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# Acylated and Non-Acylated Flavonol Monoglycosides from the Indian Minor Spice Nagkesar (*Mammea longifolia*)

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A methanol extract of nagkesar (buds of *Mammea longifolia*), which showed strong radical scavenging activity, yielded 13 compounds by separations using column chromatography and HPLC. Structure elucidation of these compounds was achieved by $^{1}H$ and $^{13}C$ NMR, including DQF−COSY, TOCSY, DEPT, HMQC, HSQC, and HMBC. They include two new compounds, quercetin 3-*O*-(2″,4″di-*E*-*p*-coumaroyl)-α-L-rhamno-pyranoside and quercetin 3-*O*-(3″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside, along with known compounds kaempferol, quercetin, the isopropylidenedioxy derivative of shikimic acid, kaempferol 3-*O*-(2″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside, kaempferol 3-*O*-(3″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside, kaempferol 3-*O*-α-L-rhamnopyranoside, quercetin 3-*O*-α-L-rhamnopyranoside, shikimic acid, kaempferol 3-*O*-β-D-glucopyranoside, quercetin 3-*O*-β-D-glucopyranoside, and β-sitosterol 3-*O*-β-D-glucopyranoside.

**KEYWORDS:** ***Mammea longifolia*; Guttiferae; nagkesar; radical scavenging activity; flavonoids; quercetin 3-O-(2″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside; quercetin 3-O-(3″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside; shikimic acid derivative**

## INTRODUCTION

Buds of *Mammea longifolia* Planch & Triana syn (Guttiferae) are well-known in India as nagkesar (Hindi) and used as a minor spice. *M. longifolia* is a large tree, growing to a height of 12−18 m, and is found in southwestern India from Khandla southward to Malabar and Coimbatore region. It bears flowers once a year, and the flower buds are globose and white or pink. The flower buds are stimulant, carminative, and astringent, and they are used in the treatment of dyspepsia and hemorrhoids (*1*). Its dried flower buds resemble enlarged clove buds, and they are extensively used in culinary preparations, especially in spice blends and *Garam Masala* powders. The buds are also used as a substitute for cloves in making *Pan Masala*, which is a chewing product in India used to improve digestion. Earlier investigations on *M. longifolia* showed the presence of vitexin and *meso*-inositol in the flowers (*2*); amentoflavone and its mono- and di-methyl ethers, quercetin 3-*O*-glucoside, 3,4,5-trihydroxy benzoic acid, and vitexin in fresh leaves (*3*); and surangin A, B, and C, which are alkylated coumarins, in the roots and the bark (*4*, *5*). Proximate composition of the flower buds, physical and chemical properties of the volatile oil, and chemical composition of the volatile oil from the flower buds have been reported (*6*).

Currently, the use of some naturally occurring antioxidant molecules and their equivalents in foods, as well as in preventive and therapeutic medicine, is gaining popularity. The hexane and methanol extracts from the buds of *Mammea longifolia* were subjected to radical scavenging assay. It was found that the methanol extract showed strong radical scavenging activity, in the xanthine−xanthine oxidase system using chemiluminescence assay (*7*). We have now investigated the chemical components of the methanol extract and isolated thirteen compounds, which include two new acylated quercetin monoglycosides.

## MATERIALS AND METHODS

**Plant Material.** Dried samples of plant material were purchased from a local market in Mysore, India. Samples were identified, and voucher specimens were deposited in the herbarium of the Botany Department, University of Mysore, India.

**Equipment.** Chemiluminescence was measured on an Atto Luminescencer AB-2000 (Tokyo, Japan). HPLC separations were made using a Jasco 880-51 2-line degasser (Tokyo, Japan), Shimadzu SCL-10A VP system controller (Kyoto, Japan), Shimadzu LC-6AD pumps (Kyoto, Japan), a Jasco 875-UV Intelligent UV/Vis detector (Tokyo, Japan), and a Shimadzu C-R6A Chromatopac recorder (Kyoto, Japan). The reverse-phase preparative column was a 250 × 20 mm i.d. Cosmosil 5C18-MS (Nacalai Tesque, Kyoto, Japan). NMR spectra were recorded on Bruker DRX600 and Bruker Avance 800 instruments (Karlsruhe, Germany). TMS was used as the internal standard.

**Extraction of Plant Material.** The dried buds were powdered, and the powder (250 g) was successively extracted with hexane and methanol. The solvent was evaporated to yield 1.7% and 8.5 wt% of extract, respectively.

**Chemiluminescence Assay.** These extracts were subjected to radical scavenging activity using chemiluminescence assay as previously reported (*7*).

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*0.5 M Tris−HCl Buffer Solution*. Tris(hydroxymethyl)amino methane (15.1 g) is dissolved in 250 mL of EDTA (100 $\mu$M).

*Reagent A*. Xanthine (1.52 mg) is dissolved in 0.5 M Tris−HCl buffer solution (10 mL).

*Reagent B*. Reagent A (1.5 mL) is mixed with 0.5 M Tris−HCl buffer (13.5 mL) and 3 mM MCLA [2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo[1,2-a] pyrazin-3-one hydrochloride; Tokyo Kasei Kogyo Company Limited, Japan] in $H_2O$ (5 $\mu$L).

*Reagent C (Enzyme)*. A 5 units/mL suspension of xanthine oxidase (Wako Chemicals, Japan) (1.5 $\mu$L) is mixed with 0.5 M Tris−HCl buffer (2 mL) and diluted to 20 mL with water.

*Preparation of Extract Solutions*. Extracts (1 mg/mL in MeOH) were prepared; 300 $\mu$L of this solution was taken in the first column of wells of the microplate, and wells in the other columns were filled with extracts on serial dilution.

*Measurement of Chemiluminescence*. A total of 10 $\mu$L each of the above solutions was transferred to three other microplates for chemiluminiscence measurement. Reagents B (100 $\mu$L) and C (100 $\mu$L) were added, and chemiluminescence was measured after 19−20 s.

**Isolation of the Compounds.** The methanol extract (21 g) was subjected to silica gel column chromatography using chloroform and methanol mixtures as solvents. Compounds **1**−**13** were isolated in the same order from chloroform−methanol (9:1 and 17:3) fractions on further chromatography. Final purification of **4**, **5**, **6** and **7** mixture, **10**, and **11** was achieved by HPLC on reversed phase with water and methanol mixtures (3:2, 11:9, and 1:1; flow rate 5 mL/min; monitoring wavelength 280 nm) as eluting solvents. The yields of the compounds were as follows: **1**, 16 mg; **2**, 10 mg; **3**, 10 mg; **4**, 12 mg; **5**, 7 mg; **6**, 8 mg; **7**, 10 mg; **8**, 5 mg; **9** and **10**, 10 mg; **11**, 20 mg; **12**, 200 mg, and **13**, 15 mg.

**Preparation of Compound 3.** Shikimic acid (10 mg) in 4 mL of acetone was stirred for 5 h at room temperature in the presence of *p*-toluenesulfonic acid. After removal of the solvent, the reaction mixture was subjected to column chromatography on silica gel using hexane and acetone mixtures (9:1, 4:1, 7:3, 3:2, and 1:1) as eluting solvents. Synthetic compound **3** (10 mg) was obtained after removal of the solvents from column fractions.

## RESULTS AND DISCUSSION

Radical scavenging activities studies on the extracts of nagkesar showed that the methanol extract was active with an activity of 1 mg of rutin equivalent to the activity of 3.2 mg of methanol extract. Thirteen compounds (**1**−**13**) were isolated in the same order from methanol extract of the dried buds by column chromatography on silica gel and reversed-phase HPLC, and their structures were elucidated by $^1$H and $^{13}$C NMR spectroscopic analysis, including DQF−COSY, TOCSY, DEPT, HMQC, HSQC, and HMBC (**Figure 1**). The compounds were identified as kaempferol (**1**), quercetin (**2**), kaempferol 3-*O*-(2″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (**4**), kaempferol 3-*O*-(3″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside (**5**), kaempferol 3-*O*-α-L-rhamnopyranoside (**8**), quercetin 3-*O*-α-L-rhamnopyranoside (**9**), shikimic acid (**10**), kaempferol 3-*O*-*β*-D-glucopyranoside (**11**), quercetin 3-*O*-*β*-D-glucopyranoside (**12**), and *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (**13**) on the basis of the above spectroscopic data.

Compounds **6** and **7** (found C 63.22; H 4.36; $C_{39}H_{32}O_{15}$; requires C 63.24; H 4.35) could not be separated using various chromatographic techniques, as these were interconverted during the separation and isolation. A similar phenomenon was reported earlier with an analogous type of compounds (*8*). From NMR spectra of the mixture (**Tables 1** and **2**), compounds **6** and **7** were considered to be quercetin 3-*O*-rhamnopyranosides disubstituted with *p*-coumaroyl groups. A DQF−COSY experiment showed clear coupling connections around the rhamnopyranoside ring, and assignment of the protons was achieved. Downfield shifts were observed at H-2″ ($\Delta\delta$ 1.33 ppm) and H-4″ ($\Delta\delta$ 1.64 ppm) in compound **6** and H-3″ ($\Delta\delta$ 1.63 ppm) and H-4″ ($\Delta\delta$ 1.89 ppm) in compound **7**, compared with those on quercetin 3-*O*-α-L-rhamnopyranoside **9**, which suggested the acyl-substitution at these positions. Comparison of $^{13}$C NMR data (**Table 2**) of compounds **6** and **7** with those of **9** showed expected upfield shifts of the signal of the carbon adjacent to acylation sites, i.e., C-1″ ($\Delta\delta$ −4.38 ppm), C-3″ ($\Delta\delta$ −3.52 ppm), and C-5″ ($\Delta\delta$ −2.24 ppm) in **6**, and C-2″ ($\Delta\delta$ −2.33 ppm) and C-5″ ($\Delta\delta$ −2.03 ppm) in **7**. Downfield shifts at the acylation site were observed for C-2″ ($\Delta\delta$ +1.00 ppm) and C-4″ ($\Delta\delta$ +1.45 ppm) in **6**. All the above shifts observed for **6** and **7** are in conformity with those of other acylated sugars reported earlier (*9*−*11*), as well as with **4** and **5** in this study. However, downfield shifts for C-3″ and C-4″ in **7** were not observed. The *E*-configurations of the *p*-coumaroyl groups and α-linkage of the rhamnose were assigned from the $^1$H NMR coupling constants of 16 Hz ($J_{\text{coumH7−H8}}$), and 1.69 ($J_{\text{H1″−H2″}}$) Hz, respectively. Carbon and proton connections were confirmed from DEPT and HSQC experiments. Hence, the structures of compounds **6** and **7** are established as quercetin 3-*O*-(2″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside and quercetin 3-*O*-(3″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside, respectively. This is the first report of quercetin rhamnoside with two coumaroyl ester substituents. This is the fourth and third report, respectively, for compounds **4** and **5** in nature. However, this is the first report for both the compounds **4** and **5** from the Guttiferae family.

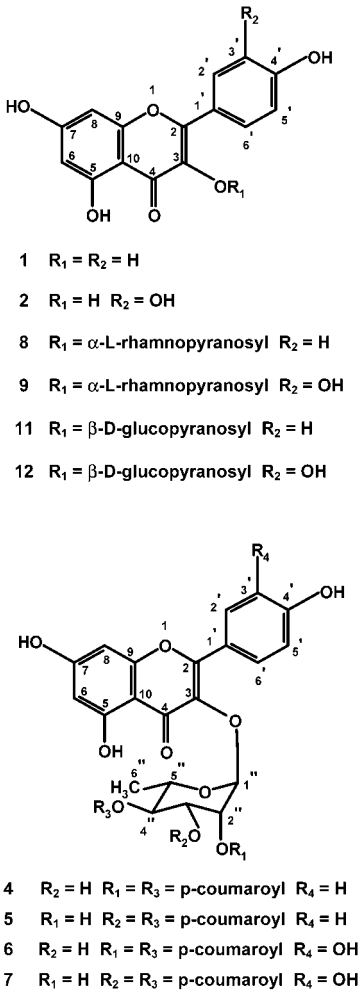



**Figure 1.** Structures of flavonoids **1**, **2**, **4**–**9**, **11**, and **12** isolated from *Mammea longifolia*.

**Table 1.** $^1$H NMR Data of **6** and **7** (methanol-$d_4$; 600 MHz)

| | **9** | | | **6** | | | **7** | | |
|---|---|---|---|---|---|---|---|---|---|
| proton | $\delta$ | multiplicity | $J$ (Hz) | $\delta$ | multiplicity | $J$ (Hz) | $\delta$ | multiplicity | $J$ (Hz) |
| 6 | 6.19(1H) | d | 2.1 | 6.22 | d | 2.1 | 6.21 | d | 2.1 |
| 8 | 6.36 (1H) | d | 2.1 | 6.39 | d | 2.1 | 6.38 | d | 2.1 |
| 2′ | 7.33 (1H) | d | 2.1 | 7.37–7.39 | m | | 7.37 | d | 2.2 |
| 5′ | 6.90(1H) | d | 8.3 | 6.99 | d | 8.3 | 7.01 | d | 8.6 |
| 6′ | 7.30 (1H) | dd | 2.1, 8.3 | 7.37–7.39 | m | | 7.31 | dd | 2.2, 8.6 |
| 1″ | 5.34(1H) | d | 1.6 | 5.74 | d | 1.7 | 5.66 | d | 1.7 |
| 2″ | 4.21(1H) | dd | 1.6, 3.3 | 5.54 | dd | 1.7, 3.4 | 4.46 | dd | 1.7, 3.1 |
| 3″ | 3.74(1H) | dd | 3.3, 9.5 | 4.19 | dd | 3.4, 9.8 | 5.37 | dd | 3.1, 10.0 |
| 4″ | 3.33(1H) | t | 9.5 | 4.97 | t | 9.8 | 5.22 | t | 10.0 |
| 5″ | 3.41(1H) | m | | 3.33 | m | | 3.43 | m | |
| 6″ | 0.93(3H) | d | 6.2 | 0.87 | d | 6.2 | 0.85 | d | 6.1 |
| 2‴,6‴ coum | | | | 7.49 | d | 8.5 | 7.40 | d | 8.5 |
| 2⁗,6⁗ coum | | | | 7.55 | d | 8.4 | 7.48 | d | 8.4 |
| 3‴,5‴ coum | | | | 6.80 | d | 8.5 | 6.76 | d | 8.5 |
| 3⁗,5⁗ coum | | | | 6.83 | d | 8.4 | 6.78 | d | 8.4 |
| 7‴coum | | | | 7.60 | d | 16.0 | 7.53 | d | 15.9 |
| 7⁗coum | | | | 7.69 | d | 15.8 | 7.63 | d | 15.9 |
| 8‴coum | | | | 6.32 | d | 16.0 | 6.22 | d | 15.9 |
| 8⁗coum | | | | 6.42 | d | 15.8 | 6.29 | d | 15.9 |

**Table 2.** $^{13}$C NMR Data of **4**, **5**, **6**, **7**, and **9** (methanol-$d_4$; 150 MHz; $\delta$ in ppm)

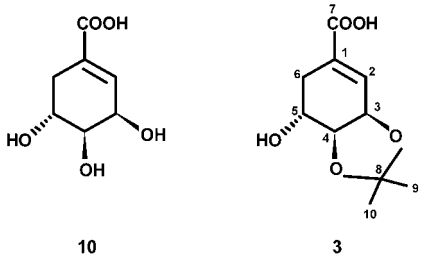
| carbon | **9** | **4** | **5** | **6** | **7** |
|---|---|---|---|---|---|
| 2 | 158.52 | 158.63 | 158.71 | 158.60 | 158.58 |
| 3 | 136.24 | 134.64 | 134.08 | 134.65 | 135.15 |
| 4 | 179.65 | 179.21 | 179.29 | 179.41 | 179.25 |
| 5 | 163.21 | 163.27 | 163.19 | 163.27 | 163.27 |
| 6 | 99.81 | 99.13 | 100.31 | 99.92 | 99.92 |
| 7 | 165.87 | 166.33 | 167.03 | 165.95 | 165.93 |
| 8 | 94.71 | 94.91 | 95.10 | 94.80 | 94.78 |
| 9 | 159.31 | 159.39 | 158.73 | 159.55 | 159.53 |
| 10 | 105.90 | 105.83 | 105.67 | 105.93 | 105.89 |
| 1′ | 122.97 | 122.49 | 122.56 | 122.83 | 123.03 |
| 2′ | 116.93 | 133.95 | 133.93 | 116.81 | 117.01 |
| 3′ | 146.41 | 116.77 | 116.78 | 146.75 | 146.73 |
| 4′ | 149.79 | 161.38 | 161.41 | 150.00 | 150.03 |
| 5′ | 116.37 | 116.77 | 116.78 | 116.79 | 116.76 |
| 6′ | 122.86 | 133.95 | 133.93 | 122.66 | 122.76 |
| 1″ | 103.54 | 100.08 | 101.51 | 99.16 | 101.94 |
| 2″ | 72.11 | 73.12 | 69.86 | 73.11 | 69.78 |
| 3″ | 72.03 | 68.48 | 71.74 | 68.51 | 71.77 |
| 4″ | 73.25 | 74.62 | 72.71 | 74.70 | 72.95 |
| 5″ | 71.90 | 69.75 | 69.71 | 69.66 | 69.87 |
| 6″ | 17.65 | 17.69 | 17.59 | 17.71 | 17.60 |
| 1‴coum | | 127.17 | 127.10 | 127.55 | 127.23 |
| 1⁗coum | | 127.19 | 127.04 | 127.20 | 127.10 |
| 2‴,6‴coum | | 131.28 | 131.28 | 131.50 | 131.45 |
| 2⁗,6⁗coum | | 131.97 | 131.95 | 131.38 | 131.29 |
| 3‴,5‴coum | | 115.87 | 115.88 | 116.60 | 116.52 |
| 3⁗,5⁗coum | | 116.82 | 116.81 | 115.87 | 115.89 |
| 4‴coum | | 161.40 | 161.43 | 161.39 | 161.37 |
| 4⁗coum | | 161.86 | 161.85 | 161.35 | 161.34 |
| 7‴coum | | 146.96 | 147.29 | 147.44 | 147.44 |
| 7⁗coum | | 147.46 | 147.44 | 147.39 | 147.03 |
| 8‴coum | | 114.68 | 114.66 | 114.98 | 114.70 |
| 8⁗coum | | 114.95 | 114.50 | 114.68 | 114.52 |
| 9‴coum | | 168.23 | 168.15 | 168.59 | 168.55 |
| 9⁗coum | | 168.48 | 168.48 | 168.26 | 168.24 |

On comparison of $^1$H and $^{13}$C NMR spectra of **3** (**Table 3**) with that of shikimic acid **10**, **3** was identified as an isopropylidenedioxy derivative of shikimic acid (**Figure 2**). The structure was confirmed by study of DEPT, DQF−COSY, HSQC, and HMBC spectra. The isopropylidenedioxy group could be placed on either the 3,4- or 4,5-positions. Couplings from the HMBC spectra indicated its attachment to the 3,4-positions. The configurations at C-3, 4, and 5 are deduced as *rel-R, S, R-*, which is same as natural shikimic acid, from the proton coupling constants. Further, the structure of **3** was confirmed by its preparation from shikimic acid and acetone in the presence of *p*-toluenesulfonic acid. Hence, compound **3** is 3,4 isopropylidenedioxyshikimicacid (i.e., 3a,6,7,7a-tetrahydro-7-hydroxy-2,2-dimethyl-[3a*R*-(3aα,7α,7aα)]-1,3-benzodioxole-5-carboxylic acid). It was concluded that this compound is present as such and not an artifact formed during the isolation, as acetone was not used in the process. A similar structure was assigned earlier to a compound isolated from *Excoecaria cochinchinensis* var. *viridis* without stereochemical details (*12*).

**Table 3.** $^1$H and $^{13}$C NMR Data of **3**

| | $^1$H | | | $^{13}$C $\delta$ | |
|---|---|---|---|---|---|
| carbon | $\delta$ | multiplicity | $J$ (Hz) | **3** | SYN[a] |
| 1 | | | | 130.25 | 130.50 |
| 2 | 6.79 (1H) | ddd | 3.4, 1.6, 1.5 | 135.08 | 135.34 |
| 3 | 4.73(1H) | ddddd | 6.2, 3.4, 1.5, 1.5, 0.3 | 72.67 | 72.93 |
| 4 | 4.13(1H) | t | 6.2 | 77.64 | 77.91 |
| 5 | 3.96(1H) | ddd | 6.2, 6.4, 4.4 | 67.99 | 68.23 |
| 6a | 2.28 (1H) | ddddd | 17.4, 6.4, 1.6, 0.3 | 29.59 | 29.42 |
| 6b | 2.56(1H) | ddddd | 17.4, 4.4, 1.5, 0.3 | | |
| 7 | | | | 167.57 | 167.78 |
| 8 | | | | 109.32 | 109.57 |
| 9 | 1.31 (3H) | s | | 25.86 | 26.13 |
| 10 | 1.32 (3H) | s | | 28.00 | 28.27 |
| OH | 4.23 | br | | | |

[a] SYN, Compound prepared from shikimic acid.




**10** **3**

**Figure 2.** Structures of shikimic acid, **10**, and the 3,4-isopropylidine derivative **3** isolated from *Mammea longifolia*.

Ten flavonoids, shikimic acid and its isopropylidenedioxy derivative, and β-sitosterol 3-*O*-β-D-glucopyranoside were isolated from the methanol extract, which showed strong radical scavenging activity. Their structures were identified with the

help of NMR study. Among the flavonoids, quercetin 3-*O*-(2″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside **6** and quercetin 3-*O*-(3″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside **7** are reported for the first time in nature; while this is the first report for both the compounds kaempferol 3-*O*-(2″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside **4** and kaempferol 3-*O*-(3″,4″-di-*E*-*p*-coumaroyl)-α-L-rhamnopyranoside **5** from the family Guttiferae. It is also the first report for *rel*-3a,6,7,7a-tetrahydro-7-hydroxy-2,2-dimethyl-[3a*R*-(3aα,7α,7aα)]-1,3-benzodioxole-5-carboxylic acid **3** from the Guttiferae, and its stereochemical structure has been established with the help of NMR studies.

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