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PHYTOCHEMICAL INVESTIGATION OF THE STEM BARKS OF *ADENIA RUMICIFOLIA* (PASSIFLORACEAE)

Kabran Aka Faustin[1*], Kablan Ahmont Landry Claude[2], Adiko N'dri Marcelline[3], Akoubet Ouayogode Aminata[3], Kablan Richmond Jean-François[4], Kodjo Charles Guillaume[5,6], Konan Dibi Jacques[1], Degny Edomtchi Anne-Lise[7], Aka Any-Grah Sandrine[7] and Attioua Koffi Barthélémy[1]

[1]Laboratoire De Chimie Organique et de Substances Naturelles, UFR Sciences Des Structures De La Matière et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[2]UFR Des Sciences Biologiques, Université Péléforo Gon Coulibaly, BP 1328 Korhogo, Côte d'Ivoire.
[3]Laboratoire de Pharmacognosie, Botanique, Biologie végétale et Cryptogamie, UFR des Sciences Pharmaceutiques et Biologiques, Université Félix Houphouët-Boigny, 22 BP 714 Abidjan 22, Côte d'Ivoire.
[4]Laboratoire De Cristallographie et Physique Moléculaire, UFR SSMT, Université Félix Houphouët Boigny, 01 BP V34 Abidjan 01, Côte d'Ivoire.
[5]Laboratoire De Chimie Bio Organique Et De Substances Naturelles, Université Nangui Abrogoua, UFR-SFA, 02 B.P. 801 Abidjan 02, Côte d'Ivoire.
[6]Laboratoire De Thermodynamique et Physicochimie Du Milieu, Université Nangui Abrogoua, UFR-SFA, 02 B.P. 801 Abidjan 02, Côte d'Ivoire.
[7]Laboratoire De Pharmacie Galénique, Cosmétologie Et Législation, UFR Des Sciences Pharmaceutiques Et Biologiques, Université Félix Houphouët-Boigny, 22 BP 714 Abidjan 22, Côte d'Ivoire.

***Corresponding Author: Kabran Aka Faustin**
Laboratoire de Chimie Organique et de Substances Naturelles, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire.

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ABSTRACT
GC-MS investigation on the hexanic extract of *Adenia rumicifolia* (Passifloraceae) led to the identification of 11 compounds including 9 fatty acid esters and 2 fatty acids. The major compounds in the extract were methyl palmitate and palmitic acid. These compounds were all identified for the first time from this species.

KEYWORDS: Passifloraceae, *Adenia rumicifolia*, fatty acids, fatty acid esters, GC-MS.

INTRODUCTION
Adenia (Passifloraceae) are used for the treatment of many diseases including dysentery, rheumatic pain relief (**Schmelzer et al., 2008**), hypertension, numbness (**Nyarko et al., 2006**), malaria and leprosy (**Kofi Annan et al., 2012**; **Adedapo et al., 2008**). Otherwise, some species of this genus have been used as fish poisons and have also been implicated in stock losses, homicide and suicide (**Van Wyk et al., 2002**; **Kellerman et al., 1988**; **Verdcourt et Trump, 1969**; **Steyn, 1934**). *Adenia* are known for their richness in cyanogenic glycosides (**Olafsdottir et al., 1989**; **Morah, 1988**; **Spencer et Seigler, 1982**; **Gondwe et al., 1978**).

Adenia rumicifolia is a woody climber. The leaves are ovate to almost round, shiny dark, green above, paler below, 3-7 veined from near the base, hairless; the apex is pointed to rounded with base shallowly cordate, sometimes with two hastate lobes. Two fleshy glands of 3 mm wide are present on the petiole at the base of the lamina. Flowers (10 units) are unisexual, axillary heads and creamy yellow. Fruits are capsules (up to 6 x 3 cm), blackish when dry. To the best of our knowledge, no phytochemical study has been reported on this plant species, hence the interest of this study. In this paper, we present the identification by GC-MS of fatty compounds from the *n*-hexane fraction of *A. rumicifolia* stem barks.

MATERIAL AND METHODS
Silica 120 g cartridge was used for flash chromatography using an Armen Instrument spot liquid chromatography flash apparatus. Chemicals and solvents were purchased from Sigma-Aldrich. Thin-layer chromatographies were carried out on aluminium plates coated with silica gel 60 F254 (Merck) and visualized with UV light then sprayed with vanillin-H_2SO_4. IR spectrum was recorded on a Bruker Vector 22 (Champs-sur-Marne, France) spectrometer. GC-MS analyzes were performed on a Thermo Scientific Trace-GC Ultra gas chromatograph with mass detection performed on a Thermo Scientific ITQ900® (Courtaboeuf, France). The injector was set with a split ratio of 1:10 at 230°C. Compounds were separated with an Agilent Technologies DB5HT column (30 m x 0.250 mm x 0.1 µm) and carrier gas was high-

purity helium at 1.1 mL.min^{-1} flow. The oven temperature was initially held at 110°C for 2 min, then raised to 360°C at a rate of 7°C min^{-1} and held for 5 min. Compounds were detected by Electronic Impact (EI) ionization, with the source temperature set at 200°C. Data analysis was performed with Xcalibur^{TM} software using NIST and a homemade data-base. Stem barks were ground using a Retsch apparatus (Eragny sur Oise, France).

Plant material

Stems of *A. rumicifolia* were harvested in January 2013, in « Petit Yapo » forest, Agboville Department, (South-east of Côte d'Ivoire). The plant samples were identified by the botanist TEHE Henry of Centre Suisse de Recherche Scientifique (Côte d'Ivoire), where a voucher specimen is deposited under the reference AR-KABRAN-Agboville 2013-1.

Extraction

Stem barks powder of *A. rumicifolia* (600g) were extracted by maceration three times with 2 L of ethanol for 72 h. After filtration and the removal of the solvent under reduced pressure, a residue of 120 g was obtained. This residue was suspended in water/ethanol (1: 1) and extracted sequentially with increasing polarity solvents to give after evaporation 15 g of *n*-hexane, 23g of dichloromethane and 5 g of ethyl acetate extracts. A part of the hexane extract (5 g) was subjected to flash chromatography using a silica 120 g Grace cartridge with a gradient of hexane-CH_2Cl_2 (100:0 - 0:100) at 80 mL/min to afford six fractions (F1-F6), according to their TLC profiles. Fraction F1 (10 mg) was then analyzed by IR and GC-MS.

Identification of compounds

Methyl palmitate (**I**): RT 12.78 min; SMIE *m/z* (%) 270 $[M]^+$ (20), 241 (22), 227 (65), 213 (25), 199 (34), 185 (35), 171 (35), 157 (23), 143 (42), 129 (19), 115 (13), 101 (47), 87 (38), 83 (26), 73 (22), 59 (18), 55 (100); ($C_{17}H_{34}O_2$, 270 g/mol).

Ethyl palmitate (**II**): RT 13.21 min; SMIE *m/z* (%) 284 $[M]^+$ (23), 255 (22), 241 (53), 227 (22), 213 (33), 199 (25), 185 (27), 171 (18), 157 (28), 143 (15), 129 (13), 115 (15), 101 (11), 87 (19), 73 (72), 61 (57), 55 (100); ($C_{18}H_{36}O_2$, 284 g/mol).

Methyl heptadecanoate (**III**): RT 13.41 min; SMIE *m/z* (%) 284 $[M]^+$ (45), 255 (31), 241 (69), 227 (25), 213 (20), 199 (35), 185 (29), 171 (17), 157 (20), 143 (39), 129 (17), 115 (9), 101 (23), 87 (44), 83 (18), 55 (100); ($C_{18}H_{36}O_2$, 284 g/mol).

(9E, 12E)-methyl octadeca-9, 12-dienoate (**IV**): RT 13.84 min; SMIE *m/z* (%) 294 $[M]^+$ (5), 263 (12), 262 (14), 255 (7), 178 (7), 150 (10), 135 (12), 121(9), 107 (10), 95 (23), 93 (17), 91 (33), 81 (50), 79 (86), 77 (67), 67 (100), 65 (32), 55 (16); ($C_{19}H_{34}O_2$, 294 g/mol).

Methyl oleate (**V**): RT 13.90 min; SMIE *m/z* (%) 296 $[M]^+$ (8), 265 (41), 264 (42), 246 (7), 235 (13), 222 (18), 221 (11), 207 (5), 193 (8), 179 (7), 166 (13), 148 (12), 141 (15), 121 (14), 98 (25), 97 (34), 96 (42), 95 (47), 83 (66), 81 (53), 79 (47), 77 (31), 69 (25), 67 (83), 65 (15), 55 (100); ($C_{19}H_{36}O_2$, 296 g/mol).

Methyl 16-methylheptadecanoate (**VI**): RT 14.03 min; SMIE *m/z* (%) 298 $[M]^+$ (38), 269 (17), 255 (84), 241 (24), 227 (18), 213 (26), 199 (45), 185 (26), 171 (15), 157 (26), 143 (41), 129 (14), 115 (9), 101 (38), 97 (14), 87 (42), 83 (19), 69 (20), 55 (100); ($C_{19}H_{38}O_2$, 298 g/mol).

Ethyl linoleate (**VII**): RT 14.2 min; SMIE *m/z* (%) 308 $[M]^+$ (3), 263 (25), 262 (47), 245 (5), 231 (5), 220 (5), 191 (7), 179 (12), 164 (12), 163 (13), 150 (15), 131 (16), 121 (13), 107 (12), 93 (11), 91 (27), 81 (51), 79 (100), 77 (85), 67 (86), 65 (43); ($C_{20}H_{36}O_2$, 308 g/mol).

Ethyl oleate (**VIII**): RT 14.28 min; SMIE *m/z* (%) 310 $[M]^+$ (7), 265 (35), 227 (6), 221 (10), 207 (6), 180 (6), 151 (12), 135 (12), 121 (13), 109 (23), 97 (31), 96 (35), 95 (51), 91 (28), 83 (53), 81 (54), 79 (64), 77 (38), 67 (87), 55 (100); ($C_{20}H_{38}O_2$, 310 g/mol).

Ethyl stearate (**IX**): RT 14.43 min; SMIE *m/z* (%) 312 $[M]^+$ (27), 283 (18), 269 (83), 255 (32), 241 (29), 227 (28), 213 (52), 199 (23), 185 (17), 171 (18), 157 (40), 143 (14), 129 (17), 115 (14), 97 (11), 87 (25), 73 (67), 61 (44), 55 (100); ($C_{20}H_{40}O_2$, 312 g/mol).

Palmitic acid (**X**): RT 13.05 min; SMIE *m/z* (%) 256 $[M]^+$ (14), 227 (10), 213 (19), 199 (12), 185 (17), 171 (16), 157 (18), 143 (14), 129 (20), 115 (14), 97 (9), 87 (49), 73 (39), 59 (37), 55 (100); ($C_{16}H_{32}O_2$, 256 g/mol).

Stearic acid (**XI**): RT 14.27 min; SMIE *m/z* (%) 284 $[M]^+$ (40), 260 (20), 245 (24), 241 (32), 227 (21), 213 (18), 199 (22), 185 (36), 171 (27), 157 (20), 143 (23), 129 (32), 115 (20), 91 (15), 87 (78), 77 (26), 73 (40), 59 (30), 55 (100); ($C_{18}H_{36}O_2$, 284 g/mol).

RESULTS AND DISCUSSION

The identified molecules (**Fig. 1**) appeared at 12-15 min on the spectra. Spectra A and B (**Fig. 2**) indicated that the hexane extract of the stems of *Adenia rumicifolia* studied consisted mainly of fatty acids and esters of these acids. That was in agreement with the infrared spectrum (**Fig. 3**) of the fraction F1 from which the compounds were derived. Indeed, this spectrum showed strong C-H elongation bands at 2851-2957 cm^{-1} and characteristic bands of carbonyl groups at 1710-1741 cm^{-1}. The molecules were identified by the analysis of *m/z* fragments, and by comparison with the reference spectra in the spectrometer data-base. It was for fatty acids (spectrum B), palmitic acid (**X**) (RT = 13.05 min, C_{16}) and stearic acid (**XI**) (RT = 14.27 min, C_{18}), and for fatty esters (spectrum A) methyl palmitate (**I**) (RT = 12.78 min, C_{17}), ethyl palmitate (**II**) (RT = 13.21 min, C_{18}),

methyl heptadecanoate (**III**) (RT = 13.41 min, C_{18}), (9*E*, 12*E*)-methyl octadeca-9, 12-dienoate (**IV**) (RT = 13.84 min, C_{19}), methyl oleate (**V**) (RT = 13.90 min, C_{19}), methyl 16-methylheptadecanoate (**VI**) (RT = 14.03 min, C_{19}), ethyl linoleate (**VII**) (RT = 14.2 min, C_{20}), ethyl oleate (**VIII**) (RT = 14.28 min, C_{20}) and ethyl stearate (**IX**) (RT = 14.43 min, C_{20}). The identified fatty esters consisted of unsaturated fatty esters (**IV**, **V**, **VII** and **VIII**) and saturated fatty esters (**I**, **II**, **III**, **VI** and **IX**). In addition, the fatty acids were all saturated. Furthermore, many detected fatty acids (spectrum B) could not be identified. Spectrum A indicated that the major ester in the studied fraction was methyl palmitate (**I**) while methyl heptadecanoate (**III**) was the minor ester. On spectrum B, palmitic acid (**X**) was predominantly in relation to stearic acid (**XI**).

Methyl palmitate, a naturally occurring fatty acid ester (**Palu et al., 2006**), showed many biological activities such as hepatoprotective (**Shoeib et al., 2016**) and anti-inflammatory (**Saeed et al., 2012**). It was also shown to suppress isolated Kupffer cells, rat peritoneal macrophages and RAW cells (**Cai et al., 2005**; **El-Demerdash, 2011**; **Sarkar et al, 2006**), and has the ability to inhibit NF-κB and downstream inflammatory cascades (**El-Demerdash, 2011**; **Mantawy et al., 2012**). Methyl palmitate is a putative regulator from perivascular fat, on the contractility of pregnant human myometrium (**Crankshaw et al., 2014**) and has protective effects against silica-induced pulmonary fibrosis in rats (**Sharawy et al., 2013**). Otherwise, this molecule is a potential biodiesel (**Altaie et al., 2017**).

Superposition of spectra A and B showed that palmitic acid appeared at a longer retention time (13.05 min) than its methylated ester, methyl palmitate, which was detected at TR = 12.78 min. That was not the case for stearic acid and its ethyl ester, ethyl stearate. Indeed, stearic acid appeared at a shorter retention time (14.27 min) than ethyl stearate which was detected at TR = 14.43 min. That observation indicated that a fatty acid could be detected before its corresponding ester (methylated or ethylated), and reciprocally. In our case, the methylated ester (methyl palmitate) appeared before its corresponding acid (palmitic acid); that was not the case of the ethyl ester (ethyl stearate) and its corresponding acid (stearic acid). Unfortunately, the fatty acids of the other identified esters were not found in our extract for further comparisons. A similar comparative study is underway on the dichloromethane extract.

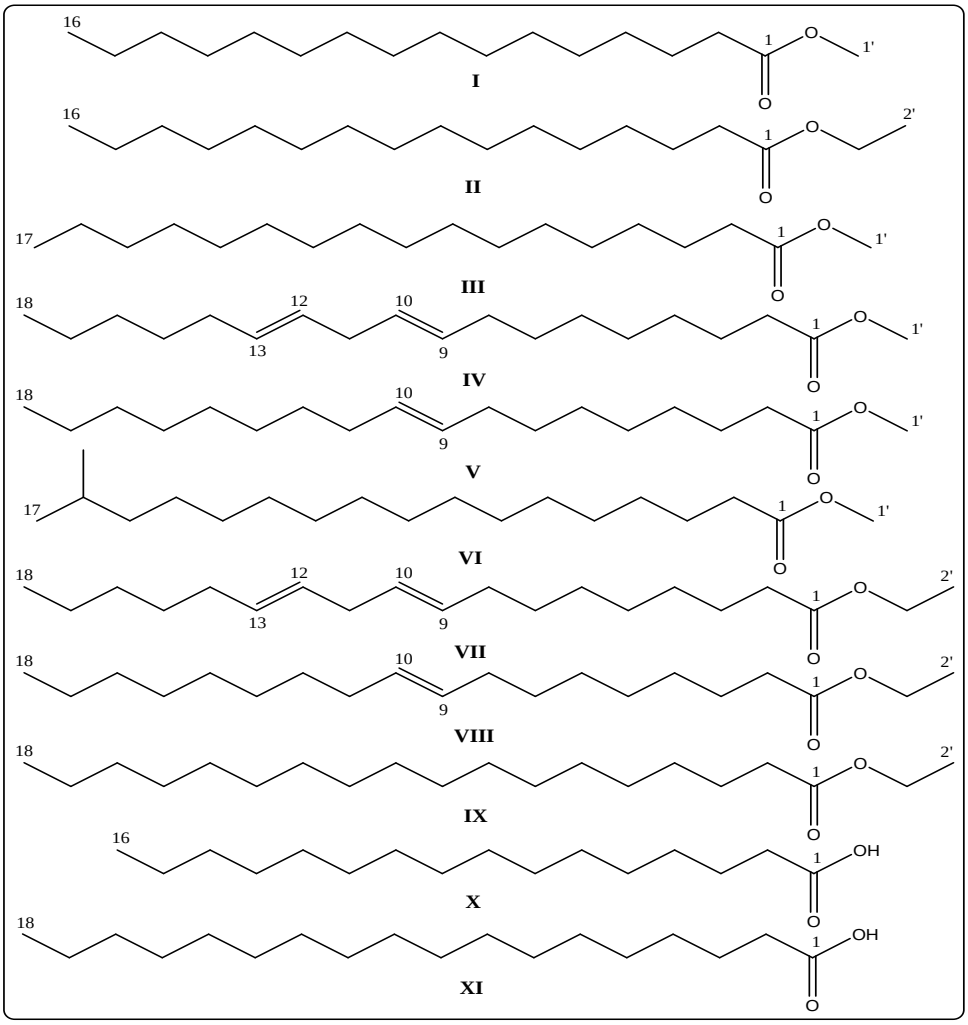

Figure 1: Structures of identified molecules from the hexane extract of *A. rumicifolia*.

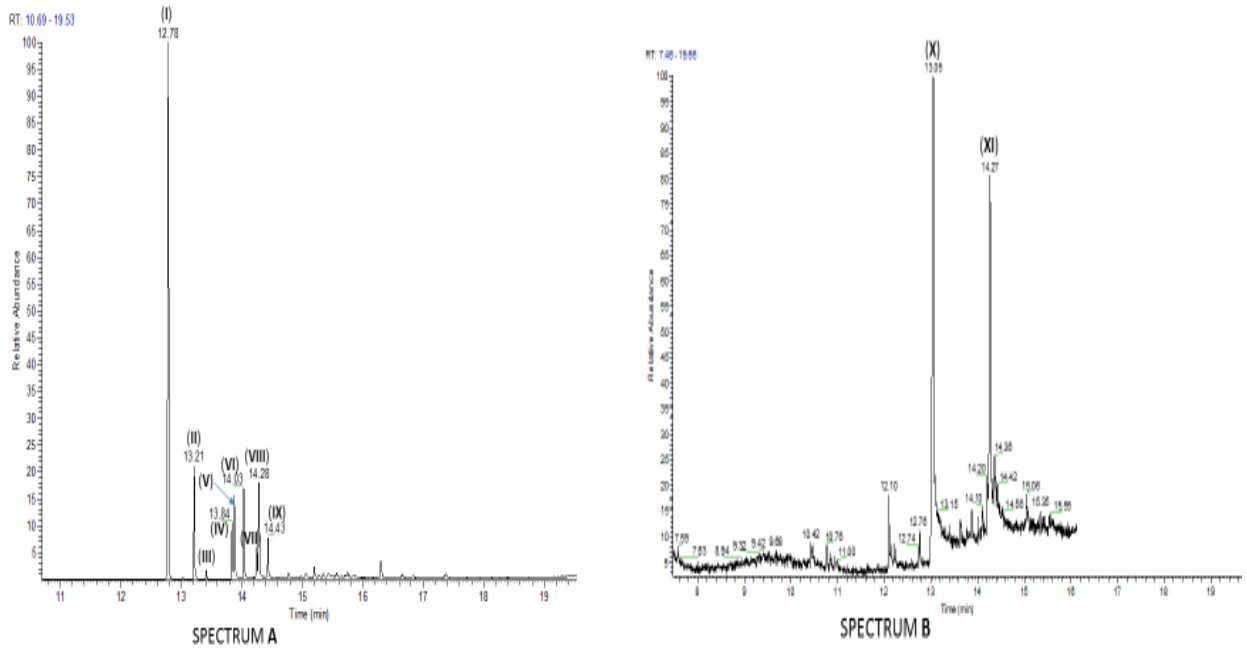

Figure 2: Fatty acid ester (spectrum A) and fatty acid (spectrum B) profiles in the hexane extract of *A. Rumicifolia* stems.

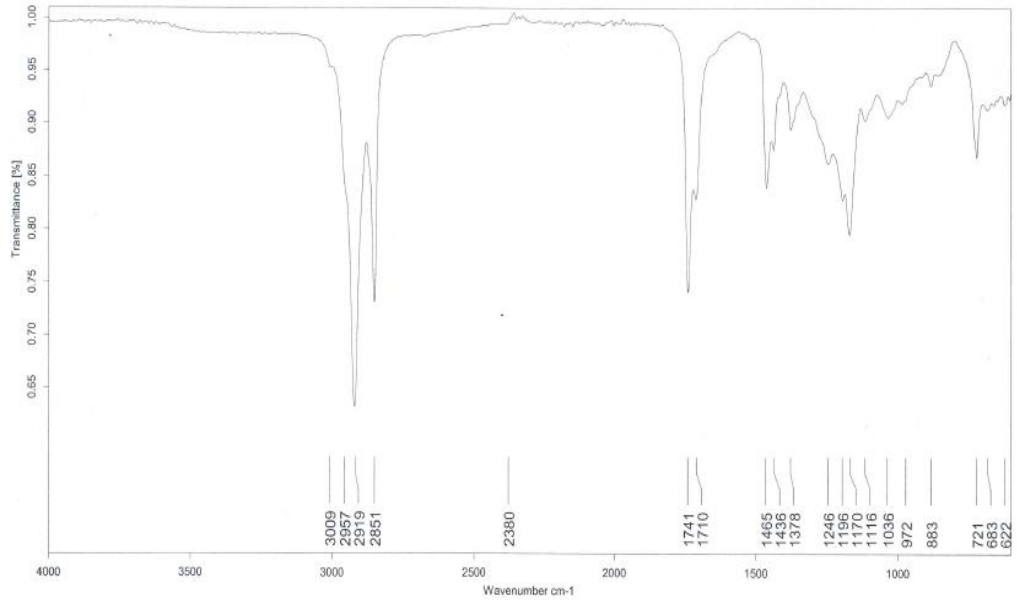

Figure 3: IR spectrum of fraction F1.

CONCLUSION

The GC-MS study of the hexane extract of *Adenia rumicifolia* stems allowed the identification of two C_{16} and C_{18} fatty acids, and nine C_{17}, C_{18}, C_{19} and C_{20} fatty acid esters. The major compounds were palmitic acid for fatty acids, and methyl palmitate for fatty esters. Other chemical studies deserve to be carried out in order to enrich the chemical knowledge on this species.

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