Ethyl tetratriacontanoate and non-polar compounds from Mitragyna inermisstem bark

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Abstract

Mitragyna inermis is widely reported for its biological activities and phytochemical components. In this report, we investigated, the non-polar compounds from stem bark by thin layer chromatography and GC-MS analysis. The thin layer chromatography on DCM fraction yielded ethyl tetratriacontanoate (**1'**). That compound is on the first isolation from *M. inermis*. Besides, gas chromatography allowed identification of thirteen major compounds as well as alkanes, phytol, esters, ester-alcohol, carboxylic acids, ester-carboxylic acids and cyanate. All compounds are on the first report on *M. inermis* and most are fatty acids and essential oils. These compounds might contribute to stem potent antioxidant, antidiabetic and antimicrobial effects. Further investigation for drug research with these non-polar compounds might allow to know their specific activities.

Introduction

Mitragyna inermis is the tropical plant from *Rubiaceae* family (Arbonnier 2002). Traditionally, this plant is widely used to treat microbial infection, diabetes, hypertension, epilepsy, malaria and so on (Arbonnier 2002; Zerbo et al. 2007; Kinda et al. 2017). Due to its potent biochemical and the abundance of some kind of compounds like terpenoid, alkaloids, phenolic compounds, many study still believe on that plant for natural drugs research (Shellard and Sarpong 1969; Shellard and Sarpong 1970; Cheng et al. 2002; Fiot et al. 2005; Asase et al. 2008; Karou et al. 2011; Adoum et al. 2012; Donfack et al. 2012; Toklo et al. 2020; Ouédraogo et al. 2022). Herein, several reported on its effectiveness *in vitro* and *in vivo* against hyperglycemia, hypertension, antifungal, antibacterial, antimalarial, its ability to abolish seizures and brain impairment with different extracts and compounds from stem bark (Traore et al. 2000; Valentin et al. 2000; Ouédraogo et al. 2004; Asase et al. 2008; Adoum et al. 2012; Serge et al 2012; Atinga et al. 2015; Pahaye et al. 2017; Alowanou et al. 2020; Ouédraogo et al. 2020; Ouédraogo et al. 2022). Therefore, these studies were focused on polar and big molecules from *M. inermis*. Until now, only root bark non-polar compounds were reported and speculated to contribute on antimicrobial activities (Mukhtar et al. 2016). Several studies reported that non-polar compounds might reveal beneficial effects in human body (Politeo et al. 2007; Hsieh et al. 2012; Fomani et al. 2015; Sharma et al. 2022). In our knowledge, there is not any study on *M. inermis* stem bark non-polar compounds like essential oils that might contribute to the most bioactivity reported and could justify some traditional using.

Know stem bark active extract non-polar compounds by thin layer chromatography and gas chromatography might able to reach the aim of this report.

Experimental

Plant material

Raw materials were *Mitragyna inermis* stem bark. Voucher specimen (UNB 939) is deposited at the Herbarium of Université Nazi Boni.

General methods

TLCs were carried out on precoated Kieselgel 60 F254 (Merck) plates developed with DCM-Acetone and plates visualized under UV 254 and 365 nm. The isolated compound was subject to ^{1}H NMR, 2D NMR HMBC, HSQC, COSY and NOESY, ^{13}C NMR and ESI-MS (*+ve*) analyses. The 1D and 2D NMR spectra were run using Bruker Avance Neo NMR spectrometers operating at 500 and 800 MHz for ^{1}H. Chemical shifts (δ) are given in ppm relative to the residual $CDCl_3$ signal, and the coupling constants (J) are in Hz. The GC/MS was performed with Agilent technology 7890A and 7000 Triple Quad system, respectively.

Raw material collection

Mitragyna inermis stem bark raw materials were collected from three areas over two months according to the previous studies that revealed their potent antioxidant (Ouédraogo et al. 2022).

Extract preparation

This study aimed to report phytochemical components of active extract. *M. inermis* stem bark ethanolic extract was previously reported to have potent antiseizure properties. Therefore, stem bark powder 120 g was macerated twice for 24 hours with 1200 ml of ethanol (99%) (the second with the recovered solvent). Then, the filtrates were evaporated in a rotary evaporator 45°C to dry and stored at 4°C (Timothy et al. 2014). Ethanolic extract were treated with ammonia then sulfuric acid 10% and submit to liquid-liquid fractionating with DCM to yield DCM fraction.

Isolation and structure elucidation

A preparative thin layer chromatography (TLC) of the last DCM fraction on precoated silica F254 plate with the DCM:Acetone (9:1) system afforded an only UV 365 active compound.

Ethyl tetratriacontanoate (1'): White amorphous powder. ESI-MS gave $[M + Na]^+$ m/z = 559.391 and $[M-H]^+$ m/z = 537.525. Molecular weight appeared to be 536. HMBC data showed correlation δH = 4.04 (t, J = 6.6 Hz), δH = 2.29 (t, J = 7.4 Hz) and δH = 1.63 (q, J = 7.5 Hz) with the carbonyl carbon at δC = 173.5. That compound is an ester (Fomani et al. 2015). No any double bond chemical shift was observed. Protons at chemical shift 0.86 (t, J = 7.2 Hz) and 0.84 (t, J = 6.6 Hz) were both terminal methyls (Table 1). ESI-MS revealed the plausible fragment $[M-15]^+$ m/z = 521.381, $[M-29]^+$ m/z = 507.387, $[M-45]^+$ m/z = 491.502, $[M-87]^+$ m/z = 449.335, $[M + H-101]^+$ m/z = 436.002, $[M-115]^+$ m/z = 421.275, $[M-129]^+$ m/z = 407.175, $[M-143]^+$ m/z = 393.245, $[M-171]^+$ m/z = 365.124, $[M-185]^+$ m/z = 351.169, $[M-213]^+$ m/z = 323.126, $[M-227]^+$ m/z = 309.832, $[M-241]^+$ m/z = 295.251, $[M-269]^+$ m/z = 267.126, $[M-297]^+$ m/z = 239.001. Therefore, calculated formula appeared $[M(C_{36}H_{72}O_2) + Na]^+$ = 559.391.

Table 1

^{1}H NMR (500 MHz, $CDCl_3$) and 2D NMR (800 MHz, $CDCl_3$) data of compound **1'**

δH	HSQC	COSY	HMBC	TOCSY	NOESY	C
4.04 (t, *J* = 6.6 Hz)	64.40	1.63	25.6, 28.0, 173.5	1.36, 1.63, 2.29	1.36, 1.63	64.40
2.29 (t, *J* = 7.4 Hz)	34.25	1.63	25.6, 173.5	1.36, 1.63, 4.04	1.36, 1.63	34.25
1.63 (q, *J* = 7.5 Hz)	28.13, 24.24	4.04, 1.36, 2.29	25.6, 64.2, 28.0, 34.2, 173.5	1.36, 2.29, 4.04, 1.27	1.36, 2.29, 4.04	28.13, 24.24
1.36 (t, *J* = 7.6Hz)	25.48	1.63	25.6, 28.0	1.63, 2.29, 4.04	1.63, 2.29, 4.04	25.48
1.27 (d, *J* = 6.6 Hz)	32.0	0.86	32.5	1.63		32.0
1.23 (s)	29.54, 23.08	0.84	29.78, 32.5, 28.0	0.86		29.54, 23.08, 28.0
0.86 (t, *J* = 7.2 Hz)	14.42	1.23	23.0, 32.5	1.23	1.23	14.42
0.84 (t, *J* = 6.6 Hz)	14.42	1.27	28.0		1.23	14.42

GC-MS analysis

Besides, the DCM fraction show before was subject to GC-MS analyses. A GC-MS analysis was performed using a 7890A gas chromatograph (Agilent technologies, Santa Clara, CA, USA), equipped with an Agilent Technology GC autosampler 120 (PAL LHX-AG12) and coupled with an Agilent 7000 Triple Quad system (Agilent technologies, USA). An HP-5MS 30 m–250 mm (i.d.) fused-silica capillary column (Agilent J &W Scientific, Folsom, CA, USA), chemically bonded with a 5% diphenyl and 95% dimethylpolysiloxane cross-linked stationary phase (0.25 mm film thickness), was used. Helium (99.99%) was used as carrier gas at a constant flow rate of 1.129 mL/min and injection volume 2 µL. The MS interface and ion source were set to 280°C. Separation was achieved using a temperature program of 80°C for 2 min, which was then ramped at 5°C min^{-1} to 300°C and held for 1 min, at a constant flow of 1 $mL{\cdot}min^{-1}$. NIST library match was employed for the identification of the compounds. Identification was carried out by comparison of retention times and EI-MS fragments to library database (supplementary data).

Table 2
Compounds characterized by GC-MS, retention times and their abundances

Peak Number	Compounds	RT	Molecular Weight (g/mol)	Area %	Molecular Formula
1	Tetradecanoic acid	15.15	228	1.9	C14H28O2
2	Phthalic acid, butyl undecyl ester	16.37	376	1.15	C23H36O4
3	Hexadecanoic acid, methyl ester	17.32	270	1.26	C17H34O2
4	n-Hexadecanoic acid	18.02	256	20.46	C16H32O2
5	Hexadecanol	20.65	242	2.98	C16H34O
6	Heptadecanoic acid, 16-methyl-, methyl ester	21.47	298	0.96	C19H38O2
7	Hexanedioic acid, mono (2-ethylhexyl) ester	25.19	258	3.07	C14H26O4
8	1,2-Benzenedicarboxylic acid, mon o(2-ethylhexyl) ester	26.65	278	29.55	C16H22O4
10	Tetradecane, 2,6,10-trimethyl-	28.15	240	1.58	C17H36
11	Octadecane, 1-isocyanato-	28.24	295	16.52	C19H37NO
12	Eicosanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	28.8	386	12.98	C23H46O4
13	12-Methyl-E ,E-2 ,13-octadecadien-1-iol	29.33	280	3.84	C19H36O
14	Heptacosane	29,69	380	1.51	C27H56
RT: retention time in minutes, Area %: percentage area					

Results And Discussion

M. inermis as others species of *Rubiaceae* family exhibit potent biochemical and pharmacological abilities. The aim of this study was to provide some phytochemical data focused on the non-polar and volatile compounds from de most used part as well as stem bark. Therefore, the thin layer chromatography on antiseizures derived DCM fraction yielded ethyl tetratriacontanoate (1'). That compound is already described in some synthesis (Francis et al. 1937) but first isolated from natural source like *M. inermis*. Besides, gas chromatography on the whole DCM fraction allowed identification of thirteen major non-polar compounds with their relative content wiz tetradecanoic acid (1.9%); phthalic acid, butyl undecyl ester (1.15%); hexadecanoic acid, methyl ester (1.26%); n-hexadecanoic acid (20.46%); hexadecanol (2.98%); heptadecanoic acid, 16-methyl-, methyl ester (0.96%); hexanedioic acid, mono(2-ethylhexyl)ester (3.07%); 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (29.55%); tetradecane, 2,6,10-trimethyl- (1.58%); octadecane, 1-isocyanato- (16.52%); eicosanoic acid, 2-hydroxy-1-

(hydroxymethyl)ethyl ester (12.98%); 12-methyl-E,E-2,13-octadecadien-1-ol (3.84%); heptacosane (1.51%) (Fig. 1&Table 2). Therefore, some compounds are on the little relative amount. The variability of the abundance would be due to the difference of biosynthesis by the plant but also to their difference of importance for the plant. These constituents could contribute to the chemotaxonomy of *M. inermis*. Most of the identified compounds are reported to be volatile from natural sources and exhibited some bioactivities as antioxidant, antimicrobial and antidiabetic (Golovnya et al. 1976; Peng 2000; Pino and Marbot 2001; Blagojević et al. 2006; Radulović et al. 2006; Rezazadeh et al. 2006; Zhao et al. 2006; Paolini et al. 2007; Politeo et al. 2007; Zeng et al. 2007). Furthermore, essential oils are mostly reported to exhibit antifungal, antibacterial, antioxidant and antidiabetics activities (Hsieh et al. 2012; Sharma et al. 2022). Herein, the non-polar compounds from the *M. inermis* might have these biological activities and could contribute to understand the biological activity shared by *M. inermis* stem bark.

Conclusions

In this report we investigated *M. inermis* non-polar compounds profile in the aim to establish these relationships with several ethnopharmacological activities like antiseizures, antimicrobials, antidiabetics and antioxidants. This report is the first on *M. inermis* stem bark non-polar phytochemical characterization. Therefore, stem bark contains fatty acids and essential oils with various chemical functions that might lead to beneficial interaction for drugs discovery. Furthermore, these compounds might be investigated for their antiseizures, antioxidant, antidiabetic and antimicrobial activities.

Declarations

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Conflicts of Interest: The authors declare no conflict of interest.

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Figures

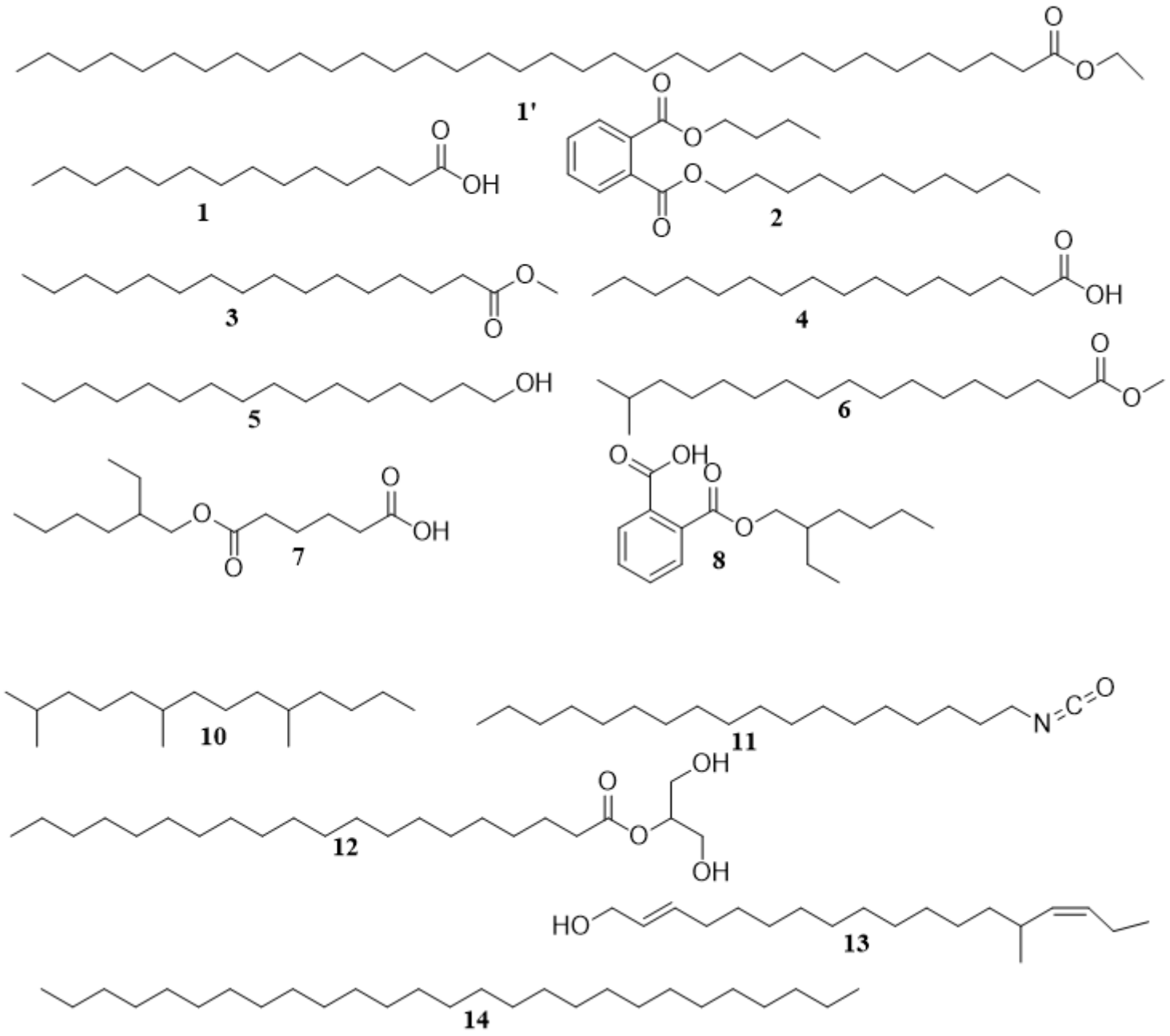

Figure 1

Compounds isolated and identified by GC-MS from stem bark DCM fraction

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