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Research article

Isolation of a new flavanone and evaluation of antidiabetic activity from *Clematis buchananiana* aerial parts

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ABSTRACT

The present study aimed to explore the phytochemistry and antidiabetic activity of *Clematis buchananiana* DC. A new compound (**1**) characterized as 3-[(11''-hydroxy, 3'',6'',10'',10''-tetramethyldodecene)] 6-[(3''',4''',4'''-trimethylhexene)] 5,7,2',4',6'pentahydroxy 8, 3',5' tri methyl flavanone was isolated from the aerial parts of the plant. The methanolic extract of the plant at a concentration of 1 mg/mL showed a 75.00% reduction in α-amylase and 73.13% in α-glucosidase enzyme activity. Moreover, the methanolic extract at 100 mg/kg b.w. showed a significant decline in blood glucose levels of alloxan-induced diabetic rats both in acute and chronic studies. The results were compared with glibenclamide, an oral hypoglycemic agent, which was used as a positive control. The present study concludes that the plant has substantial antidiabetic potential in diabetic rats. These results are indicative and further studies are needed to revalidate the findings.

INTRODUCTION

Diabetes mellitus is one of the chronic, worldwide heterogeneous and life-threatening diseases. Its prevalence will be 5.4% by the year 2025, with the global diabetic population reaching 300 million. Among all the WHO regions, the Southeast Asian region is the highest affected with the maximum global burden of the disease and, by the year 2025, there will be nearly 80 million diabetics in the region (WHO, 2016). Given the pandemic spread of type two diabetes mellitus, the identification of new therapeutic avenues in the treatment of all pathological aspects of this disorder remains a major challenge for current biomedical research. A number of plants present in nature possess marked antidiabetic activity and one of them is *Clematis buchananiana* (family Ranunculaceae), which is commonly known as Kanguli or Lagulia.

In the Garhwal region, it is found in Bhyundar valley, Mandal and Pandukeswar at a height of 1650-2300 m (Naithani, 1984). The leaf paste of *C. buchananiana* is externally applied for skin ailments (Gaur, 1999). The juice extracted by crushing fresh roots is inhaled to treat sinusitis and headache (Gaire and Subedi, 2011). Aqueous extracts of *Clematis buchananiana* leaf shows anti-inflammatory, and antipyretic properties in rats (Mostafa et al., 2010). Previous phytochemical investigation revealed that this genus contains flavonoids, saponins, phenolic derivatives, terpenoids and essential oil. Zhang et al. (2007) reported terniflonoside A together with other flavonol glycosides from the whole plant of *C. terniflora*.

The crude saponin fraction obtained from the roots of *Clematis chinensis* furnished triterpenoid prosapogenins (Kizu and Tomimori, 1980). The methanolic extract of aerial parts of *C. koreana* furnished 3-O-β-D-xylopyransoyl (1-3)-α-L-arabinopyranosyl oleanolic acid 28-O-α-L-rhamnopyranosyl (1-4)-β-D-glucopyranosyl (1-6)- β-D-glucopyranosyl ester together with other terpenoids. Shao et al. (2006) reported clematichinenol, (t)-syringaresinol, acacetin-7-α-L-rhamnopyranosyl-(1-6)-β-D-glucopyranoside and (-)-syringaresinol-4-O-beta-D-glucoside from the aerial parts of *C. chinensis*. The methanolic extract of *C. montana* roots furnished clemontanoside E together with friedelin, β-amyrin, β-sitosterol, β-sitosterol-β-D-glucoside and oleanolic acid (Thapliyal and Bahuguna, 1993; 1994). The leaf paste of *C. buchananiana* is externally applied for skin ailments (Gaur, 1999). Juice extracted by crushing fresh roots is inhaled to treat sinusitis and headache (Gaire and Subedi, 2011). The aqueous extract of the leaf showed anti-inflammatory and antipyretic activities in rats (Mostafa et al., 2010). Earlier studies revealed that *Clematis* sp. also exhibited antimicrobial (Misra and Dixit 1979; Khan et al., 2001) and antidiabetic (Eidi et al., 2006) activities in different experimental models.

MATERIALS AND METHODS

Collection and identification

The aerial parts of *Clematis buchananiana* (6 kg) were collected from Mandal, Chamoli, Uttarakhand (India) in August. The plant was authenticated at the Department of Botany, H.N.B. Garhwal University Srinagar, Uttarakhand. A voucher specimen (21345) was deposited in the department for future records.

Preparation of plant extract

The aerial parts were air-dried and ground to a moderately fine powder. The material was successively extracted with soxhlet apparatus using petroleum ether, chloroform, ethyl acetate, acetone, methanol, ethanol and water (Lin et al., 1998). Each solvent fraction was evaporated to dryness under reduced pressure using a rotary evaporator. The concentrated extracts were used for the isolation of phytochemicals and evaluation of the antidiabetic activity.

Isolation of compound

Ethyl acetate extract was chromatographed over silica gel (60-120 mesh). The elution was carried out with chloroform and the polarity was increased by methanol. The fraction eluted with chloroform and methanol (10:90) furnished compound **1**.

3-[(11''-hydroxy, 3'',6'',10'',10''-tetramethyldodecene)] 6-[(3''',4''',4'''-trimethylhexene)] 5,7,2',4',6'pentahydroxy 8, 3',5' tri methyl flavanone (Compound 1)

Compound **1** was isolated as a brownish solid powder (yield 40 mg) having m.p. 132-134°C. The IR spectrum showed absorption bands at (KBr γ_{max} cm^{-1}) 3400, 2940, 1731, 1640, 1430, 1250, 1164 and 830. The UV spectrum showed absorption at (MeOH) (λ_{max}nm) 290 and 337. The molecular weight of compound **1** was calculated to 709.6 $[M+1]^+$ on the basis of EI-MS (m/z). The elemental analysis showed C, 72.85; H, 9.10; O, 18.05% calcd. for $C_{43}H_{64}O_8$: C, 72.45; H, 9.40; O, 18.15%). NMR data of compound **1** is given in Table 1.

Table 1. ^{1}H (300 MHz), ^{13}C (75 MHz) NMR and DEPT values in CD_3OD

Position	$^{\delta}$C ppm	$^{\delta}$H ppm	DEPT	HMBC
2	84.1	4.52 (d, *J* = 3.1 Hz)	CH	
3	42.0	2.56 (dd, *J* = 8.1, 3.3 Hz)	CH	
4	178.0	-	C	
5	171.0	-	C	
6	105.2	-	C	
7	173.2	-	C	
8	105.2	-	C	
9	156.4	-	C	
10	104.7	-	C	
1'	104.7	-	C	
2'	154.9	-	C	
3'	105.2	-	C	
4'	153.0	-	C	
5'	105.2	-	C	
6'	154.9	-	C	
1''	127.0	5.12 (dd, *J* = 2.1, 1.8 Hz)	CH	C-2, C-4, C-9, C-2''
2''	128.0	5.30 (dd, *J* = 2.1, 2.1 Hz)	CH	
3''	46.0	1.98 (m)	C	C-2''
4''	37.3	1.42 (m)	CH_2	
5''	23.0	1.35 (m)	CH_2	
6''	37.6	1.85 (m)	CH	
7''	38.1	1.70 (m)	CH_2	
8''	22.6	1.49 (m)	CH_2	
9''	25.0	1.71 (m)	CH_2	
10''	46.0	1.88 (m)	C	C-11''
11''	85.0	3.35 (m)	CH	
12''	20.3	1.15 (m)	CH_3	C-11''
1'''	21.0	2.15 (m)	CH_2	
2'''	131.0	6.8 (t, *J* = 3.6 Hz)	CH	C-1''', C-4'''
3'''	139.2	-	C	
4'''	45.3	-	C	
5'''	36.7	1.45 (m)	CH_2	
6'''	9.0	1.15 (m)	CH_3	
8- CH_3	9.0	1.94 (s)	CH_3	C-7, C-9
3'- CH_3	9.0	1.93 (s)	CH_3	C-3'
5'- CH_3	9.0	1.94 (s)	CH_3	C-6'
3''- CH_3	21.0	1.68 (s)	CH_3	
6''- CH_3	21.0	1.33 (s)	CH_3	
10''- CH_3	21.0	1.33 (s)	CH_3	C-11''
10''- CH_3	21.0	1.33 (s)	CH_3	
3'''- CH_3	9.0	1.39 (s)	CH_3	C-2''', C-4'''
4'''- CH_3	21.0	1.26 (s)	CH_3	
4'''- CH_3	21.0	1.29 (s)	CH_3	

Inhibition of α-amylase

Starch solution (0.1% w/v) was prepared by stirring 0.1g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of α-amylase in 100 ml of distilled water. The colourimetric reagent was prepared by mixing sodium potassium tartrate solution and 3,5-dinitro salicylic acid solution at 96 mM concentration. Both control and plant extracts separately were added with starch solution and left to react with the α-amylase solution under alkaline conditions at 25ºC. Their action was measured after 3 minutes. The generation of maltose was quantified by the reduction of 3,5-dinitro salicylic acid to 3-amino-5- nitro salicylic acid. This reaction was detectable at 540 nm (Malik and Singh, 1980).

Inhibition of α-glucosidase

The inhibitory activity of the α-glucosidase enzyme was determined by incubating 1 ml solution of the starch substrate (2% w/v maltose or sucrose) with 0.2 M tris buffer pH 8.0 and plant extracts separately for 5 minutes at

37°C. Their action was initiated by adding 1 ml of the α-glucosidase enzyme (1U/ml) to it followed by incubation for 40 minutes at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm (Krishnaveni et al., 1984).

Experimental animals

Mature adult albino rats weighing (180-210g) were purchased from the Central animal house facility (CPCSEA Regd. No. 245/CPCSEA, dated 11 March 2015) and acclimatized for seven days to faculty animal house, and maintained at standard conditions of temperature and relative humidity, with a 12-hour light-dark cycle. Water and commercial rat feed *ad libitum* were provided. The current study was carried out with prior sanction from Institutional Animal Ethical Committee and proposal no. 583.

Acute toxicity Study

To determine the minimum lethal dose, acute oral toxicity studies were performed as per OECD guidelines (OECD Guidelines 2006). Adult albino rats of either sex weighing 180-210gm were used.

Induction of diabetes

Alloxan was used to induce diabetes in experimental rats. The animals were fasted for 12h prior to the induction of diabetes with slight modification. Alloxan (ALX), freshly prepared in 0.5% Tween 80 was administered intra-peritoneal at a single dose of 140mg/kg body weight (Barkat et al. 2013). The development of diabetes was confirmed by measuring blood glucose concentration 5 days after the administration of ALX. Rats with blood glucose levels above 200 mg/dl were considered to be diabetic and used for the studies.

Experimental design

In the present experiment, a total of 30 rats (24 diabetic surviving rats; 6 normal rats) were used. The extracts were diluted to prepare the specific doses. The rats were randomized into five groups comprising of six animals in each group as given below.

Group I: Normal control rats received distilled water

Group II: Diabetic rats received standard drug GLB (10 mg/kg p.o.), 5 days after ALX treatment.

Group III: Diabetic rats received petroleum ether extract prepared in distilled water (100 mg/kg p.o.), 5 days after ALX treatment.

Group IV: Diabetic rats received ethyl acetate extract prepared in distilled water (100 mg/kg p.o.), 5 days after ALX treatment.

Group V: Diabetic rats received methanol extract prepared in distilled water (100 mg/kg p.o.), 5 days after ALX treatment.

Biochemical assays

Blood samples were collected from retro-orbital plexus of each rat under mild anaesthesia at 0, 1, 2 and 4h (Acute study) as well as on 0^{th}, 7^{th}, 14^{th} and 21^{st} days after administration (Chronic study) of extracts. Blood glucose level was estimated by the enzymatic glucose oxidase method. The reduction in blood glucose level was calculated with reverence to the initial level. The bodyweight of all animals was quantified on the 0, 7^{th}, 14^{th} and 21^{st} days after 1h of treatment with the plant extracts and GLB (Barkat and Mujeeb, 2013).

Data and statistical analysis

Results are expressed as the mean ± SEM of six independent experiments. The data were analysed for statistical significance by one-way ANOVA test; P values <0.05 were considered to be significant.

RESULTS AND DISCUSSION

Characterization of compound 1

Compound 1 (Fig. 1) was obtained as a brownish powder (40 mg) through column elution of MeOH:$CHCl_3$ (10:90) and its molecular formula $C_{43}H_{64}O_8$ was proposed on the basis of its molecular ion at m/z 709.6 $[M+1]^+$ in EI-MS (Fig. 6). The IR spectrum shows absorption due to hydroxyl, carbonyl and aromatic groups. The presence of a flavanone substructure in compound **1** was demonstrated by UV absorptions at 290 and 337 nm, respectively (Monira et al., 1994). The NMR resonance (Fig. 2) of H-3 in compound **1** was observed at δ2.56 (1H, dd, *J*=8.1, 3.3Hz) and presence of a double of doublets for H-1'' at δ5.12 (1H, dd, *J*=2.1, *J*=1.8 Hz) and for H-2'' at δ5.30 (1H, dd, *J*=2.1, *J*=1.8 Hz) indicates the presence of a side chain at C-3, which is further supported by HMBC spectrum (Fig. 5) which shows a correlation of H-1''→C-4,C-2,C-9,C-2''. The cluster of overlapping signals are observed in the NMR of compound 1 at δ1.15-1.35, 1.42-1.70, 1.70-1.98. These signals were attributed to the C-12 side chain (Fatope et al., 2003). The presence of a triplet at δ6.8 (1H, t, *J*=3.6 Hz) is observed. HMBC correlates the proton H-2''' (δ6.8) → C-1''' (δ 21.0) & C-4''' (δ 45.3) which indicates the presence of another side chain at C-6 which is further confirmed by HMBC correlation (Fig. 7). The ^{13}C NMR (Fig. 3) DEPT-135 spectrum (Fig. 4) of compound **1** indicates the presence of 7 methylene, 7 methine, 12 methyl and 17 quaternary carbons. On the basis of the above spectral data the structure of compound **1** was elucidated as 3-[(11''-hydroxy, 3'',6'',10'',10''-tetramethyl dodecene)] 6-[(3''',4''',4'''-trimethyl hexene)] 5,7,2',4',6'pentahydroxy 8, 3',5' trimethyl flavanone.

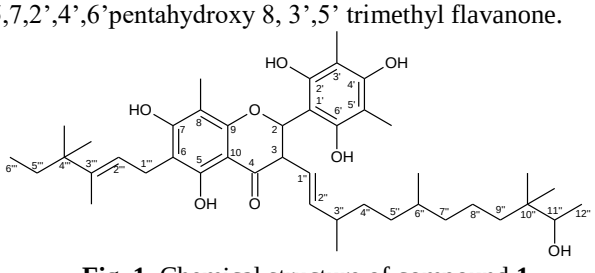

Fig. 1. Chemical structure of compound **1**

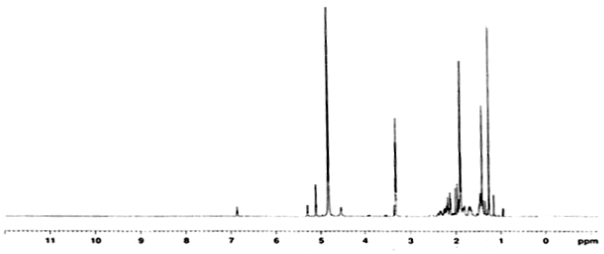

Fig. 2. ^{1}H NMR spectrum of compound **1**

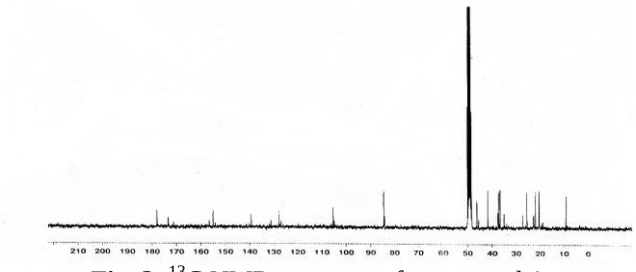

Fig. 3. ^{13}C NMR spectrum of compound **1**

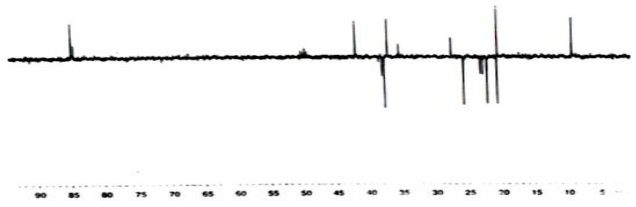

Fig. 4. DEPT (135) spectrum of compound **1**

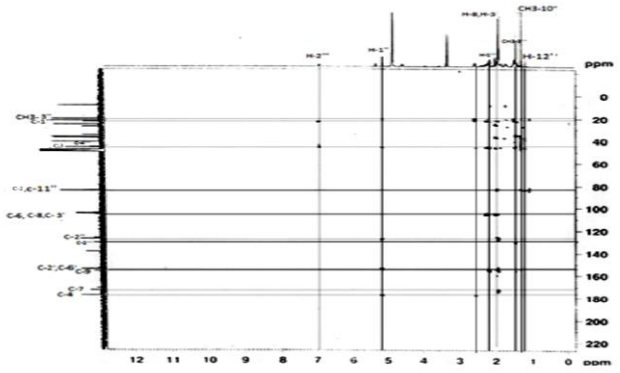

Fig. 5. HMBC spectrum of compound **1**

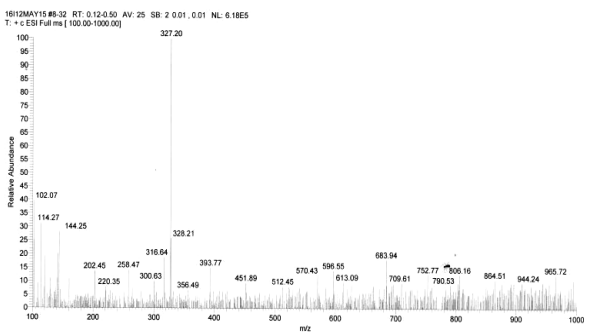

Fig. 6. Mass spectrum of compound **1**

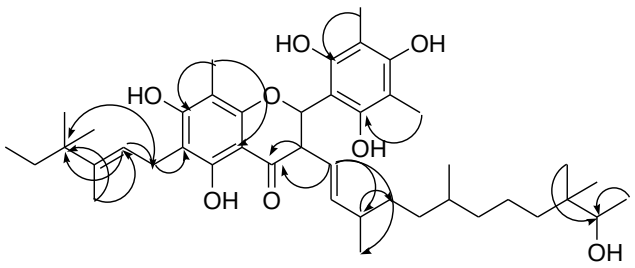

Fig. 7. HMBC correlation of compound **1**

In vitro antidiabetic activity

Medicinal plants are an important source of potentially bioactive constituents for the development of new therapeutic agents. The first step towards this goal is *in vitro* and *in vivo* antidiabetic studies. The results of *in vitro* antidiabetic activity are tabulated in Table 2 and 3 which were evaluated against two enzymes (α-amylase and α-glucosidase). The results revealed that the plant extracts possessed significant antidiabetic activity when compared with control. The *in vitro* assay revealed that out of three extracts of *C. buchananiana*, the ethyl acetate extract showed 76.56% reduction in α-amylase and 74.78% reduction in α-glucosidase enzyme activities.

Table 2. Percent reduction in α-amylase enzyme activity

Extract Name	Concentration	Percent reduction in α-amylase activity
Pet. ether	1 mg/mL	56.34%
Ethyl acetate	1 mg/mL	76.56%
Methanol	1 mg/mL	75.00%

Table 3. Percent reduction in α-glucosidase enzyme activity

Extract Name	Concentration	Percent reduction in α-glucosidase activity
Pet. ether	1 mg/mL	55.23%
Ethyl acetate	1 mg/mL	74.78%
Methanol	1 mg/mL	73.13%

In vivo antidiabetic activity

Different extracts of *C. buchananiana* were evaluated for their antidiabetic effect against alloxan-induced diabetic rats. The results (Table 4 and 5) showed that the petroleum ether extract of the plant exhibited a maximum reduction in blood glucose levels of diabetic rats as compared to the normal control group in the acute study. The results were also compared with glibenclamide, a positive control. However, the chronic study revealed that the methanolic extract of *C. buchananiana* had a maximum reduction in blood glucose levels of diabetic rats.

On the basis of the present experiment, it is suggested that the extracts of *C. buchananiana* potentially reduced the activity of α-amylase and α-glucosidase enzymes *in vitro*, whereas significantly lowered the blood glucose levels *in vivo* in diabetic rats.

Table 4. Acute antidiabetic effect of different extracts of *Clematis buchananiana* in alloxan-induced albino rats.

Treatment/ Group	Dose	Blood glucose level (mg/dl)			
		0 Hour	+1 Hour	+2 Hour	+4 Hour
Normal Control/ Group l	10 ml/kg	095.12±0.45	095.56±0.56	095.08±0.62	096.53±0.73
GLB treated/ Group II	10 ml/kg	275.67±1.12*	263.16±2.43**	224.06±1.20**	160.53±1.43**
Pet ether extract/ Group III	100 mg/kg	276.23±1.12*	253.23±2.43**	234.43±1.20**	152.33±1.43**
Ethyl acetate extract/ Group IV	100 mg/kg	252.23±1.12*	235.34±2.12**	227.03±1.23**	173.03±0.86**
Methanolic extract/ Group V	100 mg/kg	265.56±1.25*	245.67±1.45**	230.32±0.88**	156.34±0.83**

Table 5. Chronic antidiabetic effect of different extracts of *Clematis buchananiana* in alloxan-induced rats.

Treatment/Group	Dose	Blood glucose level (mg/dl)			
		4th day	7th day	14^{th} day	21th day
Normal Control/ Group l	10 ml/kg	182.12±0.45	185±0.56	189.23±0.62	197.56±0.73
GLB treated/ Group II	10 ml/kg	195.45±1.12*	192.34±2.43**	195.67±1.20**	185.34±1.43**
Pet ether extract/ Group III	100 mg/kg	185.58±1.12*	174.56±2.43**	153.32±1.20**	148.45±1.43**
Ethyl acetate extract/ Group IV	100 mg/kg	196.43±1.12*	183.45±2.12**	168.67±1.23**	148.23±0.86**
Methanolic extract/ Group V	100 mg/kg	198.45±1.25*	185.34±1.45**	157.56±0.88**	145.67±0.83**

The data are expressed in mean ± S.E.M. n = 6 in each group. P values were analyzed using One way ANOVA [*p<0.05- less significant; **p<0.05- most significant

CONCLUSION

The present finding reveals that *Clematis buchananiana* efficiently inhibits both α-amylase and α-glucosidase enzymes *in vitro* in a dose-dependent manner. The methanolic and ethyl acetate extracts showed 75.0% and 76.56% reduction against α-amylase and 73.13% and 74.78% reduction against the α-glucosidase activity, respectively. The *in vivo* experiments of the methanolic extract showed a maximum reduction of the blood glucose level of diabetic rats as compared to glibenclamide, a standard drug in a chronic study. The flavanone isolated from the aerial parts of the plant might be responsible for the antidiabetic effect. However, it needs further studies to make a final conclusion.

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CONFLICTS OF INTEREST

The author(s) declare(s) no conflicts of interest.

DECLARATION

The contents of this paper are published after receiving a signed copyright agreement from the corresponding author declaring that the contents of this paper are original. In case of any dispute related to the originality of the contents, editors, reviewers and publisher will remain neutral.

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