

Supplementary Material

RP-HPLC-DAD analysis of mitragynine content in *Mitragyna speciosa* Korth. (Ketum) leaf extracts prepared using Ultrasound Assisted Extraction Technique and their cytotoxicity

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Table S1. Chemicals and Reagents.

Chemicals and Reagents	Vendors
Methanol (HPLC grade), Silica gel 60, and Deuterated acetone (acetone- <i>d</i> ₆) used for NMR analysis,	Merck (Darmstadt, Germany)
Methanol (Analytical grade) and formic acid (Analytical grade)	Fisher Scientific (Loughborough, UK)
Ethyl acetate (Analytical grade) and acetonitrile (HPLC grade)	QReC (Asia) Chemical Co. Ltd (Selangor, Malaysia).
Ethanol (Analytical grade) and hexane (Analytical grade)	R&M Chemicals (Essex, UK)
Sephadex LH-20	Sigma-Aldrich Co. (St. Louis, Missouri, USA)
Mitragynine standard (purity ≥ 99 %)	ChromaDex Inc. (California, USA).
Elga Classic UVF ultrapure water purifier system	Elgastat, Bucks, UK
Sodium carbonate (Na ₂ CO ₃), sodium nitrite (NaNO ₂), aluminium chloride hexahydrate (AlCl ₃ .6H ₂ O), and sodium hydroxide (NaOH)	Sigma-Aldrich Co. (St. Louis, Missouri, USA).
Folin-Ciocalteu (FC) reagent	R&M Chemicals (Essex, UK).
Dimethyl sulfoxide (DMSO)	Bio Basic (Markham, Canada);
Eagle's minimum essential medium (EMEM)	American type culture collection (ATCC) (Rockville, MD, USA)
3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide (MTT), fetal bovine serum (FBS), and phosphate buffered saline (PBS)	Gibco/BRL Life Technologies Inc. (Eggenstein, Germany).
Doxorubicin hydrochloride	EMD Chemicals (Darmstadt, Canada).

Table S2. MS/MS data of mitragynine congeners identified tentatively in UAE MeOH ketum leaves extract using UHPLC-ESI-QTOF-MS/MS.

Identification	Calculated <i>m/z</i> [M+H]⁺	Precursor ion experimental <i>m/z</i> [M+H]⁺	Elemental composition	Major ions in MS/MS spectra (key fragment ions)	UAE MeOH RT, min	Ref
7- hydroxymitragynine	415.2227	415.2083	C ₂₃ H ₃₀ N ₂ O ₅	190.0739, 110.0865	8.47	[15, 16]
Corynantheidine	369.2170	369.2031	C ₂₂ H ₂₈ N ₂ O ₃	238.1297, 226.1310, 110.0844	8.89	[15, 16]
Mitragynine	399.2278	399.2136	C ₂₃ H ₃₀ N ₂ O ₄	238.1295, 226.1297, 174.0790, 110.0838	9.06	[15, 16]
Paynantheine	397.2122	397.1977	C ₂₃ H ₂₈ N ₂ O ₄	236.1135, 224.1141, 174.0788, 159.0555	9.43	[15, 16]
Speciogynine	399.2278	399.2128	C ₂₃ H ₃₀ N ₂ O ₄	238.1290, 226.1296, 174.0784, 110.0853	9.65	[15, 16]
Speciociliatine	399.2278	399.2138	C ₂₃ H ₃₀ N ₂ O ₄	238.1292, 226.1299, 174.0787, 110.0855	10.25	[15, 16]

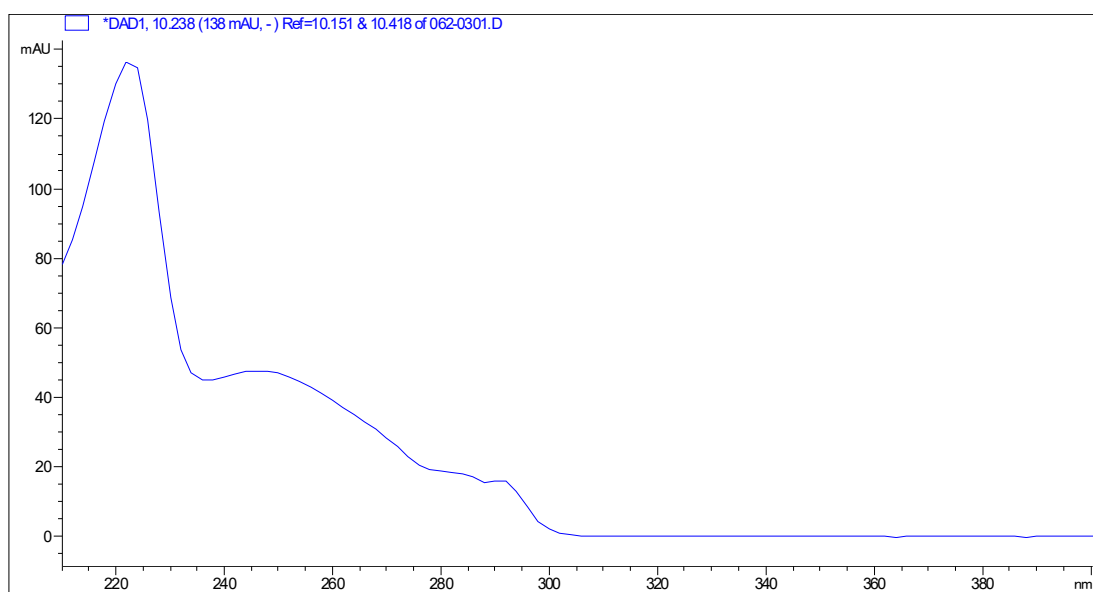


Figure S1. Peak purity UV spectrum (HPLC-DAD) of isolated mitragynine.