Antiviral activity and Chemical Constituent of *Cressacretica* L.

Abdelaaty A. Shahat[1,2], Mansour S. Alsaid[1], Rasmeia A. Hassan[2] and EssamEzzeldin[3]

[1]Pharmacognosy Department, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Kingdom of Saudi Arabia
[2]Phytochemistry Department, National Research Centre, 12311 Dokki, Cairo, Egypt.
[3]Drug Bioavailability Laboratory, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Kingdom of Saudi Arabia
aashahat@hotmail.com

Abstract: Different compounds i.e. syringaresinolglucoside as well as flavonoid compounds were isolated from the CH_2Cl_2/MeOH extract of the aerial parts of *Cressacretica* L. (Convolvulaceae). Different fractions containing these compounds gave different activities against Herpes simplex virus type1 (*HSV-1*), Poliomyelitis virus type 1 (*Polio 1*) and Vescular stomatitis virus (*VSV*). The isolated compounds were identified by spectroscopic methods including UV, ^1H and ^{13}C -NMR, and EI- or FAB-Mass spectroscopy and compared with the data published before
[Abdelaaty A. Shahat, Mansour S. AlsaidRasmeia A. Hassan, Essam E. Hassan. **Antiviral activity and Chemical Constituent and of *Cressacretica*** L Life Sci J 2013; 10(4): 2193-2196]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 292

Keywords: *Cressacretica* L. (Convolvulaceae), chemical constituents, antiviral activity.

1. Introduction

Cressacretica L. belongs to the Convolvulaceae family, the Arabic name is Molleh or Nadwa (Tackholm, 1974). *C. cretica* has been reported to have many traditional uses, dry leaves of *C. cretica* crushed with sugar are used as emetic in Sudan (Hocking, 1997). The plant is used as alterative, anthelmintic, stomachic, tonic and aphrodisiac purposes, enriches the blood and is useful in constipation, asthma and urinary discharges (Satakopan and Karandikar, 1961; Rizk and El-Ghazaly, 1995). The plant is traditionally used in Bahrain as expectorant and antibilious agent (Rizk and El-Ghazaly, 1995). Little has been reported on the chemical constituents of *Cressacretica*. Phytochemical constituents of *C. critica* led to the isolation of flavonoid compounds and Syringaresinolglucoside (Shaht et al., 2004, 2005) Phytochemical screening of the plant growing in Qatar revealed the presence of alkaloids, coumarins and sterols (Rizk, 1982). The present study represents a part of our continuous research for plant derived antiviral activity. The CH_2Cl_2/MeOH extract and different fractions from the air dried of the aerial parts of *C. cretica* were investigated for their chemical constituents and antiviral activity

2. Experimental

2.1. Plant materials

The aerial part of *Cressacretica* L. was collected at Helwan, South Cairo, Egypt.The plants were kindly authenticated by Prof. Dr. Ibrahim El-Garf, Department of Botany, Faculty of Science, Cairo University. Voucher samples are deposited at the Herbarium of the National Research Centre (NRC), Cairo, Egypt

2.2. Materials and techniques

2.2.1. Analytical method:

- Thin layer chromatography (TLC): Silica gel 60 F_{254} (plastic sheets 20x20) (precoated with layer of 0.2mm) (Merck).
- Column chromatography (CC):Silica gel 60 (230-400 mesh) was purchased from Merck (Darmstadt, Germany) and Sephadex-LH20 was bought at Amersham Pharmacia Biotech AB (Uppsala, Sweden)

2.2.2. Solvent system:
-Hexane-EtOAc-MeOH-H_20 (3:7:5:5) (A)
-EtOAcHOAc HCOOH H_2O (30:0.8:1.2:8 (B)

2.3. Detection:

-Spray reagent (a): vanillin H_2SO_4 [Mixture of 1% vanillin in methanol and 5% H_2SO_4 in EtOH],
-Spray reagent (b): Diphenyl-boric acid-ethanolamine complex (Naturstoff-reagenz A, NA Reagent) 1% solution in MeOH

2.4. Spectroscopy

2.4.1. FAB-MS spectral analysis in negative or positive mode was performed on a VG 70-SEQ Hybrid Mass Spectrometer. EI-MS were recorded on a Fisons VG 70-SEQ instrument by means of direct insertion probe ionization energy of 70 ev.

2.4.2. H-and ^{13}C-NMR spectra were recorded in CD_3OD, $CDCl_3$ or DMSO-d_6 on a Bruker DRX-400 instrument. The chemical shifts were reported in δ values (ppm) with TMS as the internal standard.

2.4.3. UV detection at 254 nm (short wavelength) and 366 nm (long wavelength)

2.5. Micro-organism

The selected viruses for testing were Herpes simplex virus type 1 (*HSV-1*), Poliomyelitis (*Polio.1*), and Vesicular stomatitis virus (*VSV*). Herpes simplex is a worthy representative of the DNA viruses, whereas Poliomyelitis and Vesicular stomatitis viruses are good prototypes of the RNA virus group (VandenBerghe et al., 1986).

2.6. Isolation and identification

The air dried, powdered whole plant material of *C. cretica* (1kg) was defated with n-hexane (CH) and then extracted subsequently with $CHCl_3$ (CC), CH_2Cl_2/MeOH (1:1) (CM) and water (CW). The CM extract was evaporated under reduced pressure and dissolved in 20% aqueous methanol. The aqueous methanolic solution was partitioned first against $CHCl_3$ (CMC), EtOAc (CME) and then against n-BuOH (CMB).

The CMC fraction was subjected to column chromatography (Silica gel 60, Merck). Elution of the column was initiated with n-hexane followed by EtOAc then EtOAc/MeOH mixtures with increasing the polarity to give 4 fractions (CMC1-CMC4). Throughout the isolation procedure fractions were collected and combined after monitoring by TLC using solvent (A). The spots were detected in UV light (254 and 366 nm) before and after spraying with reagent (a). From fraction CMC2, compound 1 was isolated. Repeated column chromatography of fraction CMC3 yielded compound 2.

The EtOAc fraction was subjected to CC on Sephadex LH-20 using propanol with increasing amount (10%) of MeOH, 4 fractions (CME1-CME4) were combined after monitoring with TLC. Repeated column chromatography of fraction CME2 and MCE2 using silica gel afforded compounds 3-5 and 6-7 respectively. Sephadex LH-20 and MeOH were used for purified these compounds. The structures were elucidated by comparison of their TLC, FAB-MS, UV and NMR spectral data with that of the authentic samples.

Compound 2 (Syringaresinolglucoside)

Compound 2 appears as dark under UV light at 254nm change to reddish brown after spraying with reagent (a) and heating.

EI-Ms spectroscopy exhibited a molecular ion at m/z 418 (molecular weight of the aglycone)

^{1}H–NMR (CD_3OD, 400 MHz): δ 6.72 (2H, s, H-2 and H-6), 4.77 (1H, d, J= 4.6 Hz, H-7), 3.14 (1H, m, H-8), 3.91/ 4.28 (1H, m, H-9), 6.65 (2H, s, H-2', H-6') 4.72 (1H, d, J=4.5 Hz, H-7'), 3.14 (1H, m, H-8'), 3.91/4.28 (2 H, m, H-9'), 3.86 (6H, s, 2x -OCH3), 3.84 (6H, s, 2x -OCH3) 4.85 (1H, d, J =7.5 Hz, H-1''), 3.47 (1H, m, H-2''), 3.40 (2H, m, H-3'' and H-4''), 3.20 (1H, m, H-5''), 3.76 (1H, m, H-6''), 3.65 (1H, dd, J=12.0 Hz, 5.2Hz, H-6'').

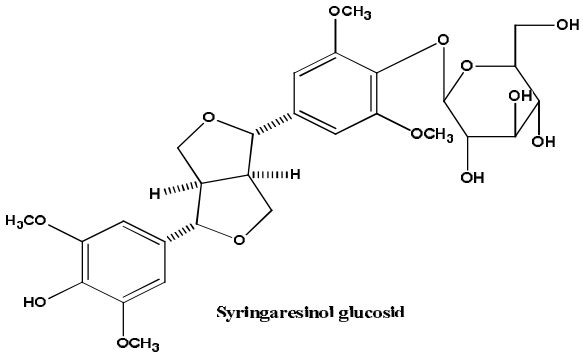

Figure 1: Chemical structure of Syringaresinolglucosid

Table 1: ^{13}C- NMR (CD_3OD, 100 MHz) assignments for compound 2

C No.	δ	CNo.	δ
1	139.62	5'	149.44
2	104.96	6'	104.66
3	154.49	7'	87.65
4	135.72	8'	55.78[a]
5	154.49	9'	72.99[b]
6	104.96	glu 1'''	105.43
7	87.26	2	75.43
8	55.57[A]	3	77.90
9	72.93[B]	4	71.43
1'	133.17	5	78.40
2'	104.66	6	62.67
3'	149.44	2x-OCH_3	56.90[c]
4'	136.35		57.16[c]

[a, b,c]Assignments bearing the same superscript may beReversed

2.7. Antiviral testing

The in vitro antiviral screening method estimated the inhibition of the cytopathic effects (CPE) of the extract and the fractions on a VERO cell monolayer infected with Herpes simplex virus using the end-point titration technique (EPTT) (Vanden Berghe et al., 1986). Confluent monolayers of VERO cells were grown in 96-well microtiter plates, which were infected with serial tenfold dilutions of a virus suspension. The virus was allowed to adsorb for 60 min at 37 OC, after which serial twofold dilutions of plant extracts or test compounds in maintenance medium, supplemented with 2% serum and antibiotics, were added. The plates were incubated at 37 OC and the viral cytopathic effect was recorded by light microscopy after 3 to 8 days. (Virus suspension are characterised by their virus titers, which are expressed as the smallest amount of virus

capable of producing a reaction in the host cells. The antiviral activity is expressed as a reduction factor (Rf), being the ratio of the viral titers in the virus control and in the presence of the maximal non-toxic dose of test substance.

Table 2: Antiviral activity of the different extract of *C. cretica*

Fractions	Herpes simplex virus (*HSV-1*)	
	[a]MNTD	[b]Rf_{MNTD}
CMC1	20	Rf 10
CMC2	20	Rf 10^2
CMC3	20	Rf 10^4
CMC4	20	Rf10
CME1	1000	Rf1
CME2	500	Rf 10^4
CME3	100	Rf 10
CME4	1000	Rf 1

[a]: MNTD = maximal non toxic dose (in µg/ml)
[b]: Rf = titer reduction factor = the ratio between virus titer of control and sample dilution

Table 3: Antiviral activity of sub-fractions isolated from *C. cretica*

Extracts	*HSV-1*		*Polio.1*		*VSV*	
	[a]MNTD	[b]Rf_{MNTD}	[a]MNTD	[b]Rf_{MNTD}	[a]MNTD	[b]Rf_{MNTD}
Hexane (CH)	100-10	Rf 1	500	Rf 1	500-10	Rf 10
Chloroform (CC)	500	Rf 10	500	Rf 10	500	Rf 10
CH_2Cl_2/MeOH(CM)	1000 500	Rf 10^4 Rf 10^2	1000-10	Rf 1	1000-10	Rf 1
Water (CW)	1000-10	Rf1	1000-10	Rf 10	1000-10	Rf 1

[a]: MNTD = maximal nontoxic dose (in µg/ml)**[b]:** Rf = titer reduction factor = the ratio between virus titer of control and sample dilution.

3. Results and Discussion

No previous antiviral studies of *Cressacretica* have been reported. The CH_2Cl_2/MeOH extract of the whole plant of *C. cretica* which had the most activity against herpes simplex virus (*HSV-1*) was repeatedly separated by silica gel to afford scopoletin 1, Syringaresinolglucoside 2 and known flavonoid vice quercetin 3, quercetin-3-O-glucoside 4, kampferol-3-O-glucoside 5, kampferol-3-O-rhamnoglucoside 6 and rutin 7. Although these compounds have been isolated from different plants but they have not been reported previously from this species, except scopoletin and quercetin. The structures of these compounds were elucidated by co-TLC with authentic samples available in the Lab. and by comparison of their spectral data with those reported in the literature (Markham, 1982; Shahat et al, 2004, 2005).

The ^{1}H-NMR indicated that compound 2 was a monoglycosylatedtetrahydrofuranlignan. Each aromatic moiety appeared to be symmetrically substituted with a 3,5-dimethoxy, 4-hydroxy substitution pattern, one of the hydroxyl being glycosylated. 1H- and ^{13}C-NMR chemical shifts were in good agreement with that reported (in DMSO-D_6) for syringaresinol-β-D glucoside (Kobayashi et al., 1985; Kinjo et al., 1991); (+)- As well as (-)-syringaresinolglucoside has been described. ^{1}H and ^{13}C-NMR do not allow discriminating between both diesterio-isomers. ^{1}H and ^{13}C-NMR assignments (see experimental) are based on published data, and on a series of 2D NMR analyses, including HSQC and HMBC (Shahat et al., 2004). These 2D NMR analyses learned that some of the published ^{13}C-NMR assignment had to be reversed. Indeed, in a symmetrical syringaresinol-diglucoside, the chemical shift of C-1/ C-1' was reported as 134.1 ((+)- isomer) and 133.8 ((-) –isomer), whereas C-4/ C-4' resonated at 137.1 ((+) –isomer) and 137.2 ((-)-isomer). Hence, in the monoglucosidic analogues, C-1 and C-4 of the glycosylated moiety showed a ^{13}C NMR chemical shift of 134.1 and 137.2 respectively, for the (+)-isomer, and 131.2 and 134.7, respectively, for the (-)-isomer. However, 2D NMR experiments, recorded in CD_3OD showed unambiguously that the most downfield resonance line at 139.62 ppm had to be

assigned to C-1 of the glycosylated aromatic moiety, and not to C-4, as expected from literature data. Also in 13C NMR spectroscopy of the flavonoids it has been observed that glycosylation of phenolic groups cause relatively large chemical shift differences in the para-position. Most probably many published ^{13}C-NMR assignments for C-1 and C-4 in the glycosylated aromatic moieties of mono- and di-glycosylated syringaresinol and related compounds have to be reversed.

Copmunds 1 and 3-7 showed chromatographic, UV absorption NMR and hydrolytic data identical with reported for scopoletin, quercetin, quercetin-3-O-glucoside, kampferol-3-O-glucoside, kampferol-3-O-rhamnoglucoside, and rutin.

Antiviral activity of the CH_2Cl_2/MeOH crude extract of the whole plant showed good activity against Herpes simplex 1 (HSV-1), the water extract was not active (Table 2). The sub-fractions CMC2, CMC3 and CME2 of the subsequent $CHCl_3$ (CMC) and EtOAc (CME) fractions were found to have good activities, the sub-fraction CME3 was weakly active (Table 3).

Acknowledgement

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no RGP- VPP -262. The author is grateful to the Pharmaceutical Sciences Department, University of Antwerp, Belgium.

Corresponding Author
Abdelaaty A. Shahat
[1]Pharmacognosy Department, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Kingdom of Saudi Arabia
[2]Phytochemistry Department, National Research Centre, 12311 Dokki, Cairo, Egypt.
aashahat@hotmail.com

10/21/2013

References

1. Barbera, O., Marco, J. A., Sanz, J. F., Sanchez-Parareda, J. (1986) Phytochemistry 25 (10), 2357-2360
2. George Macdonald Hocking, (1997), A Dictionary of Natural Product Plexas Publishing U. S. A
3. Kobayashi, H., Karasawa, H., Miyase T., and Fukushma S., (1985), Chem. Phar. Bull. 33 (4) 1452-1457
4. Kinjo, J., Fukui, K., Higuchi H., and Nohara T. (1991) Chem. Phar. Bull. 39 (6) 1623-1625
5. Markham,K. R. in Methods in Plant Biochemistry, Vol. 1, Plant Phenolics, J. B. Harborne, Ed., Academic Press, London (1989), Chapt. 6.
6. Rizk , A. M. (1982) Fitoterapia, 52, 35-44
7. Rizk, A. M. El-Ghazaly, G. A. (1995) Medicinal and Poisonous Plants of Qatar, Scientific and Applied Research Centre, University of Qatar.
8. Satakopan, S. Karandikar, G.K. (1961) J. Sci. Ind. Res. Section C, 20, 156-160
9. Shahat A. Abdelaaty, Nazif M. Naglaa, Abdel-Azim S. Nahla, Pieters Luc and Vlietinck J. A., (2005). Flavonoids from *Cressacretica*. Pharmaceutical Biology 4-5, pp.349-352
10. Shahat, A. A., Abdel-Azim, N. S., Pieters L. and Vlietinck, A. J. (2004).Isolation and Complete NMR Assignment of Syringaresinol -β- D glucoside from *Cressacretica* L. (Convolvulaceae). Fitoterapia 771-773.
11. Tackholm, V. (1974) Students Flora of Egypt, second edition,
12. VandenBerghe, D. A., Vlietinck, A. J., Van Hoof, L. (1986) Bull. Inst. Pasteur, 84, 101-147.
13. Vermes, B., Seligmann O., and Wagner H. (1991) Synthesis of biologically active tetrahydro-furofuranlignan-(Syringin, Pinoresinol)-mono-and bis-glucosides, phytochemistry 30, 3089-89