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Flavonoid Identification and Hypoglycaemic Studies of the Butanol Fraction from *Gynura procumbens*

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Abstract

The methanol extract of the leaves of *Gynura procumbens* was partitioned between chloroform, ethyl acetate and *n*-butanol. Qualitative HPLC identification of the major flavonoid constituents in the *n*-butanol fraction and separation of standard mixture of isolated compounds from the *n*-butanol fraction are given. Blood glucose levels in streptozotocin-induced type 2 diabetic rats were reduced by the administration of 1 g/kg of the *n*-butanol fraction. The results were compared with glibenclamide used as standard drug. The fraction produced no significant effect in normal rats.

Keywords: Streptozotocin, glibenclamide, Compositae, flavonoid glycoside, kaempferol, quercetin.

Introduction

Many investigations of oral anti-hyperglycemic agents of plant origin used in traditional medicine have been conducted and many of the plants show positive activity (Bailey & Day, 1989; Rahman & Zaman, 1989). Though the active principles of various classes of chemical compounds have been isolated from plants some remain to be identified (Rahman & Zaman, 1989).

Gynura procumbens (Lour,) Merr. (Compositae) is known in Indonesia and Malaysia as "Sambung nyawa" and is a traditional medicinal herb. It is believed to be useful as a febrifuge in eruptive fevers, a remedy for kidney troubles, a prevention of rheumatism, and a treatment of colon cancer, haemorrhoids and diabetes (Perry & Metzger, 1980). In Malaysia, *Gynura procumbens* is used in the form of decoction by traditional medicinal practioners to control the blood glucose levels of diabetic patients. Sterols and sterol glycosides were isolated and characterised from the leaves (Sadikun et al., 1996). Previous hypoglycaemic studies of the methanol extract from the plant showed significant hypoglycaemic effect ($P < 0.05$) in streptozotocin-induced diabetic rats but exerted no significant reduction in blood glucose level of normal rats (Akowuah et al., 2001). The methanol extract was partitioned between chloroform, ethyl acetate and *n*-butanol and we have studied the flavonoid content and hypoglycaemic studies of the *n*-butanol fraction (the active fraction).

The present study reports RP-HPLC identification of flavonol glycosides and hypoglycaemic effect of the *n*-butanol fraction of the leaves of the plant in normal and streptozotocin-induced diabetic rats.

Materials and methods

Materials for hypoglycaemic studies

Oven-dried powdered of the plant leaf, Male Sprague Dawley rats (190–250 g), streptozotocin (Sigma), distilled water, saline (0.9%), glibenclamide, YSI 2357 buffer concentrate, YSI 1531 standard D-glucose 900 mg/dl (CAS 50997) were obtained from commercial sources.

Apparatus

Melting points were determining on an electrothermal apparatus and are uncorrected. UV spectra were recorded on a Shimadzu UV-2000 ultraviolet spectrophotometer. Infrared spectra were recorded by IR Shimadzu 420 spectrophoto-

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meter and "BOMEN" spectrophotometer. ^{1}H-NMR spectra were determined on "BRUKER" BZH 300 MHz using TMS as internal standard and ^{13}C-NMR were determined on "BRUKER" BZH 75 MHz-using TMS as internal standard. The ms/ms spectra were recorded with a Triple-Stage-Quadruple mass spectrometer (TSQ) 7000 (Finnigan). Blood glucose levels were determined in mg/dl using a glucose analyser (YSI Model 23A).

Adsorbent

Silica gel 60 F_{254} (Merck), Precoated TLC sheets, silica gel 60 F_{254} (Merck), Sephadex LH-20.

Plant material

Gynura procumbens was collected in August 1998, from Penang, Malaysia, and a voucher specimen (No. 10117) was deposited at the herbarium of the School of Biological Sciences, Universiti Sains Malaysia. The leaves were dried in an oven below 40°C and the dried leaves milled into powder.

Preparation of plant extract

Powdered leaves (660 kg) were extracted with pet. ether (60–80 °C), 5 L at room temperature, followed by methanol in a Soxhlet extractor for 36 h each. The methanol residue (110 g) was dissolved in water (300 ml) and extracted with chloroform (3 × 250 ml), ethyl acetate (3 × 250 ml) and *n*-butanol (3 × 250 ml). The *n*-butanol soluble fraction was evaporated under vacuum and afforded 46.3 g.

Hypoglycaemic studies

50 g of n-butanol fraction was freeze-dried until the extracts achieved a constant weight. The dried extract was dissolved in saline for administration to experimental rats.

Induction of experimental diabetes

Diabetes was induced in male Sprague-Dawley rats (190–250 g) by intravenous injection of streptozotocin (55 mg/kg body weight) dissolved in saline (0.9%) (Theoudorou et al., 1980). The diabetic state was confirmed on day 7, after the administration of streptozotocin by determining the blood glucose concentration and only those with high blood glucose levels above 250 mg/dl were used in the experiments.

Investigation of the hypoglycaemic effect of the extracts

Normal and diabetic rats were fasted for 24 h. Normal rats were then divided into 3 groups (I–III) of 6 animals. Group I served as control and received oral saline (10 ml per kg body weight). Group II received a suspension of the *n*-butanol fraction dissolved in saline (1 g/10 ml per kg body weight), and group III received 0.025 mg/kg of glibenclamide (Sharma et al., 1984). The diabetic rats were also divided into 3 groups on the same pattern and the experiment was repeated. Blood samples were drawn from the tail vein at 0, 1, 2, 3, 5 and 7 h after the administration of the extracts. Blood glucose levels were determined using a glucose analyser (YSI Model 23A).

Statistical analysis

All grouped data were shown as means ± sem. The statistical differences between means of all groups were determined by the Student's *t*-test and n was the number of animals. A probability level of $P < 0.05$ was considered as the level of significance.

Phytochemical analysis

Isolation of compounds

20 g of the *n*-butanol fraction was subjected to column chromatography over Sephadex LH-20 and eluted with varying proportion of methanol and water. Further repeated chromatography on Sephadex LH-20 gave the compounds kaempferol 3-*O*-glucoside (**1** = 10 mg), kaempferol 3-*O*-rhamnosyl (1→6) glucoside (**2** = 10 mg), quercetin 3-*O*-rhamnosyl (1→2) galactoside (**3** = 13 mg), and quercetin 3-*O*-rhamnosyl (1→6) glucoside (**4** = 15 mg). The structures were determined by acid hydrolysis and spectral data (UV, MSMS, ^{1}H and ^{13}C NMR).

Materials for qualitative HPLC analysis of the *n*-butanol fraction

Analytical balance accurate to ± 0.1 mg, Volumetric flasks, Filter, 0.45 µm, polypropylene, sonicator, the system consist of HPLC pump model 305 (Gilson), D-2500 chromato-integrator (Hitachi), model 811B Dynamic Mixer (Gilson), Spectral System Detector UV 2000 (Spectra Physics), LiCHrosorb RP-18 column 5 µm particle size, 4.6 × 250 mm I.D., (Merck, Darmstadt Germany). Methanol (HPLC grade), water (HPLC grade) phosphoric acid, 85%, reagent grade. Quercetin (Sigma, St. Louis, MO), kaempferol (Sigma, St. Louis, MO).

Sample preparation for HPLC analysis

100 mg of the *n*-butanol fraction were transfer to a 25 mL volumetric flask, 20 mL of methanol: water (1 : 1) and sonicated for 30 min and diluted to the volume and mixed. This solution was subjected to HPLC (Brolis et al., 1998). The concentration of the reference solutions of isolated compounds was; **1** = 0.2 mg/mL; **2** = 0.2 mg/mL; **3** = 0.2 mg/mL; **4** = 0.2 mg. The solutions were filtered through membrane filters prior to injection.

HPLC conditions

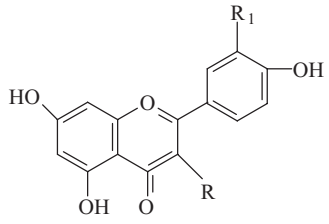
Preliminary HPLC investigation of the *n*-butanol fraction was carried out for the separation and determination of flavonoids on a reverse phase column with isocratic elution. Column: LiCHrosorb RP-18 column 5 um particle size, 4.6 × 250 mm I.D.; Flow rate: 1 mL/min; Eluent: Solvent A (water with 0.55 v/v orthophosphoric acid) and B (methanol) = 6.5 : 3.5; Flow rate was 1 ml/min; detection at 250 nm. Column temperature 25 °C; Sample volume 20 µL (Pieta & Mauri, 1987; Pieta et al., 1988).

Results and discussion

The HPLC chromatogram of the n-butanol fraction was characterized by 8 main constituents in the R_t range of 0–15 min. (Fig. 1). Column chromatography of the *n*-butanol fraction afforded four compounds. The structures of the compounds were elucidated by TLC, UV, ^{1}H-NMR, ^{13}C-NMR (Agrawal, 1989), ms/ms and hydrolytic methods (Table 1). The respective retention times of the reference compounds (isolated) chromatographed were: kaempferol 3-*O*-glucoside (**1** = 15.96 min), kaempferol 3-*O*-rhamnosyl (1→6) glucoside (**2** = 10.64 min), quercetin 3-*O*-rhamnosyl (1→2) galactoside (**3** = 5.27 min), quercetin 3-*O*-rhamnosyl (1→6) glucoside (**4** = 3.07 min) (Fig. 2). The reference compounds (isolated) were identified in the *n*-butanol fraction by comparing the retention times in the chromatogram of the reference compounds (Fig. 2) with the chromatogram of the *n*-butanol fraction (Fig. 1).

Compounds **3** and **4** were isolated as yellow powder. They were shown to possess a flavonol skeleton by characteristic absorption bands in their respective UV spectrum. Compounds **3** and **4** do not show any change in the UV spectrum on addition of $CaCl_2$ solution (in methanol) compared with the UV spectrum in methanol; this confirmed the C-3 as the site of glycosylation (Raihan, 1994). The ^{13}C-NMR showed 27 carbon signals, which were assigned by DEPT experiments. The molecular formula $C_{27}H_{30}O_{16}$ (MW = 610.1) was deduced for **3** from the ms/ms spectrum. The molecular formula of **4** was the same as **3** from the ms/ms spectrum of **4**. The ms/ms spectra of **3** and **4** showed peaks at *m*/*z* 633, 611.1, 464.9 302.7, respectively, corresponding to the molecular ion $[M+Na]^+$, quasimolecular ion $[M+1]^+$, the loss of terminal rhamnose and the loss of rhamnosegalactosyl or rhamnoseglucosyl unit. The peak at 464.9 indicates loss of 146 amu, which is indicative of rhamnose as terminal sugar. They also exhibit a peak at 302.7, which indicate loss of rhamnosylgalactose or rhamnosylglucose, and also confirm the base peak component of quercetin. To determine whether *m*/*z* 302.7 ion is related to the *m*/*z* 464.9 ion an MS^3 experiment performed on the *m*/*z* 464.9 ion showed an ion at *m*/*z* 302.7 which was attributed to the loss of hexose

Table 1. Structures of the flavonol glycosides present in the *n*-butanol fraction from *Gynura procumbens* leaf.



Peak number	Compound	R	R_1
4	**1**	Glucose	H
3	**2**	Glucose (6→1) rhamnose	H
2	**3**	Galactose (2→1) rhamnose	OH
1	**4**	Glucose (6→1) rhamnose	OH

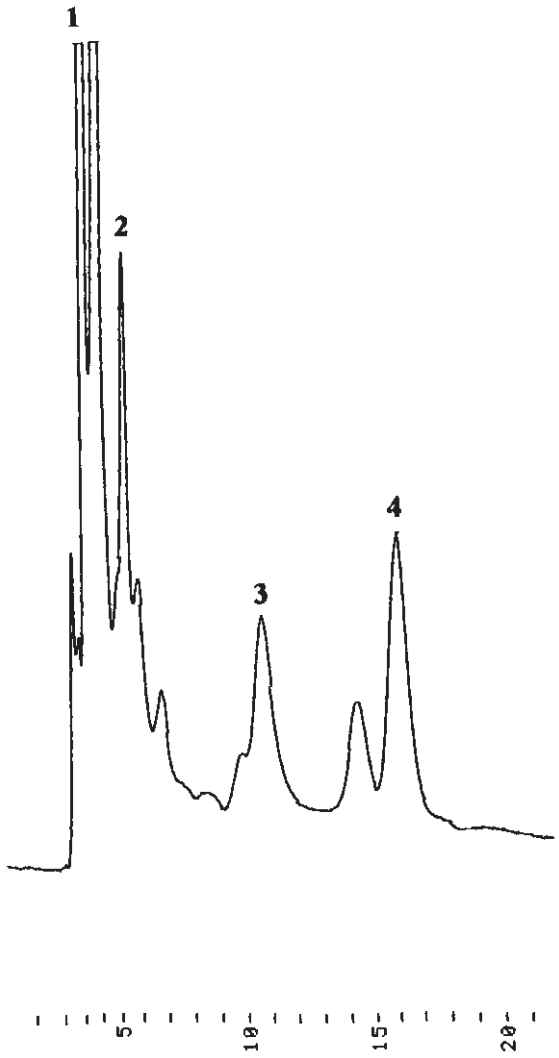


Figure 1. HPLC determination of flavonoids in the *n*-butanol fraction from *Gynura procumbens* leaf. Chromatographic condition is described in the text. For the identification of the peaks, see Table 1.

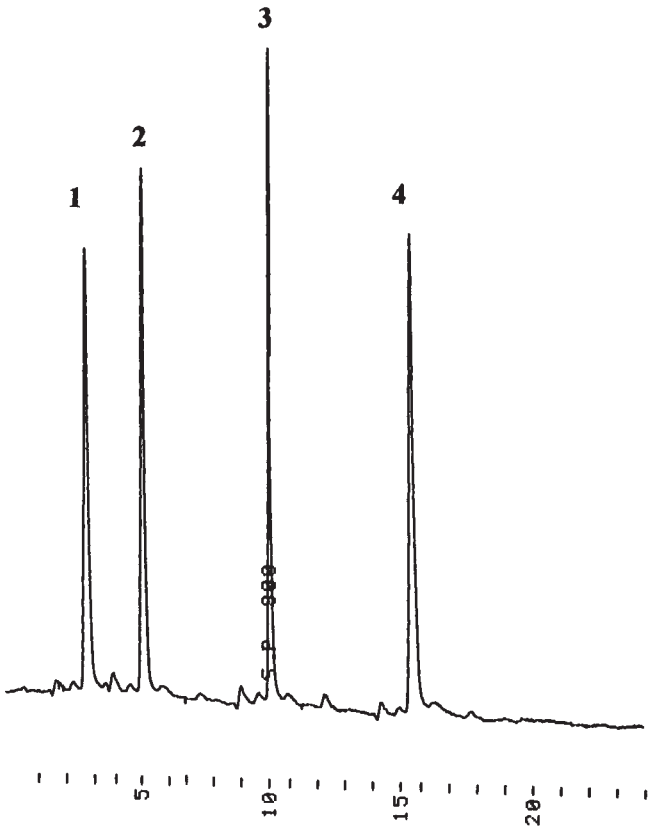


Figure 2. HPLC separation of flavonoids standard (isolated). Chromatographic condition is described in the text. For the identification of the peaks, see Table 1.

sugar unit from the precursor ion at m/z 464.9. The MS^4 experiments on the m/z 302.7 ions in the respective spectrum of **3** and **4** gave an exact match of the spectrum generated by ms/ms experiment of quercetin (Sigma, St. Louis, USA). This confirmed that the aglycone moiety of **3** and **4** as quercetin.

Acid hydrolysis of **3** with 2 M HCl, quercetin, galactose and rhamnose were identified to be present in the hydroxylate by TLC comparison with authentic samples. Compound **4** on hydrolysis with 2 M HCl gave quercetin, glucose and rhamnose by TLC comparison with authentic samples. The ^{13}C-NMR spectra showed the sugars (-D-galactopyranose and (-L-rhamnopyranose forms in 3 and (-D-glucopyranose and (-L-rhamnopyranose in **4**. Compounds **3** and **4** failed to give positive test with aniline phthalate indicated that both sugars are linked through their respective reducing groups.

Compound **3** gave data identical to those reported for quercetin 3-*O*-rhamnosyl (1→2) galactoside (Yasukawa & Takido, 1987) and the data of **4** were analogues to those reported earlier for quercetin 3-*O*-rhamnosyl (1→6) glucoside (Mabry et al., 1970). Kaempferol 3-*O*-glucoside (**1**) and kaempferol 3-*O*-rutinoside (**2**) were also identified by comparison of their spectral data (UV, IR, ^{1}H-NMR, ^{13}C-NMR, ms/ms) with published results (Markham & Mohan, 1982). These flavonoids glycosides have been shown to be responsible for the blood glucose lowering activity collectively (Chattopadhyay, 1999).

Kaempferol 3-*O*-glucoside (**1**) elution with 100% methanol and on crystallization (methanol-acetone) gave yellow powder of mp 171–172°C. The compound gives dark green colour with $FeCl_3$ and pale red colour with Mg + HCl; UV (λmax nm: 226, 294, 351, (MeOH); 275, 325, 400 (MeOH + NaOMe); 274, 300, 346, 396 (MeOH + $ALCl_3$); 226, 294, 347, 351, (MeOH + $AlCl_3$ + HCl); 275, 301, 364, (MeOH + NaOAc); 266, 294, 351 (MeOH + NaOAc + H_3BO_3); 258, 350 (MeOH + $CaCl_2$): IR (KBr) cm^{-1} 3400 (-OH), 1650 (C = O), 1580 (C = C), 1150, (C-O). ^{1}H-NMR (DMSO-d_6, 300 MHz): 7.99 (2H, d, J = 8.7 Hz H-2′ and H-6′), 6.86 (2H, d, J = 8.5 Hz, H-3′, H-5′), 6.19 (1H, d, J = 2 Hz H-8), 5.99 (1H, d, J = 2 Hz H-6); 5.37 (1H, d, J = 7.0 Hz glucosyl H-1), 3.40 (m, glucosyl protons).^{13}C-NMR (DMSO-d_6, 75 MHz): 154.1 (C-2), 132.6 (C-3), 175.4(C-4), 160.2 (C-5), 100 (C-6), 160.8 (C-7), 95.2 (C-8), 102.0 (C-10), 120.9 (C-1′), 130.5 (C-2′, C-6′), 115.1 (C-3′, C-5′), 157.3 (C-4′). ms/ms: 472 $[M + Na]^+$, 449 $[M + H]^+$, 287 $[M + H - glucose]^+$.

Kaempferol 3-*O*-rhamnosyl(1→6)glucose (**2**) elution with 2% water in methanol and on crystallization (methanol) gave a yellow powder with mp 185–187 °C. The compound gives a dark green colour with $FeCl_3$ and a pale red colour with Mg + HCl. UV (λmax nm: 266, 294, 350 (MeOH); 275, 325, 400 (MeOH + NaOMe); 274, 300, 349, 397 (MeOH + $ALCl_3$); 274, 300, 347, 396 (MeOH + $AlCl_3$ + HCl); 275, 301, 365 (MeOH + NaOAc); 266, 299, 352 (MeOH + Na OAc + H_3BO_3); 265, 351 (MeOH + $CaCl_2$): IR (KBr) cm^{-1} 3400 (-OH), 1650 (C = O), 1580, (C = C), 1150 (C-O); 941, 879, 805 (C-H of aromatic ring). ^{1}H-NMR (DMSO-d_6, 300 MHz): 7.97 (2H, d, J = 8.7 Hz H-2′ and H-6′), 6.83 (1H, d, J = 8.2 Hz H-3′ and H-5′), 6.02 (1H, d, J = 2 Hz H-8), 5.91 (1H, d, J = 2 Hz H-6), 5.22 (1H, d, J = 6.2 Hz glucosyl H-1), 4.41 (rhamnosyl H-1), 3.35 (m, rhamnosyl glucosyl protons), 1.0 (3H, d, J = 6.1 Hz) rhamnosyl CH_3. ^{13}C-NMR (DMSO-d6, 75 MHz): 155.2 (C-2), 132.9 (C-3), 175.6 (C-4), 161.0 (C-5), 99.9 (C-6), 160.8 (C-7), 94.9 (C-8), 103.2 (C-10), 120.7 (C-1′), 130.5 (C-2′, 6′), 115.2 (C-3′, C-5′), 157.2 (C-4′), 101.6 (C-1″), 74.2 (C-2″), 76.7 (C-3″), 70.4 (C-4″), 75.6 (C-5″), 66.9 (C-6″), 100.7 (C-1‴), 70.2 (C-2‴), 70.4 (C-3‴), 72.5 (C-4‴), 68.2 (C-5‴), 17.7 (C-6‴). ms/ms: 617 $[M + Na]^+$, 595.1 $[M + H]^+$, 449 $[M + H - rhamnose]^+$, 287. $[M + H - rhamnosylglucosyl]^+$.

Quercetin 3-*O*-rhamnosyl (1→2) galactoside (**3**) Elution with 5% water in methanol and on crystallization (methanol-acetonitrile) gave yellow needles with mp 209–211°C; dark green with $FeCl_3$ and pale red with Mg + HCl. UV (λmax nm: 257, 357 (MeOH); 270, 330, 409 (MeOH + NaOMe); 278, 428, (MeOH + $ALCl_3$); 269, 402 (MeOH + $AlCl_3$ + HCl); 269, 374 (MeOH + NaOAc); 261, 379 (MeOH + Na OAc + H_3BO_3); 258, 359 (MeOH + $CaCl_2$): IR (KBr) cm^{-1} 3234(-OH), 1655 (C = O), 1601, 1501, 1457, (C = C), 941,

879, 805 (C-H of aromatic ring). ^{1}H-NMR (DMSO-d_6, 300 MHz): 7.66 (1H, dd, J = 1.9 Hz, 8.5 Hz H-6′), 7.52 (H, d, J = 1.7 Hz H-2′), 6.83 (1H, d, J = 8.2 Hz H-5′), 6.36 (1H, d, J = 1.7 Hz H-8), 6.17 (1H, d, J = 1.7 Hz H-6), 5.64 (1H, d, J = 7.0 Hz galactosyl H-1), 4.99 (1H, s, rhamnosyl H-1) 3.35 (m, rhamnosyl galactosyl protons), 0.09 (3H, d, J = 6.1 Hz) rhamnosyl CH_3. ^{13}C-NMR (DMSO-d_6, 75 MHz): 156.43 (C-2), 133.25 (C-3), 177.27 (C-4), 161.18 (C-5), 98.76 (C-6), 164.09 (C-7), 93.68 (C-8), 156.23 (C-9), 103.96 (C-10), 121.58 (C-1′), 115.27 (C-2′), 144.78 (C-3′), 148.25 (C-4′), 116.19 (C-5′), 121.46 (C-6′); 102.37 (C-1″), 71.74 (C-2″), 73.85 (C-3″), 68.73 (C-4″), 75.87 (C-5″), 66.00 (C-6″), 100.47 (C-1‴), 70.96 (C-2‴), 70.55 (C-3‴), 72.38 (C-4‴), 68.24 (C-5‴), 17.74 (C-6‴); ms/ms: 633 $[M + Na]^+$, 611.1 $[M + H]^+$, 464.9 [M + H − rhamnose]$^+$, 302.7 [M + H − rhamnosylgalactosyl]$^+$.

Quercetin 3-*O*-rhamnosyl (1→6) glucoside (**4**) Elution with 5% water in methanol and on crystallization (methanol) gave a yellow powder with mp 228–230 °C; dark green with $FeCl_3$ and pale red with Mg + HCl. UV (λmax nm: 255, 354 (MeOH); 267, 328, 411 (MeOH + NaOMe); 270, 425 (MeOH + $ALCl_3$); 267, 369, 402 (MeOH + $AlCl_3$ + HCl); 268, 378 (MeOH + NaOAc); 255, 365, 373 (MeOH + NaOAc + H_3BO_3); 256, 355 (MeOH + $CaCl_2$): IR (KBr) cm^{-1} 3285 (-OH), 1654 (C = O), 1601, 1503, 1457, 1381, 1285 (C = C), 878, 807 (C-H of aromatic ring). ^{1}H-NMR (DMSO-d_6, 300 MHz): 7.53 (2H, d, J = 8.2 Hz H-6′), 7.51 (H, d, J = 1.7 Hz H-2′), 6.83 (1H, d, J = 7.7 Hz H-5′), 6.38 (1H, d, J = 1.2 Hz H-8), 6.18 (1H, d, J = 1.2 Hz H-6), 5.34 (1H, d, J = 7.0 Hz glucosyl H-1), 4.38 (rhamnosyl H-1) 3.35 (m, rhamnosyl glucosyl protons), 0.98 (3H, d, J = 6.1 Hz) rhamnosyl CH_3. ^{13}C-NMR (DMSO-d_6, 75 MHz): 156.43 (C-2), 133.30 (C-3), 177.37 (C-4), 161.23 (C-5), 98.69 (C-6), 164.09 (C-7), 93.59 (C-8), 156.60 (C-9), 103.97 (C-10), 121.18 (C-1′), 115.23 (C-2′), 144.75 (C-3′), 148.92 (C-4′), 116.27 (C-5′), 121.60 (C-6′); 101.08 (C-1″), 74.08 (C-2″), 76.49 (C-3″), 70.38 (C-4″), 75.91 (C-5″), 67.00 (C-6″), 100.75 (C-1‴), 70.70 (C-2‴), 70.56 (C-3‴), 71.85 (C-4‴), 68.25 (C-5‴), 17.74 (C-6‴); ms/ms: 633 $[M + Na]^+$, 611.1 $[M + H]^+$, 464.9 [M + H − rhamnose]$^+$, 302.7 [M + H − rhamnosylglucosyl]$^+$.

The n-butanol fraction did not cause any significant effect on the blood glucose lowering when compared with the control group of normal rats ($P > 0.05$) (Table 2). The *n*-butanol fraction exerted a significant hypoglycaemic effect ($P < 0.05$) in streptozotocin-induced diabetic rats at hour 5 and hour 7 after the administration of the extracts (Table 2). At hour 3, after the administration of *n*-butanol fraction, the blood glucose level of the diabetic rats (286.68 ± 10.89 mg/dl) and that of control diabetic rats (332.09 ± 10.38 mg/dl) (Table 1) were not significantly different and the percentage decrease is 13.67% (45.41 mg/dl). The butanol fraction exerted significant hypoglycaemic effect ($P < 0.05$) in streptozotocin-induced diabetic rats at hour 5 and hour 7, after the administration of the extract (Table 1) when compared with control groups of diabetic rats. The blood glucose levels in the diabetic rats treated with *n*-butanol fraction were lowered by 32.42% and 40.77% at hour 5 and hour 7, respectively.

The glibenclamide caused significant decrease in blood glucose levels of normal rats, but failed to cause significant effect on blood glucose levels of streptozotocin-induced diabetic rats.

Table 2. Blood glucose levels in normal and streptozotocin-diabetic rats at various time intervals after oral administration of the *n*-butanol fraction of *Gynura procumbens* leaf (1 g/kg).

	Normal rats			Streptozotocin-diabetic rats		
Time (Hours)	Control	Glibenclamide	*n*-butanol fraction	Control	Glibenclamide	*n*-butanol fraction
0	99.98 ± 1.88 (100)	98.61 ± 1.62 (98.86)	99.79 ± 1.82 (99.81)	333.50 ± 10.92 (100)	334.28 ± 11.73 (100.2)	336.07 ± 12.02 (100.77)
1	103.94 ± 1.53 (100)	90.90 ± 1.54 (87.45)	103.79 ± 2.30 (99.86)	335.62 ± 11.34 (100)	325.54 ± 11.62 (97.00)	336.19 ± 12.70 (100.17)
2	100.38 ± 2.04 (100)	86.62 ± 1.44 (86.29)	102.92 ± 2.88 (100.53)	325.58 ± 10.38 (100)	316.26 ± 11.58 (97.14)	331.93 ± 12.09 (101.95)
3	100.03 ± 2.44 (100)	74.13 ± 1.32 (74.11)	101.31 ± 2.61 (101.28)	332.09 ± 10.09 (100)	318.13 ± 11.55 (95.80)	286.68 ± 11.89 (86.33)
5	100.81 ± 2.54 (100)	69.34 ± 1.26 (68.78)*	98.31 ± 2.56 (97.52)	341.80 ± 11.77 (100)	315.37 ± 11.49 (92.27)	230.83 ± 12.03 (67.53)*
7	100.14 ± 2.33 (100)	61.94 ± 1.23 (61.85)*	97.77 ± 1.78 (97.63)	340.40 ± 11.52 (100)	313.21 ± 11.51 (92.01)	201.63 ± 11.56 (59.23)*

Each value represents the mean ± s.e.m. for six rats (n = 6).
Experimental group has beeen compared with the control groups.
Figures in parenthesis are the percentages of the control values.
* Significantly different from the control value: $P < 0.05$.

The sulfonylureas, including glibenclamide, are reported to regulate blood glucose homeostastais by stimulating pancreatic secretion of insulin. These drugs do not decrease blood glucose in streptozotocin-diabetic animals (Goth, 1885; Swanson, 1991), but oral administration of insulin is known to produce produce hypoglycaemia in both normal and streptozotocin-induced animals (Larner, 1985). From the results of this experiment, it is shown that the extract cannot act indirectly by stimulating the release of insulin, since streptozotocin-treatment causes permanent destruction of β-cells. Therefore, the extract may be effective in independent insulin also**.**

In conclusion, the results of the pharmacological screening showed that the *n*-butanol fraction from the *Gynura procumbens* leaf has a hypoglycaemic effect. The RP-HPLC eluent allows isocratic separation of the flavonoids in the *n*-butanol fraction from *Gynura procumbens*. Further investigations are going on in our laboratory to elucidate in detail the mechanism of action of the extract and also to determine other not yet identified chemical components from this plant.

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