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Isolation of phytoconstituents from the flowers of *Couroupita guianensis*

Velliangiri Prabhu*[a] & Subban Ravi[b]

[a] Department of Chemistry, Sri Shakthi Institute of Engineering and Technology, Coimbatore 641 062, India
[b] Department of Chemistry, Karpagam University, Karpagam Academy of Higher Education, Coimbatore 641 021, India
E-mail: prabhunmr@gmail.com

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Couroupita guianensis is used extensively as an ingredient in many Ayurveda preparations which cure gastritis, scabies, bleeding piles, dysentery, and scorpion poison. The flower has been subjected to sequential extraction using petroleum ether, chloroform, ethyl acetate and methanol solvents. A new compound **I** Cycloart-24-en-3-ol-4'-exomethylene heptadeconate along with stigmasterol **II**, *p*-coumaric acid **III**, *o*-coumaric acid **IV**, caffeic acid **V** and quercetin **VI** have been isolated by column chromatography and characterised using IR, ^{1}H and ^{13}C NMR and MS spectral data. Compound **I, III**, **IV** and **V** are reported for the first time from *C. guianensis*.

Keywords: *Couroupita guianensis*, *Lecythidaceae*, flower, cycloart-24-en-3-ol-4'-exo methylene heptadeconate

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 other[1,2] applications. The fruit pulp is used as a cure for 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, immunomodulatory, anthelmintic, antimicrobial, wound healing, antioxidant and antinociceptive activities[3-8]. 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, farnesol, nerol, tryptanthrine, indigo, indirubin, isatin, linoleic acid, α,β-amyrins, carotenoids and sterols[9-12]. Even though the uses of the plant parts and their extracts are well known 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 six compounds (**I**) to (**VI**) from *C. guianensis* by chromatographic and spectral methods.

Results and Discussion

Fresh flowers of *C. guianensis* were collected from Palakkad district in Kerala and extracted with petroleum ether under cold percolation and concentrated *in vacuo* to yield 6.3 g of the residue (% on w/w basis). It was further subjected to sequential extraction with chloroform, ethyl acetate and methanol to yield 11.6, 12.2 and 18.2 g of residue respectively. The obtained residues were subjected to coloumn chromatography separately which led to the isolation of compounds **I** to **VI**. From the petroleum ether extract, compounds **I** and **II** were isolated and identified as cycloart-24-en-3-ol-4′-exo methylene hepta deconate and stigmasterol respectively by spectral methods. Compound **I** was isolated from *C. guianensis* for the first time. Compounds **III**, **IV** and **V** were also isolated for the first time from the

methanol extract of *C. guianansis* and identified as *p*-coumaric acid **III**, *o*-coumaric acid **IV**, caeffic acid **V**. Compound **VI** was isolated from the ethyl acetate extract and identified as quercetin **VI** (Figure 1) by IR, NMR and MS data, and by comparison of spectral data with the reported values[13-17].

The compound **I** analysed for the molecular formulae $C_{48}H_{82}O_2$ exhibited a molecular ion peak at *m/z* 690.6315 in its EI mass spectra. The IR spectrum of compound **I** showed the presence of carbonyl group (1688 cm^{-1}), C=C (1600 cm^{-1}), −C=CH_2 (1413 cm^{-1}), C-O (1220 cm^{-1}) and C-H stretching at 3040 cm^{-1} (cyclopropane ring), 2916 and 2848 cm^{-1}. ^{1}H NMR spectra revealed the presence of four singlet methyls at δ 1.18 (H-19), 1.19 (H-28), 0.82 (H-29), 0.83 (H-30), three secondary methyls at δ 0.89 (d, J = 7 Hz, H-21), 1.55 (d, J = 7 Hz, H-26, 27), a pair of doublets in the up-field area (δ_H 0.26, d, J = 4.1 Hz and at δ 0.50, d, J = 4.1 Hz), characteristics of cycloartane cyclopropane ring. A double doublet carbinolic proton at δ 4.4 (dd, $J_{ax,ax}$ = 11.4 and $J_{ax,eq}$ = 4.4 Hz) suggested the β-orientation of the oxygen function and its appearance in the downfield region suggests that it was esterified. For biogenetic reasons the oxygen function was placed at C-3 position (the spectral data of ^{1}H NMR, ^{13}C NMR and DEPT-135 spectra of compound **I** is shown in Table I). The signal at δ 80.35 in ^{13}C NMR spectra supported the presence of the carbinolic carbon (C-3). The esterification was confirmed by the presence of the carbonyl carbon at δ 178.00. A multiplet signal at δ 5.01 which integrated for only one proton indicates the presence of a double bond and also suggests that one carbon is trisubstiuted and the other carbon is tetrasubstiuted. It was supported by the appearance of carbon signals at δ 125.27 and 130.86 in the ^{13}C NMR spectra and the disappearance of the signal at δ 130.86 in DEPT-135 spectrum. This along with two methyl groups at δ 1.55 indicates the presence of a terminal isopropylidene group. This suggests that the compound is 24,25-dihydrocycloartane type derivative.

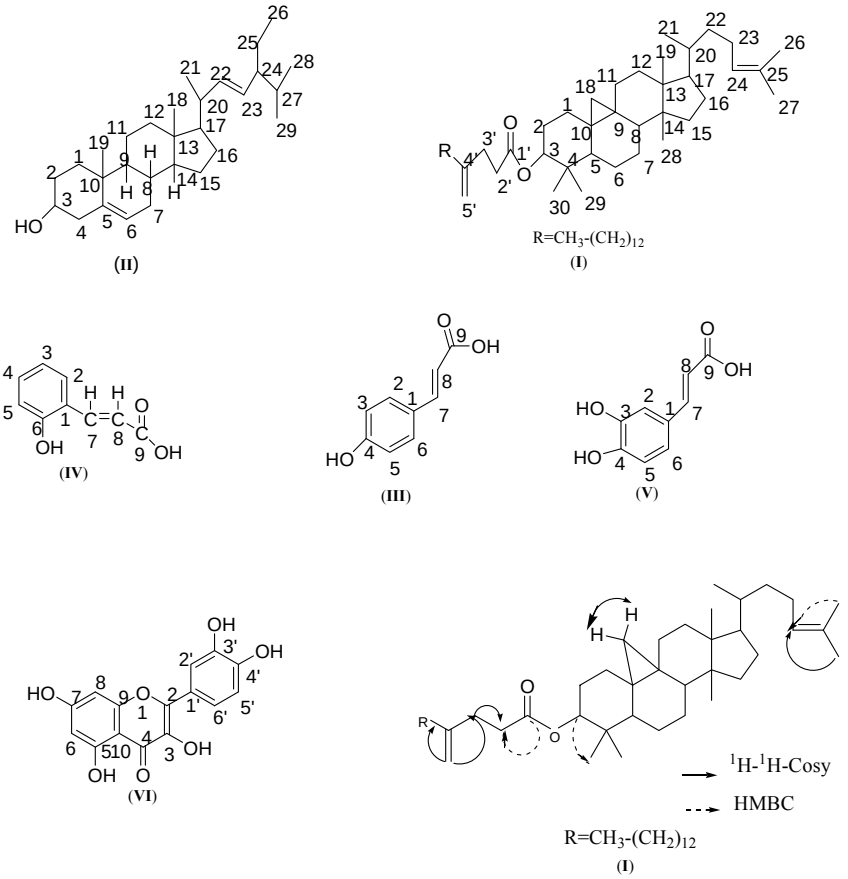

Figure 1 — Structure of the compounds (**I**), (**II**), (**III**), (**IV**), (**V**) and (**VI**) and key HMBC (^{1}H ->^{13}C), ^{1}H-^{1}H-COSY correlations for compound (**I**)

Table I — ^{1}H (400 MHZ) and ^{13}C (100 MHZ) NMR, DEPT-135 spectral data of compound (I) in $CDCl_3$.

Position	Signal (δ)		DEPT-135	Position	Signal (δ)		DEPT-135
	Carbon	Proton			Carbon	Proton	
1	31.92		Down	26	32.87	1.551(3H, d, *J* = 7.0 Hz)	Up
2	30.45		Down	27	19.28	1.551(3H, d, *J* = 7.0 Hz)	Up
3	80.35	3.9(1H, t, *J* = 11.4, 4.4 Hz)	Up	28	25.70	1.19 (3H, s)	Up
4	40.48		–	29	18.24	0.823 (3H, s)	Up
5	47.85		Up	30	20.15	0.831 (3H, s)	Up
6	21.14		Down	1′	178		–
7	28.13		Down	2′	17.97	2.30(2H, t, *J* = 7.0 Hz)	Down
8	47.19		Up	3′	21.87	1.6 (2H, t)	Down
9	20.41		–	4′	155		–
10	26.00		–	5′	105.95	4.52, 4.59 (2H, d, d, *J* = 0.8 Hz)	Up
11	29.58		Down	6′	25.17		Down
12	34.87		Down	7′	25.44		Down
13	45.31		–	8′	29.26		Down
14	48.81		–	9′	29.36		Down
15	31.33		Down	10′	29.46	1.29 (2H, m)	Down
16	26.85		Down	11′	29.69	1.29 (2H, m)	Down
17	52.27	1.536 (1H,s)	Up	12′	29.77	1.29 (2H, m)	Down
18	29.18	0.26 (1H, d, *J* = 4.1 Hz 0.50 (1H, d, *J* = 4.1 Hz)	Down	13′	31.61	1.29 (2H, m)	Down
19	15.21	1.185 (3H, s)	Up	14′	33.82	1.29 (2H, m)	Down
20	36.12		Up	15′	35.54	1.29 (2H, m)	Down
21	17.62	0.892(3H, d, *J* = 7.0 Hz)	Up	16′	35.89	1.29 (2H, m)	Down
22	22		Down	17′	36.35	1.29 (2H, m)	Down
23	22.68	1.60 (2H, m)	Down	18′	20.93	0.8 (3H, m)	Up
24	125.27	5.01 (m)	Up				
25	130.86		–				

Further, along with the peak at δ 178 for the carbonyl group a bunch of carbon signals in the ^{13}C NMR were observed between δ 21.87 to 20.93 suggesting the presence of a long chain fatty acid moiety in the compound. This was complemented by the presence of a broad singlet at δ 1.29 and a triplet at δ 2.3 for two protons (α protons to C=O group), in the ^{1}H NMR spectra. The mass spectrum indicates it may be a C_{18} fatty acid by exhibiting peaks at *m/z* 283.26, 238.75, 212.81, 198.80, 184.77, 170.83, 156.87, 142.87 and 128.89 showing the presence of long chain methylene group in the ^{1}H NMR. The presence of a exo methylene group in the acid moiety was indicated by the presence of carbon signals at δ 105.95 and 155.00 in the ^{13}C NMR spectra[18]. The absence of the signal at δ 155.00 in the DEPT-135 spectra, suggests that it is a quaternary carbon atom. The signal at δ 105.95 appeared as a methylene group signal in DEPT-135. Further HMQC spectrum indicates that both the protons at δ 4.59 and 4.52 belong to the same carbon atom which resonated at δ 105.95.

A critical comparison of the ^{13}C NMR signals of the compound **I** with the spectral values of the cycloartane ring systems in the literature suggests the presence of cycloartane ring[19]. This indicates that the exomethylene group should be present in the esterifying acid moiety. In the ^{1}H-^{1}H-COSY the terminal methylene protons at δ 4.5 showed cross peak with the H-3′ protons at δ 1.6. The H-3′ signal at δ 1.6 showed cross peak with the signal at δ 2.30 (α protons- H-2′) suggesting that the position of the terminal methylene group will be at C-5′. Further, cross peaks were observed between the signals at δ 0.26 (d) and 0.50 (d), indicating the presence of cyclopropane methylene group. Cross peaks were also observed between the signals of the terminal methylene group at δ 4.52 (H-5′) and the long chain methylene group protons at δ 1.6 (H-3′). Additional cross peaks were observed between the signals at δ 1.29 (H-17′) and 0.8 (H-18′) showing the presence of a terminal methyl group Long range couplings (HMBC) were observed between the carbonyl carbon

at δ 178.00 and the α-C-2 carbon atom, the quartenary carbon at C-26 showed correlations with the H-24 confirming the presence of isopropylidene group. Further, the signal at δ 80.35 (C-3) showed long range coupling with the methyl group H-30. Based on the above evidences the structure of the molecule was identified as **I** cycloart-24-en-3-ol-4′-exomethylene heptadeconate.

Materials and Methods

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. Voucher specimen was preserved in our Department (No. KU11CHE1934).

Extraction and Isolation

The 5.2 Kg of powdered, dried flower was extracted thrice (3×72 h) with petroleum ether under cold percolation. The combined extract was subjected to distillation and concentrated *in vacuo* to yield a residue A of 6.3 g. When run on TLC using (8:2) petroleum ether:ethyl acetate it revealed the presence of 3 major compounds with R_f values 0.62, 0.54 and 0.42 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 g silica gel. Initially the column was eluted with petroleum ether and with increasing amount of ethyl acetate, 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) and 18 to 23, compound CGA-**III (I)** (120 mg) were found to be homogeneous by TLC.

After defatting, the plant material was subjected to sequential extraction with chloroform (2×72 h), ethyl acetate (2×72 h) and methanol (2×72 h) to yield residue B (11.6 g), D (12.2 g) and C (18.2 g) respectively. Residue B on column chromatography and on elution with petroleum ether:ethyl acetate (8:2) yielded 89 g of residue in the fractions 3-5 with on R_f value 0.58 compound **CGA-IV (II)** (89 mg) and compound **CGA-V** (46 mg) of reduce from fractions 6-8 and are homogeneous on TLC. 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 on TLC. The ethyl acetate extract D (12.2 g) on column chromatographic separation using 120 g silica gel as the adsorbent led to the isolation of compound **CGA-IX (III)** (98 mg) in fractions 43-58. The solvent used for the elution of the compound is chloroform:methanol (5:5). The methanolic extract C (18.2 g) on chromatographic separation and when eluted with chloroform:methanol (8:2), yielded a mixture (E), major compound with R_f value 0.79 and 3 minor compounds were eluted with chloroform:methanol (7:3) solvent system. Fractions 91-120 (F) showed four spots in TLC (solvent system Toluene:chloroform: acetone (40:25:35) with R_f values 0.58 (major compound) and 3 minor compounds with R_f value 0.84, 0.76 and 0.44. Compound **CGA-X (V)** (67 mg) was isolated by preparative TLC of E (using silica gel G as the stationary phase and toluene: chloroform:acetone (40:25:35)) as the solvent system and compounds **CGA-XI (VI)** (124 mg) and **CGA-XII (IV)** (78 mg) were isolated by the preparative TLC of 'F' and 'G' using the solvent system toluene: acetone: formic acid (18:18:4) and chloroform:diethyl ether (27:3).

Experimental Section

^{1}H and ^{13}C NMR spectra and DEPT-135, ^{1}H-^{1}H-Cosy, HMBC and HSQC were recorded on a Bruker AM-400 (400 MHz) instrument; chemical shifts δ were measured in ppm with TMS as internal standard and coupling constants *J* in Hz. Electrospray Ionization-MS data were recorded on a Bruker Esquire 3000+ Ion-trap mass spectrometer and Electron Impact-MS instrument was performed on a Finnigan MAT-95 mass spectrometer. Perkin-Elmer model 1650 IR instrument was used to obtain the IR spectral data.

Conclusions

A new compound **I** Cycloart-24-en-3-ol-4′-exomethylene heptadeconate along with stigmasterol **I**, *p*-coumaric acid **III**, *o*-coumaric acid **IV**, caffeic acid **V** and quercetin **VI** were isolated from *C. guianensis* and characterized by IR, ^{1}H and ^{13}C NMR, and MS spectral data. Compound **I**, **III**, **IV** and **V** were reported for the first time from *C. guianensis* flower extract.

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