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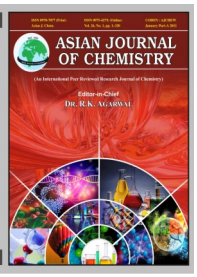

Phytochemical Investigation on *Eucalyptus globulus* Labill

M. IBRAHIM[1], S. AMBREEN[1], A. HUSSAIN[2], N. HUSSAIN[2], M. IMRAN[2], B. ALI[2], S.H. SUMRRA[3], M. YOUSUF[2] and F.S. REHMANI[1,*]

[1]Department of Applied Chemistry, Government College University, Faisalabad, Pakistan
[2]International Centre for Chemical and Biological Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan
[3]Department of Chemistry, Institute of Chemical and Biological Sciences, University of Gujrat, Gujrat 50700, Pakistan

*Corresponding author: E-mail: fsrehmani@uok.edu.pk

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Twelve compounds have been isolated from the methanolic extract of the whole plant of *Eucalyptus globulus* Labill namely, stigmasterol (**1**), stigmasterol 3-O-β-D-glucopyranoside (**2**), ursolic acid (**3**), α-amyrin (**4**), α-amyrin acetate (**5**) 4',5,7-trimethoxykaempferol (**6**), genistein (**7**), naringenin (**8**), catechin (**9**), apicatechin (**10**), octyl-β-D-glucopyranoside (**11**), 7,8-dihydroxycoumarin (**12**), respectively. Their chemical structures were established on the basis of spectroscopic methods 1D (^{1}H and ^{13}C) NMR and 2D (COSY, HMQC and HMBC) NMR in addition to mass spectrometry and comparison with literature data.

Keywords: *Eucalyptus globulus* Labill., Myrtaceae.

INTRODUCTION

Eucalyptus L'He'ritier (Myrtaceae) is one of the world's most important and most widely growing genera. In Australia, this genus is the second largest genus, after Acacia and contains about 750 species[1]. Myrtaceous plants are known to be rich source of biologically active terpenoids and polyphenols, including flavonoids, phloroglucinol derivatives and tannins[2].

Shifting of literature revealed that sesquiterpenes aromadendrene[1], acetogenin-sesquiterpenes, acetogenin monoterpenes[3-6], phloroglucinol sesquiterpenes[7] and C-methyl flavones[8] have previously been reported from this species. The phloroglucinol compounds exhibit interesting biological activities[9]. *Eucalyptus globulus* Labill. (Myrtaceae) is used as a traditional remedy in many parts of the world for treatment of a wide variety of diseases including microbial infections[5]. *Eucalyptus* oil is used as a stimulant and antiseptic gargle. It increases cardiac action. In croup and spasmodic throat troubles the oil may be freely applied externally. The oil is an ingredient of 'Catheder oil' used for sterilizing and lubricating urethral catheters. Eucalyptus oil is also administered to horses, dogs and animals in septicemia. Its extract is commonly applied for parasitic skin infections, treat pyorrhea (gum disease) and on burns to prevent infections[10]. The chemotaxonomic and ethnopharmacological significance of the genus, Eucalyptus prompted us to reinvestigate the constituents of *Eucalyptus globulus* Labill. As a result of these studies we are going to report the isolation and structural elucidation of some compounds isolated from this plant namely, stigmasterol (**1**), ursolic acid (**2**), 4',5,7-trimethoxy-kaempferol (**3**), naringenin (**4**), genistein (5), catechin (**6**), epicatechin (7), octyl-β-D-glucopyranoside (**8**), α -amyrin (**9**), stigmasterol 3-O-β-D-glucopyranoside (**10**), 7,8-dihydroxycoumarin (**11**), α -amyrin acetate (**12**) respectively.

EXPERIMENTAL

Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a 460 Shimadzu spectrometer. EI-MS and HR-FAB-MS were recorded on JMS-HX-110 and JMS-DA 5000 mass spectrometers. The ^{1}H-NMR, ^{13}C-NMR, HMQC and HMBC spectra were recorded on Bruker spectrometers operating at 400 MHz for ^{1}H- and 100 MHz for ^{13}C-NMR, respectively. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are in Hz. Aluminum sheets precoated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick; E-Merck) were used for TLC and silica gel (230-400 mesh) was used for column chromatography. Visualization of the TLC plates was carried out under UV at 254 and 366 nm and by spraying with ceric sulfate reagent (with heating). Melting points were determined on a Gallenkemp apparatus and are uncorrected. For antioxidant assay all the chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

The whole plant of *Eucalyptus globulus* Labill was collected in June 2006, from Karachi (Pakistan) and identified

by the Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited.

Extraction and isolation: The air-dried plant material (20 kg) was ground into powder and extracted with 100 % methanol at room temperature (40 L × 4 days × 4). The combined extract was concentrated under reduced pressure to afford a dark greenish brown residue (200 g), which was then partitioned between *n*-hexane and water. The water soluble fraction was further extracted with chloroform, ethyl acetate and *n*-butanol. Each fraction was concentrated under reduced pressure to give *n*-hexane fraction (30 g), ethyl acetate fraction (25 g), *n*-butanol fraction (43 g) and aqueous fraction (58 g) residue. The ethyl acetate fraction was subjected to vacuum liquid chromatography [silica gel (250 g)] using *n*-hexane-ethyl acetate gradients, six major fractions were obtained: IBR-1 to IBR-6. [(100:0, Fr. IBR-1 (2.5g)], [(9: 1, Fr. IBR-2 (3.8 g)], [(4:1, Fr. IBR-3 (2.8 g)], [(1:100, Fr. IBR-4 (1.89 g)], [(3: 7), Fr. IBR-5 (1.6 g)], [(0:100), Fr. IBR-6 (1.0 g)]. Fraction IBR-2 was subjected to silica gel column chromatography [silica gel (100 g), column (30 × 3 cm)] using dichloromethane-methanol (98:2) to afford compounds **1** (15 mg) and **2** (13 mg). Fraction IBR-3 was subjected to vacuum liquid chromatography [silica gel (250 g)] using dichloromethane-methanol gradients to obtain four sub fractions IBR-3-A to IBR-3-D. Subfraction IBR-3-C was subjected to silica gel column chromatography [silica gel (50 g), column (30 × 2 cm)] using dichloromethane-methanol 98:2 to afford compounds **3** (10 mg) and **4** (5 mg) and 5 (13 mg). Fraction IBR-4 was loaded over Sephadex LH-20 [Sephadex (50 g), column (30 × 2 cm)] column using methanol-water 1:1 as an eluent to obtain three main subfractions IBR-4-A to IBR-4- C. Subfraction IBR-4-B was subjected to silica gel column chromatography [silica gel (50 g), column (30 × 3 cm)] using dichloromethane-methanol 88:12 to afford compounds **6** (16 mg) and **7** (20 mg). Fraction IBR-5 was subjected to HPLC (HPLC gradient program: 60:40 methanol/water at 0 and 5 min; 100:0 methanol/water at 38 and 45 min and a flow rate 5.0 mL/min) to yield compound **8** (14 mg) and **9** (21 mg). Fraction IBR-6 was subjected to silica gel column chromatography [silica gel (50 g), column (30 × 3 cm)] using dichloromethane-methanol 88:12 gave compound 10 (23 mg), 11(12 mg) and 12 (20 mg).

Stigmasterol (1): White needles; R_f = 0.86 (solvent system I); m.p. 169-170 °C, EI-MS *m/z* (rel. int. %): 412 $[M]^+$ (100). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.34 (1H, dd, *J* = 2.8, 5.1 Hz, H- 6), 5.15 (1H, dd, *J* = 8.8, 15.1 Hz, H-22), 5.01 (1H, dd, *J* = 8.8, 15.1 Hz, H-23), 3.15 (1H, m, H-3), 1.02 (3H, s, CH_3-19), 1.00 (3H, d, *J* = 6.2 Hz, CH_3-21), 0.84 (3H, t, *J* = 6.3 Hz, CH_3-29), 0.79 (3H, d, *J* = 7.5 Hz, CH_3-26), 0.78 (3H, d, *J* = 7.5 Hz, CH_3-27), 0.69 (3H, s, CH_3-18). ^{13}C NMR (125 MHz, $CDCl_3$): δC 140.71 (C-5, s), 138.29 (C-22, d), 129.24 (C-23, d), 121.69 (C-6, d), 71.79 (C-3, d), 56.83 (C-17, d), 55.92 (C-14, d), 51.21 (C-24, d), 50.12 (C-9, d), 42.23 (C-13, s), 40.47 (C-20, d), 39.65 (C-4, t), 37.22 (C-1, t), 36.48 (C-10, s), 31. 85 (C-8, 25, d), 31.59 (C-2, t), 31.50 (C-7, t), 28.89 (C-28, t), 25.38 (C-16, t), 24.34 (C-15, t), 21.19 (C-21, q), 21.07 (C-11, t), 21.05 (C-27, q), 19.37 (C-26, q), 18.96 (C-19, q), 12.23 (C-29, q), 12.02 (C-18, q)[11].

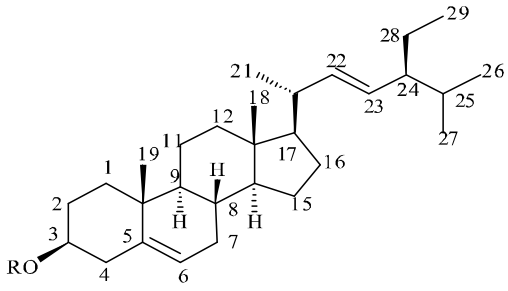
Ursolic acid (2): White amorphous powder; R_f = 0.71 (solvent system I); m.p. 292-293 °C, EI-MS *m/z* (rel. int. %): 456 $[M]^+$ (93). ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 11.92 (1H, s, 28-COOH), 5.11 (1H, dd, *J* = 3.8, 6.9 Hz, H-12), 4.28 (1H, d, J = 15.0 Hz, 3-OH), 2.98 (1H, m, H3), 2.10 (1H, d, *J* = 11.0 Hz, H-18), 1.02 (3H, s, CH_3-23), 0.90 (3H, d, *J* = 9.7 Hz, CH_3-29), 0.88 (3H, s, CH_3-27), 0.85 (3H, s, CH_3-26), 0.80 (3H, d, *J* = 6.6 Hz, CH_3-30), 0.73 (3H, s, CH_3-23), 0.66 (3H, s, CH_3-25). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 178.27 (C-28, s), 138.17 (C-13, s), 124.55 (C-12, d), 76.81 (C-3, d), 54.76 (C-5, d), 52.35 (C-18, d), 47.00 (C-17, s), 46.80 (C-9, d), 41.62 (C-8, 14, s), 38.48 (C-4, s), 38.42 (C-19, d), 38.37 (C-20, d), 38.21 (C-1, t), 36. 51 (C-22, t), 36.30 (C-10, s), 32.69 (C-7, t), 30.17 (C-21, t), 28.25 (C-23, q), 27.53 (C-15, t), 26.98 (C-2, t), 23.79 (C-16, t), 23.26 (C-27, q), 22.84 (C-11, t), 21.07 (C-30, q), 17.98 (C-6, t), 17.07 (C-26, q), 16.89 (C-29, q), 16.08 (C-25, q), 15.22 (C-24, q)[12].

4',5,7-Trimethoxykaempferol (3): Yellow needles; R_f = 0.54 (solvent system I); m.p. 152 °C, Positive-ion ESI-MS *m/z* (rel. int. %): 329 $[M + H]^+$ (100). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 8.19 (2H, dd, *J* = 1.9, 6.9 Hz, H-2', 6'), 7.05 (2H, dd, *J* = 1.9, 6.9 Hz, H-3', 5'), 6.57 (1H, d, *J* = 2.2 Hz, H-8), 6.36 (1H, d, *J* = 2.2 Hz, H-6), 3.88 (3H, s, 5-OCH_3), 3.91 (3H, s, 7-OCH_3), 3.98 (3H, s, 4'-OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 171.93 (C-4, s), 164.29 (C-7, s), 160.61 (C-5, s), 160.53 (C-4', s), 158.85 (C-2, s), 142.25 (C-9, s), 137.43 (C-3, s), 128.86 (C-2', 6', d), 123.56 (C-1', s), 113.99 (C-3', 5', d),106.24 (C-10, s), 95.64 (C-6, d), 92.39 (C-8, d), 56.39 (4'- OCH_3, q), 55.79 (7-OCH_3, q), 55.37 (5-OCH_3, q)[13,14].

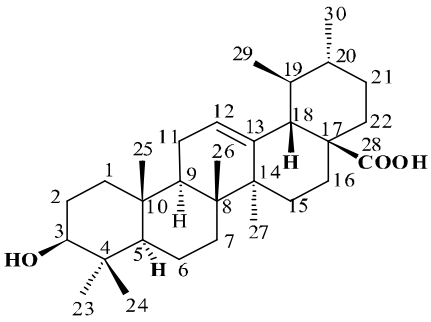
Naringenin (4): Yellowish white amorphous powder; R_f = 0.86 (solvent system II); m.p. 257-258 °C, Positive-ion ESI-MS *m/z* (rel. int. %): 273 $[M + H]^+$ (100).^{1}H NMR (500 MHz, DMSO-d_6): δ_H 12.14 (1H, s, 5-OH), 10.77 (1H, s, 7-OH), 9.57 (1H, s, 4'-OH), 7.30 (2H, d, *J* = 8.5 Hz, H-2', 6'), 6.78 (2H, d, *J* = 8.5 Hz, H-3', 5'), 5.86 (2H, br s, H-6, 8), 5.42 (1H, dd, *J* = 2.8, 12.6 Hz, H-2), 3.26 (1H, dd, *J* = 12.9, 17.0 Hz, H-3ax), 2.67 (1H, dd, *J* = 2.8, 17.0 Hz, H-3eq). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 196.40 (C-4, s), 166.62 (C-7, s), 163.47 (C-5, s), 162.93 (C-9, s), 157.71 (C-4', s), 128.83 (C-1', s), 128.34 (C-2', 6', d), 115.14 (C-3', 5', d), 101.75 (C-10, s), 95.76 (C-6, d), 94.94 (C-8, d), 78.41 (C-2, d), 41.95 (C-3, t)[15].

Genistein (5): Yellow amorphous powder; R_f = 0.77 (solvent system II); m.p. 303-304 °C, Positive-ion ESI-MS *m/z* (rel. int. %): 271 $[M + H]^+$ (100). ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 12.94 (1H, s, 5-OH), 10.87 (1H, s, 7-OH), 9.58 (1H, s, 4'-OH), 8.31 (1H, s, H-2), 7.30 (2H, dd, *J* = 1.9, 6.6 Hz, H-2', 6'), 6.78 (2H, dd, *J* = 1.9, 6.6 Hz, H-3', 5'), 6.37 (^{1}H, d, *J* = 2.2 Hz, H-8), 6.21 (1H, d, *J* = 2.2 Hz, H-6). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 180.20 (C-4, s), 164.25 (C-7, s), 161.98 (C-5, s), 157.57 (C-4', s), 157.40 (C-9, s), 153.98 (C-2, d), 130.15 (C-2', 6', d), 122.25 (C-3, s), 121.18 (C-1', s), 115.04 (C-3', 5', d), 104.45 (C-10, s), 98.95 (C-6, d), 93.64 (C-8, d)[13,14].

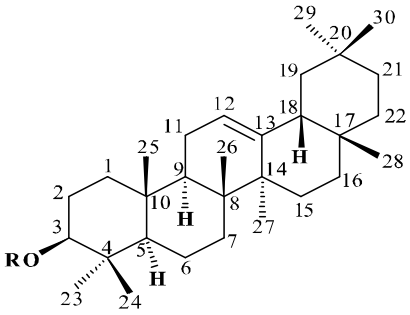
Catechin (6): Whitish powder; R_f = 0.69 (solvent system II); m.p. 244-245 °C, Positive-ion ESI-MS *m/z* (rel. int. %): 291 $[M + H]^+$ (100). ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 9.17 (1H, s, 5- OH), 8.93 (1H, s, 7-OH), 8.86 (1H, s, 4'-OH), 8.81 (1H, s, 3'-OH), 6.70 (1H, d, *J* = 1.6 Hz, H- 2'), 6.67 (1H, d, *J* = 8.2 Hz, H-6'), 6.57 (1H, dd, *J* = 1.6, 8.2 Hz, H-5'), 5.87 (1H, d, *J* = 2.2 Hz, H-8), 5.67 (1H, d, *J* = 2.2 Hz, H-6), 4.86 (1H, d, *J* = 5.86 Hz, H-2), 4.47 (1H, d, *J* = 7.6 Hz, 3-OH), 3.80 (1H, ddd, *J* = 5.3, 7.5, 12.9 Hz, H-3), 2.64 (1H, dd, *J* = 5.3, 16.1



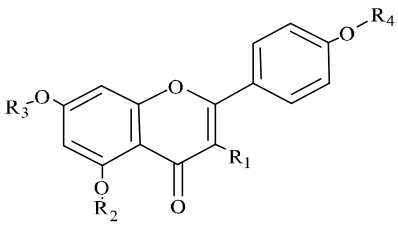
Stigmasterol (**1**), R = H
Compound (**2**), R = β-D-glucopyranoside



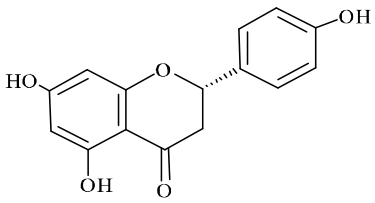
Ursolic acid (**3**)



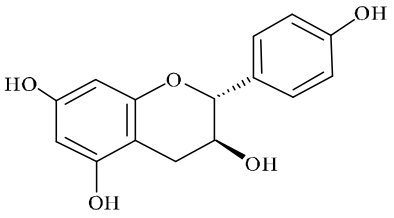
α-Amyrin (**4**), R = H
α-Amyrin acetate (**5**), R = acetate



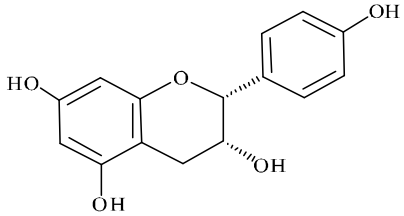
4',5,7-Trihydroxyflavanone (**6**), = R_1 = OH, R_2, R_3, R_4 = Me
Genistein (**7**), = R_1, R_2, R_3, R_4 = H



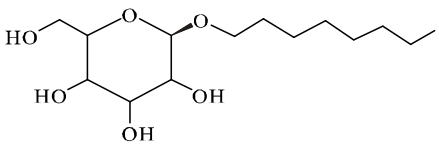
Naringenin (**8**)



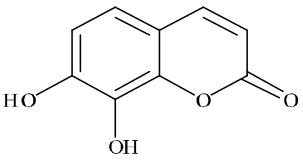
Catechin (**9**)



Epicatechin (**10**)



Octyl-β-D-glucopyranoside (**11**)



7,8-Dihydroxy coumarin (**12**)

Chemical structures of the isolated compounds

Hz, H-4eq), 2.33 (1H, dd, *J* = 7.9, 15.7 Hz, H-4ax). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 156.45 (C-5, s), 156.17 (C-7, s), 155.35 (C-9, s), 144.83 (C-3', 4', s), 130.58 (C-1', s), 118.34 (C-6', d), 115.06 (C-5', d), 114.15 (C-2', d), 99.05 (C-10, s), 95.09 (C-6, d), 93.83 (C-8, d), 80.99 (C-2, d), 66.30 (C-3, d), 27.87 (C-4, t)[15,16].

Epicatechin (7): Yellowish residue; R_f = 0.69 (solvent system II); m.p. 241-242 °C, Positive-ion ESI-MS *m/z* (rel. int. %): 291 $[M + H]^+$ (100). ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 9.17 (1H, s, 5-OH), 8.93 (1H, s, 7-OH), 8.85 (1H, s, 4'-OH), 8.75 (1H, s, 3'-OH), 6.96 (1H, d, *J* = 1.6 Hz, H-2'), 6.75 (1H, d, *J* = 8.2 Hz, H-6'), 6.71 (1H, dd, *J* = 1.6, 8.2 Hz, H-5'), 5.94 (1H, d, *J* = 2.2 Hz, H-8), 5.75 (1H, d, *J* = 2.2 Hz, H-6), 4.82 (1H, s, H-2), 4.69 (1H, d, *J* = 5.42 Hz, 3- OH), 4.07 (1H, d, *J* = 3.4 Hz, H-3), 2.64 (1H, dd, *J* = 4.1, 16.1 Hz, H-4ax), 2.33 (1H, dd, *J* = 2.2, 15.4 Hz, H-4eq). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 156.79 (C-5, s), 156.47 (C-7, s), 156.03 (C- 9, s), 144.73 (C- 4', s), 144.66 (C-3', s), 130.90 (C-1', s), 118.27 (C-6', d), 115.12 (C-2', d), 115.06 (C-5', d), 98.79 (C-10, s), 95.36 (C-6, d), 94.38 (C-8, d), 78.31 (C-2, d), 65.19 (C-3, d), 28.47 (C-4, t)[15,16].

Octyl-β-D-glucopyranoside (8): White powder; R_f = 0.62 (solvent system III); m.p. 107 °C, FAB-MS *m/z* (rel. int. %): 315 $[M + Na]^+$ (100). ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 4.92

(1H, s, 2'-OH), 4.89 (1H, s, 3' or 4'-OH), 4.86 (1H, s, 5'-OH), 4.08 (1H, d, *J* = 7.9 Hz, H-1'), 3.72, 3.41 (2H, m, H-6'), 3.72 (1H, m, H-8A), 3.40 (1H, m, H-8B), 3.10 (1H, m, H-5'), 3.06-3.03 (2H, m, H-3', 4'), 2.90 (1H, m, H-2'), 1.49 (2H, quintet, *J* = 7.2 Hz, H-7), 1.29-1.24 (12 H, br s, -$(CH_2)_6$-), 0.84 (3H, d, *J* = 7.2 Hz, H_3-1). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 102.81 (C-1', d), 76.79 (C-3', d), 76.75 (C-5', d), 73.41 (C-2', d), 70.05 (C-4', d), 68.52 (C-8, t), 61.06 (C-6', t), 31.24 (C-7, t), 29.29 (C-6, t), 28.88 (C-5, t), 28.68 (C-4, t), 25.52 (C-3, t), 22.02 (C-2, t), 13.95 (C-1, q)[17].

β-Amyrin (9): Crystallized from ethanol (33 mg); m.p.: 197-198 °C; $[\alpha]_D^{25}$: +100 (c = 0.21, $CHCl_3$); IR (KBr, ν_{max}, cm^{-1}): 3510, 3055, 1635 and 820; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 5.11 (1H, m, H-12), 3.19 (1H, dd, *J* = 10.0, 4.5 Hz, H-3), 1.02, 1.01, 1.08, 0.96, 0.93, 0.88, 0.85 and 0.80 (3H, each s, Me); EI-MS *m/z* (rel. int.): 426 $[M]^+$ (15), 411 (18), 408 (16), 393 (32), 257 (20), 218 (100), 207 (10), 203 (40) and 189 (55); HR-EI-MS *m/z*: 426.3825 (calcd. for $C_{30}H_{50}O$, 426.3861). The physical and spectral data were in close agreement to the reported values[18,19].

Stigmasterol 3-O-β-D-glucopyranoside (10): Colorless crystals (38 mg); m.p.: 289-290 °C; $[\alpha]_D^{25}$: -51.5 (c = 0.22, CH_3OH) IR (KBr, ν_{max}, cm^{-1}): 3454 (OH), 3024, 1646 (C=C); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.23 (lH, br d, *J* = 5.4 Hz, H-6), 5.14 (lH, dd, *J* = 15.2, 8.4 Hz, H-22), 5.02 (lH, dd, *J*= 15.2, 8.6 Hz, H-23), 4.78 (lH, d, *J* = 7.4 Hz, H-l′), 3.83 (lH, m, H-3), 3.84-4.44 (m, Glc-H′), 1.01 (3H, s, Me-19), 0.90.(3H, d, *J* = 6.2 Hz, Me-21), 0.83 (3H, d, *J* = 6.5 Hz, Me-26), 0.82 (3H, t, *J* = 7.0 Hz, Me-29), 0.80 (3H, d, *J* = 6.5 Hz, Me-27), 0.67 (3H, s, Me-18); ^{13}C-NMR (CD_3OD, 125 MHz) δ: 141.5 (C-5), 138.9 (C-22), 129.1 (C-23), 121.1 (C-6), 102.8 (C-1'), 79.8 (C-3), 76.9 (C-3'), 76.7 (C-5'), 74.2 (C-2'), 70.6 (C-4'), 62.2 (C-6'), 57.0 (C-14), 56.1 (C-17), 52.1 (C-24), 50.8 (C-9), 43.9 (C-4), 43.1 (C-13), 40.5 (C-20), 39.9 (C-12), 37.8 (C-l), 36.9 (C-10), 32.9 (C-25), 32.8 (C2), 31.9 (C-7), 31.7 (C-8), 28.9 (C-16), 25.6 (C-28), 24.5 (C-15), 21.9 (C-21), 21.7 (C-27), 21.5 (C-11), 19.5 (C-19), 19.1 (C-26), 12.6 (C-18), 12.1 (C-29); EI-MS *m/z* (rel. int.): 412 $[M\text{-}Glu]^+$ (72), 397 (15), 394 (22), 379 (28), 369 (35), 351 (71), 300 (67), 327 (55), 301 (15), 273 (21), 271 (26); HR-FAB-M\S *m/z*: 575.4231 $[M + H]^+$(calcd. for $C_{35}H_{59}O_6$, 575.4233). It was identified through physical and spectral data as Stigmasterol 3-O-β-D-glucopyranoside[20,21].

7,8-Dihydroxycoumarin (11): Amorphous solid (14 mg), m.p. 113-114 °C; UV ($CHCl_3$) λ_{max} (log ε) nm: 312 (3.77), 243 (3.82), 218 (4.08); IR (KBr, ν_{max}, cm^{-1}): 3108, 1713, 1607, 1595, 1525, 1503; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.61 (1H, d, *J* = 9.5 Hz, H-4), 6.85 (1H, d, *J* = 8.4 Hz, H-5), 6.75 (1H, d, *J* = 8.4 Hz, H-6), 6.10 (1H, d, *J* = 9.5 Hz, H-3); HR-EI-MS, *m/z*: 178.0261 (calcd. for $C_9H_6O_4$, 178.0267), El-MS *m/z* (rel. int.): 178 (100), 150 (84), 122 (14), 94 (28), 66 (43), 51 (14). The physical and spectral data were much closed to the reported values[22].

α-Amyrin acetate (12): Crystallized from MeOH (34 mg); m.p.: 244-245 ºC; $[\alpha]_D^{25}$: + 81.4 (c = 0.20, $CHCl_3$); IR (KBr, ν_{max}, cm^{-1}): 3055, 1710, 1660, 1460, 1382, 1180 and 810; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 5.10 (1H, m, H-12), 4.05 (1H, dd, *J* = 10.0, 4.5 Hz, H-3), 2.13 (3H, s, OAc), 1.00, 1.07, 0.98, 0.96 (3H, each s, Me), 0.90 (6H, s, H-29 and H-30), 0.86 and 0.81 (3H, each s, Me); EI-MS *m/z* (rel. int.): 468 $[M]^+$ (27), 426 (35), 411 (11), 408 (12), 257 (26), 218 (100), 207 (8), 203 (55) and 189 (75); HR-EI-MS *m/z*: 468.3931 (calcd. for $C_{32}H_{52}O_3$, 468.3916). The physical and spectral data coincided with the literature[23].

RESULTS AND DISCUSSION

The methanolic extract of the whole plant of *Eucalyptus globulus* Labill. With *n*-hexane, ethyl acetate, *n*-butanol and water to get subsequent fractions. The ethyl acetate fraction was subjected to a series of column and flash chromatographic techniques as described in the experimental to obtain twelve compounds reported for the first time from this species. These could be identified as stigmasterol (**1**), ursolic acid (**2**), 4',5,7-trimethoxykaempferol (**3**), naringenin (**4**), genistein (**5**), catechin (**6**), epicatechin (**7**), octyl-β-D-glucopyranoside (**8**), α-amyrin (**9**), stigmasterol 3-O-β-D-glucopyranoside (**10**), 7,8-dihydroxycoumarin (**11**) and α-amyrin acetate (**12**), respectively, on the basis of their respective spectral data.

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