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Metabolite Profiling of Ethanol Extract of Radix *Uvaria rufa* Blume by UPLC-QToF-MS/MS and Its Potential Aphrodisiac Activity

Maximus M. Taek [1], Burhan Ma'arif [2, *], Dian Nurmawati [3], Novia Maulina [2], Diva Dahayu [2], Muhammad Alauddin Isyrafi [2], Gita Firli Rahmawati [2], Faisal Akhmal Muslikh [4]

[1]*Department of Chemistry, Faculty of Science and Technology, Widya Mandira Catholic University, Kupang, Indonesia*
[2]*Department of Pharmacy, Faculty of Medicine and Health Science, Maulana Malik Ibrahim State Islamic University, Malang, East Java, Indonesia*
[3]*Department of Pharmacy, Faculty of Health Science, Kadiri University, Kediri, Indonesia*
[4]*Department of Pharmacy, Faculty of Pharmacy, Hang Tuah University, Surabaya, Indonesia*

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UPLC-QToF-MS/MS
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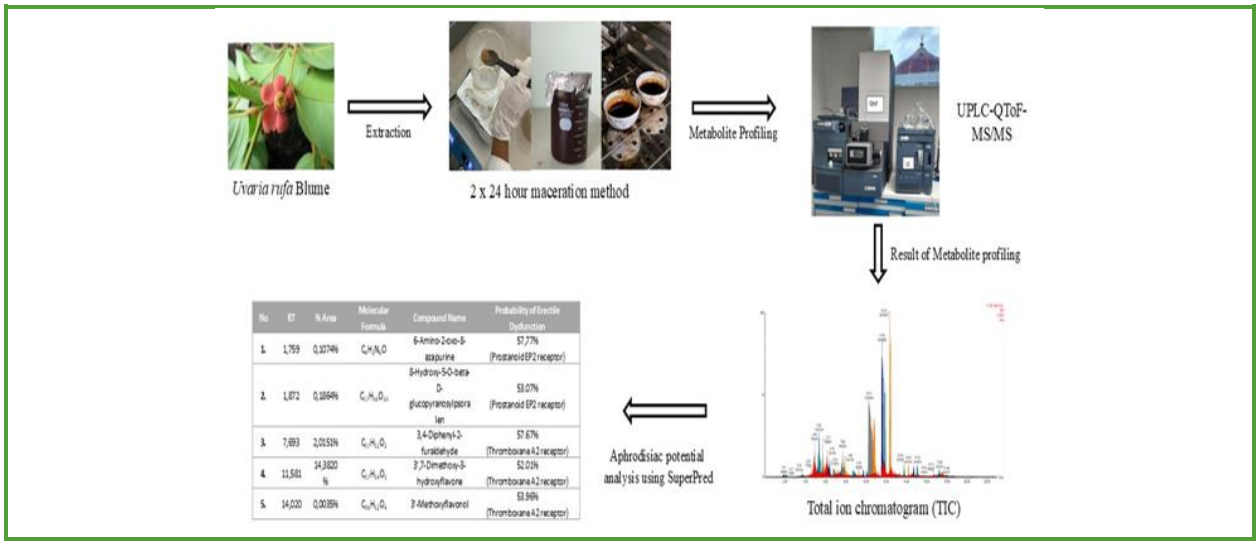
ABSTRACT

Uvaria rufa Blume, locally called Lelak, has been traditionally used by people in East Nusa Tenggara (NTT), Indonesia, as an herbal medicine to treat erectile dysfunction. Despite its relevance in ethnomedicine, comprehensive scientific data on the metabolite profiling of *U. rufa* radix is still limited. This study aims to determine the metabolite profile of 70% ethanol extract of U. rufa radix using the Ultra Performance Liquid Chromatography-Quadrupole-Time-of-Flight–Mass Spectrometry (UPLC-QToF-MS/MS) method and to determine compounds predicted as aphrodisiacs based on literature studies and preliminary result *in silico* studies using the SuperPred website. A total of 48 metabolites were identified, with 7-Methoxy-2H-1,3-benzodioxole-5-carboxylic acid (22.14%), Formononetin (15.57%), and 3′,7-Dimethoxy-3-hydroxyflavone (14.38%) being the most abundant compounds. Review of relevant literature shows that several identified compounds including Formononetin, 3',7-Dimethoxy-3-hydroxyflavone, and Boldine are known to have important biological activities, particularly antioxidant properties. Additionally, prediction results obtained through the SuperPred website indicate that five metabolites may exhibit potential interactions with molecular targets associated with erectile dysfunction. Collectively, these findings provide initial scientific support for the traditional use of U. rufa radix and provide a basis for further pharmacological studies on its prospective aphrodisiac activity.

Corresponding author, E-mail: burhan.maarif@farmasi.uin-malang.ac.id (B. Ma'arif).
Tel.: +081335555725.

Graphical Abstract



Introduction

Indonesia is a tropical country with diverse climates and topography, contributing to its rich and unique biodiversity. Its tropical forests cover approximately 143 million hectares, and around 80% of the world's 28,000 medicinal plant species originate from Indonesia. This biodiversity includes various endemic flora and fauna adapted to their respective habitats [1,2]. Approximately 100 to 150 plant families are distributed across Indonesia, most of which have the potential to be utilized in various industries as sources of fruits, spices, and medicinal raw materials [3]. Additionally, based on empirical data and traditional practices passed down through generations, Indonesians have long utilized plants and animals as alternative medicines for various diseases [4]. *Uvaria rufa* Blume, commonly known as Lelak, is a species of liana belonging to the Annonaceae family. These plants are distributed across tropical to subtropical regions at altitudes of up to 1500 meters above sea level. Their distribution includes Indonesia, China, Laos, Thailand, Malaysia, Papua New Guinea, the Philippines, Singapore, Vietnam, and India [5]. In Indonesia, one of the traditional uses of this plant can be found in the East Nusa Tenggara (NTT) region. In this area, *U. rufa* is locally known as Lekem and thrives in lowland forests as well as in the mountainous areas of Ruteng. The local community utilizes its bark and roots for traditional medicinal purposes [6]. Additionally, this plant is incorporated into herbal concoctions aimed at maintaining physical fitness, preventing illness, and restoring vitality and stamina after sickness including enhancing male stamina. For generations, people have prepared decoctions from the cortex, stems, and roots of *U. rufa* for consumption [7,8]. Previous research reported that *U. rufa* contains various secondary metabolite compounds, such as flavonoids, terpenoids, steroids, aromatic compounds, and alkaloids [9]. Metabolite profiling is an analytical technique within the metabolomics approach that is used to characterize the profiles of secondary metabolites in plants [10]. This method provides a comprehensive overview of metabolite composition and enables the simultaneous detection of multiple compounds, providing valuable insights for future research.

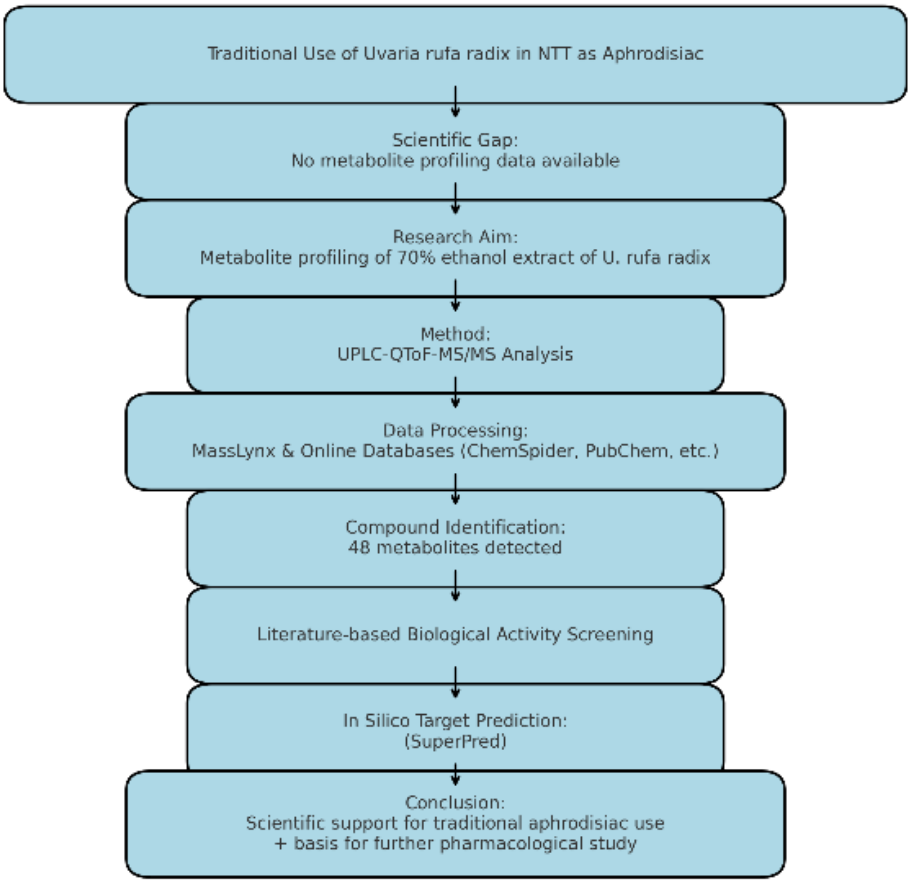


Figure 1. Overview of the research workflow

Such identification has become a widely adopted strategy for revealing secondary metabolite profiles without the need for extensive metabolite isolation processes [11]. The overall experimental workflow of this study is illustrated in Figure 1.

The identification of secondary metabolites in plants can be conducted using UPLC-QToF-MS/MS instruments. This instrument integrates two different methods UPLC and QToF-MS/MS which function to map metabolite profiles, mass spectra, and chromatograms. UPLC-QToF-MS/MS generates qualitative data in the form of metabolite profiles through chromatogram peaks and quantitative data by measuring the percentage concentration of each compound based on chromatogram peak areas [12].

In view of the lack of comprehensive scientific data on the secondary metabolites of *U. rufa* radix and its traditional use as an aphrodisiac in NTT this study aimed to profile the metabolites of a 70% ethanol extract of *U. rufa* radix using UPLC-QToF-MS/MS. Furthermore, potential bioactive compounds associated with aphrodisiac activity were identified through a literature review and predictive pharmacological analysis. The findings are expected to provide a scientific rationale for its traditional application and to support future pharmacological studies, particularly in the management of erectile dysfunction.

Experimental

Materials

The root of *U. rufa* were collected from Kupang, East Nusa Tenggara, Indonesia, and identified at the Phytochemistry Laboratory, Faculty of Pharmacy, Widya Mandira University, Kupang with number 234/WM.H9/KET/XI/2024. The chemicals used in this study included 70% ethanol, dichloromethane, methanol, acetonitrile, and formic acid (all from Merck, Germany). Sterile distilled water (aquadest) was prepared in the laboratory. Instruments used in this study consisted of an ultrasonic cleaner (Sonica, Italy), a rotary evaporator (Heidolph, Germany), and an ACQUITY UPLC® H-Class System coupled with a Xevo G2-S QToF Mass Spectrometer (Waters Corp., USA). The UPLC-QToF-MS/MS analysis was conducted at the Forensic Laboratory, Indonesian Police Criminal Investigation Agency, East Jakarta.

Methods

Plant material and authentication

The roots of *Uvaria rufa* were collected from Kupang, East Nusa Tenggara, Indonesia. The plant material was authenticated at the Phytochemistry Laboratory, Faculty of Pharmacy, Widya Mandira University, Kupang. A voucher specimen was deposited under the registration number 234/WM.H9/KET/XI/2024.

Extraction

The *U. rufa* radix dried plant material extract was obtained using the maceration method. The dried plant material was soaked in a solvent at a ratio of 1:15. During the first maceration, it was immersed in nine parts of the solvent, stirred for 30 min, and subsequently left to stand for 24 h at ambient temperature in a container covered with aluminum foil. The second maceration was performed by adding six parts of the solvent and allowing it to stand for another 24 h. The macerated filtrate was then filtered and concentrated using a rotary evaporator at 50 °C, with a pressure of 175 psi and a rotation speed of 70 rpm, until the solvent volume was significantly reduced. The remaining solvent was further evaporated in an oven at 50 °C to obtain a thick extract. Once the dry extract was obtained, the percentage yield was calculated as the final step. The yield was determined using Equation (1) [13]:

$$\text{Yield (\%)} = \frac{Weight\ of\ Extract}{Weight\ of\ dried\ plant\ material} \text{x } 100\% \quad (1)$$

Metabolite profiling

The metabolite profiling process was conducted using UPLC-QToF-MS/MS instrument. The steps taken were a 70% ethanol extract of *U. rufa* radix as much as 10 mg dissolved with 10 ml of solvent and inserted into the conditioned Solid Phase Extraction (SPE) column. An aliquot of 5 µL of the prepared sample was injected into the UPLC system using a micro syringe. Chromatographic separation was performed on an ACUITY UPLC® HSS C18 column (1.8 µm, 2.1 × 150 mm; Waters). The mobile phases consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The elution was conducted using a gradient system at a flow rate of 0.2 mL/min for 23 minutes. The separation was conducted under reversed-phase conditions, using a non-polar stationary phase (C18 column) and polar mobile phases. Mass spectrometric analysis was performed using a Xevo G2-S QToF mass spectrometer (Waters, USA) equipped with an electrospray ionization (ESI) source operating in positive ion mode. The instrument was

operated in Quadrupole followed by Time-of-Flight (ToF) mode, with a collision energy of 4 V and 25–70 V, a mass analysis range of m/z 30–1200, a source temperature of 100 °C, a desolation temperature of 350 °C, and a desolation gas flow of 793 L/h.

Data identification and result interpretation

The raw data from the analysis, in the form of chromatograms and spectra, were further analyzed using *MassLynx 4.1* software to obtain information on peak area, retention time, measured mass, calculated mass, and the molecular formula of each detected compound [4]. The confirmation of compound formulas was performed using online database websites such as ChemSpider, MassBank, and PubChem. The selection and confirmation of compounds in the database were based on the highest number of relevant publications and the obtained spectra. The probability of the predicted compounds with activity against erectile dysfunction was determined using the SuperPred website by entering the Simplified Molecular Input Line Entry System code (SMILES).

Results and Discussion

The extraction of *U. rufa* radix in this study was conducted using the maceration method. Maceration is an extraction technique that isolates filtrates from solid samples using specific solvents. This method operates based on the "like dissolves like" principle, where the solvent penetrates plant cell walls and saturates intracellular spaces. The concentration gradient between intracellular and extracellular environments facilitates the diffusion of active compounds into the solvent [14]. The final obtained extract weighed 22.4 g, while the initial weight of dried plant material was 309 g, resulting in a 7.2% yield of the 70% ethanol extract of *U. rufa* radix (Table 1).

Table 1. Yield of 70% ethanol extract of *U. rufa* radix

Dried Plant Material (g)	Extract (g)	Yield (%)
309	22.4	7.2

Metabolite profiling is a type of analysis that utilizes a metabolomics approach to describe or map the profile of secondary metabolite compounds in plants [10]. Based on phytochemical studies, ethanol extracts of *Uvaria* rufa roots contain flavonoids, alkaloids, highly oxidized cyclohexane, rutin, isoquercetin, kaempferol 3-O-β-D-galactopyranoside, astragalin, isoquercitrin-6-acetate, benzoylated derivatives, flavonols, kaempferol, quercetin, and lignan glycosides [15]. Prior to injection into the instrument, the sample was pretreated with methanol using the SPE method. The purpose of SPE in the 70% ethanol extract of *U. rufa* radix is to enhance sensitivity and selectivity in sample analysis, thereby enhancing the efficiency of analyte separation from the matrix [16]. Methanol with its high polarity index is able to attract polar secondary metabolites such as flavonoid glycosides, tannins, and some alkaloids. Additionally, the solvent is effective for phenolic compounds with low molecular weight and intermediate polarity [17]. The analysis process in this study employed a reversed-phase system, where the stationary phase is nonpolar, and the mobile phase is polar. In reversed-phase chromatography, more polar substances elute first and have shorter Retention time (Rt) compared to nonpolar substances [18]. The C18 stationary phase, consisting of an 18-carbon hydrocarbon chain, provides strong hydrophobic interactions, making it highly effective for retaining nonpolar or slightly polar analytes [19].

The processing and interpretation of chromatogram and spectral data were performed using *MassLynx 4.1* software, which is specifically designed to analyze the chromatographic patterns and spectral characteristics of each detected peak. This software facilitates the identification and prediction of the molecular formula of each compound present in the sample. The chromatogram interpretation results include chromatographic peaks, retention times, and m/z values [20]. The confirmation of compound formulas was conducted using online databases such as ChemSpider, MassBank, and PubChem. The selection and validation of compounds from these databases were based on the number of relevant publications and the spectral data obtained [18]. The identification of metabolite profile compounds was further supported by literature studies. Literature searches were conducted using secondary sources that discuss compounds found in *U. rufa* and their associated metabolite profiles in the 70% ethanol extract of *U. rufa* radix. The retrieved data were then classified according to the research formula, processed through reference citation, and compiled into structured abstractions to obtain comprehensive data. These data were subsequently interpreted to generate knowledge that supports the formulation of conclusions. The results of the Total Ion Chromatogram (TIC) and the predicted compounds analyzed from *U. rufa* radix extract with methanol preparation are presented in Figure 2 and Table 2.

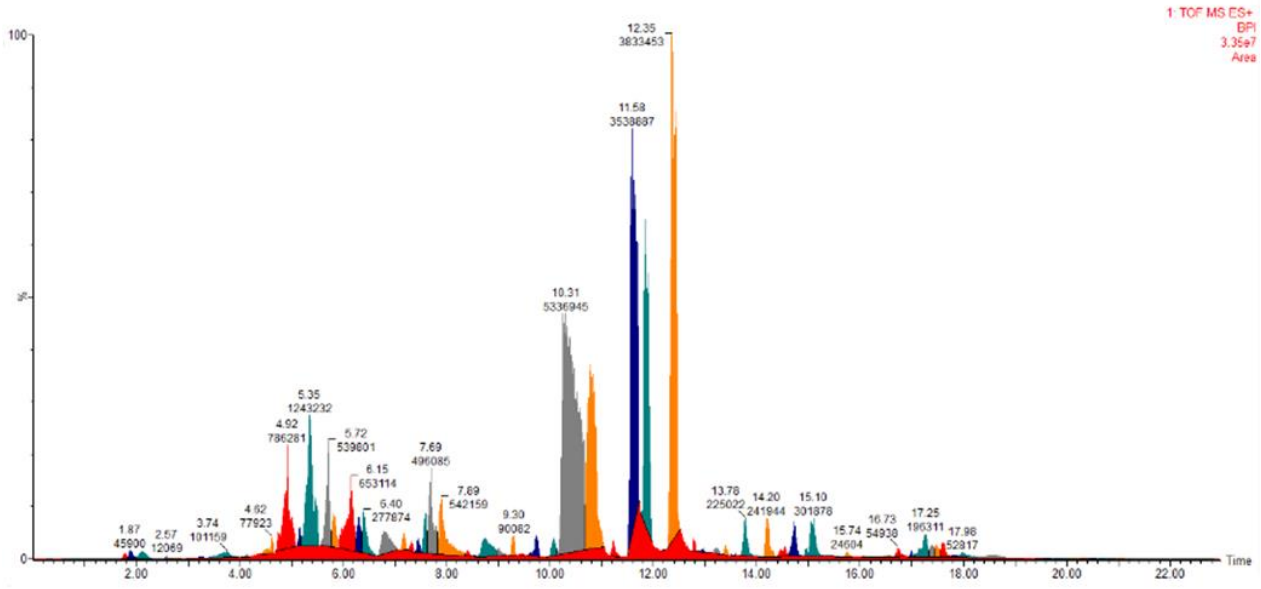
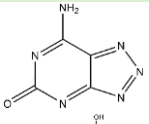
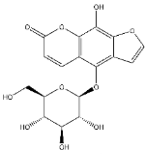
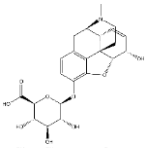
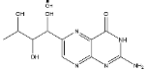
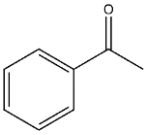
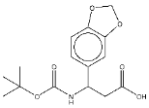
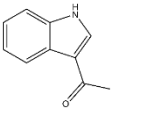
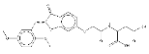
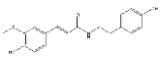
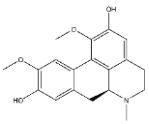
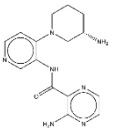
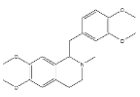
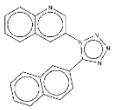
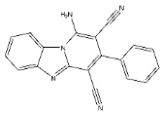
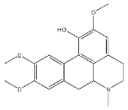
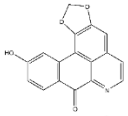
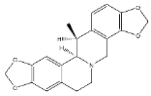
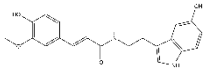
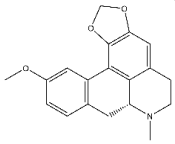
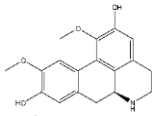
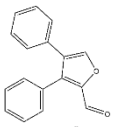
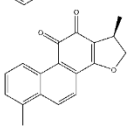
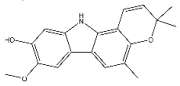
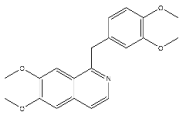
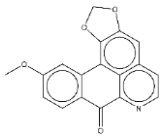
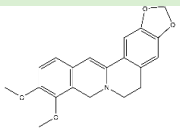
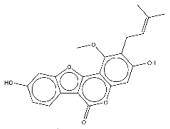
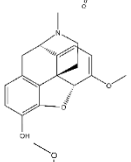
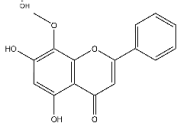
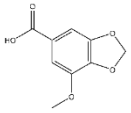
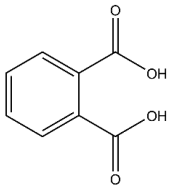
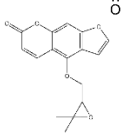
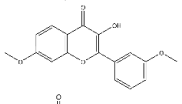
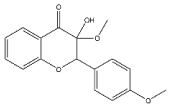
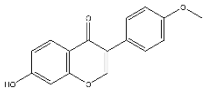
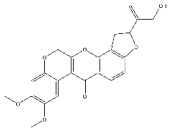
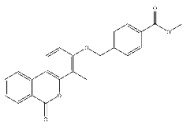


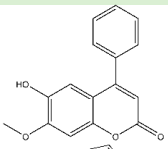
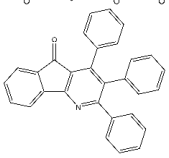
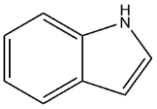
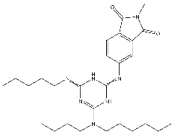
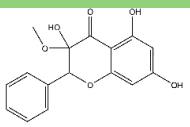
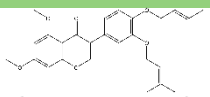
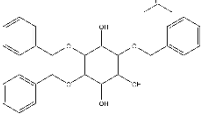
Figure 2. Total ion chromatogram (TIC) of 70% ethanol extract of *U. rufa* Radix

Table 2. Predicted compounds in 70% ethanol extract of *U. rufa* with methanol solvent

No.	RT	% Area	Measured Mass	Calculated Mass	Molecular Formula	Compound Name	Structure	Activity
1	1.759	0.1074 %	150.0286	150.0290	$C_4H_2N_6O$	6-Amino-2-oxo-8-azapurine		Antiplatelet [21]
2	1.872	0.1864 %	380,0807	380.0728	$C_{17}H_{16}O_{10}$	8-Hydroxy-5-O-beta-D-glucopyranosylpsoralen		-
3	2.110	0.3080 %	461.1693	461.1695	$C_{23}H_{27}NO_9$	Morphine-3-glucuronide		-
4	2.575	0.0490 %	267.0976	267.0981	$C_{10}H_{13}N_5O_4$	Rhamnopterin		-
5	2.792	0.0267 %	120.0579	120.0575	C_8H_8O	Acetophenone		Antioxidant and anti-inflammatory [22,23]
6	2.989	0.0073 %	309.1214	309.1213	$C_{15}H_{19}NO_6$	3-Benzo [1,3] dioxol-5-yl-3-tert-butoxycarbonylamino-propionic acid		-
7	3.257	0.0396 %	159.0687	159.0684	$C_{10}H_9NO$	3-Acetylindole		Antibacterial, anti-HIV, and anti-inflammatory [24,25]
8	3.742	0.4109 %	*Unknown*	*Unknown*	*Unknown*	Unknown	-	-
9	4.136	0.0400 %	193.1110	193.1110	$C_4H_{15}N_7S$	Unknown	-	-
10	4.621	0.3165 %	471.1168	471.1166	$C_{23}H_{21}NO_{10}$	N-({[2-(2,5-Dimethoxybenzylidene)-3-oxo-2,3-dihydro-1-benzofuran-6-yl] oxy} acetyl) aspartic acid		-
11	4.923	3.4558 %	313.1318	313.1314	$C_{18}H_{19}NO_4$	N-feruloyltyramine		Antioxidant, (Reduces ROS levels), and antidiabetic [26-28]
12	5.345	5.0500 %	327.1477	327.1478	$C_{12}H_{21}N_7O_2S$	Boldine		Antioxidant, neuroprotective, analgesic, and anticancer [29]
13	5.718	2.1927 %	313.1691	313.1688	$C_{15}H_{19}N_7O$	3-amino-N-[4-[(3S)-3-amino-1-piperidyl]-3-pyridyl] pyrazine-2-carboxamide		-
14	5.801	0.6892 %	313.1686	357.1955	$C_{21}H_{27}NO_4$	1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-		-

No.	RT	% Area	Measured Mass	Calculated Mass	Molecular Formula	Compound Name	Structure	Activity
						tetrahydroisoquinoline		
15	6.153	2.6529 %	323.1168	323.1171	$C_{20}H_{13}N_5$	3-[5-(2-Naphthyl)-1H-tetrazol-1-yl] quinoline		-
16	6.287	0.8211 %	309.1008	309.1013	$C_{19}H_{11}N_5$	1-Amino-3-phenylpyrido[1,2-a] benzimidazole-2,4-dicarbonitrile		-
17	6.400	1.1287 %	313.1686	341.1682	$C_{20}H_{23}NO_4$	Thaliporphine		Antioxidant and anti-inflammatory [30]
18	6.793	1.1985 %	291.0534	291.0532	$C_{17}H_9NO_4$	10-Hydroxyliriodenine		-
19	7.166	0.3661 %	337.1314	337.1314	$C_{20}H_{19}NO_4$	Tetrahydrocorysamine		Anti-cancer [31]
20	7.320	0.1571 %	352.1425	352.1428	$C_{20}H_{20}N_2O_4$	Feruloylserotonin		-
21	7.454	0.2332 %	309.1365	309.1365	$C_{19}H_{19}NO_3$	Laureline		-
22	7.588	0.8181 %	313.1319	313.1314	$C_{18}H_{19}NO_4$	Laurolistine		-
23	7.693	2.0151 %	248.0843	248.0838	$C_{17}H_{12}O_2$	3,4-Diphenyl-2-furaldehyde		-
24	7.890	2.2022 %	278.0954	278.0950	$C_{18}H_{14}O_3$	Dihydrotanshinone I		-
25	8.396	0.1137 %	309.1359	309.1359	$C_{19}H_{19}NO_3$	Koenigine		-
26	8.551	0.0202 %	339.1471	339.1471	$C_{20}H_{21}NO_4$	Papaverine		Pulmonary vasoconstriction, anti-viral, anti-inflammatory, and antiviral [32,33]
27	8.747	1.0114 %	305.0692	305.0688	$C_{18}H_{11}NO_4$	Oxolaureline		-

No.	RT	% Area	Measured Mass	Calculated Mass	Molecular Formula	Compound Name	Structure	Activity
28	9.015	0.1943 %	337.1314	337.1314	$C_{20}H_{19}NO_4$	Dihydroberberine		Antidiabetic (increase in insulin) [34]
29	9.296	0.3659 %	366.1103	366.1104	$C_{21}H_{18}O_6$	Glycyrol		Immunomodulator of inflammatory arthritis [35]
30	9.450	0.0358 %	297.1369	297.1365	$C_{18}H_{19}NO_3$	Oripavine		Analgesics [36]
31	10.070	0.4188 %	284.0689	284.0685	$C_{16}H_{12}O_5$	Wogonin		Antioxidant, anti-inflammatory, and anticancer [37,38]
32	10.308	22.1439%	196.0379	196.0381	$C_9H_8O_5$	7-Methoxy-2H-1,3-benzodioxole-5-carboxylic acid		-
33	10.773	9.1982 %	166.0270	166.0266	$C_8H_6O_4$	Phthalic acid		Anticancer, immunomodulatory, antimicrobial, and insecticidal activity [39,40]
34	11.229	0.3207 %	304.0953	304.0952	$C_{16}H_{16}O_6$	Oxypeucedanin		Antiproliferative [41]
35	11.581	14.3820%	298.0846	298.0841	$C_{17}H_{14}O_5$	3',7-Dimethoxy-3-hydroxyflavone		Antioxidant and anti-invasive [42-44]
36	11.848	8.1381 %	298.0847	298.0846	$C_{17}H_{14}O_5$	3′,4′-Dimethoxyflavonol		Neuroprotective [45]
37	12.355	15.5714%	268.0739	268.0736	$C_{16}H_{12}O_4$	Formononetin		Antioxidant, anti-inflammatory, anti-tumour, and agent that enhances adipocyte thermogenesis [46,47]
38	12.790	0.3223 %	408.1208	407.1214	$C_{23}H_{20}O_7$	6a,12a-Didehydroamorphigenin		-
39	12.966	0.0665 %	374.1153	374.1157	$C_{23}H_{18}O_5$	methyl 4-{[(4-methyl-6-oxo-6H-benzo[c]chromen-3-yl) oxy] methyl} benzoate		-

No.	RT	% Area	Measured Mass	Calculated Mass	Molecular Formula	Compound Name	Structure	Activity
40	13.058	0.0062 %	268.0740	268.0736	$C_{16}H_{12}O_4$	Dalbergin		Antifungal, antidiabetic, and antiosteoporotic [48]
41	13.233	0.1362 %	409.1471	409.1467	$C_{30}H_{19}NO$	2,3,4-Triphenyl-5H-indeno[1,2-b] pyridin-5-one		-
42	13.514	0.0221 %	117.0582	117.0579	C_8H_7N	Indole		-
43	13.585	0.0243 %	495.3325	495.3322	$C_{27}H_{41}N_7O_2$	5-({4-[Butyl(hexyl)amino]-6-(pentylamino)-1,3,5-triazin-2-yl} amino)-2-methyl-1H-isoindole-1,3(2H)-dione		-
44	13.782	0.9140 %	993.6625	993.6627	$C_{53}H_{87}N_9O_9$	Unknown		-
45	14.020	0.0035 %	268.0741	268.0736	$C_{16}H_{12}O_4$	3'-Methoxyflavonol		-
46	14.196	0.9828 %	108.3661	108.3661	$C_{58}H_{96}N_{10}O_{10}$	Unknown		-
47	14.548	0.3120 %	450.2039	450.2036	$C_{19}H_{34}N_2O_8S$	5,7-Dimethoxy-3',4'-diprenyloxyisoflavone		-
48	14.723	0.8230 %	450.2039	450.2043	$C_{27}H_{30}O_6$	3,5,6-Tris(benzyloxy)-1,2,4-cyclohexanetriol		-

The compound identification results revealed 44 known compounds and 4 unknown compounds based on a database literature search. The unknown compounds were detected at retention times (RT) of 3.742, 4.136, 13.782, and 14.196 minutes. These unknown compounds may represent impurity compounds that were still detectable by the method or potentially new compounds that have not yet been registered in online databases such as ChemSpider, particularly if their concentrations are high. The results of the analysis obtained several major compounds or dominant compounds identified from data interpretation in Table 2. Major compounds are compounds that have higher levels, as indicated by the percentage of area, compared to other compounds contained in the extract [10]. The major compound in the 70% ethanol extract of *U. rufa* radix with preparation using methanol is the compound 7-Methoxy-2H-1,3-benzodioxole-5-carboxylic acid with a percentage area of 22.1439%, while several compounds with the highest percentage area are Formononetin having a percentage area of 15.5714% and 3',7-Dimethoxy-3-hydroxyflavone with a percentage area of 14.3820%. There has been no research on the compound 7-Methoxy-2H-1,3-benzodioxole-5-carboxylic acid. Meanwhile, formononetin is an isoflavone belonging to the

phytoestrogen group [49,50]. As a result of the literature search, there are several compounds with biological activity. The major compound, 7-Methoxy-2H-1,3-benzodioxole-5-carboxylic acid, is a derivative of piperonylic acid, which is known to have various biological activities. Piperonylic acid has been reported to have anticancer, antioxidant, and antibacterial activities [51-54]. Formononetin compounds have activities as antioxidants, anti-inflammatory [46], antitumor, and agents that increase adipocyte thermogenesis [47]. A study explains that formononetin has an antioxidant mechanism by reducing Reactive Oxygen Species (ROS) produced due to exposure to H_2O_2, as well as stabilizing the redox conditions of cells [55]. The compound 3',7-Dimethoxy-3-hydroxyflavone has biological activity as an antioxidant that protects cells from free radical damage [41,42], as well as an anti-invasive that plays a role in inhibiting the spread of invasive cells, such as in cancer cases [44]. Additionally, the compounds identified in the 70% ethanol extract of *U. rufa* radix also have several activities, including: Phthalic acid compound with an area percentage of 9.1982% has anticancer, immunomodulatory [39], and antimicrobial activity [40], Boldine compound with an area percentage of 5.0500% has antioxidant and neuroprotective activity [29], N-feruloyltyramine compound with an area percentage of 3.4558% has antioxidant and antidiabetic activity [28], Wogonin compound with an area percentage of 0.4188% has anti-inflammatory and anticancer activity [37,38], and Dihydroberberine compound with an area percentage of 0.1943% has antidiabetic activity by increasing insulin [34]. The presence of antioxidants is essential in counteracting oxidative stress and maintaining normal penile function. Compounds such as superoxide dismutase (SOD), vitamin C, vitamin E, melatonin, alpha-lipoic acid, peroxynitrite decomposition catalysts, and gamma-linolenic acid have been recognized for their ability to minimize diabetic vasculopathy and autonomic neuropathy affecting the penile tissue, which in turn contributes to the improvement of erectile performance at various physiological level [42,56-61]. Evidence from animal studies involving arteriogenic erectile dysfunction models has indicated that pomegranate juice may reduce oxidative stress and enhance erectile function [56]. These favorable effects are largely associated with the high content of polyphenolic antioxidants present in pomegranate, which are believed to support vascular integrity and erectile capacity [62]. Antioxidant activity plays a crucial role in erectile dysfunction treatment by reducing oxidative stress caused by Reactive Oxygen Species (ROS) and enhancing blood flow to erectile tissue, thereby offering therapeutic potential [63]. Although the antioxidant activity in several secondary metabolites of 70% ethanol extract of *Uvaria rufa* radix has not been proven to directly improve erectile function, the presence of compounds related to antioxidants indicates the potential that can be developed and analyzed more deeply regarding the therapeutic effects of erectile dysfunction. There are five compounds identified in the 70% ethanol extract of *U. rufa* radix, namely 6-Amino-2-oxo-8-azapurine, 8-Hydroxy-5-O-beta-D-glucopyranosylpsoralen, 3,4-Diphenyl-2-furaldehyde, 3',7-Dimethoxy-3-hydroxyflavone, and 3'-Methoxyflavonol. Several of these compounds were simulated using the *SuperPred* website regarding the percentage of probability of indications of erectile dysfunction. The website uses the principle of similarity of physicochemical properties and biological activity.

Table 3. Predicted probability of compounds against erectile dysfunction

No.	RT	% Area	Molecular Formula	Compound Name	Probability of Erectile Dysfunction
1.	1.759	0.1074%	$C_4H_2N_6O$	6-Amino-2-oxo-8-azapurine	57,77% (Prostanoid EP2 receptor)
2.	1.872	0.1864%	$C_{17}H_{16}O_{10}$	8-Hydroxy-5-O-beta-D-glucopyranosylpsoralen	53.07% (Prostanoid EP2 receptor)
3.	7.693	2.0151%	$C_{17}H_{12}O_2$	3,4-Diphenyl-2-furaldehyde	57.67% (Thromboxane A2 receptor)
4.	11.581	14.3820%	$C_{17}H_{14}O_5$	3',7-Dimethoxy-3-hydroxyflavone	52.01% (Thromboxane A2 receptor)
5.	14.020	0.0035%	$C_{16}H_{12}O_4$	3'-Methoxyflavonol	53.96% (Thromboxane A2 receptor)

Its use can be done by uploading a molecular structure or entering the SMILE code, then the system will simulate the reaction of compound and protein interactions with the data in the database [64]. The SuperPred analysis results Table 3 identified five compounds with a probability percentage indicating their potential relevance to erectile dysfunction. Several receptors were found to predict the probability of compounds against erectile dysfunction, namely Prostanoid EP2 receptor and Thromboxane A2 receptor [65,66]. The EP2 receptor is a member of the G protein-coupled receptor (GPCR) family involved in the prostanoid signaling pathway. This receptor regulates blood flow to erectile tissue by activating Gs protein-mediated signaling, which increases the production of cyclic adenosine monophosphate (cAMP). Elevated cAMP levels activate protein kinase A (PKA), leading to a reduction in intracellular calcium levels and promoting smooth muscle relaxation in the corpus cavernosum, thereby facilitating penile erection [67,68]. Thromboxane A2 receptor (TP) is a receptor that belongs to the G protein-bound receptor (GPCR) group. This receptor plays a role in various physiological and pathophysiological processes, such as platelet aggregation and smooth muscle contraction. Thromboxane A2 (TXA2) receptor binding to TP receptors can trigger smooth muscle contraction in penile arteries through the activation of Rho kinase (ROCK). If TXA2 and the ROCK pathway are inhibited, smooth muscle contraction will be reduced. This causes the smooth muscle in the penile arteries to relax, resulting in increased blood flow to the erectile tissue [69,70]. The percentage probability of the compound 3',7-Dimethoxy-3-hydroxyflavone is 52.01% with a possible target receptor TXA2. The compound has a percentage area of 14.3820%, and could be a potential compound responsible for the therapeutic effect of *U. rufa* radix. The compound 3',7-Dimethoxy-3-hydroxyflavone is included in the secondary metabolites of the flavonoid group. Flavonoid compounds can be antagonists for TXA2 by reducing the mechanism of platelet aggregation induced by TXA2 agonists [71]. The percentage of probability generated by the SuperPred website is still a prediction based on simulations from the website's machine learning model based on available databases such as CheEMBL. The percentage generated from the 5 compounds is still relatively low, and further confirmation must be done [64,72]. This confirmation can be done through *in silico* analysis using a molecular docking approach to obtain more accurate parameters as a reference for the next test. High probability results, such

as (>80%) for the interaction of a compound and a biological target, indicate high confidence in predictions based on simulated data patterns. High probabilities support the belief that the compound interacts with the target, while low probabilities indicate the need for further validation [72].

Conclusion

This study successfully characterized the metabolite profile of a 70% ethanol extract of *Uvaria rufa* radix using UPLC-QToF-MS/MS, resulting in the identification of 48 secondary metabolites. The major compounds identified included 7-methoxy-2H-1,3-benzodioxole-5-carboxylic acid, Formononetin, and 3′,7-dimethoxy-3-hydroxyflavone. Literature based evidence suggests that several of these compounds possess biological properties, particularly antioxidant activity. Furthermore, target prediction through the *SuperPred* platform indicated potential interactions between five metabolites and targets related to erectile dysfunction. These findings provide preliminary scientific support for the traditional use of *U. rufa* radix and serve as a foundation for future pharmacological and mechanistic studies exploring its potential aphrodisiac effects.

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Disclosure Statement

The authors declare no conflict of interest.

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Authors' Contributions

Conceptualization, Burhan Ma'arif and Maximus M. Taek; Methodology, Burhan Ma'arif, Dian Nurmawati, and Novia Maulina; Investigation, Diva Dahayu, Muhammad A. Isyrafi, and Gita F. Rahmawati; Data curation, Diva Dahayu, Muhammad A. Isyrafi, and Gita F. Rahmawati; Formal analysis, Maximus M. Taek and Burhan Ma'arif; Writing—original draft preparation, Burhan Ma'arif and Maximus M. Taek; Writing—review and editing, Faisal A. Muslikh and Burhan Ma'arif; Supervision and Project administration, Burhan Ma'arif; All authors have read and agreed to the published version of the manuscript.

Orcid

Maximus M. Taek
https://orcid.org/0000-0002-4597-2167
Burhan Ma'arif
https://orcid.org/0000-0001-9182-343X
Dian Nurmawati
https://orcid.org/0000-0003-0750-0545
Novia Maulina
https://orcid.org/0000-0002-7948-0101
Diva Dahayu
https://orcid.org/0009-0001-4544-6693
Muhammad A. Isyrafi
https://orcid.org/0009-0008-9634-0849
Gita F. Rahmawati
https://orcid.org/0009-0006-3572-5249
Faisal A. Muslikh
https://orcid.org/0000-0002-9611-7937

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