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## A new gnidiflavanone-flavonol dimer and other constituents from *Gnidia apiculata*

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### ABSTRACT

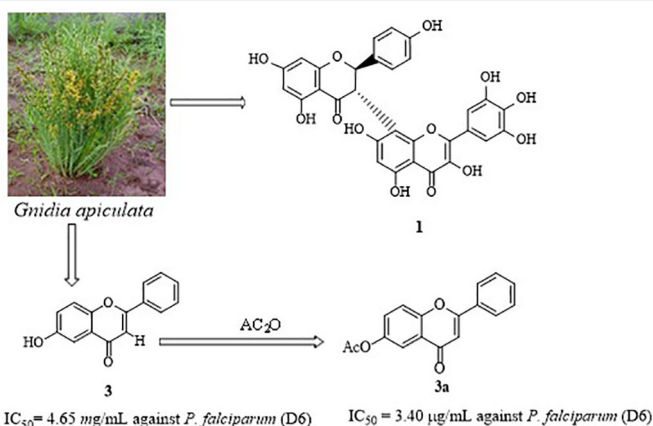
A new 3,8''-flavanone-flavonol dimer gnidiflavanone-flavonol (**1**) and 10 known compounds (**2–11**), including four rare primula-type flavones **2–5**, were isolated from the roots of *Gnidia apiculata*. Compounds **2–5** and **7** were reported for the first time from the plant family Thymelaeaceae. Structures of the isolated compounds were established by spectroscopic data, including 1D and 2D NMR (COSY, HMBC, HSQC and ROESY) and mass spectrometry, as well as by the comparison with literature data. The crude roots extract and isolated compounds were evaluated for antimicrobial and antiplasmodial activities. Among isolated compounds, 6-hydroxyflavone (**4**) and 6-*O*-acetylflavone (**4a**) showed antiplasmodial activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*.

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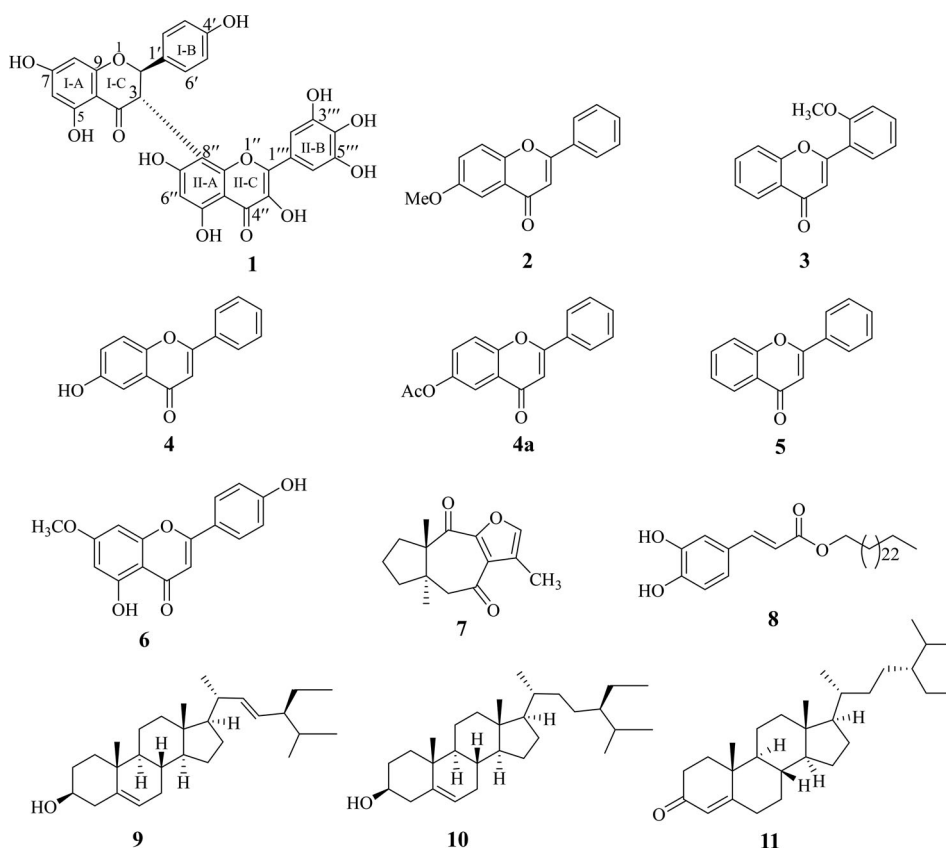
## 1. Introduction

*Gnidia* is the largest genus of the plant family Thymelaeaceae, with about 140–160 species, distributed mostly in Southern and tropical East Africa (Rogers 2009). Plants of this genus are used in folk medicine to manage fever, cancer, snake bites, pulmonary tuberculosis, toothache and other ailments (Bhandurge et al. 2013). Previous phytochemical studies on this genus have reported daphnane-type diterpenoids with ring-C orthoesters (De Mieri et al. 2015; Kalbessa et al. 2019). Other classes of compounds including primula-type flavonoids, bi-flavonoids (Ferrari et al. 2003; De Mieri et al. 2015; Kalbessa et al. 2019), benzophenone glycosides (Ferrari et al. 2000), sesquiterpenes (De Mieri et al. 2015), phenylpropanoid glycosides (Munkombwe et al. 2003), phenylpyranones, biscoumarins (De Mieri et al. 2015) and flavone-coumarin hybrids (Franke et al. 2002) were also reported from this genus. Some of the diterpenoids isolated from this genus have shown anticancer activities (Bhandurge et al. 2013).

The phytochemical constituents and biological activities of *G. apiculata* were not reported previously. Therefore, the roots extract (MeOH:CH<sub>2</sub>Cl<sub>2</sub>; 1:1) was subjected to chromatographic separation which yielded a new flavanone-flavonol dimer (**1**), rare primula-type flavones (**2–5**), and other known compounds **6–11**. Compounds **2–5** and **7** have not been previously reported from plant family Thymelaeaceae. The structure elucidation of Compound **1** was carried out using spectroscopic data and by comparison with previously reported data of an isomeric compound (2*R*,3*S*)-buchananiflavonol (Stark et al. 2013). The crude extracts and isolated compounds were evaluated for antimicrobial and antiplasmodial activities, of which 6-hydroxyflavone (**4**) and 6-*O*-acetylflavone (**4a**) showed inhibitions against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*.

## 2. Results and discussion

Compound **1** (Figure 1) was isolated as yellow powder and found to be dextrorotatory ( $[\alpha]_D^{26} + 30.3$  ( $c$  0.33, MeOH)). Its ESI-HRMS (Figure S1) showed a protonated molecular ion peak at  $m/z$  589.0979  $[M + H]^+$ , attributed to molecular formula C<sub>30</sub>H<sub>20</sub>O<sub>13</sub>. The UV spectrum of **1** showed absorption bands at  $\lambda_{max}$  280 and 380 nm, suggesting a flavone chromophore, and the FTIR spectrum (Figure S2) displayed bands at  $\nu_{max}$  3275 and 1601 cm<sup>-1</sup>, attributed to hydroxy and conjugated carbonyl groups, respectively. The <sup>13</sup>C NMR data (Table S1 and Figure S3) of Compound **1** revealed 30 signals between  $\delta_C$  50.4 and 198.1, which were assigned to 2 carbonyls ( $\delta_C$  198.1 and 177.2), 2 methines ( $\delta_C$  50.4, C-3 and 82.8, C-2) typical of a flavanone moiety, and 26 sp<sup>2</sup> hybridized carbon atoms (9 hydrogenated, 12 oxygenated and 5 quaternary carbons). The <sup>1</sup>H NMR spectrum (Table S1 and Figure S5) revealed two doublets at  $\delta_H$  5.73 ( $J=15.0$  Hz, H-2) and  $\delta_H$  4.83 ( $J=15.0$  Hz, H-3), suggesting a *trans* arrangement between H-2 and H-3 protons for a C-3 substituted flavanone skeleton (Ito et al. 1999). In addition, two deshielded intramolecular hydrogen bonded peaks were observed at  $\delta_H$  12.2 (C-5-OH) and 12.6 (C-5''-OH), characteristic for hydroxyl groups. These data so far suggested a dimer, comprising of a flavanone unit linked to a flavonol moiety.



**Figure 1.** Structures of Compounds (1–11) isolated from roots of *G. apiculata*.

The spectral data of Compound **1** were closely comparable to two corresponding diastereomeric dimers [(*2R,2''R, 3S,3''R*)-3'',3''',4',4''',5,5'',5''',7,7''-nonahydroxy-3,8''-biflavonol and (*2S,2''R, 3R,3''R*)-3'',3''',4',4''',5,5'',5''',7,7''-nonahydroxy-3,8''-biflavonol], isolated from the aerial parts of *Gnidia involucrata* (Ferrari et al. 2003). The difference was that, unlike these diastereomers, Compound **1** had unsaturated bond at C-2''/C-3'' as shown from the  $^{13}\text{C}$  NMR for two  $\text{sp}^2$  hybridized carbons at  $\delta_{\text{C}}$  147.7 and 137.0, which were assigned to C-2'' and C-3'', respectively, by the evidence of HMBC correlations (Figures S8 and S9). The unsaturation of the C-2''/C-3'' bond in Compound **1** was further supported by the significantly shielded carbonyl carbon at C-4'' ( $\delta_{\text{C}}$  177.2), compared to that of the diastereomers ( $\delta_{\text{C}}$  197.8 ppm) (Ferrari et al. 2003). The unsaturation in Compound **1** at C-2''/C-3'' allows vinylogous electronic transmission, from the oxygen atom at position-1 to the carbonyl group C-4'' through the conjugated system leading to a more shielded carbonyl carbon.

Further examination of the spectroscopic data of Compound **1** revealed that it was closely related to the dimer (*2R,3S*)-buchananiflavonol (Stark et al. 2013). The two compounds have a total of nine hydroxylated carbon atoms and unsaturation at C-2''/C-3''. The difference was that Compound **1** had a single hydroxyl group in ring I-B and three hydroxyl groups in ring II-B, while (*2R,3S*)-buchananiflavonol had two hydroxyl groups in both rings. This difference was observed from their respective NMR data.

The  $^1\text{H-NMR}$  data of (2*R*,3*S*)-buchananiflavonol had three sets of signals at  $\delta_{\text{H}}$  6.70 (*s*, 2H), 6.73 (*d*,  $J = 8.0$  Hz, 2H) and 6.56 (*d*,  $J = 7.8$  Hz, 2H), each two protons were assigned to H-2'/2''', H-5'/5''' and H-6'/6''', respectively. However, in Compound **1**, an AA'XX' spin system was observed for the two doublets at  $\delta_{\text{H}}$  7.13 (*d*,  $J = 10.0$  Hz, 2H) and  $\delta_{\text{H}}$  6.38 (*d*,  $J = 10.0$  Hz, 2H) (Table S1 and Figure S5), which were assigned to H-2'/6' and H-3'/5' of the symmetric ring I-B. This was consistent with the HMBC spectrum, which showed correlations between H-C2'/6' and C-2, C-1' and C-4' (Figures S8 and S9). Furthermore, a sharp singlet at  $\delta_{\text{H}}$  7.24 (*s*, 2H) was assigned to two aromatic protons H-2'''/6''' of the trisubstituted ring II-B. The relative configuration of Compound **1** was determined by analysis of its ROESY spectrum (Figure S6). No ROESY cross peak was observed between H-2 and H-3, which was consistent with a *trans* arrangement of ring I-B and flavonol substituents. This was in agreement with the *trans* configuration deduced from the coupling constant of H-2 and H-3 ( $J = 15.0$  Hz). Based on the foregoing evidence, Compound **1** was assigned to a positional isomer of the previously known dimer (2*R*,3*S*)-buchananiflavonol. Therefore, **1** was established as *trans*-2-(4-hydroxyphenyl)-2,3-dihydro-3'-5,5'-7,7'-pentahydroxy-2'-(3,4,5-trihydroxyphenyl)-[3,8'-bi-1-benzopyran]-4,4'-dione, a new dimer named gnidiflavanone-flavonol.

In addition, 10 known compounds were isolated and identified as 6-methoxyflavone (**2**) (Freeman et al. 1981; Zhang et al. 2019), 2'/6'-methoxyflavone (**3**) (Budzianowski et al. 2005), 6-hydroxyflavone (**4**) (Ren et al. 2010; Singh et al. 2017), unsubstituted flavone (**5**) (Colombo et al. 2014), genkwanin (**6**) (Li et al. 2013), chlorantene A (**7**) (Yuan et al. 2008), pentacosanyl caffeate (**8**) (Freire et al. 2007), stigmasterol (**9**) and  $\beta$ -sitos-terol (**10**) mixture (Chaturvedula and Prakash, 2012), and stigmast-4-en-3-one (**11**) (Su et al. 2009). In addition, 6-hydroxyflavone (**4**) was acetylated to its corresponding 6-acetoxyflavone (**4a**) (Table S5 and Figures S30–32).

The crude roots extract of *G. apiculata*, Compounds **1–11** and **4a** were tested for *in vitro* antimicrobial and antiplasmodial activities against selected bacteria, fungi and *Plasmodium falciparum*. All the isolated compounds were found inactive towards bacteria and fungi. However, 6-hydroxyflavone (**4**) and its acetylated derivative **4a** showed activities against chloroquine-sensitive (D6) and -resistant (W2) strains of *P. falciparum*. Compound **4** exhibited  $\text{IC}_{50}$  values of 4.6 and  $>4.7$   $\mu\text{g/mL}$  against D6 and W2 strains, respectively, while **4a** had  $\text{IC}_{50}$  values of 3.4 and 4.6  $\mu\text{g/mL}$  against D6 and W2, compared to reference standards chloroquine and artemisinin ( $\text{IC}_{50}$  0.02, 0.16, and  $\text{IC}_{50}$  0.005 and 0.003  $\mu\text{g/mL}$ , respectively).

### 3. Experimental

#### 3.1. General experimental procedures

Optical rotation was determined by AUTOPOL® IV polarimeter. The UV spectra were recorded using Shimadzu UV-VIS spectrophotometer and the IR spectra were measured by Shimadzu (IRAfinity-1S) FTIR spectrophotometer. The  $^1\text{H}$ - and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Avance 400 or 500 MHz spectrometers. HMBC, HSQC and ROESY were measured on an Agilent DD2-500 NMR spectrometer. The ESI-HRMS spectral data were obtained by utilizing an Agilent 6545 LC/Q-ToF system. LC/MS data was measured using Agilent 1290 Infinity series UHPLC instrument, coupled to an Agilent

6120 quadrupole mass spectrometer with a dual ESI and APCI interface. TLC analysis was done using analytical silica gel 60 PF<sub>254+366</sub> pre-coated alumina plates (Merck, 0.25 mm thick). CC was done on Silica Gel (Loba Chemie, 70-230 mesh) or (CDH, 60-120 mesh) and sephadex LH-20 (Merck). Solvents for extraction and CC were distilled before use.

### 3.2. Plant material

The roots of *G. apiculata* were collected from Machakos County, about 40 km from Nairobi city, Kenya, and a voucher specimen (RWK 2017/03) was deposited in the University herbarium in the Department of Botany, University of Nairobi.

### 3.3. Extraction and isolation

The plant material (2 kg) was air dried, ground to fine powder and extracted by cold percolation with MeOH:CH<sub>2</sub>Cl<sub>2</sub> (1:1) for 24 h (× 3) to yield 160 g of crude extract. A 150 g of the extract was fractionated by column chromatography using a gradually increasing polarity of EtOAc/*n*-C<sub>6</sub>H<sub>14</sub> solvent mixture which yielded six combined fractions (labelled as IA-IF). Further analysis was not done on fraction IA (2.01 g) because it contained oils with poor TLC separations. The mixture of Compounds **9** and **10** (20 mg, *R<sub>f</sub>* 0.39 (10% EtOAc/*n*-C<sub>6</sub>H<sub>14</sub>)) was obtained from crystallization of the second major fraction IB. The mother liquor of fraction IB (1.14 g) was subjected to column chromatography using CH<sub>2</sub>Cl<sub>2</sub>:*n*-C<sub>6</sub>H<sub>14</sub> to yield **7** (15 mg), as a white solid (*R<sub>f</sub>* 0.42, 70% CH<sub>2</sub>Cl<sub>2</sub>/*n*-C<sub>6</sub>H<sub>14</sub>), and **11** (15 mg), as a colorless oil (*R<sub>f</sub>* 0.25, 80% CH<sub>2</sub>Cl<sub>2</sub>:*n*-C<sub>6</sub>H<sub>14</sub>). The third major fraction (IC, 4.6 g) was further separated using EtOAc/*n*-C<sub>6</sub>H<sub>14</sub> with gradually increasing polarity to yield subfractions IIA-IIC. Crystallization of subfraction IIB in CH<sub>2</sub>Cl<sub>2</sub>/*n*-C<sub>6</sub>H<sub>14</sub> afforded **8**, as a yellow powder (20 mg, *R<sub>f</sub>* = 0.28; 2% MeOH/CH<sub>2</sub>Cl<sub>2</sub>). Also, crystallization of RWK55C yielded another yellow powder (**6**; 18 mg, *R<sub>f</sub>* = 0.26, 30% EtOAc/*n*-C<sub>6</sub>H<sub>14</sub>). Chromatography of mother liquor of RWK55C (0.5 g) using CH<sub>2</sub>Cl<sub>2</sub>:*n*-C<sub>6</sub>H<sub>14</sub>, followed by crystallization yielded white needles (**5**, 28 mg, *R<sub>f</sub>* = 0.28, 1% MeOH/CH<sub>2</sub>Cl<sub>2</sub>).

A 4.9 g fraction (1D) from the major column was chromatographed repeatedly using MeOH/CH<sub>2</sub>Cl<sub>2</sub> mixtures to yield a yellow solid (**2**, 12 mg, *R<sub>f</sub>* = 0.37, 1% MeOH/CH<sub>2</sub>Cl<sub>2</sub>), followed by a mixture of **2** and **3** (20 mg, *R<sub>f</sub>* = 0.22, 30% EtOAc/*n*-C<sub>6</sub>H<sub>14</sub>). The last major fraction (IE, 7 g) was subjected to chromatography using EtOAc/*n*-C<sub>6</sub>H<sub>14</sub> solvent mixture to yield **4** and **1**. Compound **4** (9 mg, *R<sub>f</sub>* 0.32, 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>), brown crystals, was obtained from crystallization of the second subfraction IIIB. Finally, chromatographic separation of subfraction IIIC over sephadex, using MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1:1) as eluent, yielded **1** (30 mg, *R<sub>f</sub>* 0.41, 20% MeOH/CH<sub>2</sub>Cl<sub>2</sub>).

Gnidiflavanone-flavonol (**1**): Yellow powder; [ $\alpha$ ]<sub>D</sub><sup>26</sup> + 30.3 (c 0.33, MeOH); ESI-HRMS *m/z* 589.0979 [M+H]<sup>+</sup> (calculated for *m/z* 589.0977, [C<sub>30</sub>H<sub>21</sub>O<sub>13</sub>]<sup>+</sup>) (Figure S1); UV (MeOH):  $\lambda_{\max}$  (log  $\epsilon$ ) 380 (3.19), 280 (4.00) nm; IR (neat)  $\nu_{\max}$  3275, 1601, 1319, 1161, 1028, 829 cm<sup>-1</sup> (Figure S2); <sup>1</sup>H-NMR (500 MHz, DMSO);  $\delta_{\text{H}}$  12.6 (1H, s, 5''-OH), 12.2 (1H, s, 5-OH), 7.24 (2H, s, H-2''', 6'''), 7.13 (2H, d, *J* = 10.0 Hz, H-2', 6'), 6.38 (2H, d, *J* = 10.0 Hz, H-3', 5'), 6.20 (1H, s, H-6''), 5.95 (2H, m, H-6, 8), 5.73 (1H, d, *J* = 15.0 Hz, H-

2), 4.83 (1H, d,  $J = 15.0$  Hz, H-3), 3.41 (brs, 3'',3''', 4', 4''', 5''', 7, 7'',-OH) (Table S1, Figure S5);  $^{13}\text{C}$ -NMR (125 MHz, MeOD);  $\delta_{\text{C}}$  198.1 (C-4), 177.2 (C-4''), 168.2 (C-7), 165.7 (C-9''), 164.9 (C-5), 162.8 (C-7''), 161.8 (C-5''), 158.5 (C-4'), 156.1 (C-9), 147.7 (C-2''), 146.6 (C-3''', 5'''), 137.0 (C-3''), 137.0 (C-4'''), 130.4 (C-1'), 129.5 (C-2', 6'), 122.9 (C-1'''), 115.5 (C-3', 5'), 108.5 (C-2''', 6'''), 104.1 (C-10''), 103.2 (C-10), 101.4 (C-8''), 99.0 (C-6''), 97.3 (C-6), 96.4 (C-8), 82.8 (C-2), 50.4 (C-3) (Table S1 and Figure S3).

### 3.4. O-Acetylation of 6-hydroxyflavone (4)

The acetylation of Compound **4** was performed using acetic anhydride (Ranu et al. 2003). A neat mixture of Compound **4** (10 mg) and acetic anhydride (12 mL) was placed in a 100 mL round bottomed flask and heated at 100 °C with constant stirring for 2 hours. Progress of the reaction was monitored by TLC analysis. The product was extracted with  $\text{CH}_2\text{Cl}_2$  followed by crystallization, to yield a white solid of 6-O-acetylflavone (**4a**) (Table S5 and Figures S30–32) in 98% yield (9.8 mg,  $R_f = 0.35$  under 1.5% MeOH/ $\text{CH}_2\text{Cl}_2$ ).

### 3.5. Antimicrobial assay

The *in vitro* antimicrobial activity of crude extract and pure compounds was determined against selected standard ATCC organisms (bacteria and fungi) from ATCC, using protocol as described in literature (Kumarihamy et al. 2021). The crude extract was tested at 200  $\mu\text{g}/\text{mL}$ , while the pure compounds were dissolved to make a stock solution of 2 mg/mL. The pure compounds were tested at concentrations of 20, 4 and 0.8  $\mu\text{g}/\text{mL}$  and the respective  $\text{IC}_{50}$ 's determined. The  $\text{IC}_{50}$  values were calculated using XLFit software. Amphotericin B (92% pure, MP Biomedical, CA, USA) and vancomycin (90% pure, from Sigma-Aldrich, MO, USA) were used as positive controls against fungi and bacteria, respectively.

### 3.6. Antiplasmodial assay

The *in vitro* antiplasmodial activity of the crude extract and pure compounds was done by parasite Lactate Dehydrogenase Assay (Nogueira and Rosário 2010) on chloroquine -resistant W2 and -sensitive D6 strains of *Plasmodium falciparum* (Kumarihamy et al. 2021). The compounds were dissolved in dimethyl sulfoxide and pre-diluted in RPMI 1640 medium to make concentrations between 528.9 to 4760 ng/mL. Chloroquine and artemisinin were used as positive controls. The percent inhibition of the parasites by the phytochemicals was determined from the absorbance at 650 nm, shown by SpectraMAX ELISA Reader. The XLFit software was used to calculate  $\text{IC}_{50}$ ; the concentration of the sample that caused inhibition of 50% of the tested parasites.

## Conclusions

Phytochemical investigation of *G. apiculata* led to the isolation of a new 3,8''-flavone-flavonol dimer, *trans*-2-(4-hydroxyphenyl)-2,3-dihydro-3'-5,5'-7,7'-pentahydroxy-2'-

(3,4,5-trihydroxyphenyl)-[3,8'-bi-1-benzopyran]-4,4'-dione (**1**), and ten previously reported compounds (**2-11**). Compounds, **2-5** and **7** are reported for the first time in the family Thymelaeaceae. Therefore, these findings have enriched the chemical diversity of genus *Gnidia*.

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## Disclosure statement

The authors declare no conflict of interest.

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## References

- Bhandurge P, Rajarajeshwari N, Ganapaty S, Pattanshetti S. 2013. The *Gnidia* genus: a review. *Asian J. Biomed. Pharm. Sci.* 3(19):1–31.
- Budzianowski J, Morozowska M, Wesołowska M. 2005. Lipophilic flavones of *Primula veris* L. from field cultivation and *in vitro* cultures. *Phytochemistry.* 66(9):1033–1039.
- Chaturvedula VSP, Prakash I. 2012. Isolation of stigmaterol and  $\beta$ -sitosterol from the dichloromethane extract of *Rubus suavissimus*. *Int. Curr. Pharm. J.* 1(9):239–242.
- Colombo PS, Flamini G, Christodoulou MS, Rodondi G, Vitalini S, Passarella D, Fico G. 2014. Farinose alpine *Primula* species: phytochemical and morphological investigations. *Phytochemistry.* 98:151–159.
- De Mieri M, Du K, Neuburger M, Saxena P, Zietsman PC, Hering S, van der Westhuizen JH, Hamburger M. 2015. HERG channel inhibitory daphnane diterpenoid orthoesters and polycephalones A and B with unprecedented skeletons from *Gnidia polycephala*. *J Nat Prod.* 78(7):1697–1707.
- Ferrari J, Terreaux C, Sahpaz S, Msonthi JD, Wolfender JL, Hostettmann K. 2000. Benzophenone glycosides from *Gnidia involucreta*. *Phytochemistry.* 54(8):883–889.
- Ferrari J, Terreaux C, Kurtán T, Szikszai-Kiss A, Antus S, Msonthi JD, Hostettmann K. 2003. Isolation and on-line LC/CD analysis of 3,8''-linked biflavonoids from *Gnidia involucreta*. *HCA.* 86(8):2768–2778.
- Franke K, Porzel A, Schmidt J. 2002. Flavone-coumarin hybrids from *Gnidia socotrana*. *Phytochemistry.* 61(7):873–878.



- Freeman PW, Murphy ST, Nemorin JE, Taylor WC. 1981. The constituents of Australian *Pimelea* species. The isolation of unusual flavones from *P. simplex* and *P. decora*. *Aust J Chem.* 34(8): 1779–1784.
- Freire CSR, Silvestre AJD, Neto CP. 2007. Demonstration of long-chain-alkyl caffeates and  $\Delta^7$ -steryl glucosides in the bark of *Acacia* species by gas chromatography–mass spectrometry. *Phytochem Anal.* 18(2):151–156.
- Ito C, Itoigawa M, Miyamoto Y, Rao KS, Takayasu J, Okuda Y, Mukainaka T, Tokuda H, Nishino H, Furukawa H. 1999. A new biflavonoid from *Calophyllum panicflorum* with antitumor-promoting activity. *J Nat Prod.* 62(12):1668–1671.
- Kalbessa A, Dekebo A, Tesso H, Abdo T, Abdissa N, Melaku Y. 2019. Chemical constituents of root barks of *Gnidia involucrata* and evaluation for antibacterial and antioxidant activities. *J Trop Med.* 2019:8486214–8486218. DOI:.
- Kumarihamy M, Tripathi SK, Khan S, Muhammad I. 2021. Schottiiin, a new prenylated isoflavones from *Psoralea schottii* and antibacterial synergism studies between methicillin and fremontone against methicillin-resistant *Staphylococcus aureus* ATCC 1708. *Nat. Prod. Res.* :1–9.
- Li S, Chou G, Hseu Y, Yang H, Kwan H, Yu Z. 2013. Isolation of anticancer constituents from flos genkwa (*Daphne genkwa* Sieb. et Zucc.) through bioassay-guided procedures. *Chem Cent J.* 7(1):159.
- Munkombwe NM, Galebotswe P, Modibesane K, Morebodi N. 2003. Phenylpropanoid glycosides of *Gnidia polycephala*. *Phytochemistry.* 64(8):1401–1404.
- Nogueira F, Rosário VE. 2010. Methods for assessment of antimalarial activity in the different phases of the *Plasmodium* life cycle. *Rev Pan-Amaz Saúde.* 1(3):109–124.
- Ranu BC, Dey SS, Hajra A. 2003. Highly efficient acylation of alcohols, amines and thiols under solvent-free and catalyst-free conditions. *Green Chem.* 5(1):44–46.
- Ren L, Wang F, Xu Z, Chan WM, Zhao C, Xue H. 2010. GABA(A) receptor subtype selectivity underlying anxiolytic effect of 6-hydroxyflavone. *Biochem Pharmacol.* 79(9):1337–1344.
- Rogers ZS. 2009. A revision of Malagasy *Gnidia* (Thymelaeaceae, Thymelaeoideae). *Ann Mo Bot Gard.* 96(2):324–369.
- Singh B, Sahu PM, Sharma RA. 2017. *In vitro* and *in vivo* evaluation of flavonoids from *Heliotropium ellipticum* exudate for antioxidant, antineoplastic and cytotoxic activities II. *Ind J Pharm Sci.* 79(6):939–947.
- Stark TD, Germann D, Balemba OB, Wakamatsu J, Hofmann T. 2013. New highly *in vitro* antioxidative 3,8''-linked biflav(an)ones and flavanone-C-glycosides from *Garcinia buchananii* stem bark. *J Agric Food Chem.* 61(51):12572–12581.
- Su K, Gong M, Zhou J. 2009. Study on chemical composition of *Nauclea officinalis* leaves. *Int J Chem.* 1(2):77.
- Yuan T, Zhang CR, Yang SP, Yin S, Wu WB, Dong L, Yue JM. 2008. Sesquiterpenoids and phenylpropanoids from *Chloranthus serratus*. *J Nat Prod.* 71(12):2021–2025.
- Zhang JW, Yang WW, Chen LL, Chen P, Wang YB, Chen DY. 2019. An efficient tandem synthesis of chromones from *o*-bromoaryl ynones and benzaldehyde oxime. *Org Biomol Chem.* 17(32): 7461–7467.