Suzy A. El-Sherbeni et al /J. Pharm. Sci. & Res. Vol. 8(9), 2016, 1034-1041

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

A New Clerodane Diterpene, Flavonoids and Sterols from *Cassia nodosa* Growing in Egypt: Anti-inflammatory Activity of Plant Extracts

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Abstract

Column chromatography of different fractions of leaves, flowers and stem bark of *Cassia nodosa* Buch.-Ham. ex Roxb. afforded seven compounds. The purified compounds included one triterpenoidal and two steroidal compounds were identified as lupeol **1**, β-sitosterol **2** and β-sitosterol-3-*O*-*β*-D-glucopyranoside **3;** a homoisoflavanone which was identified as 5,3`,4`-trimethoxy-7-methyl-3-benzyl chromanone **4**; two flavonoids which were identified as: kaempferol-3-*O*-α-L-rhamnoside **5** and quercetin-3-*O*-α-L-rhamnoside **6;** a new diterpene which was identified as 2-methoxy-3-en-15-*O*-(4`-chlorobenzoyl) clerodane **7.** Compound **4** was isolated for the first time from *Cassia nodosa*. The structural elucidation of the isolated compounds was based on physical characters, chromatographic behavior as well as spectroscopic analysis. ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC experiments were used to identify compound **7**. The anti-inflammatory activity of the methanolic extracts of leaves, flowers and stem bark, as well as different fractions of leaves methanolic extract was carried out. The total methanolic extract of leaves exhibited the highest activity. The data revealed that the ethyl acetate fraction of the leaves possessed the highest and most significant anti-inflammatory activity.

Keywords: Anti-inflammatory activity, *Cassia nodosa*, homoisoflavanone, kaempferol-3-*O*-α-L-rhamnoside, 2-methoxy-3-en-15-*O*-(4`-chlorobenzoyl) clerodane, quercetin-3-*O*-α-L-rhamnoside.

1. INTRODUCTION

Cassia nodosa Buch.-Ham. ex Roxb. [1] is one of species of family Fabaceae [2]. It is also called flowering or pink cassia. It is scattered in India, Malay Peninsula, Sumatra and Thailand as ornamental large deciduous trees [3]. It is considered as a sub-species of *Cassia javanica* L. [4, 5]. It contains different natural products such as flavonoids, anthraquinones, chromones, coumarins, triterpens, sterols and fatty acids [6-10]. It was used in traditional medicine for treatment of different ailments as ring worms, cheloid tumor, insect bite and rheumatism [11]. Seeds, leaflets, petioles and bark of the plant showed an insecticidal activity [12]. Recent studies proved that the leaves possessed significant hypoglycemic activity [13]. Different extracts of leaves and ethanolic extract of root bark showed anti-microbial activities against different micro-organisms [10, 14]. The present study concerned with the isolation and structural elucidation of seven different compounds from leaves, flowers and stem bark of *Cassia nodosa* Buch.-Ham. ex Roxb. as well as the study of the anti-inflammatory activity of different plant extracts.

2. MATERIALS AND METHODS

2.1. Plant material

The leaves, flowers and stem bark were collected from a private garden in Meet Mohsen village in El-Dakahlia governorate, Egypt, May 2011. The plant was kindly identified by Prof. Dr. Mohammed Ibrahim Fotoh, Prof. of ornamental horticulture and landscape design, Faculty of Agriculture, Tanta University, Tanta, Egypt.

2.2. Instruments and materials

Melting points were uncorrected and determined using melting point apparatus Stuart, Bibby Scientific Limited Stone, UK. UV spectra were recorded on Shimadzu UV/Vis spectrophotometer, UV-1800, Japan. IR spectra were recorded using Jasco FT/IR-6100 (Japan) and Nexus 670 FTIR (Nicolet Co., USA) spectrophotometers. Mass spectra were obtained on Thermo Scientific ISQ Single Quadrupole MS, USA and Thermo Scientific TSQ Quantum Access MAX triple quadrupole system, USA. ^{1}H-NMR and ^{13}C-NMR measurements were obtained using NMR Jeol ECA (500 MHz), Japan and Bruker High performance Digital FT-NMR spectrophotometer Avance III (400 MHz), Germany, using solvents as $CDCl_3$, CD_3OD and DMSO-d_6. Two-Dimensional NMR experiments (^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC) were performed with standard Bruker program. Column chromatography was performed on silica gel G 60 (70-230 mesh, Merck). TLC was carried out using precoated TLC plates of silica gel 60 F_{254} (Merck) and silica gel 60 RP-18 F_{254} sheets (5 x 7.5 cm, Merck). Sheets of Whatman No.1 filter paper were used for paper chromatography (Whatman Ltd., England). Anti-infalmmatory activity was carried out using Indomethacin and carrageenan, which were purchased from Sigma Aldrich Co., in addition to normal saline and tween 80 (El-Nasr Chemical CO., Egypt).

2.3. Extraction and isolation

The dried stem bark, flowers and leaves (1.5 Kg each) of *Cassia nodosa* were extracted at room temperature with 95 % aqueous methanol (3 x 5 L) till exhaustion to yield after

evaporation under vacuum dry extracts of 100.43 g, 120.72 g and 172.8 g, respectively. Fractionation of each crude methanolic extract was carried out successively using petroleum ether, methylene chloride, ethyl acetate and *n*-butanol.

Petroleum ether fraction of stem bark was saponified (1 g) to obtain the unsaponifiable matter (0.69 g) which was divided according to solubility into two fractions. One fraction was dissolved in petroleum ether-methylene chloride (1:1) mixture and the second fraction was dissolved in methylene chloride-methanol (1:1) mixture. The first fraction was chromatographed on a silica gel column (1.5x30 cm, 20 g). Elution was started with petroleum ether 100% then petroleum ether - methylene chloride mixture (1% gradual increments of methylene chloride) for the first column. The collected fractions (20 ml each) were pooled according to TLC behavior. Fractions eluted with petroleum ether- methylene chloride (96:4) and (94:6) were chromatographed separately on silica gel column (1x30 cm, 10 g) to yield pure white powder of compound **1 (**6 mg) and pure white needle crystals of compound **2** (22 mg), respectively. The second fraction was chromatographed on silica gel column (1.5x30 cm, 20 g) and eluted with methylene chloride – methanol mixture (2% gradual increments of methanol). The collected fractions (20 ml each) were pooled in according to TLC behavior. Fractions obtained with eluent ratio of methylene chloride - methanol (94:6) was chromatographed on silica gel column (1x30 cm, 10 g) to afford pure 5 mg of compound **3**.

Chromatographic investigation of 5 g of ethyl acetate fraction of total methanolic extract of flowers was carried out using a silica gel column (4x40 cm, 150 g), eluted by methylene chloride-methanol mixture. Fractions of 100 ml volume were pooled according their TLC pattern to afford seven main groups of fractions. The third group (147 mg) was eluted with methylene chloride - methanol (93:7) was then re-chromatographed on silica gel column (1x30 cm, 10 g), followed by sephadex LH-20 column (1x20 cm, 5 g), respectively to produce pure white powder (7 mg) of compound **4**. The fourth group (500 mg) was eluted with methylene chloride : methanol (92:8) was re-chromatographed on silica gel column (1.5x30 cm, 20 g) followed by reversed phase ODS column (1x30 cm, 10 g) starting with 100% water and decreasing polarity with methanol. At 70 % methanol a pure yellow powder of compound **5 (**30 mg) was obtained. The fifth group (670 mg) was eluted with methylene chloride- methanol (91:9) and re-chrmatographed on silica gel (1.5x35 cm, 25 g) then ODS columns (1x30 cm, 10 g) starting with 100% water and decreasing polarity with methanol. Compound **6** (35 mg) was obtained from ODS column at 50 % methanol-water.

Methylene chloride fraction (2 g) of crude methanolic extract of leaves was chromatographed on silica gel column (2x50 cm, 70 g) starting with methylene chloride. Fractions obtained at 100% methylene chloride were pooled, concentrated and re-chromatographed on silica gel column (1x30 cm, 10 g) and purified several times with acetone to afford (33 mg) of compound **7**.

2.4. Method of preparation of unsaponifiable matter

Petroleum ether fraction of crude methanolic extract of stem bark (1 g) was saponified by refluxing at 100°C, with 40 ml of 10 % alcoholic KOH for 5 hours. The alcoholic portion of the saponified mixture was distilled off, then diluted with water. The resultant solution was extracted with ether till exhaustion. The combined ether extract was washed with distilled water and dehydrated over anhydrous Na_2SO_4. The ether extract was distilled off to give the unsaponifiable matter [15].

2.5. Acid hydrolysis Method

Complete acid hydrolysis was carried out by mixing 5 mg of each glycoside with 10 ml of 1.5 N H_2SO_4 in aqueous methanol. The mixture was refluxed for 2 hours at 100^o C. The hydrolysate was then extracted with ethyl acetate, and washed with water, then evaporated to obtain the aglycone. The mother liquor of hydrolysate was neutralized with $BaCO_3$ and used for the identification of the sugar moiety [16].

2.6. Biological activity: Anti-inflammatory activity

2.6.1. Animals

Male albino rats of 100-120 g were housed and acclimatized for 1 week under laboratory conditions. They were fed with standard diet and water. The anti-inflammatory study was conducted in accordance with the ethical guidelines of the Research Ethics Committee of Faculty of Pharmacy- Tanta University (REC-FPTU).

2.6.2. Carrageenan induced rat hindpaw oedema

Two experiments were carried out [17]. The first experiment was done to investigate anti-inflammatory activity of methanolic extract of leaves, flowers and stem bark using 85 male albino rats (100-120) g, which were divided to 17 groups (5 rats each). Different doses of 10, 50, 100, 150, 200 mg/kg body weight (0.5 ml each) were injected into rats as well as the 0.5 ml of 2% tween 80 in normal saline (-ve control) and 0.5 ml of 5 mg/kg body weight of indomethacin (standard).

The second experiment was performed for testing anti-inflammatory activity of different fractions (petroleum ether, methylene chloride, ethyl acetate and *n*-butanol) of leaves methanolic extract. In this study 120 male albino rats (100-120 g) were divided into 24 groups of 5 animals each and the different doses of 15, 30, 50, 75, 100 and 150 mg/kg body weight of each fraction were injected intraperitoneally.

2.6.3. Statistical analysis

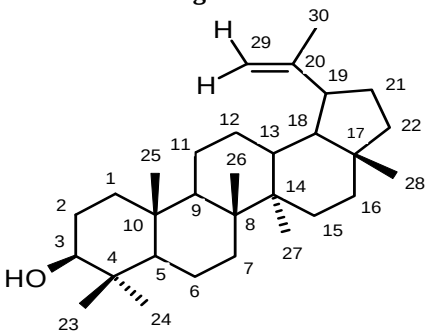
Results were reported as mean $\pm$ standard error of mean (SEM) for n=5 animals. The statistical analysis of data was evaluated with one-way ANOVA followed by Dunnett's post test. P-values less than 0.05 were considered statistically significant.

3. RERULTS

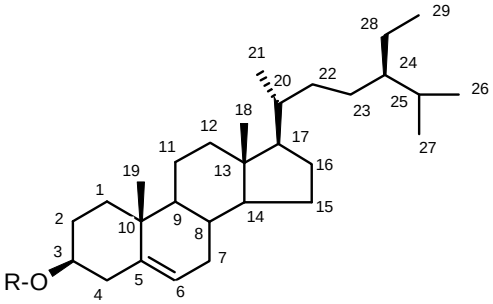
3.1. The physical and spectral data of the isolated compounds 1-7

Compounds 1-7 are shown in Figure 1

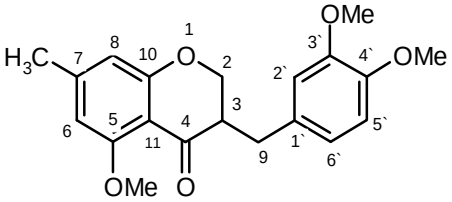
Figure 1



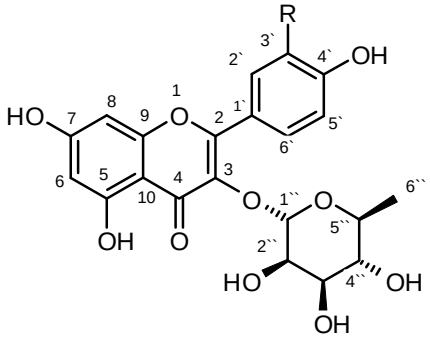
Compound 1



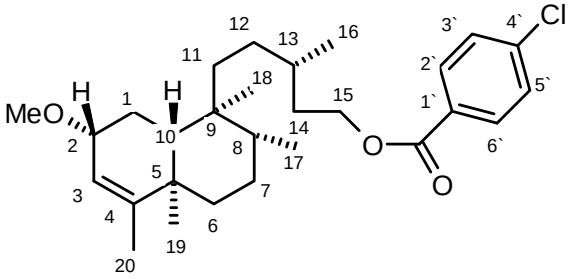
Compound 2, R= H
Compound 3, R= glucose



Compound 4



Compound 5, R=H
Compound 6, R= OH



Compound 7

3.1.1. Compound 1

White powder; mp 212-213 °C; FTIR (KBr): 3319, 2927, 2858, 1639, 1460, 1076 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 3.12 (1H, m, H-3), 2.3 (1H, m, H-19), 1.98 (2H, m, H-21), 4.62 (1H, s, H-29a), 4.51 (1H, s, H-29b), 1.62 (3H, s, H-30); ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 38.7 (C-1), 22.6 (C-2), 78.8 (C-3), 38.8 (C-4), 55.3 (C-5), 18.3 (C-6), 34.3 (C-7), 40.8 (C-8), 50.4 (C-9), 37.1 (C-10), 20.9 (C-11), 25.1 (C-12), 38.0 (C-13), 42.8 (C-14), 27.4 (C-15), 35.6 (C-16), 43.0 (C-17), 48.3 (C-18), 47.9 (C-19), 150.0 (C-20), 29.6 (C-21), 40.0 (C-22), 27.9 (C-23), 16.1 (C-24), 15.9 (C-25), 15.3 (C-26), 14.5 (C-27), 18.0 (C-28), 109.3 (C-29), 19.3 (C-30).

3.1.2. Compound 2

White needle crystals ($CHCl_3$-MeOH); mp 135-136 °C; FTIR (KBr) 3415, 2936, 2860, 1633, 1453, 1043 cm^{-1}; EI MS *m/z* 414 $[M]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 3.51 (1H, m, H-3), 5.18 (1H, m, H-6), 1.03 (3H, s, H-18), 0.70 (3H, s, H-19), 0.93 (3H, d, *J*=6.5 Hz, H-21), 0.84 (3H, s, H-26), 0.82 (3H, s, H-27), 0.86 (3H, s, H-29); ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C 37.2 (C-1) , 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7),31.6 (C-8), 50.1 (C-9), 37.2 (C-10), 21.2 (C-11), 39.7(C-12), 42.2 (C-13), 56.7 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 11.8 (C-18), 19.4 (C-19), 36.5 (C-20), 19.0 (C-21), 33.9 (C-22), 26.0 (C-23), 45.8 (C-24), 29.3 (C-25), 18.7 (C-26), 19.8 (C-27), 23.0 (C-28), 12.2 (C-29).

3.1.3. Compound 3

White powder; mp 285-287 °C; FTIR (KBr): 3427, 2934, 2871, 1631, 1462, 1375, 1025 cm^{-1}; positive ESI-MS displayed $[M+H]^+$ peak at *m/z* 577.2; EI MS *m/z* 414 $[M\text{-glucose}]^+$.

^{13}C-NMR (125 MHz, DMSO, d_6): δ_C 37.3 (C-1) , 30.3 (C-2), 77.4 (C-3), 42.3 (C-4), 140.9 (C-5), 121.7 (C-6), 31.9 (C-7), 29.7 (C-8), 50.1 (C-9), 36.7 (C-10), 21.1 (C-11), 39.7(C-12), 42.3 (C-13), 56.6 (C-14), 24.3 (C-15), 28.3 (C-16), 55.9 (C-17), 12.1 (C-18), 19.6 (C-19), 36.4 (C-20), 19.4 (C-21), 33.8 (C-22), 25.8 (C-23), 45.6 (C-24), 28.3 (C-25), 19.1 (C-26), 20.2 (C-27), 23.1 (C-28), 12.3 (C-29), 101.2 (C-1`), 73.9 (C-2`), 77.2 (C-3`), 70.6 (C-4`), 77.2 (C-5`), 61.6 (C-6`).

3.1.4. Compound 4

White powder; mp 173-175 °C; FTIR (KBr): 3060, 1696, 1629, 1452, 1379, 1276, 1092 cm^{-1}; UV λ_{max} (MeOH) 274; +NaOH 285 nm; positive ESI-MS displayed $[M+H]^+$ at *m/z* 342.96; EI-MS *m/z* 341.53 $[M]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ_H 4.62 (2H, br s, H-2) , 2.48 (1H, m, H-3), 5.89 (1H, d, *J*= 2.3 Hz, H-6), 5.91 (1H, d, *J*= 2.3 Hz, H-8), 2.83 (1H, dd, *J*= 16.8, 4.6 Hz, H-9a), 2.73 (1H, dd, *J*= 16.8, 2.3 Hz, H-9b), 7.2 (1H, s, H-2`), 6.76 (1H, d, *J*= 8.4 Hz, H-5`), 7.3 (1H, d, *J*= 8.4 Hz, H-6`), 3.28 (3H, s, 3`-O-CH_3), 3.32 (3H, s, 4`-O-$CH_3$), 4.15 (3H, s, 5-O-$CH_3$), 2.1 (3H, s, Ar-$CH_3$); $^{13}$C-NMR (125 MHz, $CD_3OD$): $\delta_C$ 78.5 (C-2), 47.4 (C-3), 192.5 (C-4), 156.6 (C-5), 95.0 (C-6), 99.9 (C-7), 94.5 (C-8), 28.0 (C-9), 156.5 (C-10), 114.3 (C-11), 127.8 (C-1`), 125.1 (C-2`), 156.0 (C-3`), 156.3 (C-4`), 127.1 (C-5`), 130.2 (C-6`), 47.4 (3`-O-CH_3), 47.8 (4`-O-CH_3), 66.1 (5-O-CH_3), 22.9 (Ar- CH_3).

3.1.5. Compound 5

Yellow powder; mp 152-153 °C; FTIR (KBr): 3433, 2926, 1655, 1609, 1454, 1367, 1176 and 1063 cm^{-1}; UV λ_{max} MeOH 265, 297, 341; +NaOH 273, 324,389; +$AlCl_3$ 273, 303, 346, 393; + $AlCl_3$/HCl 274, 302, 342, 391; +NaOAc 273, 307, 375; +NaOAc/boric acid 265, 340 nm; positive ESI-MS displayed $[M+H]^+$ at *m/z* 433; EI-MS *m/z* 286.3 [M-rhamnose]$^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ_H 6.18 (1H, d, *J*= 2.3 Hz, H-6), 6.36 (1H, d, *J*= 1.5 Hz, H-8), 7.74 (2H, d, *J*= 8.4 Hz, H-2`,6`), 6.90 (2H, d, *J*= 8.4 Hz, H-3`,5`), 5.35 (1H, s, H-1``), 0.90 (3H, d, *J*= 5.5 Hz, H-6``); ^{13}C-NMR (125 MHz, CD_3OD): δ_C 157.2 (C-2), 134.8 (C-3), 178.2 (C-4), 157.9 (C-5), 98.5 (C-6), 164.6 (C-7), 93.4 (C-8), 160.2 (C-9), 104.5 (C-10), 121.3 (C-1`), 130.5 (C-2`, 6`), 115.2 (C-3`, 5`), 161.8 (C-4`), 102.1 (C-1``), 70.70 (C-2``), 70.78 (C-3``), 71.8 (C-4``), 70.5 (C-5``), 16.3 (C-6``).

3.1.6. Compound 6

Yellow powder; mp 181-183 °C; FTIR (KBr) 3318, 2933, 1652, 1604, 1455, 1358, 1198 and 1065 cm^{-1}; UV λ_{max} MeOH 257, 360; +NaOH 273, 413; +$AlCl_3$ 273, 413; + $AlCl_3$/HCl 269, 400; +NaOAc 215, 272, 400; +NaOAc/boric acid 227, 260, 375 nm; positive ESI-MS displayed $[M+H]^+$ at *m/z* 449; EI-MS *m/z* 302.2 [M-rhamnose]$^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ_H 6.18 (1H, d, *J*= 2.2 Hz, H-6), 6.35 (1H, d, *J*= 2.3 Hz, H-8), 7.31 (1H, d, *J*=2.3 Hz, H-2`), 6.88 (1H, d, *J*= 8.4 Hz, H-5`), 7.29 (1H, dd, *J*= 8.4, 2.3 Hz, H-6`), 5.32 (1H, s, H-1``), 0.90 (3H, d, *J*= 6.2 Hz, H-6``); ^{13}C-NMR (125 MHz, CD_3OD): δ_C 157.2 (C-2), 134.8 (C-3), 178.3 (C-4), 161.8 (C-5), 98.4 (C-6), 164.6 (C-7), 93.3 (C-8), 157.9 (C-9), 104.5 (C-10), 121.5 (C-1`), 115.0 (C-2`), 145.0 (C-3`), 148.4 (C-4`), 115.5 (C-5`), 121.6 (C-6`), 102.2 (C-1``), 70.6 (C-2``), 70.7 (C-3``), 71.9 (C-4``), 70.5 (C-5``), 16.3 (C-6``).

3.1.7. Compound 7

Faint yellow solid mass; elemental analysis: C%= 72.14, H%= 9.36, Cl%= 7.5, O%= 11; positive ESI-MS displayed $[M+H]^+$ at *m/z* 461.15; EI-MS m/z 461 $[M+1]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 7.63 (2H, br s, H-2`, 6`), 7.45 (2H, br s, H-3`, 5`), 5.04 (1H, br s, H-3), 4.24 (2H, m, H-15), 4.22 (1H, m, H-2), 3.47 (3H, s, O-CH3), 1.18 (3H, s, H-19), 0.81 (H-16), 0.81 (3H, s, H-18), 0.80 (3H, s, H-20), 0.78 (H-17); ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C 29.3 (C-1) , 69.5 (C-2), 125.6 (C-3), 145.3 (C-4), 37.4 (C-5), 36.4 (C-6), 27.4 (C-7), 35.4 (C-8), 38.4 (C-9), 46.6 (C-10), 31.9 (C-11), 35.2 (C-12), 29.7 (C-13), 39.3 (C-14), 64.2 (C-15), 22.2 (C-16), 14.1 (C-17), 22.5 (C-18), 22.7 (C-19), 19.5 (C-20), 127.4 (C-1`), 130.9 (C-2`,6`), 128.8 (C-3`,5`), 132.3 (C-4`), 167.6 (C=O), 56.0 (O-CH_3).

3.2. Anti-inflammatory activity

3.2.1. Anti-inflammatory activity of total methanolic extract of leaves, flowers and stem bark

The present study revealed that methanolic extract of flowers reduced oedema significantly at a dose of 150 mg/kg body weight of rats and the percent of inhibition was 39.66 %. Leaves showed significant activity at 100 and 150 mg/kg body weight compared to - ve control mean ± SEM, with percent inhibition of 44.83 % and 32.75 %, respectively. The obtained results were comparable to indomethacin (standard). The results are illustrated in Figures (2-4).

3.2.2. Anti-inflammatory activity of different fractions of leaves methanolic extract

In the second study the anti-inflammatory activity was assessed by comparing different mean of differences ± SEM of different fractions. It was found that ethyl acetate fraction possessed significant anti-inflammatory activity at doses: 50, 75, 100 and 150 mg/kg body weight, with percent of inhibition of 41.38%, 48.28%, 37.93% and 31.03%, respectively. Petroleum ether, methylene chloride and *n*-butanol fractions displayed significant activity at dose: 50 and 75 mg/kg body weight. Ethyl acetate fraction showed the best anti-inflammatory activity. The results are illustrated in Figures (5-8).

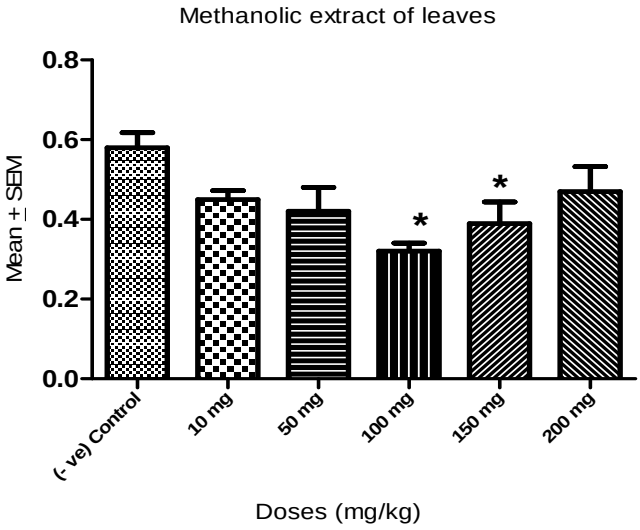


Figure 2: Anti-inflammatory activity of leaves methanolic extracts of *Cassia nodosa*
(mean ± SEM for *n* = 5 animals versus doses mg/kg, * significant at $P < 0.05$)

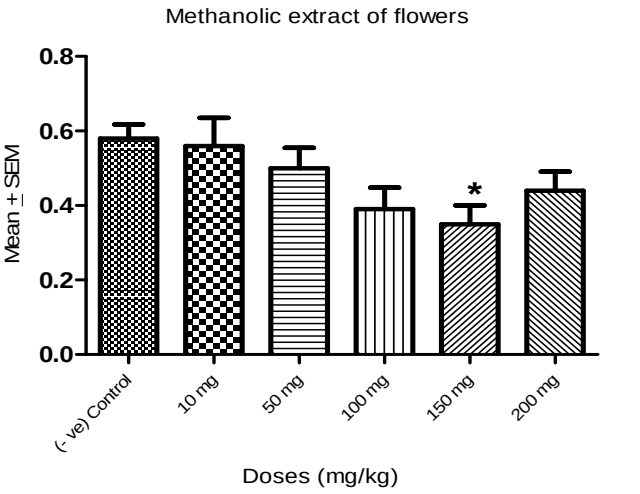


Figure 3: Anti-inflammatory activity of flowers methanolic extracts of *Cassia nodosa*
(mean ± SEM for *n* = 5 animals versus doses mg/kg, * significant at $P < 0.05$)

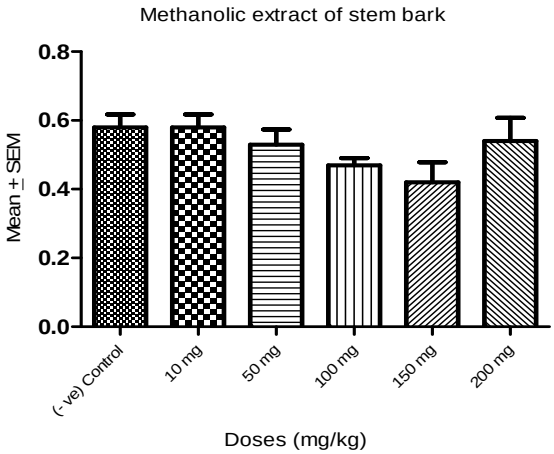


Figure 4: Anti-inflammatory activity of stem bark methanolic extracts of *Cassia nodosa* (mean ± SEM for $n = 5$ animals versus doses mg/kg, * significant at $P < 0.05$)

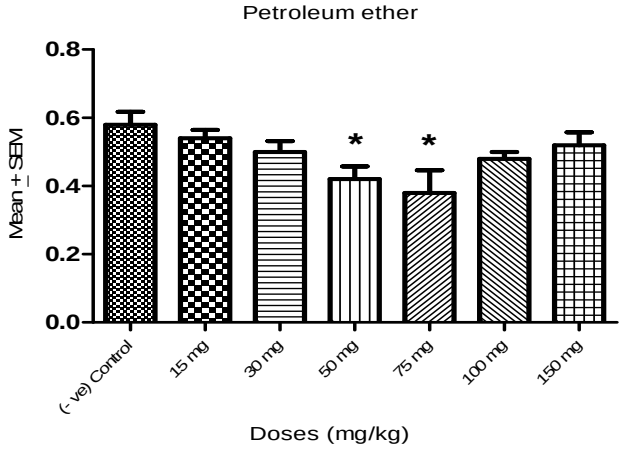


Figure 5: Anti-inflammatory activity of petroleum ether fraction of leaves methanolic extract of *Cassia nodosa* (mean ± SEM for $n = 5$ animals versus doses mg/kg, * significant at $P < 0.05$)

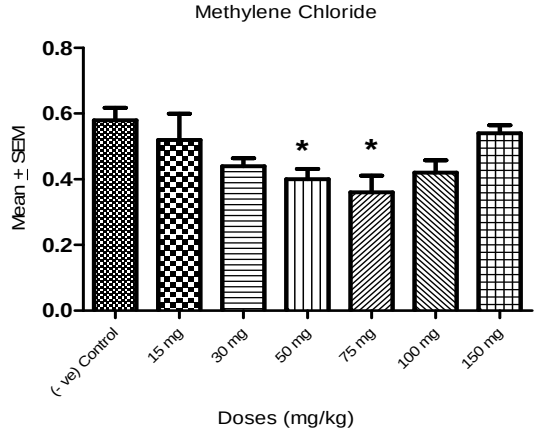


Figure 6: Anti-inflammatory activity of methylene chloride fraction of leaves methanolic extract of *Cassia nodosa* (mean ± SEM for $n = 5$ animals versus doses mg/kg, * significant at $P < 0.05$)

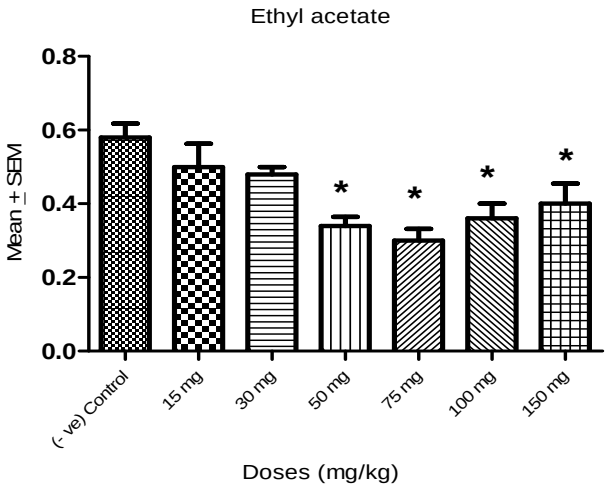


Figure 7: Anti-inflammatory activity of ethyl acetate fraction of leaves methanolic extract of *Cassia nodosa* (mean ± SEM for $n = 5$ animals versus doses mg/kg, * significant at $P < 0.05$)

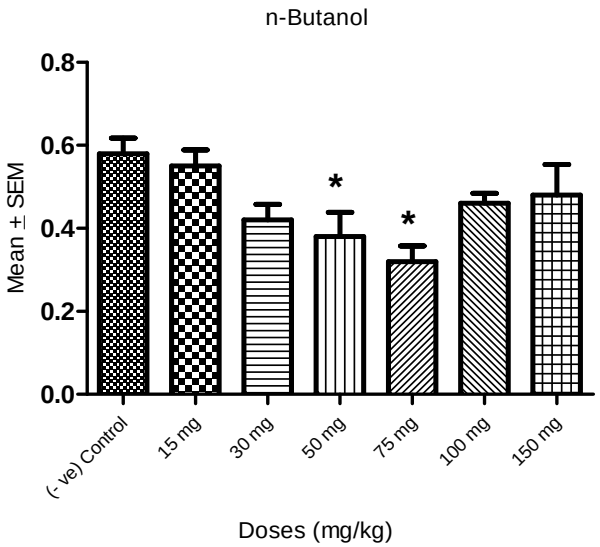


Figure 8: Anti-inflammatory activity of *n*-butanol fraction of leaves methanolic extract of *Cassia nodosa* (mean ± SEM for $n = 5$ animals versus doses mg/kg, * significant at $P < 0.05$)

4. DISCUSSION

4.1. Structural elucidation of isolated compounds

Compounds 1-3 were isolated from unsaponifiable portion of petroleum ether fraction of stem bark. They were tested with Liebermann's and Salkowski's tests to give positive results. This indicated that they may be terpenes or sterols. They exhibited the exact TLC and co-TLC pattern with authentic samples which confirm their identity as well as the different physical (mp and mmp) and by comparison of the obtained spectral data with the published data [18-22]. Compounds **1-3** were identified as lupeol, β-sitosterol and β-sitosterol-3-*O*-*β*-D-glucopyranoside, respectively.

Compound 4 was isolated as a white powder (aggregates of needles)**.** UV spectrum of compound **4**

showed single peak at λ_{max} 274 nm in methanol and with NaOH showed peaks at λ_{max} 285 nm. It didn't show UV shift neither with $AlCl_3$- $AlCl_3$/HCl nor with NaOAc-NaOAc/boric acid mixture. This indicates absence of free OH groups at 5, 3`and 4`.

IR spectral analysis exhibited bands at υ_{max} cm^{-1} : 3060 for aromatic (C-H), 1696 for carbonyl group, 1629 for C=C stretching vibration (aromatic), 1452 for CH_2 bending vibration, 1379 for CH_3 bending vibration, 1276 and 1092 for (C-O). In the positive-ion mode, the ESI-MS exhibited a pseudomolecular ion $[M+H]^+$ at *m/z* 342.96, presumably consistent with molecular formula of $C_{20}H_{22}O_5$. Also EI-MS spectral data showed molecular ion peak at m/z 341.53 $[M]^+$.

^{1}H-NMR spectral analysis revealed presence of signals at δ_H 7.3, 7.20, 6.76 ppm for aromatic protons of H- 6`, 2`, 5`. The doublet signals of aromatic protons (H-6, 8) resonating as at δ_H 5.89 and 5.91 ppm. Significant signals resonating at δ_H 2.83 and 2.73 ppm as a douplet of doublet assigned for the two different protons of C-9, each proton coupled with vicinal proton at C-3 and with the other geminal proton at C-9. Coupling constant confirming this with *J*= 16.8 Hz for geminal protons, 4.6 and 2.3 Hz for vicinal protons. Signals of O-CH_3 protons resonating at δ_H 3.28, 3.32 and 4.15 ppm.

^{13}C-NMR spectrum provided important spectral analysis information. Signal resonating at δ_C 192.5 ppm ascribed for carbonyl group. Signals shown at δ_C 156.0, 156.3, 156.6 ppm were for C- 3`, 4`, 5. The carbons of aromatic rings were shown at 95.0, 94.5 ppm ascribed for C-6, 8, while C-1`, 2`, 5`, 6` displayed signals resonating at δ_C 127.8, 125.1, 127.1, 130.2. Carbons of 3`, 4`O-CH_3 resonating at δ_C 47.4 and 47.8 and at δ_C 66.1 for 5-OCH_3.

From the above mentioned data which were compared with the published spectral data [8, 23, 24], it was found that compound **4** is 5,3`,4`-trimethoxy-7-methyl-3-benzyl chromanone. It is the first report for the isolation of this compound from *Cassia nodosa*.

Compound 5: The physical and chemical properties suggested that it may be flavonoid glycoside [25]. IR spectrum data showed that signals at υ_{max} (cm^{-1})= 3433 was for OH group, 2926 was for aliphatic CH_3 stretching, 1655 was for C=O conjugated, 1609 was for C=C stretching (aromatic), 1454 was for CH_2 bending, 1367 was for CH_3 bending, 1176 and 1063 were for C-O.

UV spectral properties of compound **5** showed typical flavonol glycosides substituted at C-3 hydroxyl (the presence of absorption maxima at 341 and 265 nm). Bathochromic shift with NaOH, NaOAc and $AlCl_3$ indicated the presence of free hydroxyl groups at 4`, 7 and 5 positions. Spectra obtained with $AlCl_3$, $AlCl_3$/HCL and NaOAC/$H_3BO_3$ revealed absence of ortho-dihydroxyl system at 3` and 4` positions of ring B [25]. Rhamnose was obtained by acid hydrolysis, which was confirmed using paper chromatography alongside authentic sugar.

^{1}H-NMR spectrum exhibited the presence of six aromatic (H-6, 8, 2`, 3`, 5` and 6`) and one anomeric proton at δ_H 5.35 ppm. Protons of methyl group of rhamnose were exhibited as s doublet signal at (δ_H 0.90 ppm, *J*= 5.50 Hz).

^{13}C-NMR spectrum showed signals at δ_C 102.1 ppm for anomeric carbon of rhamnose, beside signal at δ_C 16.3 ppm for CH_3 of rhamnose. The positive mode ESI-MS confirmed the identity of this compound as it displayed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 433, consistent with molecular formula of $C_{21}H_{20}O_{10}$. Compound **5** was identified as kaempferol-3-*O*-α-L-rhamnoside (afzelin), by comparison of spectral data with the published data [26, 27].

Compound 6: The physical and chemical properties suggested it may be flavonoid glycoside [25]. The broad band in IR spectrum showed strong bands at 3318 cm^{-1} for OH groups (aglycone and sugar part) and at 1652 cm^{-1} for conjugated carbonyl. The UV spectral data in methanol showed the characteristic absorption of flavonols at λ_{max} 360 and 257 nm. This data suggesting quercetin type flavonol glycoside [25, 28].

Acid hydrolysis afforded quercetin as aglycone and rhamnose as a sugar moiety which was identified alongside authentic sugar using paper and TLC chromatography.

^{1}H-NMR spectrum exhibited signal resonating at δ_H 7.29 ppm was assigned for H-6` as doublet of doublet signal due to meta coupling with H-2` (*J*= 2.3 Hz) and ortho coupling with H-5` (*J*= 8.4 Hz). There are two doublets resonating at $\delta_H$ (6.88, 7.31 ppm) for H-5` and 2`, respectively with *J*= 2.3 Hz for meta coupling in case of H-2` and *J*= 8.4 Hz for ortho coupling in case of H-5`. The meta coupled protons H-6, 8 had their doublet signals at δ_H 6.18, 6.35 ppm, respectively. The anomeric proton of rhamnose resonating at δ_H 5.32 while the de-oxy sugar (C-6``) signal was resonating at δ_H 0.90 ppm.

^{13}C-NMR spectrum showed signal at δ_C 178.3 ppm assigned to carbonyl carbon at C-4, and downfield signals at δ_C 145.0, 148.4, 157.2, 157.9, 161.8, 164.6 ppm were assigned to carbons attached to oxygen atom at C- 3`, 4`, 2, 9, 5, 7. The anomeric carbon of sugar moiety was resonating at δ_C 102.2 ppm, and the other signals of C- 2``, 3``, 4``, 5``, 6`` were assigned for rhamnose with signal at δ_C 16.3 ppm assigned for methyl group of the de-oxy sugar. Positive mode ESI-MS confirmed the structure of this compound as it displayed an $[M+H]^+$ peak at *m/z* 449, consistent with molecular formula of $C_{21}H_{20}O_{11}$.

The aforementioned physical, chemical and spectral data when compared with the published data [29], it revealed that compound **6** is quercetin-3-*O*-α-L-rhamnoside (quercetrin).

Compound 7: EI-mass spectrum showed $[M+1]^+$ peak at m/z= 461 This was confirmed by positive mode ESI-MS analysis, which displayed a pseudomolecular ion peak $[M+H]^+$ at *m/z* = 461.15

Based on ^{1}H-NMR and ^{13}C-NMR spectral data it can be proposed that the compound may be terpenoid in nature due to signals of aliphatic CH_3 and CH_2 groups resonating at δ_H 0.78-1.65 in ^{1}H-NMR and δ_C 14.1-37.1 in ^{13}C-NMR spectrum.

^{1}H-NMR signals at δ_H 7.63 (H 2`, 6`) and 7.45 (H 3`, 5`) were for para-substituted benzene ring which appeared in ^{13}C-NMR spectrum as 4 signals resonating at δ_C 128.8 (C-3`, 5`), 130.9 (C- 2`, 6`), 127.4 (C- 1`) and at 132.3 (C- 4`). ^{1}H-NMR spectrum showed H-2 assignment at δ_H 4.22,

while H-3 (C3-C4 double bond) was assigned at δ_H 5.04. The signal at δ_C 167.6 indicates the presence of C=O group (ester) conjugated with benzene ring. Signals resonating at δ_C 56.0 and 69.5 were assigned for O-CH_3 and C-2, respectively. Also the signal resonating at δ_C 64.2 was assigned for (C-15), which is attached to oxygen atom of ester group. The presence of double bond at C-3 and 4 was displayed as signals at δ_C 125.6 and 145.3.

The presence of chlorine atom was confirmed by qualitative elemental analysis, while the molecular formula of compound **7** was deduced to be $C_{28}H_{41}O_3Cl$ based on quantitative elemental analysis.

COSY, HMQC and HMBC spectral analysis of compound **7** lent further evidence to the different ^{1}H- ^{1}H, ^{1}H-^{13}C one bond and ^{1}H-^{13}C long range correlations. COSY spectrum exhibited symmetric cross peaks at δ_H 7.63 and 7.45 indicating that nuclei 2`,6` were correlated with nuclei 3`,5`. HMBC spectrum showed ^{1}H-^{13}C correlation between the protons (H-15, H-2`, H-6`) resonating at δ_H 4.24, 7.63 and the carbonyl carbon of ester group resonating at δ_C 167.6. In addition HMBC correlations (Figure 9) proved the heteronuclear correlation between the protons of methoxyl group resonating at δ_H 3.47 with C-2 and C-3 resonating at δ_C 69.5 and 125.6, respectively. Furthermore there were other correlations between ^{1}H and ^{13}C were observed of the known diterpene clerodane skeleton. These heteronuclear correlations emphasized the new structure of this compound. They proved that the methoxyl group was attached to C-2 and the chlorobenzoyl group was attached to C-15.

The identification of compound **7** was achieved based on the different spectral analysis as well as comparing the spectral data with the published data in literature [30-32]. Compound **7** was identified as 2-methoxy-3-en-15-*O*-(4`-chlorobenzoyl) clerodane. It was found that chlorinated diterpenoid compounds were reported to be isolated from marine and terrestrial plants [33,34].

Compound **7** was a new compound which was isolated for the first time from *Cassia nodosa* Buch.-Ham. ex Roxb.

4.2. Anti-inflammatory activity

The present study was carried out for the first time to evaluate the anti-inflammatory activity of *Cassia nodosa* Buch.-Ham. ex Roxb. The methanolic extracts of leaves and flowers showed significant activities at 100 and 150 mg/kg body weight compared to the negative control mean ± SEM. Leaves methanolic extract had the best anti-inflammatory activity and this led to investigate its fractions at different doses. In the second study by comparing the mean of differences ± SEM of different fractions it was clear that ethyl acetate fraction significantly reduced carrageenan-induced hindpaw oedema in a dose dependent manner in accordance to the (- ve) control. It displayed the most significant anti-inflammatory activity of 41.38 and 48.28 % inhibition at doses of 50 and 75 mg/kg body weight, respectively. This significant activity is due to the presence of high flavonoid content in the ethyl acetate fraction of leaves of *Cassia nodosa*, in particular kaempferol and quercetin and their glycosides [35]. Flavonoids have demonstrated anti-inflammatory [36] and antiproliferative activities, the second activity is found to cause decrease in volume and contents of granuloma in inflammation [37].

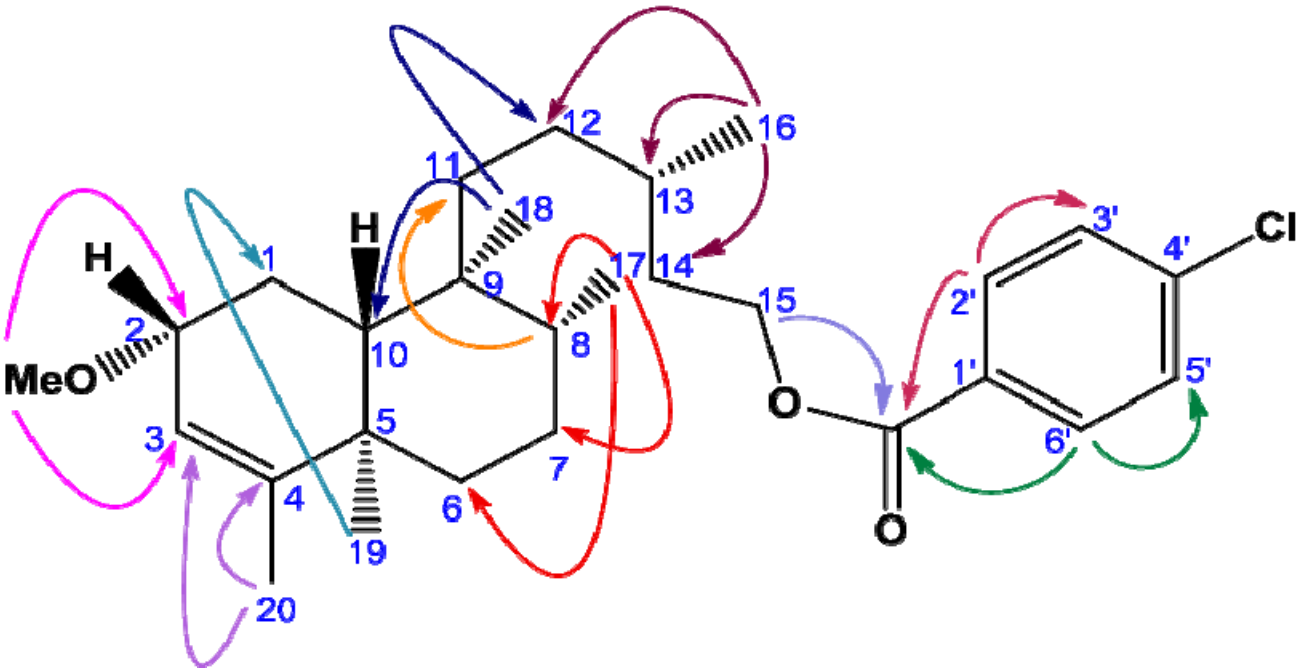


Figure 9: Selected correlations observed in the HMBC spectrum of 2-methoxy-3-en-15-*O*-(4`-chlorobenzoyl) clerodane (compound 7): (H→C)

5. CONCLUSIONS

The present study of *Cassia nodosa* Buch.-Ham. ex Roxb. growing in Egypt displayed the isolation and structure elucidation of compounds **1-7**. All compounds were previously reported to be isolated from this plant except compounds **4** and **7.** Compound **4** was isolated for the first time, while compound **7** was a new compound. Anti-inflammatory study of different plant extracts were carried out for the first time for this plant and it was concluded that the methanolic extract of leaves and its ethyl acetate fraction showed the best activity. It was deduced that the high flavonoidal content (quercetin and kaempferol) of these extracts is responsible for this significant activity.

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