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CHARACTERISATION OF FATTY ACIDS IN THE ROOTS OF *Cryptolepis sanguinolenta*

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The roots of Cryptolepis saguinolenta *were extracted with petroleum ether (40–60°C) to afford the oil in 0.7% yield to the wet mass of raw material. The fatty acid methyl esters (FAME) were separated and analyzed by GC-MS. The following fatty acids as their methyl esters in various proportions were obtained: linoleic acid (52%), palmitic acid (17%), stearic acid (5.8%), margaric acid (2.8%), oleic acid (1.8%), and arachidic acid (1.8%). The most abundant fatty acid identified was the essential dietary fatty acid linoleic acid in the proportion of 52%, which underscores the health and nutritional benefits of the plant besides its medicinal properties, which was the aim of this work.*

Keywords: *Cryptolepis sanguinolenta,* fatty acid methyl ester, gas chromatography-mass spectrometry.

Cryptolepis sanguinolenta (Lindl.) Schlechter (Asclepiadaceae) is a plant native to West Africa and used in the treatment of malaria [1]. A decoction of the roots has been used in clinical therapy, both of malaria and of urinary and upper respiratory tract infection at the Centre for Scientific Research into Plant Medicine (CSRPM) in Ghana since 1974 [2]. The roots of the plant are known to contain alkaloids, notably quindoline, cryptolepine, and other related compounds [2–4]. The aqueous root extract of the plant is biologically active and has medicinal properties. In the *in vitro* toxicity analysis of the aqueous extract of *C. sanguinolenta* using V79 cells, a Chinese hamster lung fibroblast frequently used to assess genetic toxicity and a number of organ-specific human cell lines, the aqueous extract caused a dose- and time-dependent reduction in viability of the V79 cell line. Cryptolepine, the major alkaloid of the plant and in the antimalarial decoction, is a cytotoxic DNA intercalator and a potential anticancer agent [5].

The traditional antimalarial decoction is prepared either by boiling the powder of the root in water for about two hours and strained or by maceration with ethanol in a preparation popularly called 'Bitters' in Ghana. The preparation methods of the antimalarial decoction are such that fatty acids esters are also extracted into the decoction. The antimalarial preparation is therefore a mixture of alkaloids, fatty acids, and other phytoconstituents. Besides the alkaloids therefore, fatty acid esters are also consumed in the use of the roots of the plant for malaria treatment.

The aim of this work was to determine the fatty acids in the roots of the plant *Cryptolepis sanguinolenta* and to assess the health implications from the perspective of the fatty acids intake in the use of the plant in the management of malaria fever in Ghana.

Several recent reports have focused on the structures and NMR spectroscopy of the alkaloidal constituents of the West African medicinal plant *Cryptolepis sanguinolenta* (Lindl.) Schlechter (Asclepiadaceae) [4, 6, 7]. Unlike the alkaloids, not much attention has been given to the study of fatty acids of *C. sanguinolenta* inspite of the high patronage of the plant as an antimalarial in Ghana and the West African subregion and the risk its fatty acids constituents could pose to consumers as the cause of many ailments such as heart diseases, stroke, diabetes, and hypertension [5].

Exhaustive extraction of the root of the plant with petroleum ether 40–60°C provided a pale yellow oil of 3.8 g (0.7%) to the wet mass of raw material as compared to the usually dark-red syrup of the total alkaloidal extract of 5.5% [1]. The small amount of the oil in the plant might be the reason why fatty acids of the plant have received little or no attention, resulting in the paucity of information on the fatty acids constituents in the plant.

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TABLE 1. Gas Chromatography (GC) Separation of Fatty Acid Methyl Esters (FAME) of Oil of *Cryptolepis sanguinolenta*

FAME	Fatty acid	RT*, min	%
1	16:0	45.25	16.9
2	17:0	47.81	2.8
3	(*Z,Z*)18:2	49.66	35.6
4	(*E,E*)18:2	49.79	15.9
5	18:1	49.85	1.8
6	18:0	50.29	5.8
7	20:0	54.90	1.8
8	$C_{19}H_{34}O_2$	54.94	5.6
9	$C_{19}H_{34}O_2$	62.56	8.8
10	$C_{19}H_{34}O_2$	62.74	4.6

*RT: retention time to standard Tetradecane which was added as standard.

The infrared spectrum of the oil showed absorbance at 2950 and 2925 cm^{-1} associated with stretching vibrations of methyl and methylene groups. The strong band at 1700 cm^{-1} was due to the carbonyl functional group of acids (COOH). The bending vibrations of methylene and methyl groups were observed at 1450 and 1390 cm^{-1}, respectively. The other important diagnostic bands were observed at 3000 cm^{-1} for the alkenic C–H group and 1650 cm^{-1} for the C=C functional group. The infrared spectrum was therefore characteristic of an oil with free fatty acids [8].

The transesterification of the oil was then followed by GC-MS analyses of the fatty acid methyl esters (FAME), which enabled six of the components to be characterized as shown in Table 1.

The most abundant fatty acid methyl esters in the oil of *C. sanguinolenta* were methyl-(*Z*,*Z*)-9,12-octadecadienoate (methyl linoleate), **3**, with percentage composition of 35.6%, and methyl hexadecanoate (methyl palmitate), **1**, in the proportion of 16.9%. These were followed by methyl-(*E*,*E*)-9,12-octadecadienoate, **4**, with percentage composition of 15.9%, and methyloctadecanoate (methyl stearate), **6**, with percentage composition of 5.8%. The others were, in order of abundance, as follows: methyl heptadecanoate (margaric acid methyl ester), **2**, gave 2.8% while *E*- or *Z*-methyl-9-octadecanoate (methyl elaidate or oleic acid methyl ester), **5**, was 1.8%, and methyl eicosanoate (arachidic acid methyl ester), **7**, was 1.8%.

The most abundant fatty acid methyl-(*Z*,*Z*)-9,12-octadecadienoate, **3** (35.6%), and the third abundant fatty acid methyl-(*E*,*E*)-9,12-octadecadienoate, **4** (15.9%), are from the essential dietary fatty acid linoleic acid. They together made up 52% of the fatty acid composition of the oil of *Cryptolepis sanguinolenta*. The presence and abundance of linoleic acid in *Cryptolepis sanguinolenta* is significant as there is great interest at present in conjugated dienoic fatty acids (conjugated linoleic acid or CLA) because of their apparent anticancer and antiatherogenic properties found for the Z10, E12-18:2, and E9, Z11-18:2 isomers [9]. Linoleic acids, **3** and **4**, and linolenic acid and arachidonic acid are unsaturated fatty acids that must be present in the human diet and that are used for the synthesis of prostaglandins in the body [10].

The amount of oil obtained was very small (ca. 0.7%) as compared to the alkaloidal extract (ca. 5.5%) reported in the literature [1]. The biological activity and the medicinal properties of the roots of the plant might therefore be due mainly to the alkaloids. The other minor chemical constituents in the roots of *C. sanguinolenta,* including the fatty acids, might at least play a synergistic role in the biological activities of the plant reported in the literature [7]. Three unidentified compounds (MW 308, $C_{20}H_{36}O_2$) were also indicated in the GC-MS data.

Although the plant *C. sanguinolenta* is high in the two isomers of linoleic acid, **3** and **4**, further investigation is necessary to establish their identity. The use of GC-MS alone to identify the isomers *Z*,*Z* (9,12), **3**, and *E*,*E* (9,12), **4**, of linoleic acid was insufficient. It is when this has been done that an unambiguous statement can be made about the safety of the crude drug for antimalarial treatment.

EXPERIMENTAL

Plant Material. The roots of *Cryptolepis sanguinolenta* (Lindl.) Schlechter (Asclepiadaceae) were used. They were obtained from the Herbarium of the Centre for Scientific Research into Plant Medicine (CSRPM), Mampong-Akwapim in

Ghana in August 2007, where a voucher specimen is deposited. The samples were dried for a week (30–40°C) and milled (Mallinckrodt, 100 mesh) into a powder.

Extraction and Analysis of Oil. The powdered roots (500 g) were extracted with 2.5 L of petroleum ether (40–60°C) in a Soxhlet apparatus for 11 h. The extract was cooled to room temperature and evaporated under reduced pressure at 40°C to afford 3.8 g (0.7%) of the oil, which was stored in a dark bottle and kept at 4°C until analysis and further studies. IR (ν_{max}, neat oil, cm^{-1}): 2950, 2925 (CH_2, CH_3), 1700 (C=O), 1450 (CH_2), 1390 (CH_3).

Transesterification of Oil. The crude oil (2 g) in concentrated sulfuric acid (1 mL) and methanol (9 mL) was heated under reflux in an atmosphere of nitrogen for 1 h. It was cooled to room temperature and extracted with hexane (3 × 5 mL). The hexane extract was dried over $MgSO_4$ for 20 min. The extract was reduced to a volume of 5 mL under reduced pressure at 40°C to afford the fatty acid methyl ester (FAME), which was stored in a dark bottle at 4°C for analysis.

GC/MS Analysis of the Oil. A Hewlett-Packard (HP) GC 5890 Series II gas chromatograph interfaced to an HP 5972 MSD with NBS 75K and Wiley 138 Chem. Station data system was used for MS identification of GC components.

GC conditions were INNOWAX (Carbowax 20 M, 0.25 mm film thickness) column: 50 m × 0.25 mm i.d. and HP-5 (polydimethylsiloxane, 0.25 μm film thickness) 30 m × 0.25 i.d (internal diameter). Temperature programme: 50°C (4.5°C min^{-1}), 250°C (10 min). Carrier gas, He at 78 kPa, linear velocity of 20 cm·min^{-1}, and make-up gas (nitrogen) flow 29 mL/min. FID at 250°C at injection port split/splitless (splitting ratio 1:30) at 250°C; injection volume, 0.5 μL (hexane solution of transesterified oil). MS conditions were: ionization, EI-MS at 70 eV; *m/z* range, 30–400; scan rate, sec, ionization chamber at 180°C; transfer line at 280°C. Tetradecane was used as internal standard. Components were identified by comparison of their mass spectra with those of known fatty acids.

The mass spectra of the identified fatty acid methyl esters were as follows.

Methyl Hexadecanoate (methyl palmitate or palmitic acid methyl ester) (1) [$CH_3(CH_2)_{14}COOCH_3$]. EI-MS, *m/z* (relative abundance, %): 270 [M^+] (10), 239 (5), 227 (15), 143 (20), 97 (5), 87 (80), 74 (100), 67 (20), 55 (40), 43 (50), 29 (20).

Methyl Heptadecanoate (margaric acid methyl ester) (2) [$CH_3(CH_2)_{15}COOCH_3$]. EI-MS, *m/z* (relative abundance, %): 284 [M^+] (10), 253 (5), 241 (10), 199 (5), 185 (5), 143 (10), 87 (70), 74 (100), 55 (30), 43 (40).

***E,E*-Methyl-9,12-octadecadienoate (methyl linolelaidate) (3)** [$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOCH_3$]. EI-MS, *m/z* (relative abundance, %): 294 [M^+] (10), 263 (5), 150 (5), 109 (25), 123 (10), 95 (60), 81 (80), 67 (100), 55 (60), 41 (60), 29 (20).

***Z,Z*-Methyl-9,12-octadecadienoate (methyl linoleate) (4)** [$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOCH_3$]. EI-MS, *m/z* (relative abundance, %): 294 [M^+] (10), 263 (5), 150 (5), 109 (25), 123 (10), 95 (60), 81 (80), 67 (100), 55 (60), 41 (60), 29 (20).

***E* or *Z*-Methyl-9-octadecenoate (methyl elaidate or oleic acid methyl ester) (5)** [$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOCH_3$]. EI-MS, *m/z* (relative abundance, %): 296 [M^+] (5), 264 (20), 222 (10), 180 (10), 123 (10), 111 (20), 97 (50), 83 (40), 75 (10), 69 (60), 55 (100), 41 (80), 29 (20).

Methyl Octadecanoate (methyl stearate) (6) [$CH_3(CH_2)_{16}COOCH_3$]. EI-MS, *m/z* (relative abundance, %): 298 [M^+] (10), 267 (10), 255 (15), 199 (15), 143 (20), 87 (80), 74 (100), 67 (10), 55 (30), 43 (40), 29 (20).

Methyl Eicosanoate (arachidic acid methyl ester) (7) [$CH_3(CH_2)_{18}COOCH_3$]. EI-MS, *m/z* (relative abundance, %): 326 [M^+] (10), 283 (10), 143 (20), 87 (70), 74 (100), 55 (30), 43 (40), 29 (10).

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