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A new triterpenoid saponin from *Glinus oppositifolius* with α-glucosidase inhibitory activity

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Evaluation of α-glucosidase inhibitory activity led to the isolation of six triterpene saponins from the aerial parts of *Glinus oppositifolius* including one new and five known constituents. The structure of the new saponin, glinoside C (**1**), was established as 16-*O*-(β-D-glucopyranosyl)-3β,12β,16β,21α,22-pentahydroxy hopane by extensive use of 1-D, 2-D NMR and mass spectral techniques. The other constituents identified were 3-*O*-(β-D-xylopyranosyl)-spergulagenin A (**2**), spergulacin (**3**), spergulin A (**4**), spergulacin A (**5**) and spergulin B (**6**). Compound **1** exhibited the greatest inhibition of the enzyme with IC_{50} of $127 \pm 30\,\mu M$. Kinetics study for the compound **1** demonstrated mixed type of inhibition ($Ki = 157.9\,\mu M$).

Keywords: *Glinus oppositifolius*; Molluginaceae; glinoside-C; α-glucosidase; hopanoid triterpene saponin

1. Introduction

α-Glucosidase is an intestinal enzyme that catalyses the final step in the process of carbohydrate hydrolysis. Inhibition of this enzyme plays an important role in the management of post-prandial hyperglycaemia, as it delays the digestion of carbohydrates. There are several reports describing the α-glucosidase inhibitory potential of various constituents (Feng, Yang, & Wang, 2011; Li et al., 2008; Wafo et al., 2011) including triterpene saponins (Li et al., 2009; Luo, Ma, & Kong, 2008; Mbaze et al., 2007) isolated from plants. With the aim to uncover new leads with α-glucosidase inhibitory potential, the less explored plant *Glinus oppositifolius* (L.) Aug. DC. (Syn: *Mollugo spergula* L., Family: Molluginaceae) has been investigated in this study. The plant, commonly known as Gima, is an edible plant traditionally used for the treatment of skin diseases (Anonymus, 1962) and is a good source of triterpenoid saponins (Barua et al., 1980; Sahu, Koike, Banerjee, Achari, & Nikaido, 2001; Traore et al., 2000). This article describes the isolation of six saponins, out of which one is a new one, and another one is being reported for the first time from this plant. All the constituents were evaluated for their α-glucosidase inhibitory activity, and the new constituent was observed to exert the greatest activity.

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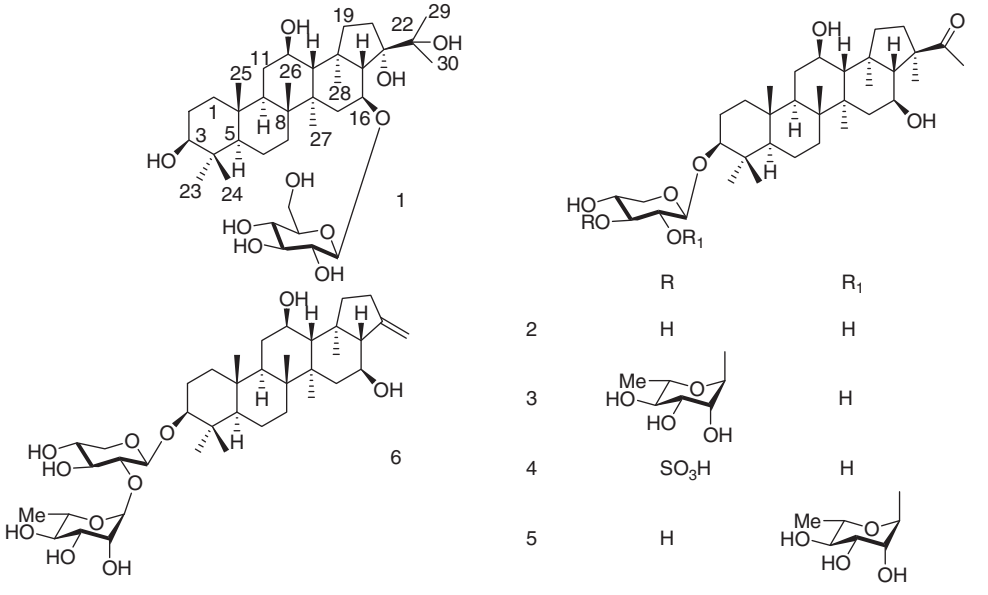

Figure 1. Structure of compounds **1**–**6**.

2. Results and discussion

Compound **1** (Figure 1) was obtained as an amorphous solid. The molecular formula was assigned as $C_{36}H_{62}O_{10}$ on the basis of its HR–FAB–MS ion at m/z 677.4242 $[M + Na]^+$ (Calcd for $C_{36}H_{62}O_{10}Na$, 677.4241). Infrared (IR) absorption bands (3389, 1067 cm^{-1}) were suggestive of a glycoside (Mimaki, Doi, Kuroda, & Yokosuka, 2007). The compound displayed 36 carbon signals in its ^{13}C–NMR spectrum, of which 6 could be assigned to a hexose unit and 30 attributable to the agylone part. The multiplicity of the individual resonances, determined using DEPT, revealed the presence of 8 methyl, 9 methylene, 12 methine and 7 aliphatic quaternary resonances. In support, the ^1H–NMR spectrum showed the presence of eight tertiary methyl signals at δ_H 0.71, 0.84, 1.00, 1.09, 1.21, 1.32, 1.52 and 1.56 (each 3H, s). Acid hydrolysis of the compound **1** with 5% HCl-dioxan gave glucose as sugar component, which was identified by TLC, and the absolute configuration was determined by the measurement of optical rotation (Hamed et al., 1996). The ^1H– and ^{13}C–NMR spectra revealed that the aglycone was a triterpenoid with a hopane skeleton (Sahu et al., 2001; Traore et al., 2000) and contained five hydroxyl groups (three secondary ones at C-3, C-12, C-16 and two tertiary ones at C-21 and C-22). The presence of eight-OH groups was indicated by HMBC correlated ^1H– and ^{13}C–NMR signals at δ_H/δ_C 7.33/76.0, 7.31/66.9, 7.28/75.8, 7.19/79.0, 6.29/72.7, 5.88/63.8, 5.80/78.4 and 4.96/86.1. The ^1H–NMR spectrum also showed a signal at δ_H 5.13 (d, $J = 7.8$ Hz), and the corresponding carbon peak appeared at δ_C 102.0, assigned to the anomeric CH. The exact linkage of the sugar was determined by the combined use of ^1H–^1H correlation spectroscopy (COSY), NOESY, HSQC and HMBC (Figure 2), where there were cross peaks between H-1′ (δ_H 5.13) and C-16 (δ_C 75.4). Chemical shift of the anomeric proton and its coupling constant suggested that the sugar linkage at C-16 was a β-D-glucopyranoside. The chemical shift of C-17 appears somewhat higher but may be explained by the contribution of C-21–OH substitution as reported for

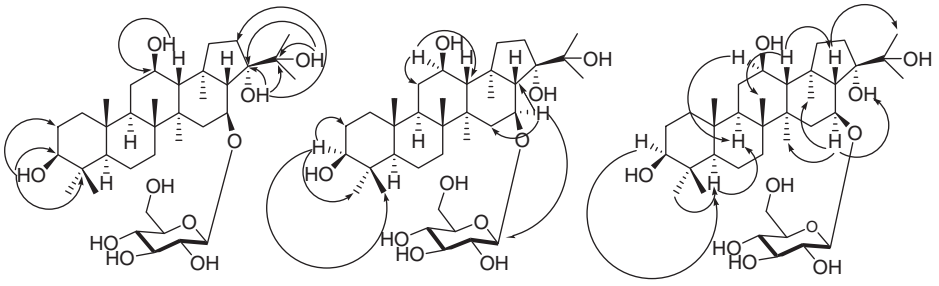

Figure 2. Key HMBC hydroxyl proton–carbon correlations, HMBC proton–carbon correlation and ^{1}H–^{1}H NOESY correlations of **1**.

Table 1. α-Glucosidase inhibitory activity of *G. oppositifolius* extract, fractions and isolated saponins.

Sample	Inhibitory concentration (IC_{50})[a]
Methanolic extract	$2.548 \pm 0.106\,mgmL^{-1}$
Ethyl acetate fraction	$2.138 \pm 0.117\,mgmL^{-1}$
n-Butanol fraction	$0.858 \pm 0.074\,mgmL^{-1}$
Aqueous fraction	$>3\,mgmL^{-1}$
Glinoside C (**1**)	$127 \pm 30\,\mu M$
3-*O*-(β-D-xylopyranosyl)-spergulagenin A (**2**)	$1654 \pm 170\,\mu M$
Spergulacin (**3**)	$628 \pm 80\,\mu M$
Spergulin A (**4**)	$143 \pm 20\,\mu M$
Spergulacin A (**5**)	$694 \pm 60\,\mu M$
Spergulin B (**6**)	$1783 \pm 290\,\mu M$
Acarbose	$15 \pm 3\,\mu M$

Note: [a]Values are expressed as mean $\pm$ SEM ($n = 3$).

glinoside A (Traore et al., 2000). The stereochemistry of **1** was determined by NOESY experiment. From the above data, we concluded that compound **1** was a glucoside with a new aglycone, 3β, 12β, 16β, 21α, 22-pentahydroxy hopane. The known compounds (Figure 1) were identified as 3-*O*-(β-D-xylopyranosyl)-spergulagenin A (**2**), spergulacin (**3**), spergulin A (**4**), spergulacin A (**5**) and spergulin B (**6**) by direct comparison of their spectral data with those in the literature (Sahu et al., 2001). Compound **2** is reported for the first time from this plant.

The *n*-BuOH fraction showed maximum inhibition of the enzyme *in vitro* among the three fractions. The isolated saponins were tested for their potential to inhibit α-glucosidase, *in vitro*, and the result showed varying degree of inhibition. The α-glucosidase exhibited Michaelis–Menten kinetics with *p*-nitrophenyl α-D-glucopyranoside at 37°C (Km: 1.513 mM; V_{max}: $0.910\,mM\,min^{-1}$). Compound **1** affected the greatest inhibition of enzyme (Table 1) and demonstrated a mixed type of enzyme inhibition ($Ki = 157.9\,\mu M$; Figure 3). The inhibition demonstrated by these constituents was less active compared to acarbose, a standard drug, hence these constituents may be classified as moderate inhibitors of the enzyme. On the basis of these results, the edible plant *G. oppositifolius* may be used for the management of diabetic complications as food additive.

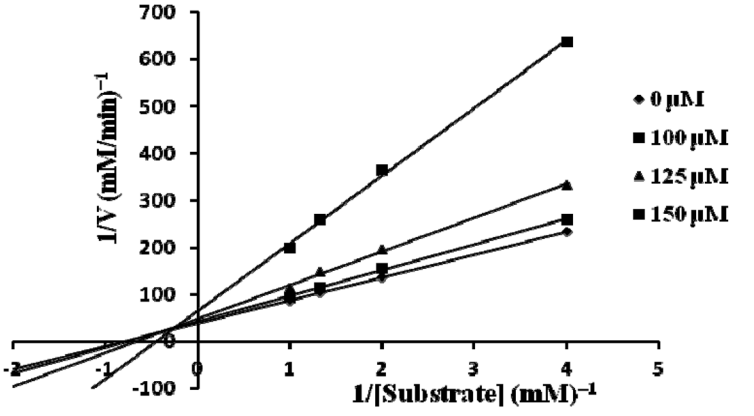

Figure 3. Kinetic analysis of α-glucosidase inhibition by glinoside C. Lineweaver–Burke plot of hydrolysis of *p*-nitrophenyl α-D-glucopyranoside (0.25, 0.5, 0.75 and 1 mM) by α-glucosidase (0.5 U mL^{-1}) in the presence or absence of glinoside C (**1**).

3. Experimental

3.1. *General experimental procedures*

Optical rotation was recorded in MeOH using a Jasco P-1020 polarimeter. IR spectra were recorded with a Jasco-FT-IR-model 410 spectrophotometer as KBr disks. ^{1}H–, ^{13}C– and 2D NMR were recorded on a Bruker Ultrashield NMR (600 MHz) in pyridine-d_5 with TMS as internal standard. HR–FAB–MS was recorded on a MStation JMS 700 (JEOL Ltd, Japan). Silica gel (Merck, 100–200 mesh) was used for column chromatography; silica gel (60 F254) was used for TLC and spots were visualised by spraying with Lieberman–Burchard reagent followed by heating.

3.2. *Chemicals and reagents*

α-Glucosidase (Maltase, ex microorganism, EC 3.2.1.20) and *p*-nitrophenyl α-D-glucopyranoside were purchased from SRL Mumbai, India. Acarbose was purchased from Sigma. Na_2HPO_4, NaH_2PO_4, DMSO, Na_2CO_3, other chemicals and solvents were of highest purity grade and purchased from Merck, India.

3.3. *Plant material*

The aerial part of the plant was collected in Kolkata (India) in August 2010 and identified by the Botanical Gardens, Howrah, India. A voucher specimen (NIP-K/BCP/004) has been deposited at National Institute of Pharmaceutical Education and Research, Kolkata.

3.4. *Extraction and isolation*

The air-dried plant (1 kg) was subjected to extraction using MeOH (3.5 L × 5) at room temperature for 48 h. The extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator at 45°C. The resultant extract was lyophilised to afford the crude MeOH extract (138 g; yield 13.8% w/w). A part (100 g) of the extract was then suspended in Milli-Q H_2O and partitioned successively with EtOAc and *n*-BuOH (Kumar, Mallick, Vedasiromoni, & Pal, 2010). Each fraction was evaporated under

vacuum to yield the EtOAc fraction (25 g), *n*-BuOH fraction (39 g) and aqueous fraction (35 g). All the fractions were stored at 4°C till further use.

The *n*-BuOH fraction (20 g) was chromatographed on silica gel (200 g) using a step gradient, petroleum ether–$CHCl_3$ 1 : 1 (500 mL, fr-1), 1 : 9 (500 mL, fr-2), $CHCl_3$ (500 mL, fr-3), $CHCl_3$–MeOH 9 : 1 (500 mL, fr-4), 8 : 2 (500 mL, fr-5) and 7 : 3 (500 mL, fr-6), to get a total of six fractions. Repeated silica gel column chromatography of fr-4 led to the isolation of compounds **2** (40 mg) and **3** (200 mg). Fr-5 was further chromatographed over a silica gel column and eluted with increasing polarity of $CHCl_3$–MeOH, 1 : 0 to 7 : 3 (v/v), to give a total 30 fractions. Among these, fractions 15–17 were mixed together because HPLC analysis (Discovery® RP amide C16, 5 µm, 25 cm × 4.6 mm, eluted with MeOH–H_2O 60 : 40, at 1 mLmin^{-1}, ELS-detector) suggested them to have similar pattern with four major peaks (t_R = 9.6, 16.2, 19.9, 29.8 min). The mixed fraction was purified using Prep-HPLC (Discovery® RP amide C16, 5 µm, 25 cm × 10 mm, MeOH–H_2O 60 : 40 at 2.5 mLmin^{-1}, RI-detector). All the four peaks were collected and evaporated under reduced pressure using rotary evaporator at 45°C to afford compounds **4** (48 mg), **1** (30 mg), **5** (67 mg) and **6** (40 mg).

3.4.1. *Glinoside C (**1**)*

Amorphous solid; $[\alpha]_D^{28} = +17.2°$ (*c* 0.02, MeOH); IR (KBr cm^{-1}) υ_{max} : 3389, 2945, 1654, 1563, 1455, 1384, 1067, 644; HR–FAB–MS *m/z*: 677.4242 $[M + Na]^+$, Calcd for $C_{36}H_{62}O_{10}Na$, 677.4241. ^{1}H–NMR (C_5D_5N; 600 MHz) aglycone moiety, δ : 1.49 (1H, m, H-1a), 0.79 (1H, m, H-1b), 1.78 (1H, m, H-2a), 1.24 (1H, m, H-2b), 3.43 (1H, m, H-3), 0.73 (1H, d, J = 12.0 Hz, H-5), 1.49 (1H, m, H-6a), 1.29 (1H, m, H-6b), 1.17 (1H, m, H-7a), 1.41 (1H, m, H-7b), 1.24 (1 H, m, H-9), 2.30 (1H, m, H-11a), 1.45 (1H, m, H-11b), 4.42 (1H, m, H-12), 1.86 (1H, d, J = 7.8 Hz, H-13), 1.67 (1H, m, H-15a), 1.89 (1H, m, H-15b), 4.44 (1H, m, H-16), 2.32 (1H, d, J = 9.0 Hz, H-17), 1.91 (1H, m, H-19a), 2.69 (1H, m, H-19b), 1.82 (1H, m, H-20a), 1.91 (1H, m, H-20b), 1.21, 1.00, 0.71, 0.84, 1.09, 1.32, 1.52, 1.56 (each 3H, s, H_3−23, −24, −25, −26, −27, −28, −29, −30), sugar moiety, 5.13 (1H, d, J = 7.8 Hz, H-1′), 4.08 (1H, m, H-2′), 4.35 (1H, m, H-3′), 4.25 (1H, m, H-4′), 4.05 (1H, m, H-5′), 4.40 (1H, m, H-6′a), 4.62 (1H, m, H-6′b), hydroxyl proton, 5.80 (1H, d, J = 4.2 Hz, 3-OH), 7.31 (1H, s, 12-OH), 4.96 (1H, s, 21-OH), 7.28 (1H, s, 22-OH), 7.33 (1H, s, 2′-OH), 7.19 (1H, s, 3′-OH), 6.29 (1H, s, 4′-OH), 5.88 (1H, br s, 6′-OH). ^{13}C–NMR (C_5D_5N; 600 MHz) aglycone moiety, δ : 39.1 (C-1), 28.6 (C-2), 78.4 (C-3), 39.8 (C-4), 56.0 (C-5), 19.1 (C-6), 33.8 (C-7), 42.0 (C-8), 48.9 (C-9), 37.6 (C-10), 27.9 (C-11), 66.9 (C-12), 54.6 (C-13), 45.5 (C-14), 46.0 (C-15), 75.4 (C-16), 74.1 (C-17), 47.9 (C-18), 42.8 (C-19), 38.1 (C-20), 86.1 (C-21), 75.8 (C-22), 29.0 (C-23), 16.6 (C-24), 16.2 (C-25), 17.2 (C-26), 20.0 (C-27), 17.2 (C-28), 26.9 (C-29), 27.6 (C-30), sugar moiety, 102.0 (C-1′), 76.0 (C-2′), 79.0 (C-3′), 72.7 (C-4′), 78.4 (C-5′), 63.8 (C-6′).

3.5. *Acid hydrolysis of 1*

A part (15 mg) of compound **1** was heated with 2 (N) HCl in Dioxan–H_2O (1 : 1 (v/v), 10 mL) for 2 h on H_2O bath (80°C). Dioxan was distilled off under reduced pressure and the product after dilution with H_2O was extracted with EtOAc (15 mL × 2). Evaporation furnished a product (5 mg), which showed three spots in TLC perhaps due to artefact formation and was not proceed with further. The aqueous layer was neutralised with Ag_2CO_3, then filtered and evaporated. The sugar was identified as D-glucose with authentic sample by TLC in EtOAc–MeOH–H_2O–AcOH (13 : 4 : 4 : 3; R_f 0.35). Sugar was isolated by preparative TLC with same solvent system and the optical rotation of sugar was measured. $[\alpha]_D^{28}$ + 103.8 (*c* 0.10, H_2O) (Hamed et al., 1996).

3.6. α-Glucosidase enzyme inhibition assay

The α-glucosidase enzyme inhibitory assay was performed in a 96 well plate as described earlier by Hou et al. (2009), with some modifications. For the analysis of the type of inhibition, kinetics of enzyme inhibition by glinoside C (**1**) was studied by double reciprocal plot at four different substrate concentrations −0.25, 0.5, 0.75 and 1 mM.

3.7. *Statistical analysis*

Statistical calculations were carried out with GraphPad Prism (GraphPad Software, Inc., USA). Results are expressed as the mean ± SEM.

4. Conclusion

α-glucosidase inhibitory activity guided fractionation and purification led to isolation of six triterpene saponins from the aerial part of the *G. oppositifolius*, including one new saponin, glinoside C (**1**). This exhibited the greatest inhibition of the enzyme (IC_{50} $127 + 30\,\mu M$) and its kinetics study showed mixed type of inhibition.

Supplementary material

Experimental details relating to this article are available online, alongside Table S1 and Figures S1–S22.

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References

Anonymous. (1962). *The wealth of India, raw materials* (Vol. 6, pp. 396–397). New Delhi: Council of Scientific and Industrial Research.

Barua, A.K., Banerjee, S.K., Gupta, C.D., Basu, K., Bose, L., & Chakrabarti, P. (1980). The structure and stereochemistry of spergulagenol, a triterpene from *Mollugo spergula*. *Phytochemistry, 19*, 1551–1553.

Feng, J., Yang, X.W., & Wang, R.F. (2011). Bio-assay guided isolation and identification of α-glucosidase inhibitors from the leaves of *Aquilaria sinensis*. *Phytochemistry, 72*, 242–247.

Hamed, A.I., Springuel, I., el-Emary, N.A., Mitome, H., Miyaoka, H., & Yamada, Y. (1996). Triterpenoidal saponin glycosides from *Glinus lotoides* var *dictamnoides*. *Phytochemistry, 43*, 183–188.

Hou, W., Li, Y., Zhang, Q., Wei, X., Peng, A., Chen, L., & Wei, Y. (2009). Triterpene acids isolated from *Lagerstroemia speciosa* leaves as alpha-glucosidase inhibitors. *Phytotherapy Research, 23*, 614–618.

Kumar, D., Mallick, S., Vedasiromoni, J.R., & Pal, B.C. (2010). Anti-leukemic activity of *Dillenia indica* L. fruit extract and quantification of betulinic acid by HPLC. *Phytomedicine, 17*, 431–435.

Li, W., Fu, H., Bai, H., Sasaki, T., Kato, H., & Koike, K. (2009). Triterpenoid saponins from *Rubus ellipticus* var. *obcordatus*. *Journal of Natural Products, 72*, 1755–1760.

Li, J., Lu, Y., Su, X., Li, F., She, Z., He, X., & Lin, Y. (2008). A norsesquiterpene lactone and a benzoic acid derivative from the leaves of *Cyclocarya paliurus* and their glucosidase and glycogen phosphorylase inhibiting activities. *Planta Medica, 74*, 287–289.

Luo, J.G., Ma, L., & Kong, L.Y. (2008). New triterpenoid saponins with strong Alpha-glucosidase inhibitory activity from the roots of *Gypsophila oldhamiana*. *Bioorganic and Medicinal Chemistry, 15*, 2912–2920.

Mbaze, L.M., Poumale, H.M., Wansi, J.D., Lado, J.A., Khan, S.N., Iqbal, M.C., ... Laatsch, H. (2007). Alpha-Glucosidase inhibitory pentacyclic triterpenes from the stem bark of *Fagara tessmannii* (Rutaceae). *Phytochemistry, 68*, 591–595.

Mimaki, Y., Doi, S., Kuroda, M., & Yokosuka, A. (2007). Triterpene glycosides from the stems of *Akebia quinata*. *Chemical and Pharmaceutical Bulletin, 55*, 1319–1324.

Sahu, N.P., Koike, K., Banerjee, S., Achari, B., & Nikaido, T. (2001). Triterpenoid saponins from *Mollugo spergula*. *Phytochemistry, 58*, 1177–1782.

Traore, F., Faure, R., Ollivier, E., Gasquet, M., Azas, N., Debrauwer, L., ... Balansard, G. (2000). Structure and antiprotozoal activity of triterpenoid saponins from *Glinus oppositifolius*. *Planta Medica, 66*, 368–371.

Wafo, P., Kamdem, R.S., Ali, Z., Anjum, S., Begum, A., Oluyemisim, O.O., ... Choudhary, M.I. (2011). Kaurane-type diterpenoids from *Chromoleana odorata*, their X-ray diffraction studies and potent α-glucosidase inhibition of 16-kauren-19-oic acid. *Fitoterapia, 82*, 642–646.