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Hypoglycemic effects of *P. palatiferum* leaf extract

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Two major compounds, stigmasterol (ST) and sitosterol-3-O-β-D-glucopyranoside (SG) were isolated from *Pseuderanthemum palatiferum* leaf extract which is used traditionally as an antidiabetic. ST and SG at doses of 0.25 and 0.50 mg/kg were fed to diabetic rats for 21 days, and the fasting blood glucose (FBG) level and biochemical data on day 0, 4, 7, 10, 14, 17 and 21 were determined and compared with the anti-diabetic drug, glibenclamine. FBG levels at all doses of ST and SG were significantly decreased (*p*<0.05) with a concomitant increase in serum insulin. SG at the dose of 0.50 mg/kg showed the highest hypoglycemic effect. ST and SG also improved the following biochemical data and hematology parameters such as total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), blood urea nitrogen, creatinine, red blood cells, platelet and white blood cells.

Key words: *Pseuderanthemum palatiferum*, acanthaceae, stigmasterol, sitosterol-3-O-β-D-glucopyranoside;, hypoglycemic activity.

INTRODUCTION

Pseuderanthemum palatiferum (Nees) Radlk is a medicinal plant belonging to the acanthaceae family. The leaves of this plant are widely use in folk medicine of Vietnam and Thailand for promoting and treating of various diseases including hypertension, diarrhea, arthritis, hemorrhoids , stomach ache , tumors, colitis, bleeding, wounds, constipation, flu, colon cancer, nephritis and diabetes (Padee et al., 2010).

Some pharmacological properties of *P. palatiferum* have been reported to support the efficacy of its traditional use. The leaf extract showed high antioxidant activity against the hydrogen peroxide radical in the

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human blood. The ethyl acetate leaf extract showed strong antibacterial activity against *Salmonella typhi* 158, *Shigella flexineri*, and *Escherichia coli*. It was also active as an antifungal against *Candida albicans* and *Candida stellatoidea*. The anti-diarrhea efficacy of *P. palatiferum* was not significantly different from two antibiotic drugs (Colinorgan and Cotrimoxazole) (Padee and Nualkaew, 2009). The 80% ethanolic leaf extract of *P. palatiferum* showed a hypoglycemic effect at a dose of 250 mg/kg in streptozotocin (STZ)-induced diabetic rats by reducing fasting blood glucose level and stimulating insulin secretion (Padee et al., 2010).

The toxicity of *P. palatiferum* was also tested in our previous study; there is no acute toxicity in rats at the dose up to 2 g/kg and no cytotoxicity in vero cells at the concentration of 50 µg/ml (Padee et al., 2009). The chemical constituents in *P. palatiferum* which have been reported are flavonoids, triterpenoid saponins, kaempferol, apigenin, phytol, palmitic acid and salicylic acid. Essential amino acids such as lysine, methionine and threonine were also reported including some minerals namely calcium, potassium, magnesium and iron (Padee and Nualkaew, 2009). After the hypoglycemic effect of *P. palatiferum* leaf extract was confirmed by our previous study, the major compounds were isolated from an 80% ethanolic leaf extract. This study presents the active compounds responsible for this activity.

MATERIALS AND METHODS

General experimental procedures

TLC was conducted using normal-phase silica gel 60 F_{254} (Merck, Germany) on precoated aluminium plates. UV light (254 nm) and anisaldehyde-sulphuric acid spray reagent were used for detection. Column chromatography (CC) was carried out using silica gel 60 (0.063 to 0.200 mm; Merck, Germany). The melting point was obtained by using a Büchi melting point meter (Switzerland). UV spectra were obtained from a JASCO V530 UV/Vis spectrophotometer (Japan), while IR spectra were recorded with a Perkin Elmer FT-IR spectrometer (Germany). The MS were obtained using a Bruker MicroTOF ESI-TOF (USA) spectrometry double-focusing probe at 70 eV, while NMR spectra (1D and 2D) were measured on a Varian Mercury Plus 400 (USA) at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectra. Solvents were of analytical grade such as n-hexane (Lab-scan, Ireland), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), methanol (MeOH) (Carlo Erba, Italy), ethanol (EtOH) (Merck, Germany).

Plant material

The leaves of *P. palatiferum* were collected from Roi Et province, Northeast of Thailand. The specimen was identified by the Plant Varieties Protection Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. The voucher specimen was deposited at the Faculty of Pharmacy, Mahasarakham University, Thailand (herbarium code: MSU.PH-ACA-P1).

Extraction

Air dried leaves of *P. palatiferum* were ground and macerated with 80% ethanol (1:10) at room temperature for 7 days. The extract was dried by a rotary evaporator followed by freeze drying to get a powder (12.7% w/w of dry leaves). The obtained extract was stored at -20°C until being used.

Isolation

The *P. palatiferum* extract (10g) was suspended in 80% MeOH (200 ml) and partitioned successively with 200 ml of n-hexane, CH_2Cl_2, and EtOAc to yield the n-hexane (16.1%), CH_2Cl_2 (24.8%), EtOAc (8.5%) and residual 80% MeOH (46.3%) extracts. The n-hexane extract (1.61 g) was chromatographed on a silica gel column and eluted with gradient of n-hexane and EtOAc (100:0, 80:20, 70:30, 50:50, 30:70, 20:80 and 0:100 v/v) each portion of 500 ml. These elutes were collected in a series of test tubes with 20 mL in each fraction. The homogeneity of the eluted was monitored by TLC and the identical fractions were combined to afford five fractions. Fraction 1 was rechromatographed on a silica gel column and eluted with n-hexane:EtOAc (8:2 v/v) to afford compound 1 (2.33 % w/w of n-hexane extract). Fraction 5 (700 mL) was precipitated with EtOAc to yield compound 2 (3.07 % w/w of n-hexane extract). Compounds 1 and 2 belong to the main constituents of each fraction. Fractions 2 to 4 were discarded.

Animals

Male wistar rats, aged 5 to 7 week (150 to 170 g) from the National Laboratory Animal Centre (NLAC), Mahidol University, Thailand were used. They were acclimatized in an air conditioned room at 25±2°C, 12 h light/12 h dark cycle and relative air humidity 40 to 60% for 7 days, and given a standard chow and water *ad libitum* prior to the experiment. This method was performed in accordance with the advice of the Institutional Animal Care and Use Committee, MSU, Thailand (License 01/2009).

Hypoglycemic activity in rats

Hypoglycemic effect in Streptozotocin diabetic rats

Animals were injected intraperiteoneally by a single dose of 65 mg/kg streptozotocin (STZ) (Sigma Chemicals, St. Louis, MO) to induce diabetes. After injection, they were provided with 2% sucrose solution as their drink for 48 h to alleviate the initial hypoglycemic phase. Three days after injection, the rats were examined to fasting blood glucose (FBG) to confirm diabetic stage. The rats with fasting blood glucose (FBG) higher than 126 mg/dL were used in the experiments (Talubmook, 2008). Rats were randomly divided into seven groups of six animals each. Groups I and II were normal and diabetic control rats administrated orally with 2% tween 80. Group III was diabetic rats treated orally with glibenclamide at the dose of 0.25 mg/kg. Group IV and V were diabetic rats treated orally with sitosterol-3-O- -D-glucopyranoside (SG) at the doses of 0.25 and 0.50 mg/kg. Group VI and VII were diabetic rats treated orally with stigmasterol (ST) at the doses of 0.25 and 0.50 mg/kg. The samples were suspended in 2% tween 80 and administered orally by gavage 1 ml each animal once a day for 21 days.

Determination of fasting blood glucose level

The rats were fasted overnight for 8 to 12 h before blood collection.

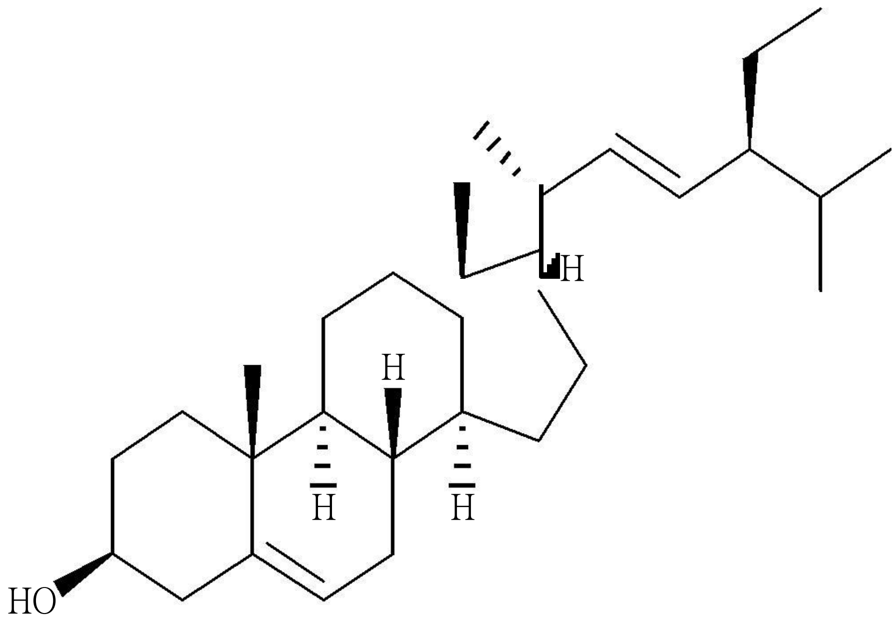

Figure 1. Structure of stigmasterol.

The blood samples were collected, taken from the tail vein. FBG was measured at days 0, 4, 7, 10, 14, 17, and 21 with Accu-chek Adventage II (Roche, Germany) (Padee et al., 2010).

Determination of serum insulin level

After 21 days of administration, the rats were fasted overnight. They were sacrificed by cervical dislocation technique, then blood samples were drawn from the rat's heart and centrifuged at 3500 rpm for 20 min to separate blood serum. The serum insulin was determined by a radioimmunoassay kit (MP Biomedicals-Orangeburg, USA) and detected by an automatic gamma counter (Wallac 1470 Wizard, Perkin Elmer instrument, Germany) (Padee et al., 2010).

Determination of biochemical data and hematological parameters

The blood samples were collected with the same technique as used for determination of serum insulin level. The biochemical data, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), blood urea nitrogen (BUN), and creatinine, were measured using an automatic blood chemical analyzer (BT 2000 plus, Germany). The hematological parameters including red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), platelet, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean platelet volume (MPV), white blood cells (WBC), lymphocytes, monocytes and neutrophils were determined using an automatic blood analyzer (Swelab Alfa, Biozen, Sweden).

Statistical analysis

All data were expressed as mean ±standard error of mean (SEM). Statistical analysis was carried out using *F* -test (One-way ANOVA) followed by Duncan's New Multiple Range Test. The criterion of statistical significance was measured at *p*-values less than 0.05.

RESULTS AND DISCUSSION

Isolation

Compound 1 was obtained as white needle crystals, mp 135 to 136°C and its molecular formula was assigned to be $C_{29}H_{48}O$ according to it mass spectrum (*m/z* 412 $[M]^+$). Compound 2 was obtained as white needle crystals, mp 297 to 298°C and its molecular formula was assigned to be $C_{35}H_{60}O_6$ according to it mass spectrum (*m/z* 599 $[M+Na]^+$). By comparing their physical and spectroscopic data of IR, NMR and MS with those reported in the literature (De-Eknamkul et.al., 2003; Kongduang et. al., 2008; Jamal et.al., 2009; Jayaprakasha et.al., 2010; Giang et.al., 2005) and analyzing their 2D NMR spectral data, the compound 1 (Figure 1) and compound 2 (Figure 2) were identified as stigmasterol and sitosterol-3-O- -D-glucopyranoside, respectively. The ^1H -NMR and ^{13}C NMR of compound 1 and 2 were showed in Table 1.

Hypoglycemic activity

The effect of SG and stigmasterol (ST) isolated from *P. palatiferum*, on fasting blood glucose and serum insulin level is shown in Table 2. At all doses of SG, ST and glibenclamide, FBG level were significantly (p<0.05) reduced while increased serum insulin level was dose

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compound 1 (in $CDCl_3$) and 2 (in CD_3OD), (δ in ppm, J in Hz).

Position	Compound 1		Compound 2	
	δ_H	C	δ_H	δ_C
1	1.15 (m)	37.24	1.25 (m)	37.17
2	1.44 (m)	31.64	1.33 (m)	29.45
3	3.52 (m)	71.79	3.78 (m)	78.99
4	2.0 (m)	42.20	2.22 (m)	38.54
5	-	140.74	-	140.3
6	5.36 (bs)	121.69	5.36 (bs)	121.94
7	1.82 (m)	31.88	1.84 (m)	31.81
8	1.44 (m)	31.86	1.22 (m)	31.81
9	1.44 (m)	50.15	1.22 (m)	50.13
10		36.49		36.60
11	1.44 (m)	21.05	1.33 (m)	20.94
12	1.44 (m)	39.67	1.33 (m)	39.68
13	-	42.28	-	42.22
14	1.44 (m)	56.85	1.42 (m)	56.68
15	1.58 (m)	24.34	1.56 (m)	24.14
16	1.58 (m)	28.89	1.56 (m)	28.11
17	1.58 (m)	55.94	1.56 (m)	55.97
18	0.68 (s)	12.03	0.65 (s)	11.62
19	1.05 (s)	19.37	0.97 (s)	19.07
20	2.26 (m)	40.46	1.33 (m)	36.03
21	0.86 (d, 6.8)	21.05	1.08 (d, 6.8)	18.54
22	5.16 (dd, 12, 8)	138.29	1.56 (m)	33.83
23	5.02 (dd, 12, 8)	129.27	1.5 (m)	25.95
24	2.22 (m)	51.22	1.12 (m)	45.79
25	1.82 (m)	31.86	1.84 (m)	29.04
26	0.84 (d, 6.8)	21.19	0.75 (d, 6.8)	18.73
27	0.78 (d, 6.8)	18.96	0.88	19.50 (d, 6.8)
28	-	25.38	1.33 (m)	22.94
29	0.82 (t, 6.8)	12.22	0.79 (t, 6.8)	11.67
1’	-	-	4.38 (d, 6.8)	101.05
2’	-	-	3.32 (m)	73.50
3’	-	-	3.58 (m)	76.45
4’	-	-	3.32 (m)	70.09
5’	-	-	3.38 (m)	75.89
6’	-	-	3.18 (m)	61.61

dependent. This result confirmed previous pharmacological investigation of Perez and Vargas (2002), who reported that SG has hypoglycemic activity and ST induces the uptake of insulin from α-cells producing an anti-hyperglycemic effect (Ivorra et al., 1990, 1998; Panda et.al., 2009). The anti-diabetic mechanism of these compounds may be connected to insulin stimulatory activity.

This was confirmed by serum insulin that was increased at all doses of SG, ST and glibenclamide; especially SG at the dose of 0.50 mg/kg showed highest serum insulin in diabetic rats. This effect is probably due to the regeneration of pancreatic -cells which are destroyed by streptozotocin (Eidi et al., 2006). The doses of SG and ST which were used in this study were calculated from percent amount of SG and ST in fresh leaf of *P. palatiferum.* Thai healers usually suggest to eat 7-9 fresh leaves of *P. palatiferum* every day for treatment of diabetes. The amount of 7 to 9 fresh leaves of *P. palatiferum* (about 2 g fresh weight) was related to 0.25 mg of SG and ST, therefore the dose 0.25 mg of SG and ST were used in this study including double dose (0.50 mg).

The result showed that the dose 0.25 and 0.50 mg/kg

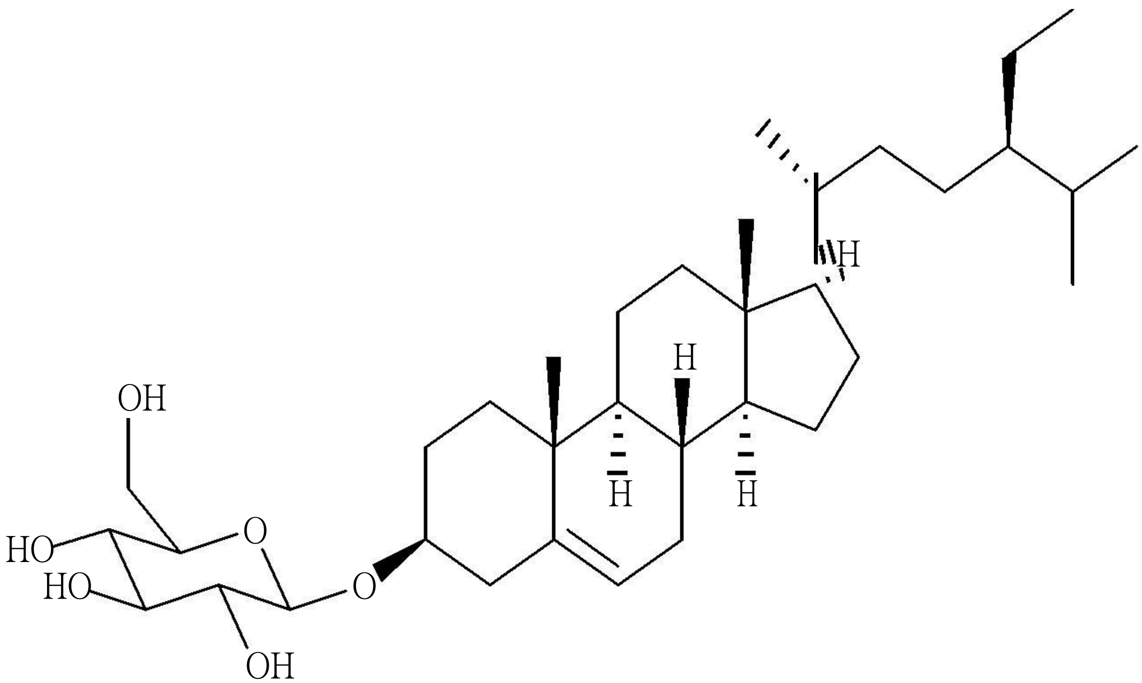

Figure 2. Structure of sitosterol-3-O- -D-glucopyranoside.

Table 2. Effect of SG and ST at the doses of 0.25 and 0.50 mg/kg isolated from *P. palatiferum* (Nees) Radlk. leaf extract and glibenclamide (Gliben) at the dose of 0.25 mg/kg on FBG and serum insulin levels in rats for 21 days.

Parameters	Normal	Diabetic	Gliben	SG		ST	
FBG (mg/dL)				0.25 mg/kg	0.50 mg/kg	0.25 mg/kg	0.50 mg/kg
day 0	89.83±0.48[a]	285.33±14.23[b]	277.17±13.40[b]	252.67±14.34[b]	255.67±15.18[b]	266.17±14.35[b]	278.33±13.07[b]
day 4	78.00±1.65[a]	320.33±6.52[b]	339.00±10.84[b]	385.17±5.68[c]	328.33±6.09[b]	353.17±8.31[bc]	320.50±6.82[b]
day 7	85.50±1.52[a]	365.00±13.38[c]	449.00±13.81[d]	376.17±10.63[c]	291.50±8.41[b]	365.50±10.21[c]	366.50±6.64[c]
day 10	85.17±1.94[a]	443.50±8.25[e]	396.17±6.12[cd]	371.33±6.23[c]	315.17±7.63[b]	414.17±7.33[d]	330.50±9.43[b]
day 14	86.17±3.52[a]	485.17±5.12[d]	436.67±8.39[c]	436.67±7.50[c]	285.00±8.81[b]	443.50±6.19[c]	438.83±6.65[c]
day 17	76.67±3.06[a]	479.33±7.80[e]	436.83±8.38[d]	386.67±4.96[c]	322.17±7.42[b]	425.83±7.46[d]	373.00±7.88[c]
day 21	80.67±2.79[a]	505.83±5.26[e]	443.00±6.47[d]	440.50±10.29[d]	313.00±6.96[b]	431.33±14.38[d]	361.17±8.29[c]
Insulin (IU/ml)	24.03±0.69[d]	11.43±0.48[a]	15.85±0.34[b]	17.41±0.60[b]	20.85±0.42[c]	17.19±0.55[b]	16.77±0.47[b]

The values represent the mean ±SEM within the same rows followed by the different superscript letters (a-e) are significantly different at p<0.05.

of SG and ST have anti-hyperglycemic effect especially 0.50 mg/kg SG; but the activity was not as strong as Thai healers mention. It may be that additional active constituents are present, a positive interaction of constituents or even a synergistic effect occurs (Jamaluddin et al., 1994). The effect of sitosterol-3-O- -D-glucopyranoside and stigmasterol on biochemical data and hematological parameter in diabetic rats after treatment of 21 days is shown in Table 3. All doses of SG, ST and glibenclamide significantly (p<0.05) improved TC, TG, HDL, LDL, BUN, creatinine, RBC, platelet and WBC. This improvement indicates that SG and ST can prevent complications resulting from diabetes and improve renal and liver function.

Conclusion

The anti-diabetic effect of these compounds support the anti-hyperglycemic effect and traditional use of *P. palatiferum* leaf extract (Padee et.al., 2010) and point on the constituents responsible for the effects. Whether SG and ST exhibit synergistic effects and whether other compounds are involved, should be clarified later.

of β-sitosterol and stigmasterol. Food Chem. 49(4):339-345.
Jayaprakasha GK, Jadegoud Y, Gowda GAN, Patil BS (2010). Bioactive compounds from sour orange inhibit colon cancer cell proliferation and induce cell cycle arrest. J. Agric. Food Chem. 58:180-186.
Kongduang D, Wungsintaweekul J, De-Eknamkul W (2008). Biosynthesis of β-sitosterol and stigmasterol proceeds exclusively via the mevalonate pathway in cell suspension cultures of *Croton stellatopilosus*. Tetrahedron Lett. 49:4067-4072.
Padee P, Nualkeaw S (2009). Current information of medicinal plants: *Pseuderanthemum palatiferum* (Nees) Radlk. J. Health Sci. 8:131-138.
Padee P, Nualkeaw S, Talubmook C, Sakuljaitrong S (2009). Acute toxicity and sub-acute toxicity of *Pseuderanthemum palatiferum* (Nees) Radlk. leaf extract. Isan. J. Pharm. Sci. 5:74-81.
Padee P, Nualkeaw S, Talubmook C, Sakuljaitrong S (2010). Hypoglycemic effect of a leaf extract of *Pseuderanthemum palatiferum* (Nees) Radlk. in normal and streptozotocin-induced diabetic rats. J. Ethnopharmacol. 132:491-496.
Panda S, Jafri M, Kar A, Meheta BK (2009). Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. Fitoterapia 80:123-126.
Perez GRM, Vargas SR (2002). Triterpens for *Agarista maxicana* as potential anti-diabetic agents. Phytother. Res. 16:55-58.
Talubmook C (2008). Effect of polysaccharide from *Phellinus ignarius* (L) Quel. on hematological values and blood cell characteristic in diabetic rats. J. Microscopy Soc. Thailand 22:42-45.