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Isolation and characterisation of phytoconstituents using low polar solvents from the flowers of *Couroupita guianensis*

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Abstract- *Couroupita guianensis is used extensively as an ingredient in many ayurvedic preparations which cure gastritis, scabies, bleeding piles, dysentery, and scorpion poison. The flower was subjected to extraction with petroleum ether and chloroform solvents. Compounds* ***(CGA-I)*** *octyl 4-(nonanoyloxy) benzoate along with myristoleic acid* ***(CGA-II)****, linoleic acid* ***(CGA-V)*** *and (8E, 10E, 12E)-icosa-8, 10, 12-triene)* ***(CGA-VIII)*** *were isolated by column chromatography and characterised with IR, ^{1}H-NMR, ^{13}C-NMR spectral data.*

Keywords- *Couroupita guianensis, Lecythidaceae, flower, octyl 4-(nonanoyloxy) benzoate, myristoleic acid, linoleic acid, (8E, 10E, 12E)-icosa-8, 10, 12-triene)*

I. INTRODUCTION

During the past decade, the indigenous or traditional system of medicine has gained importance in the field of medicine. In most of the developing countries, a large number of populations still depend on traditional practitioners, who in turn are dependent on medicinal plants, to meet their primary health care needs. Thus, it is clear that herbal medicine plays a pivotal role in therapeutic strategies in the modern world. One such plant that has been used widely in traditional medicine is *Couroupita guianensis* Aubl. belonging to the family *Lecythidaceae*. It is grown in Indian gardens as an ornamental tree. *C. guianensis*, also called as Cannonball tree is native to South India and Malaysia and is commonly known as Nagalinga pushpam in Tamil. In *Ayurveda*, it is called as ayahuma, it is used extensively as an ingredient in many preparations which cure gastritis, scabies, bleeding piles, dysentery, scorpion poison and many [10,4]. It has rubefacint and anti rheumatic properties used in Ayurvedic concepts, cold relief balm. The fruit pulp is used to cure headache. In folk medicine, the flowers are used to cure cold, intestinal gas formation and stomach ache, and also for treating diarrhoea, and when dried and powdered, used as a snuff. The fragrance of flowers is used for curing asthma. The shell of the fruit is used as a utensil. The flowers of *C. guianensis* showed analgesic and anti-inflammatory activity and immunomodulatory activity, anthelmintic activity, antimicrobial, wound healing and antioxidant activity, antinociceptive activity Geetha et al. [3], Pradhan et al. [6], Farrukh Aqil et al. [2], Rajamanickam et al. [7], Umachigi et al. [11] and Pinheiro et al.[5]. Previous work on *C. guianensis* has showed that the plant consists of several chemical constituents with novel structures and possesses bio-active moieties. This includes eugenol, linalool,

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farnesol, nerol, tryptanthrine, indigo, indirubin, isatin, linoleic acid, α, β- amyrins, carotenoids and sterols [12], Rane et al.[8], Bergman et al. [1] and Sen et al. [9]. Albeit, the known uses of the plant parts and their extracts in various disorders, especially those against microbial infections, none of the studies aimed at isolation and identification of the constituents from the flowers of *C. guianensis*. This prompted us to undertake the present work and we have isolated and identified the four compounds **CGA-I**, **CGA- II**, **CGA- V** and **CGA-VIII** from *C. guianensis* by chromatographic and spectral methods.

Experimental

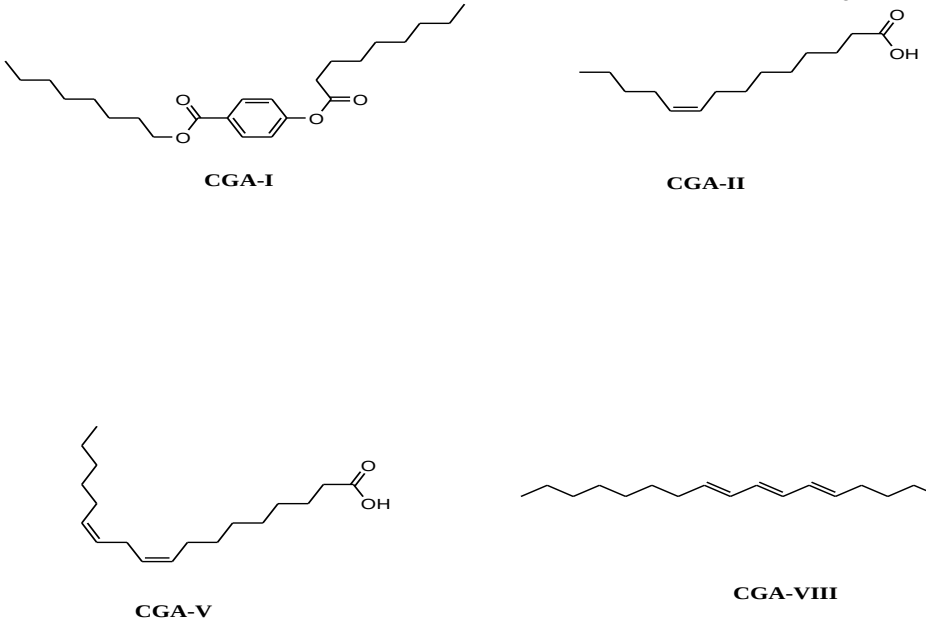
A. Plant material

Fresh flowers of *C. guianensis* was collected in February, 2010, from Palakkad district, Kerala and the plant species was authenticated in the Department of life science, Karpagam University, Coimbatore-21. Voucher specimen was preserved in our Department (No. KU11CHE1934).

B. Extraction and Isolation

The 5.2 Kg of powdered, dried flower was extracted thrice (3X72 hrs) with petroleum ether under cold percolation. The combined extract was subjected to distillation and concentrated under *vacuo* to yield a residue A of 6.3 gm. When monitored by TLC using (8:2) petroleum ether: ethyl acetate showed the presence of 2 major compounds with R_f values 0.62 and 0.54 respectively and 2 minor compounds with R_f values 0.84 and 0.18. The residue was subjected to column chromatography. The column was packed with 120 gm of silica gel. Initially the column was eluted with petroleum ether and with increasing amount of ethyl acetate and fractions of 20 ml were collected and monitored with TLC using petroleum ether and ethyl acetate (9:1) solvent system. Iodine vapour was used as the identification reagent. On eluting the column fraction 7 to 9, compound **CGA-I** (0.40 mg), 10 to 17, compound **CGA- II** (160 mg) were found to be homogeneous by TLC.

After defatting, the plant material was subjected to sequential extraction with chloroform (2X72 hrs) to yield residue B (11.6 g). Residue B on column chromatography and on elution with petroleum ether: ethyl acetate (8:2) yielded 46 gm of residue in the fractions 6-8 with on R_f value 0.49 compound **CGA-V**. Further three more compounds were isolated from the fractions 37-65 compound CGA-VI (53.2 mg), fractions 97-123 compound CGA-VII (81 mg) and fractions 124-144 compound **CGA-VIII** (76 mg) were obtained when the column was eluted with the solvent system petroleum ether and ethyl acetate (5:5) and were homogeneous by TLC. The structure of the compounds **CGA-I**, **CGA- II**, **CGA- V** and **CGA-VIII** were shown in "fig.1."



CGA-I **CGA-II**

CGA-V **CGA-VIII**

Figure 1: Structure of the compounds **CGA-I**, **CGA- II**, **CGA- V** and **CGA-VIII**

General

^{1}H-NMR and Spectra ^{13}C-NMR were recorded on a Bruker AM-400 (400 MHz) instrument; chemical shifts δ in ppm with TMS as internal standard, coupling constants J in Hz. Perkin –Elmer model 1650 IR instrument was used to carried the IR spectra.

CGA-I

IR ν_{max} (KBr): 1730, 1600, 2988, 1450 and 1375 cm^{-1}

^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, J in Hz): 7.63, 7.45 (4H, dd, J= 6.2 Hz, 2.0 Hz, H-2, H-6, H-5), 4.00 (2H, t, H-2'), 2.29 (2H, t, H-2''), 2.00 (2H, m, H-3'), 1.56, 1.25 (2H, m), 0.832(12H).

^{13}C-NMR (100 MHz, $CDCl_3$, δ in ppm):74.93 (C=O), 163.56(C=O), 128.28 (C=C) 126.66(C-3, C-5), 127.07 (C-1, C-4), 124.46 (C-1), 126.66 (C-2, C-6), 34.57(C-2'').

CGA-II

IR ν_{max} (KBr): 1712, 1581, 2924, 1456 and 1375 cm^{-1}

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^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, J in Hz): 5.20 (2H, m), 2.10 (2H, d, H-2), 2.0 (2H, m), 1.29 (16 H, s).
^{13}C-NMR (100 MHz, $CDCl_3$, δ in ppm):174.72 (C=O), 130.02 (C=C), 128.04 (C=C), 22.21(C-C), 22.33(C-C), 24.77(C-C), 25.51(C-C), 26.90(C-C), 28.81(C-C), 28.97(C-C), 29.14(C-C), 29.25(C-C), 31.18(C-C), 31.54(C-C), 33.98(C-C), 41.67 (C-C), 14.16 (C-C).

CGA-V

IR ν_{max} (KBr): 1709, 1581, 2925, 2854 and 1465 cm^{-1}
^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, J in Hz):5.29 (2H, m), 2.7, 2.8 (2H, d), 2.29 (2H, d, H-2), 2.0 (2H, m) 1.29 (16 H, s) 0.81 (6H, s).
^{13}C-NMR (100 MHz, $CDCl_3$, δ in ppm): 180.00 (COOH), 130.22, 130.02 (C=C) 128.07, 127.91 (C=C), 128.04 (C=C), 22.56 (C-C), 22.68(C-C), 24.71(C-C), 25.63(C-C), 27.18(C-C), 27.28(C-C), 27.20(C-C), 29.04(C-C), 29.14(C-C), 29.25(C-C), 29.35(C-C), 29.43(C-C), 29.58(C-C), 29.68(C-C), 31.52(C-C), 31.92(C-C), 33.94 (C-C).

CGA-VIII

IR ν_{max} (KBr): 1465, 2854, 2925, 1595 and 1375 cm^{-1}
^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm, J in Hz): 5.10 (2H, m), 2.01 and 1.93 (8H, m), 1.56 (3H, s), 1.18 (10H, m), 0.765 (12H, m).
^{13}C-NMR (100 MHz, $CDCl_3$, δ in ppm): 135.06, 134.85 (C=C), 131.18, 124.43 (C=C), 124.32, 124.29 (C=C), 22.68 (C-C), 25.66(C-C), 26.67(C-C), 26.78(C-C), 28.27(C-C), 29.35(C-C), 29.60(C-C), 29.69(C-C), 30.03(C-C), 31.92(C-C), 39.75(C-C), 39.73(C-C), 37.10(C-C), 37.02(C-C), 14.09(C-C), 15.98(C-C), 16.02(C-C), 17.65(C-C).

II. RESULT AND DISCUSSION

CGA-I

The IR spectra exhibited absorption bands at 1730 cm^{-1} showing the presence of a carbonyl group, 1600 cm^{-1} for a C=C group and at 2988 cm^{-1} and 1450 and 1375 cm^{-1} for the presence of C-H. The ^{1}H-NMR spectrum shows the presence of a multiplet of signals at δ 0.832 for 12 protons suggesting the presence of four methyl groups. This along with the complex multiplet signal at δ 1.56 (Fig 45) indicates the presence of two isopropyl groups. The broad singlet at δ 1.25 strongly suggests the presence of a long chain of methylene groups in the compound. The signal at δ 2.00 (H-3') is indicative of the presence of a methylene group in the β-position to an oxygen function. The triplet signal at δ 4.00 (H-2') is due a methylene group attached to an oxygen function. The triplet at δ 2.29 for two protons (H-2") may be assigned to a methylene group attached to a carbonyl group. Apart from this two signals were observed at δ 7.45 (H-2 and H-6) and 7.63 (H-3 & H-5) as doublet of doublets (J= 6.2 Hz, 2.0 Hz) suggests the presence of four aromatic protons. Complementing the above data the ^{13}C-NMR spectra showed two signals at δ 174.93 and 163.56 for two ester carbonyls in two different environments. In the aromatic region of the spectra it showed the presence of only signals at δ 128.28 (C-3, C-5), 126.66(C-2, C-6) (Fig 46) and a weak signal at δ 127.07 (for quaternary C-1, C-4) each for two carbons. There is only one signal is observed at δ 63.99 indicating the presence of only one carbon atom attached to the oxygen function. The signal at δ 34.57 is assigned to a carbon atom (C-2") attached to a carbonyl group. Eventhough the compound showed two carbonyl groups only one methylene group is observed under oxygen function and another methylene group is attached to the carbonyl group. This supports the assignments made in the ^{1}H-NMR spectral data for the signals at δ 2.29 and 4.00. It may be suggested that in the aromatic ring the C-1 position may be a phenolic position where it is esterified with a long chain fatty acid and the C-4 position may have a carboxylic acid group which is esterified with a long chain alcohol. Based on the above spectral data the assumption of structure of the compound may be assigned as (octyl 4-(nonanoyloxy) benzoate).

CGA-II

The IR spectra exhibited absorption bands at 1712 cm^{-1} showing the presence of a carbonyl group, 1581 cm^{-1} for a C=C group and at 2924 cm^{-1} and 1465 and 1375 cm^{-1} for the presence of C-H.

The ^{1}H-NMR spectrum shows the presence of a triplet signal at δ 0.83 for 3 protons suggesting the presence of a methyl group. The broad singlet at δ 1.29 for 16 protons strongly suggests the presence of a long chain of methylene groups in the compound. The triplet at δ 2.10 for two protons (H-2) is assigned to a methylene group attached to a carbonyl group The signal at δ 2.00 is indicative of the presence of a methylene group in the allylic position to the double bond. The multiplet signal at δ 5.20 (Fig 47) is due the protons of the double bond. This suggests the presence of a long chain unsaturated fatty acid.

The above assignment was supported by the ^{13}C-NMR spectral data by exhibiting a signal at δ 174.72 for a carbonyl group, δ 130.02 and 128.04 for the presence of a C=C double bond, 14.16 for a methyl group carbon, δ 41.67 for the α-carbon atom of the carbonyl group. A group of signals 22.21, 22.33, 24.77, 25.51, 26.90, 28.81, 28.97, 29.14, 29.25, 31.18, 31.54, 33.98 (Fig 48) are due to the long chain methylene groups carbon atom of the fatty acid. Presence of two carbon atoms in the unsaturated region of the spectrum suggests that only one double bond is present in the compound. Based on the above data the assumption of the compound is a unsaturated fatty acid (Myristoleic acid).

CGA-V

The IR spectra exhibited absorption bands at 1709 cm^{-1} showing the presence of a carbonyl group, 1581 cm^{-1} for a C=C group and at 2925, 2854 cm^{-1} and 1465 and 1375 cm^{-1} for the presence of C-H.

The ^{1}H-NMR spectrum shows the presence of a triplet signal at δ 0.81 for 6 protons suggesting the presence of two methyl group. This along with the complex multiplet signal at δ 1.56 indicates the presence of an isopropyl group. The broad singlet at δ 1.29 for 16

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protons strongly suggests the presence of a long chain of methylene groups in the compound. The triplet at δ 2.29 for two protons (H-2) is assigned to a methylene group attached to a carbonyl group The signal at δ 2.00 is indicative of the presence of a methylene group in the allylic position to the double bond. The multiplet signal at δ 5.29 (Fig 49) is due the protons of the double bond. This suggests the presence of a long chain unsaturated fatty acid. Absence of bis-allylic protons between δ 2.7 and 2.8 indicates that the double bonds are conjugated.

The above assignment was supported by the ^{13}C-NMR spectral data by exhibiting a signal at δ 180.00 for a carbonyl group, δ 130.22,130.02 128.07 and 127.91 for the presence of two C=C double bond, 11.50 for a methyl group carbon, δ 33.94 for the α-carbon atom of the carbonyl group. A group of signals 22.56, 22.68, 24.71, 25.63, 27.18, 27.28, 27.20, 29.04, 29.14, 29.25, 29.35, 29.43, 29.58, 29.68, 31.52, 31.92 (Fig 50) are due to the long chain methylene groups carbon atom of the fatty acid. Based on the above data the assumption of the compound is Linoleic acid an unsaturated fatty acid with two double bonds conjugated to each other.

CGA-VIII

The IR spectra exhibited absorption bands at 1595 cm^{-1} for a C=C group and at 2925, 2854 cm^{-1} and 1465 and 1375 cm^{-1} for the presence of C-H.

The ^{1}H-NMR spectrum shows the presence of a multiplet signal at δ 0.765 for 12 protons suggesting the presence of four methyl group. The broad singlet at δ 1.18 for 10 protons strongly suggests the presence of a long chain of methylene groups in the compound. A group of signals at δ 1.56 indicates the presence of methyl groups attached to the unsaturated carbon atoms. The triplet at δ 2.01 and 1.93 for eight protons is assigned to a methylene group attached to allylic position position of the double bonds. The multiplet signal at δ 5.10 (Fig 51) is due the protons of the double bond. The above assignment was supported by the ^{13}C-NMR spectral data by exhibiting signals at δ 135.06, 134.85, 131.18, 124.43, 124.32 and 124.29 for the presence of three C=C double bond, 14.09, 15.98, 16.02 and 17.65 for four methyl group carbons, δ 39.75, 39.73, 37.10, and 37.02 belongs to the carbon adjacent to quaternary atoms, A group of signals 22.68, 25.66, 26.67, 26.78, 28.27, 29.35, 29.60, 29.69, 30.03 and 31.92 (Fig 52) are due to methylene groups of a long chain unsaturated hydrocarbon carbon atoms. Based on the above data the assumption of the compound is an unsaturated hydrocarbon with three double bonds conjugated to each other. It may be a sesquiterpenoid (8E, 10E, 12E)-icosa-8, 10, 12-triene).

II. CONCLUSION

A compound **CGA-(I)** octyl 4-(nonanoyloxy) benzoate along with myristoleic acid **(CGA-(II)**, linoleic acid **(CGA-(V)**, (8E, 10E, 12E)-icosa-8, 10, 12-triene) **(CGA-(VIII)** were isolated from *C. guianensis* and characterized by IR, ^{1}H-NMR and ^{13}C-NMR spectral data.

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