 5 mm long. Calyx lobes are linear, twisted, persistent at the apex, about 1 mm long. Capsule-shaped fruit, when dry, will crack and open so that many small seeds are released. Each fruit has many seeds; each seed has two wings. Seeds about 0.2-0.3 mm long, seed plus wing about 4-5 mm long. The length of the embryo is about 0.1-0.2 mm. Its radicles are longer than cotyledons (POWO, 2023; Australian Tropical Rainforest Plants, 2020).

2.2 Chemical Content and Biological Activity of Uncaria callophylla

The chemical content of *Uncaria callophylla* has not been completely reported, but some research reveals that the leaves and stems contain alkaloids that have antioxidant and antidiabetic activity. The stem of *Uncaria callophylla* contains pseudoyohimbine and dihydrochorinantein, while the leaves contain dihydrocorinantein, gambirin and a dimeric alkaloid which is probably a derivative of gambirin and pseudoyohimbine (Goh *et al*., 1985; Ahmad *et al.*, 2011). Kam (1999) isolated several alkaloids from the leaves of *Uncaria callophylla,* such as isogambirine, gambireine, callophylline, dihydrocorynantheine, rotundifoline, yohimbine, pseudo-yohimbine, α-yohimbine and β-yohimbine. According to Chang *et al.* (1989), the alkaloid dihydrocorynantheine isolated from *Uncaria callophylla* can affect the cardiovascular system in both conscious and anaesthetized normotensive rats. And according to Mok *et al.* (1992), intravenous injection of alkaloid gambirine, which is a typical alkaloid from the *Uncaria* plants, isolated from *Uncaria callophylla,* can lower blood pressure in both systolic and diastolic blood pressures as well as heart rate.

3. Methodology

3.1 Plant materials

The stems of *Uncaria callophylla* (Figure 1) were collected from the peat swamp forests of Central Kalimantan and identified at Herbarium Bogoriense, Indonesian Institute of Biological Research, Cibinong, West Java, Indonesia. Once collected, the stems were cut into about 40 cm long and washed with clean water, and the bark was removed. Then the wood is air-dried in a place that is not directly exposed to sunlight.

3.2 Preparation of methanolic extract of the stems of Uncaria callophylla

The wood of *U. callophylla* was cut into pieces with a size of about 5 cm x 1 cm, then air-dried in a 50^0C oven and pulverized into powder using a grinder. A total of 500 grams of *U. callophylla* stem powder was then macerated for 3x24 hours using 4000 ml of methanol with an orbital shaker at 100 rpm. The filtrate is then concentrated using a rotary vacuum evaporator. The viscous extract was collected, weighed, and then stored in a refrigerator at 5°C until used for further analysis.

Figure 1. *Uncaria callophylla* (Rubiaceae)

3.3 GC-MS Analysis

GC-MS analysis was carried out as described in previous research (Trifani et al., 2022) with minor modifications. A total of 5 grams of *U. callophylla* stem methanolic extract (UCSME) was dissolved in 25 mL of methanol. GC-MS analysis was performed in Agilent Technologies 7890 Gas Chromatography equipped with an Auto Sampler and a 5975 Mass Selective Detector (MSD). Capillary column (HP Ultra 2) measuring 30 m × 0.20 mm with a film thickness of 0.11 m and helium gas as a carrier at a 1.2 ml/min flow rate and constant flow in column mode. The initial temperature was 80°C for 0 minutes, then increased at a rate of 3°C/min to 150°C, stabilized for 1 min, and finally rose by 20 °C/min to 280 °C, which lasted 26 min. The injector temperature, ion temperature, interface temperature, and quadrupole temperature in scan mode are set at 250 °C, 230 °C, 280 °C, and 140 °C, respectively. The electron impact ionization mode is set at 70eV. The structure of the reported compounds was assessed by comparing the fragmentation patterns obtained by 1:8 separation. The chromatograms were analyzed, and the relative percentages of the compounds were calculated. Compounds were identified by comparing the mass spectra with the reference mass spectra in the Willey 275 database. The relative content of the compounds was calculated based on the total peak area of the integrated ion chromatogram (TIC) for the co-eluting peaks, and the results were expressed as total abundances.

3.4 LC-MS/MS Analysis

LC-MS/MS analysis was carried out as described in previous research (Trifani et al., 2022) with minor modifications. A total of 0.5 grams of UCSME was dissolved in 10 ml methanol, followed by sonication for 30 minutes. Then it was diluted further with methanol, homogenized to the right concentration, and passed through a syringe filter with 0.22 m GHP/PTFE membrane. The LC-MS analysis was carried out in the C18 column of the Waters Acquity UPLC system (2.1 mm × 100 mm, 1.7 mm) equipped with an auto-sampler,

column manager, and adjustable MS detector. The mobile phase was 0.1% formic acid in acetonitrile (solvent A) and 0.1% formic acid in bidestilated water (solvent B). For gradient elution, the flow rate of the mobile phase was kept at 0.6 mL/min. The total chromatography run time was 2.0 min. The temperatures for the column and auto-sampler were maintained at 40°C and 15°C. The injection volume was 10 L. MS analysis was performed with an electrospray ionization source (ESI) in positive and negative ion modes. MS data is obtained in the m/z range of 50-1200 and MSE Tof mode operation. The compounds were identified using a UNIFI data processor with a mass spectrum library of natural active substances from the Waters Traditional Medical Scientific Library database based on UPLC/QTof MSE Data Acquisition, which is integrated with an automatic identification process.

4. Results and Discussion

4.1 GC-MS Analysis

GC-MS chromatogram of the UCSME showed the presence of 24 peaks (Figure 2), respectively, corresponding to the bioactive compounds that were recognized by relating their mass spectral fragmentation patterns to that of the known compounds described by the NIST library. In comparison with the NIST library, nine of them have a quality of 90% or above. Quality is a measurement of the level of similarity of compounds in the sample with compounds in the library. The higher the quality indicates the higher the similarity between the compound with the one in the GC-MS instrument library. The nine peaks that have high similarity, 90% or above, were identified as 1,2-Benzenedicarboxylic Acid, Diethyl Ester; 1,3,7-Trimethyl-3,7-Dihydro-1H-Purine-2,6-Dione; Hexadecanoic Acid, Methyl Ester; n-Hexadecanoic Acid; 9,12-Octadecadienoic Acid, Methyl Ester; Methyl 14-Methylheptadecanoate; (9E,12E)-9,12-Octadecadienoic Acid; Z, E-3,13-Octadecadien-1-o1; and 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexanethyl-, (all-E)- (Table 1). The biological activity of the compounds is presented in Table 2, and the chemical structures are presented in Table 3. The GC-MS analysis is the first step towards understanding the nature of active principles in natural products. According to our knowledge, the chemical composition of methanol extract from *Uncaria callophylla* stems, investigated by gas chromatography coupled with mass spectroscopy (GC/MS), was performed for the first time in this study.

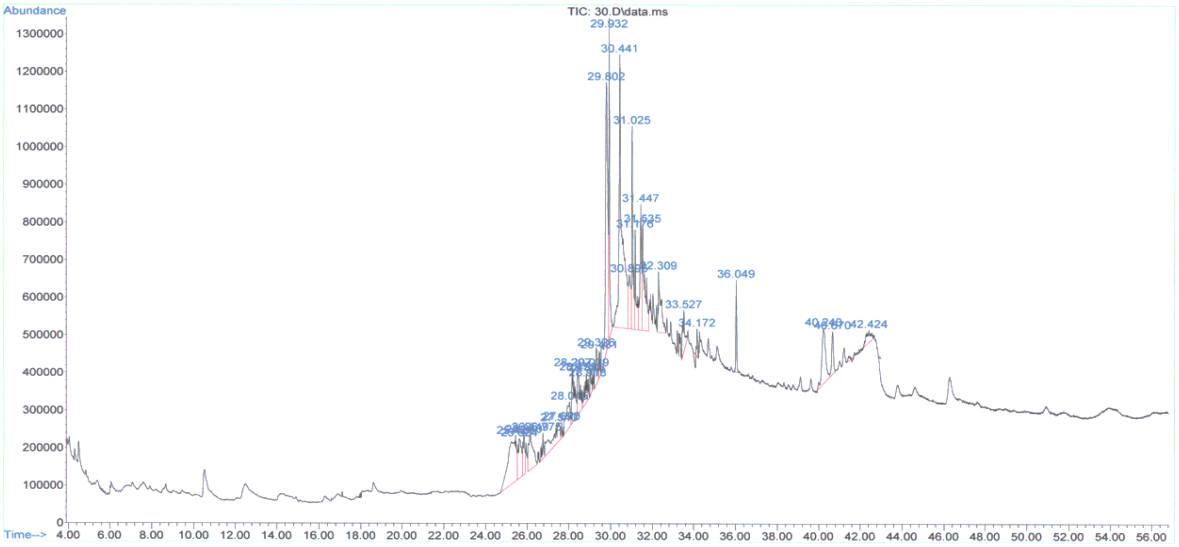


Figure 2. GC-MS chromatogram of *Uncaria callophylla* stem methanolic extract

The phytochemicals identified in GC-MS analysis of the methanolic extract of *Uncaria callophylla* stems are known to exhibit biological activities which have medicinal potencies. The predominant components in the extract were n-Hexadecanoic Acid (16.63%) and 1,3,7-Trimethyl-3,7-Dihydro-1H-Purine-2,6-Dione (13.07%). n-Hexadecanoic acid possesses some biological activities such as antioxidants, antiinflammatory, antibacterial, hypocholesterolemic, and anticancer activities (Ganesan et al., 2022; Mazumder et al., 2020; Aparna et al., 2012). Goyal et al. (2020) state that n-Hexadecanoic acid has antiinflammatory, hypocholesterolemic antispasmodic, anticancer, haemolytic, 5-alpha reductase inhibitor, antiviral, nematicide, pesticide, and potent mosquito larvicide activity. According to the PASS Software prediction database (http://www.way2drug.com/passonline/predict.php), n-Hexadecanoic acid has inhibitory activity towards several enzymes, such as acylcarnitine hydrolase, alkylacetylglycerophosphatase, and sugar-phosphatase. Acylcarnitines play an essential role in regulating the balance of intracellular sugar and lipid metabolism. They serve as carriers to transport activated long-chain fatty acids into mitochondria for β-oxidation as a major source of energy for cell activities. Inhibition of acylcarnitine hydrolase activity is one mechanism of hypolipidemic drugs such as clofibrate. n-Hexadecanoic acid also possesses activity as a platelet aggregation stimulant, macrophage stimulant, procollagen, angiogenesis stimulant, fibroblast growth factor agonist, indicating its potential in wound healing.

The methyl ester of hexadecanoic acid, which was also identified as contained in the extract at 5.51%, was reported to have many biological activities, including antibacterial and hypercholesterolemia, and also used as a flavoring agent, cosmetic, and perfumery (Balabhaskar and Vijayalakshmi, 2021; Shaaban et al., 2021). According to the PASS Software prediction database, hexadecanoic acid methyl ester also has protease inhibitor activity, especially as a saccharopepsin inhibitor, acrocylindropepsin inhibitor, and chymosin inhibitor. Protease inhibitors, or antiproteases, are molecules that inhibit the function of proteases (enzymes that aid the breakdown of proteins). In medicine, protease inhibitor is often used interchangeably with alpha 1-antitrypsin, the protease inhibitor most often involved in alpha-1 antitrypsin deficiency disease.

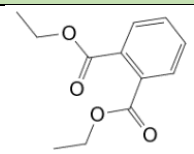
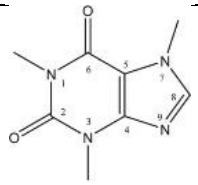
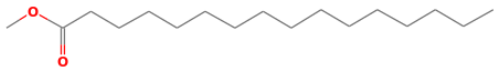
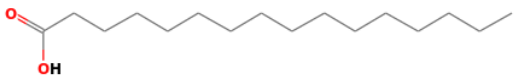
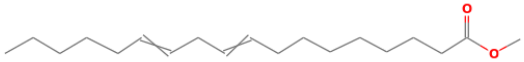
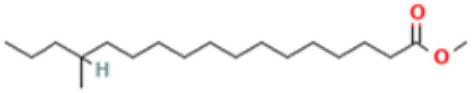
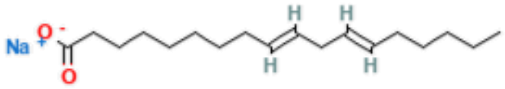
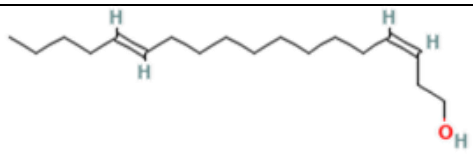
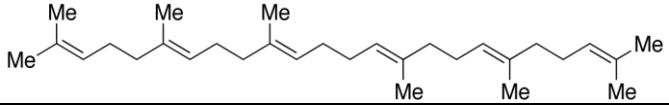
Table 1. Phytochemicals with high similarity identified in methanolic extract of *Uncaria, callophylla* stems by GC-MS analysis

Retention Time (min)	Quality	Name of Compound	Quantity (%)
26.152	90	1,2-Benzenedicarboxylic Acid, Diethyl Ester	3.35
29.799	98	1,3,7-Trimethyl-3,7-Dihydro-1H-Purine-2,6-Dione	13.07
29.931	98	Hexadecanoic Acid, Methyl Ester	5.51
30.441	99	n-Hexadecanoic Acid	16.63
31.027	97	9,12-Octadecadienoic Acid, Methyl Ester	4.91
31.179	94	Methyl 14-Methylheptadecanoate	2.42
31.447	92	(9E,12E)-9,12-Octadecadienoic Acid	3,89
33.530	90	Z,E-3,13-Octadecadien-1-ol	1.70
36.047	98	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexanethyl-, (all-E)-	1.62

Table 2. Biological activity of volatile compounds identified in the methanolic extract of *Uncaria, callophylla* stems (Based on PASS Software's prediction database)

Name of Compound	Bioactivity
1,2-Benzenedicarboxylic Acid, Diethyl Ester	2-Hydroxymuconate-semialdehyde hydrolase inhibitor, Prolyl aminopeptidase inhibitor
1,3,7-Trimethyl-3,7-Dihydro-1H-Purine-2,6-Dione	Cyclic AMP phosphodiesterase inhibitor, Xanthine-like respiratory analeptic, Kidney function stimulant, Analeptic
Hexadecanoic Acid, Methyl Ester	Saccharopepsin inhibitor, Acrocylindropepsin inhibitor, Chymosin inhibitor
n-Hexadecanoic Acid	Acylcarnitine hydrolase inhibitor, Alkylacetylglycerophosphatase inhibitor, Sugar-phosphatase inhibitor, Platelet aggregation stimulant, macrophage stimulant, procollagen, angiogenesis stimulant, fibroblast growth factor agonist
9,12-Octadecadienoic Acid, Methyl Ester	Antieczematic, All-trans-retinyl-palmitate hydrolase inhibitor, Macrophage colony stimulating factor agonist
Methyl 14-Methylheptadecanoate	Saccharopepsin inhibitor, Acrocylindropepsin inhibitor, Chymosin inhibitor
(9E,12E)-9,12-Octadecadienoic Acid	Phosphatidylglycerophosphatase inhibitor, Acylcarnitine hydrolase inhibitor, Mucomembranous protector
Z,E-3,13-Octadecadien-1-ol	Macrophage colony stimulating factor agonist, Sugar-phosphatase inhibitor, Alkenylglycerophosphocholine hydrolase inhibitor.
2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexanethyl-, (all-E)-	Fatty-acyl-CoA synthase inhibitor, Antieczematic, All-trans-retinyl-palmitate hydrolase inhibitor

Table 3. Chemical structure of compounds identified in methanolic extract of *Uncaria, callophylla* stems by GC-MS analysis

Compound	Molecular Formula	Chemical Structure
1,2-Benzenedicarboxylic Acid, Diethyl Ester	$C_{12}H_{14}O_4$	
1,3,7-Trimethyl-3,7-Dihydro-1H-Purine-2,6-Dione	$C_8H_{10}N_4O_2$	
Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	
n-Hexadecanoic Acid	$C_{16}H_{32}O_2$	
9,12-Octadecadienoic Acid, Methyl Ester	$C_{19}H_{34}O_2$	
Methyl 14-Methylheptadecanoate	$C_{19}H_{38}O_2$	
(9E,12E)-9,12-Octadecadienoic Acid	$C_{18}H_{32}O_2$	
Z,E-3,13-Octadecadien-1-ol	$C_{18}H_{34}O$	
2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexanethyl-, (all-E)-	$C_{24}H_{38}$	

The second most volatile compound in the methanolic extract of *Uncaria callophylla* stems was 1,3,7-trimethyl-3,7-Dihydro-1H-Purine-2,6-Dione (13.07%). According to the PASS Software prediction database, 1,3,7-trimethyl-3,7-Dihydro-1H-Purine-2,6-Dione has bioactivities as cyclic AMP phosphodiesterase inhibitor, xanthine-like respiratory analeptic, kidney function stimulant, and analeptic. Analeptics are central nervous system stimulants that are also convulsant drugs. Their actions are mainly on the brain stem and spinal cord, where they increase reflex excitability and stimulate the respiratory and vasomotor centers.

Although contains many compounds that have medicinal potential, the methanolic extract of *Uncaria callophylla* stems also contains compounds that are potentially toxic, especially for internal use. One of them is 1,2-Benzenedicarboxylic Acid, Diethyl Ester or diethyl phthalate (DEP). It has activity as a teratogenic agent, a neurotoxin and a plasticizer. DEP exposure may induce androgen-independent male reproductive toxicity (i.e., sperm effects) as well as developmental toxicity and hepatic effects, with some evidence of female reproductive toxicity. DEP is often used as a solvent and vehicle for fragrance and cosmetic ingredients, as well as alcohol denaturants (Darbre and Harvey, 2015; Weaver et al., 2020). The other compounds identified in the methanolic extract of *Uncaria callophylla* stems has various biological activities which might contribute to the medicinal properties of the natural product (Table 2).

4.2 LC-MS/MS Analysis

LC-MS chromatogram of the methanolic extract of *Uncaria callophylla* stems showed five peaks (Figure 3) which indicates the presence of five phytochemical compounds. The five compounds were characterized and identified as shown in Table 4, and their chemical structures and bioactivities were presented in Table 5.

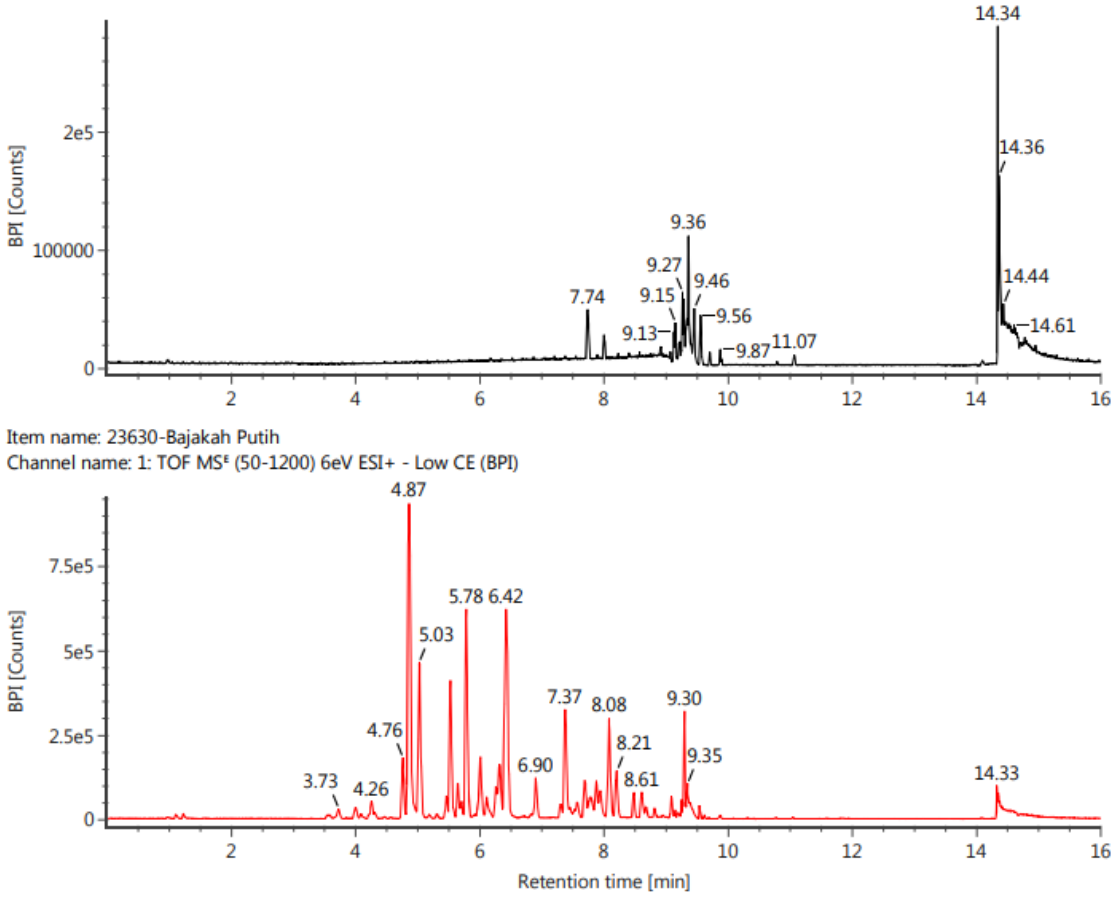


Figure 3. LC-MS chromatogram of *Uncaria callophylla* stem methanolic extract

Table 4. Five phytochemicals identified by LC-MS/MS analysis in the methanolic extract of *Uncaria callophylla* stems

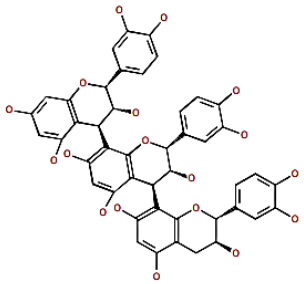
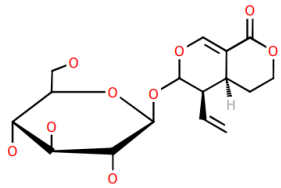
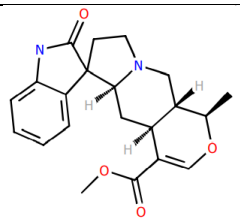
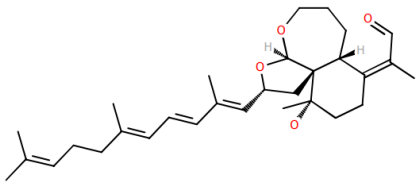
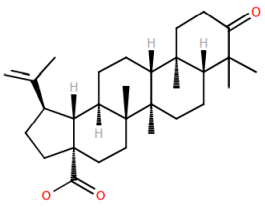
Retention Time (min)	Name of Compound	Observed m/z	Neutral Mass (Da)	Detector Counts	Response	Formula
4.04	Arecatannin A1	867.2131	866.20581	72473	70130	C45H38O18
4.26	Sweroside_2	359.1329	358.12638	46150	30834	C16H22O9
4.87	Uncarine A	369.1805	368.17361	1204424	947346	C21H24N2O4
6.43	Epianhydrobelachinal	469.3310	468.32396	1139428	809718	C30H44O4
9.30	Betulonic Acid	455.3514	454.34470	117744	87799	C30H46O3

Arecatannins are a class of condensed tannins in the sub-class procyanidins contained in the seeds of *Areca catechu*. Areca tannin has been proposed as possessing the blood pressure-controlling effect through its capability to constrain the pressor response to the hormone angiotensin I and II (Chung et al., 2007). Arecatannin A1 is a member of the class of compounds known as biflavonoids and polyflavonoids. Biflavonoids and polyflavonoids are organic compounds containing at least two flavan/flavone units. These units are usually linked through CC or C-O-C bonds. Some examples include C2-O-C3, C2-O-C4, C3'-C3''', and C6-C8''. Arecatannin A1 is practically insoluble in water and a very weakly acidic compound. Phenolic compounds, as well as flavonoids, are well-known as antioxidants and many other important bioactive agents that have long been interested due to their benefits for human health, curing and preventing many diseases (Tungmunnithum *et al.*, 2018). According to PASS Software's prediction database,

arecatannins have antihemorrhagic and antimutagenic activities, as well as membrane integrity agonists and mucomembranous protectors.

Sweroside_2 is a glycoside found in *Strychnos axillaris*, *Lonicera japonica*, and other organisms. Based on PASS Software's prediction database, sweroside_2 has antiprotozoal, antifungal, and hepatoprotective activity. According to Anyanwu *et al.* (2019), sweroside_2 and its derivatives have the potential as anti-diabetic agents due to their α-glucosidase inhibitory activity. Inhibition of α-glucosidase is a therapeutic approach for antidiabetic agents because the enzyme acts to decrease the production and absorption of glucose in the digestive tract. Chronic elevations in blood glucose are characteristic of type 2 diabetes mellitus, and α-glucosidase inhibitors decrease blood glucose levels. It is also known that sweroside significantly increases feces output. Thus, sweroside_2 can act as a potent laxative that facilitates the excretion of feces. Laxatives are known to loosen feces and increase bowel movements resulting in soft, mushy, and watery feces.

Table 5. Bioactivity of compounds identified in the methanolic extract of *Uncaria callophylla* by LC-MS (Based on PASS Software's prediction database)

Name of Compound	Formula	Chemical Structure	Bioactivity
Arecatannin A1	$C_{45}H_{38}O_{18}$		Membrane integrity agonist, TP53 expression enhancer, Mucomembranous protector, Antihemorrhagic, Antimutagenic
Sweroside_2	$C_{16}H_{22}O_9$		Antiprotozoal, CDP-glycerol glycerophosphotransferase inhibitor, gluconate 2-dehydrogenase (acceptor) inhibitor, hepatoprotectant, antifungal
Uncarine A	$C_{21}H_{24}N_2O_4$		Anesthetic general, vasodilator, peripheral, diuretic inhibitor, bilirubin oxidase inhibitor, antihypertensive, Alzheimer's disease treatment, antineoplastic, and anesthetic.
Epianhydrobelachinal	$C_{30}H_{44}O_4$		Antineoplastic, apoptosis agonist, chemopreventive, immunosuppressant, antiinflammatory
Betulonic Acid	$C_{30}H_{46}O_3$		Aspulvinonedimethylallyl transferase inhibitor, alkenylglycerophosphocholine hydrolase inhibitor, mucomembranous protector

Uncarine A or popular by its synonym isoformosanine, is an alkaloid that contains a spirooxindole ring. Many natural spirooxindole-containing compounds have been identified as bio-promising agents (Beecham et al., 1968). Based on PASS Software's prediction

database, Uncarine A has biological activity as an anesthetic, vasodilator, diuretic inhibitor, bilirubin oxidase inhibitor, antihypertensive, antineoplastic, and can be used in Alzheimer's disease treatment.

Betulonic acid is a triterpenoid that is known to have several biological activities such as diuretic, antimicrobial, antiviral, antidiabetic, antiparasitic, immunomodulatory, and anticancer activities (De Sá *et al*., 2009; Oliveira-Costa *et al*., 2022; Jiang *et al*., 2021). De Sá *et al*. (2009) showed that betulonic acid and its derivatives, betulinic acetate, betulinic acid methyl ester, and betulinic acetate methyl ester, exhibit anti-plasmodial activity against chloroquine-resistant *Plasmodium falciparum* parasite in vitro. Mice infected with *Plasmodium berghei* and treated with betulinic acid had a reduction in parasitemia. The results of this research indicated that betulinic acid and its derivatives are potential candidates for the development of new antimalarial drugs. Betulonic acid is also considered to have anticancer potential because it has been shown to be cytotoxic to various types of cancer cells and causes inhibition of tumor growth in a xenograft-mouse model (Jiang *et al*., 2021). The immune-modulatory activity of betulonic acid is also promising because it is able to modulate several types of immune system cells, such as macrophages and lymphocytes, and its anti-inflammatory activity has been demonstrated in various models of inflammation (Oliveira-Costa *et al*., 2022).

5. Conclusion

From the results of this work, it can be concluded that the methanolic extract of *Uncaria callophylla* Blume ex Korth contains various chemical compounds that have medicinal potential. Nine compounds were identified by GC-MS analysis with a quality match of 90% or above, and five compounds were identified by LC-MS/MS analysis.

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ORCID iD (if any)

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