SUPPLEMENTARY MATERIAL

A new glycosidic antioxidant from *Ranunculus muricatus* L. (Ranunculaceae) exhibited lipoxxygenasae and xanthine oxidase inhibition properties

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Abstract

Phytochemical investigation of *Ranunculus muricatus* L. (Ranunculaceae) led to the isolation of a new metabolite named as ranuncoside from the ethyl acetate fraction of the plant. Structure of the novel compound was elucidated through detailed spectroscopic analyses, using UV, IR, ¹H-, ¹³C-NMR, and 2D-NMR in combination with EIMS and HR EI-MS techniques. The compound was evaluated for antioxidant activity using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay. Its inhibitory potential was tested against lipoxxygenase and xanthine oxidase enzymes. Ranuncoside potently scavenged the DPPH free radicals (IC₅₀ = 56.7 ± 0.43 μM) and strongly inhibited the activities of lipoxxygenase (IC₅₀ = 63.9 ± 0.17 μM) and xanthine oxidase (IC₅₀ = 43.3 ± 0.22 μM).

Keywords

Ranunculus muricatus; ranuncoside; free radicals inhibition; anti-inflammatory; enzyme inhibition.

Table S1: ^1H and ^{13}C NMR data of ranuncoside

No.	Hydrogens	Carbons
1	-	202.0
2	5.88, s	126.2
3	-	165.7
4	2.68, d, ($J = 9.5$ Hz)	56.8
5	-	37.2
6	2.04, d ($J = 16.5$ Hz); 2.47, d($J = 16.5$ Hz)	48.4
7	5.75, dd ($J = 15.0, 9.0$ Hz)	131.2
8	5.57, dd ($J = 15.5, 7.5$ Hz)	137.0
9	4.48, m	75.0
10	1.27, d ($J = 6.5$ Hz)	22.2
11	1.97, s	23.9
12	1.02, s	28.0
13	0.98, s;	27.4
1'	4.27, d ($J = 8.0$ Hz)	101.2
2'	3.18, m	74.9
3'	3.23, m	71.7
4'	3.13, m	78.2
5'	3.64, m	74.7
6'	3.51, dd ($J = 11.0, 6.0$ Hz); 3.58, t($J = 5.0$ Hz)	64.4

Figure S1: COSY correlations in ranuncoside.

Figure S2: HMBC correlations in ranuncoside.

NAILA RAZIA/DR.SHAHQ/RM-01/CD3OD
 ICCBS/U.O.K
 1H

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NAME      nov30-13
EXPNO     1
PROCNO    1
Date_     20131130
Time      10.30
INSTRUM   spect
PROBHD    5 mm CPDUL 13C
PULPROG   zg30
TD         32768
SOLVENT   MeOD
NS         16
DS         0
SWH        10000.000 Hz
FIDRES     0.305176 Hz
AQ         1.6385000 sec
RG         181
DW         50.000 usec
DE         6.50 usec
TE         298.3 K
D1         2.00000000 sec
TD0        1

===== CHANNEL f1 =====
NUC1       1H
P1         16.50 usec
PL1        -0.50 dB
PL1W       14.09191513 W
SFO1       500.3335023 MHz
SI         32768
SF         500.3300145 MHz
WDW        EM
SSB        0
LB         0.30 Hz
GB         0
PC         1.00
  
```

NAILA RAZIQ/DR.SHAIQ/RM-01
BB

NAME dec19-13
EXPNO 6
PROCNO 1
Date_ 20131220
Time_ 7.37
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zgpg
TD 32768
SOLVENT MeOD
NS 10764
DS 4
SWH 35971.223 Hz
FIDRES 1.097755 Hz
AQ 0.4555391 sec
RG 32768
DW 13.900 usec
DE 6.50 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TDO 12

===== CHANNEL f1 =====
NUC1 13C
P1 12.70 usec
PL1 -1.81 dB
PL1W 81.92915344 W
SFO1 150.8950149 MHz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 3.31 dB
PL12 23.31 dB
PL13 22.50 dB
PL2W 6.79873323 W
PL12W 0.06798734 W
PL13W 0.08192718 W
SFO2 600.0336002 MHz
SI 16384
SF 150.8774509 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00

NAILA RAZIQ/DR.SHAIQ/RM-01
deptsp135

—137.021
—131.196
—126.169

101.186
78.349
78.189
78.043
77.981
74.941
74.754
73.864
71.705
64.410
62.837
56.870
49.847
49.565
49.429
49.283
49.138
49.001
48.855
48.717
48.457
28.015
27.414
23.906
22.231

NAME dec19-13
EXPNO 7
PROCNO 1
Date_ 20131220
Time_ 15.02
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG deptsp135
TD 32768
SOLVENT MeOD
NS 1913
DS 2
SWH 30303.031 Hz
FIDRES 0.924775 Hz
AQ 0.5407385 sec
RG 32768
DW 16.500 usec
DE 6.50 usec
TE 298.6 K
CNST2 145.0000000
D1 1.50000000 sec
D2 0.00344828 sec
D12 0.00002000 sec
TD0 6

===== CHANNEL f1 =====
NUC1 13C
P1 12.70 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -1.81 dB
PL0W 0.00000000 W
PL1W 81.92915344 W
SFO1 150.8927518 MHz
SP2 4.19 dB
SPNAM2 Crp60comp.4
SPOAL2 0.500
SPOFFS2 0.00 Hz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
P3 8.00 usec
P4 16.00 usec
PCPD2 80.00 usec
PL2 3.31 dB
PL12 23.31 dB
PL2W 6.79873323 W
PL12W 0.06798734 W
SFO2 600.0330002 MHz
SI 16384
SF 150.8774509 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

NAILA RAZIQ/DR.SHAIQ/RM-01
deptsp 90

—137.018
—131.195
—126.168

—101.186

78.349
78.189
78.042
77.981
74.941
74.753
73.863
71.705
64.410
62.837
56.870
49.565
49.429
49.283
49.137
49.001
48.856
48.717
48.459
28.015
27.420

NAME dec19-13
EXPNO 8
PROCNO 1
Date_ 20131220
Time_ 16.07
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG deptsp90
TD 32768
SOLVENT MeOD
NS 1009
DS 2
SWH 30303.031 Hz
FIDRES 0.924775 Hz
AQ 0.5407385 sec
RG 32768
DW 16.500 usec
DE 6.50 usec
TE 298.5 K
CNST2 145.0000000
D1 1.50000000 sec
D2 0.00344828 sec
D12 0.00002000 sec
TD0 4

===== CHANNEL f1 =====
NUC1 13C
P1 12.70 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -1.81 dB
PL0W 0.00000000 W
PL1W 81.92915344 W
SFO1 150.8927518 MHz
SP2 4.19 dB
SPNAM2 Crp60comp.4
SPOAL2 0.500
SPOFFS2 0.00 Hz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
P3 8.00 usec
P4 16.00 usec
PCPD2 80.00 usec
PL2 3.31 dB
PL12 23.31 dB
PL2W 6.79873323 W
PL12W 0.06798734 W
SFO2 600.0330002 MHz
SI 16384
SF 150.8774509 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

NAILA RAZIQ/DR.SHAIQ/RM-01
hsqc

NAILA RAZIQ/DR.SHAIQ/RM-01
hmbc

NAME dec19-13
EXPNO 5
PROCNO 1
Date_ 20131220
Time_ 1.41
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG hmbcgp1pndqf
TD 2048
SOLVENT MeOD
NS 32
DS 16
SWH 4194.631 Hz
FIDRES 2.048160 Hz
AQ 0.2442908 sec
RG 41285.1
DW 119.200 usec
DE 6.50 usec
TE 297.9 K
CNST2 145.0000000
CNST13 13.0000000
D0 0.00000300 sec
D1 2.00000000 sec
D2 0.00344828 sec
D6 0.03846154 sec
D16 0.00015000 sec
IN0 0.00001440 sec
===== CHANNEL f1 =====
NUC1 1H
P1 8.00 usec
P2 16.00 usec
PL1 3.31 dB
PL1W 6.79873323 W
SFO1 600.0321001 MHz
===== CHANNEL f2 =====
NUC2 13C
P3 11.50 usec
PL2 -1.81 dB
PL2W 81.92915344 W
SFO2 150.8950149 MHz
===== GRADIENT CHANNEL =====
GPNAM1 SINE.100
GPNAM2 SINE.100
GPNAM3 SINE.100
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 2000.00 usec
ND0 2
TD 256
SFO1 150.895 MHz
FIDRES 135.569733 Hz
SW 230.000 ppm
PnMODE OF

NAILA RAZIQ/DR.SHAIQ/RM-01
cosy

NAME dec19-13
EXPNO 2
PROCNO 1
Date_ 20131219
Time_ 16.16
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG cosydfqf
TD 2048
SOLVENT MeOD
NS 16
DS 4
SWH 4194.631 Hz
FIDRES 2.048160 Hz
AQ 0.2442908 sec
RG 14.3
DW 119.200 usec
DE 6.50 usec
TE 298.1 K
D0 0.00000300 sec
D1 1.50000000 sec
D13 0.00000400 sec
D20 0.00000200 sec
IN0 0.00023840 sec

===== CHANNEL f1 =====
NUC1 1H
P1 8.00 usec
PL1 3.31 dB
PL1W 6.79873323 W
SFO1 600.0321001 MHz
ND0 1
TD 128
SFO1 600.0321 MHz
FIDRES 32.770554 Hz
SW 6.991 ppm
FnMODE QF
SI 1024
SF 600.0300172 MHz
WDW QSINE
SSB 0
LB 0.00 Hz
GB 0
PC 4.00
SI 1024
MC2 QF
SF 600.0300172 MHz
WDW QSINE
SSB 0
LB 0.00 Hz
GB 0

NAILA RAZIQ/DR.SHAIQ/RM-01
noesy

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NAME      dec19-13
EXPNO     3
PROCNO    1
Date_     20131219
Time      17.17
INSTRUM   spect
PROBHD    5 mm CPTCI 1H-
PULPROG   noesygpph
TD        2048
SOLVENT   MeOD
NS        16
DS        4
SWH       4194.631 Hz
FIDRES    2.048160 Hz
AQ        0.2442908 sec
RG        25.4
DW        119.200 usec
DE        6.50 usec
TE        298.1 K
D0        0.00010901 sec
D1        2.00000000 sec
D8        0.80000001 sec
D16       0.00020000 sec
IN0       0.00023840 sec

===== CHANNEL f1 =====
NUC1      1H
P1        8.00 usec
P2        16.00 usec
PL1       3.31 dB
PL1W      6.79873323 W
SFO1      600.0321001 MHz

===== GRADIENT CHANNEL =====
GPNAM1    SINE.100
GPNAM2    SINE.100
GPZ1      40.00 %
GPZ2      -40.00 %
P16       1000.00 usec
ND0       1
TD        256
SFO1      600.0321 MHz
FIDRES    16.385277 Hz
SW        6.991 ppm
FrMODE    States-TPPI
SI        1024
SF        600.0300172 MHz
WDW       SINE
SSB       2
LB        0.00 Hz
GB        0
PC        4.00
SI        512
MC2       States-TPPI
SF        600.0300172 MHz
WDW       SINE
SSB       2
LB        0.00 Hz
GB        0
  
```

File: RM-01
Sample: NAILA RAZIA/DR. SHAIQ
Instrument: JEOL JMS 600-H
Inlet: My Inlet

Date Run: 12-05-2013 (Time Run: 15:57:09)

Ionization mode: EI+

Scan: 18

R.T.: 1.52

Base: m/z 192; 99.9%FS TIC: 23628418

#Ions: 283