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Phytochemical Studies on the Stem Bark of *Couroupita guianensis* Aubl.

P. SARKAR[1], F.T. ZOHORA[1], A. JABBAR[1], F.S. TAREQ[2], C.M. HASAN[1,*] and M. AHSAN[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
[2]Department of Applied Chemistry and Chemical Technology, University of Dhaka, Dhaka-1000, Bangladesh

*Corresponding author: Tel: +880 2 1819253698; E-mail: cmhasan@gmail.com

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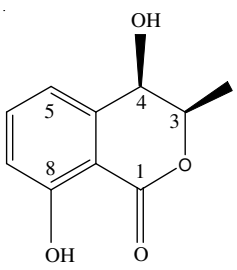
From the phytochemical investigation of the stem bark of *Couroupita guianensis* Aubl (Lecythidaceae) a total of ten compounds have been isolated. An unusual polyketide, an alkaloid, two triterpene caffeates, one diunsaturated ketosteroid, three triterpenes, one phenolic compound and a steroid have been isolated from the stem bark of *Couroupita guianensis* Aubl. The structures of these compounds were elucidated as *cis*-4-hydroxymellein (**1**), tryptanthrin (**2**), betulin-3β-caffeate (**3**), lupeol caffeate (**4**), stigmasta-4,22-dien-3-one (**5**), betulin (**6**), β-amyrin (**7**), lupeol (**8**), methyl gallate (**9**), stigmasterol (**10**) by ^{1}H NMR spectra and confirmed by comparison with previously reported values. The polyketide, *cis*-4-hydroxymellein is the first report from this family as well as lupeol caffeate and stigmasta-4,22-dien-3-one are also the first\ reports from this plant.

Key Words: *Couroupita guianensis* Aubl. (Lecythidaceae), Polyketide, Triterpenes, Phenolic compound, Steroids.

INTRODUCTION

The Lecythidaceae comprise a family of about 20 genera and 250-300 species of woody plants[1]. *Couroupita guianensis* Aubl. or the Cannonball Tree is native to the tropical forests of northeastern South America, especially the Amazon Basin and in the tropics such as in India and Thailand[2]. This plant has been the subject of interest because of the range of biological activities shown by this genus, with having antinociceptive activity[3], protective effect against oxygen reactive species, skin fibroblast stimulation[4] and antidepressant activity in mice[5]. Numerous studies undertaken on this genus revealed the presence of sterols, triterpenes, ketosteroids[6], alkaloids[7,8] and flavonoids[4]. In this paper, we report the isolation and structure elucidation of *cis*-4-hydroxymellein (**1**), tryptanthrin (**2**), betulin-3β-caffeate (**3**), lupeol caffeate (**4**), stigmasta-4,22-dien-3-one (**5**), betulin (**6**), β-amyrin (**7**), lupeol (**8**), methyl gallate (**9**) and stigmasterol (**10**).



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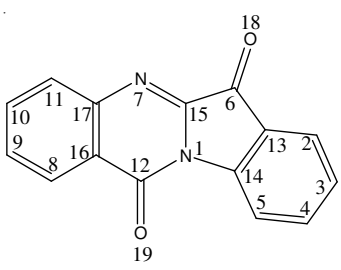

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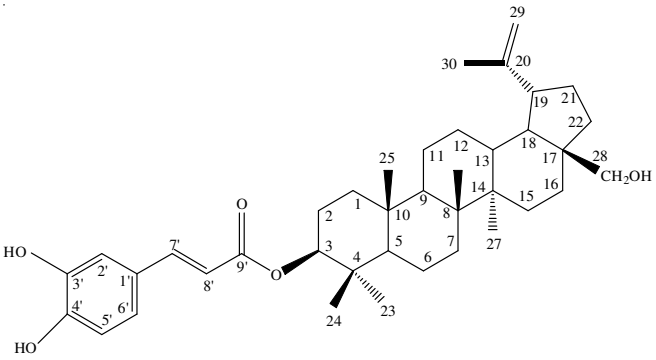

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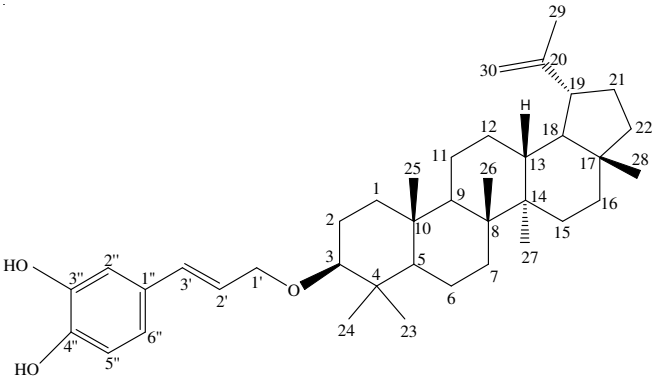

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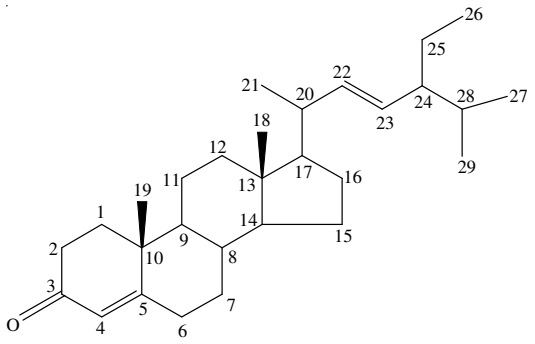

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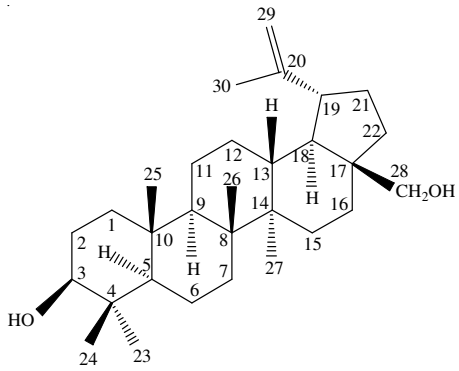

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EXPERIMENTAL

^{1}H NMR spectra were obtained using a Varian Unity 500 spectrometer (500 MHz) in $CDCl_3$ or CD_3OD. Vacuum liquid chromatography (VLC) was carried out on short column packed with TLC grade silica gel (Kiselgel 60H, Merck) that was operated under reduced pressure and column chromatography was conducted on sephadex LH-20. TLC was carried out using Merck precoated TLC plates (Silica gel 60, F_{254}), eluting with suitable solvent system. Spots on the TLC plates were visualized under UV light at 254 and 366 nm as well as by spraying with vanillin sulphuric acid followed by heating for 5 min at 110 ºC.

Plant materials: The stem bark of *Couroupita guianensis* Aubl. (Lecythidaceae) was collected in August, 2011 from Dhaka, Bangladesh. A voucher specimen had been maintained in the herbarium of the Department of Botany, University of Dhaka under the accession number DUH-7130. The samples were cut into small pieces and sun dried for 7 days followed by oven drying for 24 h at 40 ºC to facilitate grinding.

Extraction and isolation: The air-dried and powdered plant materials (700 g) were cold extracted with methanol. The whole mixture was then filtered off through a cotton plug followed by Whatman filter paper No. 1 and the filtrate thus obtained was concentrated at 40 ºC with a rotary evaporator. Then the solvent was evaporated to obtain a solid residue of around 15 g. This was then subjected to vacuum liquid chromatography[9] for the initial rapid fractionation of the crude extract. The column was first eluted with 100 % *n*-hexane. Then the mobile phases with progressively increasing polarity were passed through the column, until it reached to 100 % ethyl acetate. Thin layer chromatographic technique was used for the initial screening of the vacuum liquid chromatography fraction extracts and the compounds-**7** (9 mg), **8** (3.8 mg), **10** (5 mg) were isolated from the vacuum liquid chromatography extracts of 10 and 12 % EtOAc in hexane (compound **7**), 20 % EtOAc in hexane (compound **8**) and 8 % EtOAc in hexane (compound **10**), followed by TLC using Merck precoated TLC plates (Silica gel 60, F_{254}), eluting with toluene/EtOAc (60:40, 80:20, 90:10, respectively). Selected vacuum liquid chromatography fractions which showed mixture of several compounds were then subjected to gel permeation chromatography (GPC) over Sephadex (LH-20) using a mixed solvent system of hexane:chloroform (2:8) followed by mixtures of methanol and chloroform of increasing polarity and finally only with methanol. Depending upon the TLC behaviour fractions were mixed and compound **1** (3 mg), **2** (3.4 mg), **3** (2.8 mg), **4** (3.6 mg), **5** (3 mg), **6** (2.5 mg), **9** (3 mg) were isolated followed by TLC using Merck precoated TLC plates (Silica gel 60, F_{254}), eluting with toluene/EtOAc (85:15, 60:40, 95:5, 80:20, 80:20, 80:20, 90:10, respectively) from vacuum liquid chromatography fraction-50 % EtOAc in hexane and Sephadex fraction No. 20-25, vacuum liquid chromatography fraction-80 % EtOAc in hexane and Sephadex fraction No. 14-18, vacuum liquid chromatography fraction-30 % EtOAc in hexane and Sephadex fraction No. 8-12, vacuum liquid chromatography fraction-25 % EtOAc in hexane and Sephadex fraction No. 10-16, vacuum liquid chromatography fraction-20 % EtOAc in hexane and Sephadex fraction No. 8-14, vacuum liquid chromatography fraction-15 % EtOAc in hexane and Sephadex fraction No. 4-10, vacuum liquid chromatography fraction-40 % EtOAc in hexane and Sephadex fraction No. 20-26, respectively.

***Cis*-4-hydroxymellein (1):** (3 mg); white amorphous solid and it was visualized as a violet spot under UV light (both 254 and 356 nm) on TLC plate but it did not show any colour after spraying with vanillin-sulphuric acid reagent followed by heating at 110 ºC for 5-10 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.04 (1H, s, 8-OH), 7.53 (1H, t, *J* = 8.0,7.5 Hz, H-6), 7.03 (1H, d, *J* = 7.0 Hz, H-7), 6.92 (1H, d, *J* = 7.0 Hz, H-5), 4.69 (1H, dq, *J* = 1.5, 6.5 Hz, H-3), 4.58 (1H, br.s, H-4), 1.59 (3H, d, *J* = 6.5 Hz, H-3).

Tryptanthrin (2): (3.4 mg); yellow needles; the compound showed dark quenching spot under UV light (254 nm) on TLC plate as well as it also showed yellow colour spot after spraying with vanillin-sulphuric acid reagent followed by heating at 110 ºC for 5-10 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.63 (1H, d, *J* = 8.0 Hz, H-10), 8.45 (1H, d, *J* = 8.0 Hz, H-1), 8.03 (1H, d, *J* = 8.0 Hz, H-4), 7.91 (1H, dd, *J* = 8.0,7.0 Hz, H-3), 7.85 (1H, dd, *J* = 8.0, 7.0 Hz, H-9), 7.79 (1H, dd, *J* = 8.0,8.0 Hz, H-2), 7.43 (1H, dd, *J* = 8.0, 7.0 Hz, H-8).

Betulin-3β-caffeate (3): (2.8 mg); white amorphous solid; the compound did not show any UV sensitivity on (254 nm) TLC plate and it was evident as a pinkish red spot after spraying with vanillin-sulphuric acid reagent followed by heating at 110 ºC for 5-10 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53 (1H, d, *J* = 15.5 Hz, H-7'), 7.07 (1H,s, H-2'), 7.01 (1H, d, *J* = 8.5 Hz, H-6'), 6.85 (1H, d, *J* = 8.0 Hz, H-5'), 6.25 (1H, d, *J* = 15.5 Hz, H-8'), 4.67 (1H, br. s, H-29), 4.60 (1H, t, H-3), 4.58 (1H, br. s, H-29), 3.79 (1H, d, *J* = 10.5 Hz, H-28), 3.33 (1H, d, *J* = 10.0 Hz, H-28), 1.68 (3H, s, H-30), 1.02 (3H, s, H-

27), 0.98 (3H, s, H-26), 0.90 (3H, s, H-23), 0.87 (3H, s, H-25), 0.86 (3H, s, H-24).

Lupeol caffeate (4): (3.6 mg); off-white crystal; it did not show any UV sensitivity (254 nm) on TLC plate and was evident as a purple spot after spraying with vanillin-sulphuric acid reagent followed by heating at 110 ºC for 5-10 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.52 (1H, d, *J* = 15 Hz, H-3'), 7.07 (1H, s, H-2"), 6.95 (1H, d, *J* = 8.0 Hz, H-6"), 6.84 (1H, d, *J* = 8.0 Hz, H-5"), 6.23 (1H, d, *J* = 16 Hz, H-2'), 4.72 (1H, br. s, H-29), 4.59 (1H, t, H-3), 4.57 (1H, br. s, H-29), 1.68 (3H, s, H-30), 1.04 (3H, s, H-27), 0.98 (3H, s, H-23), 0.96 (3H, s, H-26), 0.85 (3H, s, H-25), 0.84 (3H, s, H-24), 0.78 (3H, s, H-28).

Stigmasta-4,22-dien-3-one (5): (3 mg); white amorphous solid; it showed blue fluorescence under UV light (366 nm) on TLC plate and after spraying with vanillin-sulphuric acid reagent followed by heating at 110 ºC for 5-10 min, it was evident as a purple spot. ^{1}H NMR (500 MHz, CDCl3): δ 5.81 (1H, s, H-4), 5.11 (1H, m, H-22), 5.03 (1H, m, H-23), 4.03 (1H, m, H-3), seven methyl groups from δ 0.7-1.4 ppm.

Betulin (6): (2.5 mg); white needles; it showed quenching spot under UV light (254 nm) on TLC plate and was evident as a purple spot after spraying with vanillin-sulphuric acid reagent followed by heating at 110 ºC for 5-10 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.67 (1H, br.s, H-29), 4.57 (1H, br.s, H-29), 3.79 (1H, d, *J* = 11.5 Hz, H-28), 3.32 (1H, d, *J* = 11.0 Hz, H-28), 3.18 (1H, br.d, *J* = 11.5 Hz, H-3), 1.67 (3H, s, H-30), 1.01 (3H, s, H-27), 0.97 (3H,s, H-26), 0.96 (3H, s, H-23), 0.81 (3H, s, H-25), 0.75 (3H, s, H-24).

β-Amyrin (7): (9 mg); white, needle like crystal; it was visualized as a quenching spot under UV light (254 nm) on TLC plate and showed an intense pinkish colour after spraying with vanillin-sulphuric acid reagent followed by heating at 110 ºC for 5-10 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.17 (1H, dd, *J* = 3.5 Hz and 3.5 Hz, H-12), 3.21 (1H, dd, *J* = 11.0 Hz and 6.0 Hz, H-3), 1.12 (3H, s, Me-27), 0.99 (3H, s, H-26), 0.96 (3H, s, H-23), 0.93 (3H, s, H-25), 0.86 (6H, s, H-29 and H-30), 0.82 (3H, s, H-28), 0.78 (3H, s, H-24).

Lupeol (8): (3.8 mg); white crystals; it showed blue fluorescence under UV light (366 nm) on TLC plate and was evident as a purple spot after spraying with vanillin-sulphuric acid reagent followed by heating at 110 ºC for 2 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.70 (1H, br. s, H-29), 4.59 (1H, br. s, H-29), 3.20 (1H, m, H-3), 1.67 (3H, s, H-30), 1.02 (3H, s, H-27), 0.98 (3H, s, H-26), 0.96 (3H, s, H-25), 0.82 (3H, s, H-24), 0.78 (3H, s, H-23), 0.75 (3H, s, H-28).

Methyl gallate (9): (3 mg); colourless needles; it was visualized as a purple spot under UV light (254 nm) on TLC plate and was evident as a purple spot after spraying with vanillin-sulphuric acid reagent followed by heating at 110 ºC for 5-10 min. ^{1}H NMR (500 MHz, $CDCl_3$ and CD_3OD): 7.03 (2H, s, H-2,6), 3.78 (3H, s, OCH_3).

Stigmasterol (10): (5 mg); white needles; ^{1}H NMR (500 MHz, $CDCl_3$): 5.35 (1H, d, *J* = 8.2 Hz, H-6), 5.27 (1H, dd, *J* = 15.2, 8.0 Hz, H-22), 5.18 (1H, dd, *J* = 15.2, 7.2 Hz, H-23), 3.52 (1H, m, H-3), 1.01 (3H, s, H-10), 0.91 (3H, d, *J* = 6.8 Hz, H-20), 0.83* (3H, d, *J* = 6.0 Hz, H-25), 0.83* (3H, d, *J* = 6.0 Hz, H-25), 0.82 (3H, t, *J* = 6.5 Hz, H-25), 0.67 (3H,s, H-13) (Assignments are interchangeable).

RESULTS AND DISCUSSION

Repeated chromatographic separation and purification of the methanolic extract of the stem bark of the plant of *Couroupita guianensis* Aubl. over silica gel provided ten compounds. The structures of these compounds were determined by analysis of ^{1}H NMR spectral as well as by comparison with previously reported values.

The ^{1}H NMR spectrum of compound **1** showed three downfield signals for aromatic protons which appeared as one proton doublets at δ 6.92 (1H, d, *J* = 7.0 Hz, H-5), δ 7.03 (1H, d, *J* = 7.0 Hz, H-7) and a one proton triplet at δ 7.53 (1H, t, *J* = 7 Hz, H-6). The *cis*-isomer was characterized by the presence of these three downfield signals. It also revealed the presence of a three-proton doublet at δ 1.59 (3H, d, *J* = 6.5 Hz, H -3) which was assigned to a methyl group. The proton NMR also showed a one proton doublet of quartet at δ 4.69 (1H, dq, *J* = 1.5, 6.5 Hz, H-3) and a one proton singlet at δ 4.58 (1H, br.s, H-4). Furthermore, a hydrogen bonded phenolic proton appeared as a highly deshielded one proton singlet at δ 11.04 (1H, s, 8-OH). The *cis*-configuration was assigned to H-3 and H-4 on the basis of the small coupling constant (1.5 Hz).

The above spectral features were similar to the ones reported for *cis*-4-hydroxymellein[10]. On this basis, compound **1** was characterized as *cis*-4-hydroxymellein.

The ^{1}H NMR spectrum of compound **2** displayed a complex group of signals at δ 7.2-8.7 ppm. All hydrogen atoms must, therefore, be bound to the aromatic system. Since no N-H resonance appears in the spectrum, also the two nitrogen atoms are claimed to be parts of an enlarged aromatic molecule.

After comparison of the spectral data with those published in the literature[11], the structure was elucidated as tryptanthrin.

The ^{1}H NMR spectrum of compound **3** exhibited the presence of six singlets for methyl groups at δ 0.90 (3H, s, H-23), 0.86 (3H, s, H-24), 0.87 (3H, s, H-25), 0.98 (3H, s, H-26), 1.02 (3H, s, H-27), 1.68 (3H, s, H-30). The spectrum also showed the presence of a set of geminal protons at δ 3.79 (1H, d, *J* = 10.5 Hz, H-28) and 3.33 (1H, d, *J* = 10.0 Hz, H-28) and two exocyclic methylene protons at δ 4.67 and 4.58 (1H, Br.s each, H-29). The presence of the proton with oxygenated carbon at δ 4.60 (1H, t, H-3) can be assigned to 3β position.

The spectrum also showed the presence of three aromatic protons at δ 7.07 (1H, s H-2'), δ 6.85 (1H, d, *J* = 8.0 Hz, H-5') and δ 7.01 (1H, d, *J* = 8.5 Hz, H-6'). The coupling relationship of these protons established a 1,3,4-substituted ring. The coupling constant of the proton signals at δ 7.53 (1H, d, *J* = 15.5 Hz, H-7') and δ 6.25 (1H, d, *J* = 15.5 Hz, H-8') indicate the presence of two *trans* olefinic protons. Thus, it was assumed that there is an O-caffeoyl group.

The structure of compound **3** was determined as betulin-3β-caffeate with a comparison of the above data with that published in the literature[12].

The ^{1}H NMR spectrum of compound **4** revealed the presence of two characteristic olefinic signals of lupeol. These two olefinic protons appeared as a singlet at δ 4.72 (1H, s,) and 4.59 (1H, s). The proton NMR spectrum also showed s even methyl singlets at δ 1.68, 1.04, 0.98, 0.96, 0.85, 0.84 and 0.78.

The spectrum also showed the presence of three downfield signals for aromatic protons at δ 7.07 (1H, s, H-2''), δ 6.95 (1H, d, *J* = 8.0 Hz, H-6''), δ 6.84 (1H, d, *J* = 8.5 Hz, H-5''). The coupling relationship established a 1,3,4-substituted ring. The coupling constant of the proton signals at δ 7.52 (1H, d, *J* = 16 Hz, H-3') and δ 6.23 (1H, d, *J* = 16 Hz, H-2') indicate the presence of two *trans* olefinic protons. Thus, it was assumed that there is an O-caffeoyl group. The spectral features were similar to the ones reported for lupeol caffeate[13]. On this basis, compound **4** was characterized as lupeol caffeate.

The ^{1}H NMR spectrum of compound **5** displayed a one proton multiplet at δ 4.03, the position and multiplicity of which was indicative of H-3 of the steroidal nucleus. The signal for the H-4 of the steroidal skeleton was evident from a singlet at δ 5.81. The olefinic protons (H-22 and H-23) appeared as characteristics downfield signals at δ 5.11 and δ 5.03, respectively. Furthermore, the spectrum also showed seven methyl groups from δ 0.7-1.4 ppm. With a comparison of the above data with those published in the literature[14], the structure of compound **5** was determined as stigmasta-4,22-dien-3-one.

The ^{1}H NMR spectrum of compound **6** exhibited the presence of six singlet for methyl groups at δ 1.01 (3H,s, H-27), δ 0.97 (3H, s, H-26), δ 0.81 (3H, s, H-25), δ 0.75 (3H, s, H-24), δ 0.96 (3H, s, H-23), δ 1.67 (3H, s, H-30). The spectrum also showed the presence of a set of geminal protons at δ 3.79 (1H, d, *J* = 11.5 Hz, H-28) and 3.32 (1H, d, *J* = 11.0 Hz, H-28) and also two exocyclic methylene protons at δ 4.67 and 4.57 (1H Br. s, each, H-29). The presence of the proton with oxygenated carbon at δ 3.32 (1H, d, *J* = 11.5 Hz, H-3) can be assigned to a 3β-hydroxyl functionality. The structure of compound **6** was elucidated as betulin with a comparison of the above data with that published in the literature[15].

The ^{1}H NMR spectrum of compound **7** showed the presence of eight methyl singlets at δ 0.82 (3H, s, H-28), 1.12 (3H, s, H-27), 0.99 (3H, s, H-26), 0.93 (3H, s, H-25), 0.78 (3H, s, H-24), 0.96 (3H, s, H-23), 0.86 (6H, s, H-29 and H-30) on an oleanane skeleton *i.e.*, the compound must be a pentacyclic compound. A characteristic double doublet at δ 5.17 (1H, dd, *J* = 3.5, 3.5 Hz) was assigned to H-12, suggesting an olean-12-ene skeleton. One methane proton at δ 3.21 (1H, dd, *J* = 11.05, 6.0 Hz) showed that compound **7** has at least one hydroxyl group. The coupling constant of this methane proton indicates that the hydroxyl function must be in axial position. The spectral features were similar to the ones reported for β-amyrin[16]. On this basis, compound **7** was characterized as β-amyrin.

The ^{1}H NMR spectrum of compound **8** displayed two olefinic protons at δ 4.70 and 4.57 (1H, Br.s each), a vinylic methyl at δ 1.67 (3H, s, H-30) and six tertiary methyl at δ 1.02 (3H, s, H-27), 0.98 (3H, s, H-26), 0.96 (3H, s, H-25), 0.82 (3H, s, H-24), 0.78 (3H, s, H-23) and 0.75 (3H, s, H-28).

Further the spectrum showed signal at δ 3.20 (1H, m), attributable to H-3. The compound was identified as lupeol by comparison with the published data[17].

The ^{1}H NMR spectrum of compound 9 showed signal at δ 7.03 (2H, s, H-2,6), integrating for two protons. The downfield resonance of this signal suggested the presence of a tetra substituted phenolic derivative. The spectrum also revealed the presence of a signal at δ 3.78 (3H, s, OCH_3) which could be attributable to methoxyl proton.

The structure of compound **9** was determined as methyl gallate with a comparison of the above data with those published in the literature[18].

On the basis of comparison of the ^{1}H NMR data of compound **10** with previously reported values[19], allowed to characterize it as stigmasterol. Again the identity of compound **10** was further confirmed by co-TLC with an authentic sample.

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