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Short Communication

Dicaffeoylquinic Acids from the Aerial Parts of *Artemisia ciniformis* Krasch. & Popov ex Poljakov

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ABSTRACT

Background: *Artemisia ciniformis* (*A. ciniformis*) belongs to the genus *Artemisia* and grows at northeast of Iran. The current phytochemical study was carried out on the most potent extract in cell-free antioxidant assays.
Methods: Hydroethanolic extract of the aerial parts was fractionated using vacuum-liquid chromatography (VLC). The selected fraction from the previous cell-free antioxidant study was purified by semi-preparative HPLC. The structures of isolated compounds were identified using one- and two-dimensional NMR and ESIMS techniques.
Results: Three identified compounds in this study were the known isomers of dicaffeoylquinic acid (DCQ), including 3,5- DCQ (isochlorogenic acid A), 3,4-DCQ (isochlorogenic acid B) and 4,5-DCQ (isochlorogenic acid C).
Conclusion: The outstanding free radical scavenging potential in the hydroethanolic extract of *A. ciniformis* might be partly related to the presence of isochlorogenic acid derivatives.

Introduction

Artemisia as a genus belonging to the family Asteraceae, has been used in Iranian traditional medicine because of various attributed effects like antispasmodic, vermifuge and emmenagogue[1] as well as hemostatic and laxative activities.[2] The genus is a rich source of plant secondary metabolites, including terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes.[3] *Artemisia ciniformis* Krasch. & Popov ex Poljakov is one of wildly growing species in Iran.[4] Dichloromethane and ethyl acetate extracts of *A. ciniformis* have been effective against AGS, MCF-7, and HeLa cell lines in cytotoxicity assay.[5,6] In another study on various extracts of *A. ciniformis*, dichloromethane and petroleum ether extracts exhibited the highest toxicity against HL-60 and K562 cell lines, respectively.[7] Ethanolic extract has shown leishmanicidal activity against promastigotic forms of *Leishmania major*.[8] Potential antimalarial activities of different extracts from *Artemisia* species were evaluated and the highest effect was observed in *A. ciniformis* dichloromethane extract.[9] The volatile oil of this species has shown antimicrobial and cytotoxic activity against *Acinetobacter baumannii* and HeLa cell line, respectively.[10] Another study suggested that the leaf volatile oil of *A. ciniformis* can be regarded as a bioactive source of antioxidant and antimicrobial agents.[11] Pretreatment of H9c2 cell line with ethyl acetate, ethanolic and hydroethanolic extracts of this species resulted in a significant increase in cell viability after exposure to hydrogen peroxide.[12] In another research, antioxidant capacity was evaluated in five extracts of *A. ciniformis* aerial parts via cell-free methods. Then, seven fractions were prepared from the most potent extract and their antioxidant capacities were evaluated by both cell-free and cell-based methods (in PC12 cell line). The results of cell-free methods indicated that the hydroethanolic extract and particularly one of its fractions (eluted by 60% methanol in water in a reversed phase vacuum liquid chromatography (VLC) system), possessed significant antioxidant activity and total phenolic contents.[13]

In spite of phytochemical studies conducted on the volatile oil of this species and reports on the presence of some mono and sesquiterpenes[14,15], no study has so far tried to isolate and identify polar secondary metabolites. The present research reports the presence of three known isochlorogenic acid isomers (1-3) in *A. ciniformis* aerial parts.

Materials and Methods

Reagents and chemicals

All the solvents used for purification procedures were of gradient grade and purchased from Scharlau (Spain) and Caledon (Canada).

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General experimental procedures

The chromatographic system for semi-preparative HPLC consisted of a binary pump YL 9111S, a PDA detector YL9160 and a VertiSep UPS C18 (250 × 30 mm i. d., 10 µm) column. An Ascentis ® (250 × 10 mm i. d., 5 µm) column was replaced for final purification. NMR spectra were recorded on a Bruker AVANCE III 500 MHz spectrometer in dimethyl sulfoxide-d_6 as the solvent and residual solvent signal used as internal standard. ESIMS data were obtained on an Esquire 3000 plus ion trap mass spectrometer (Bruker).

Plant materials

Aerial parts of *A. ciniformis* were collected from the Tandoreh National Park in Khorasan Razavi province in September 2011 and identified by Dr. Valiollah Mozaffarian (Iranian Research Institute of Forests and Rangelands). A voucher specimen (No. 12569) is preserved at the herbarium, Faculty of Pharmacy, Mashhad University of Medical Sciences, Iran.

Extraction and fractionation

In this study, the fraction residues obtained in the previous study[13] were used. Briefly, hydroethanolic extract was obtained by maceration after successive extraction of dried and ground aerial parts with petroleum ether (40-60), dichloromethane, ethyl acetate, ethanol, and equal mixture of water-ethanol. The fractionation was carried out by reversed phase VLC method with rising ratios of methanol in water as a mobile phase.

Isolation procedure

300 mg of fraction E (eluted by 60% methanol in water in a reversed phase VLC system and was previously reported as the most potent fraction in cell-free antioxidant assays[13]) was re-fractionated by semi-preparative HPLC (mobile phase: 0–50 min, MeOH from 20 to 70% in H2O; 50 –51 min MeOH from 70 to 100% in H_2O; 51–56 min 100% MeOH, flow rate 8 ml/min) to yield 10 subfractions. Further purification of the subfraction 3 (10.5 mg, *t*R = 25.2 min) by semi-preparative HPLC (mobile phase: 0–30 min, MeOH from 10 to 100% in H_2O; 30–35 min 100% MeOH, flow rate 3 ml/min) yielded compound **1** (7.1 mg, *t*R = 14.0 min). Further purification of the subfraction 4 (16.0 mg, *t*R = 29.0 min) by semi-preparative HPLC (mobile phase: 0–20 min, ACN from 10 to 28% in H_2O; 20 –24 min, 28% ACN in H_2O; 24 –26 min ACN from 28 to 100% in H_2O; 26–33 min 100% ACN, flow rate 3 ml/min) yielded compounds **2** (5.8 mg, *t*R = 14.2 min) and **3** (7.8 mg, *t*R = 15.3 min). The structures of isolated compounds were elucidated by means of spectroscopic analysis including ESIMS, ^{1}H- and 2D-NMR.

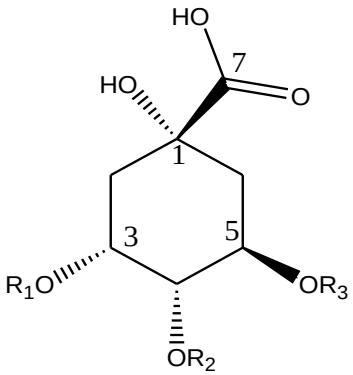
Results

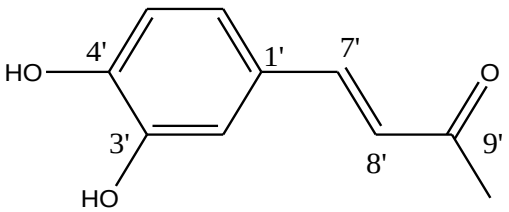
This study led to the isolation of three compounds (isochlorgenic acids A, B and C) from hydroethanolic extract of *A. ciniformis* aerial parts (Figure 1).

The structural identification of the compounds

The chemical structures of isolated compounds were elucidated unequivocally through ESIMS and NMR and also all spectroscopic data were in agreement with respective published data.[16-19]

Compound 1 (3, 5- dicaffeoylquinic acid) : brown powder. ESI-MS (m/z): 515.2 $[M-H]^-$, 1031.5 $[2M-H]^-$. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.99- 2.15 (4H, m, H-2 and H-6), 3.85 (1H, m, H-4), 5.18 (1H, m, H-5), 5.23 (1H, m, H-3), 6.18 (1H, d, *J* = 16.0 Hz, H-8”); 6.25 (1H, d, *J* = 16.0 Hz, H-8’); 6.78 (1H, overlapping signals (ov), H-5”), 6.79 (1H, ov, H-5’), 6.98 (1H, ov, H-6”), 6.99 (1H, ov, H-6’),7.06 (1H, br s, H-2”), 7.07 (1H, br s, H-2’), 7.46 (1H, d, *J* = 16 Hz, H-7”), 7.49 (1H, d, *J* = 16 Hz, H-7’); ^{13}C-NMR (data from HSQC and HMBC spectra, DMSO-d6) δ (ppm): 35.5 (C-2), 37.0 (C-6), 68.5 (C-4), 71.5 (C-3 and C-5), 114.8 (C-8”), 115.0 (C-2’), 115.1 (C-2”), 115.2 (C-8’), 116.5 (C- 5’ and C-5″), 122.0 (C-6′ and C-6”), 125.6 (C-1’), 125.7 (C-1”), 145.0 (C-7′ and C-7″), 145.6 (C-3′ and C-3″), 148.3 (C-4’), 148.4 (C-4”), 165.0 (C-9”), 166.0 (C-9’), unobserved signals (C-1 and C-7).





R_1=R_3=Caffeoyl R_2=H Iscochlorogenic acid A

R_1=R_2=Caffeoyl R_3=H Iscochlorogenic acid B

R_2=R_3=Caffeoyl R_1=H Iscochlorogenic acid C

Caffeoyl moiety

Figure 1. Chemical structures of isolated compounds from the aerial parts of *A. ciniformis.*

Compound 2 (3, 4- dicaffeoylquinic acid) : brown powder. ESI-MS (m/z): 515.2 $[M-H]^-$, 1031.5 $[2M-H]^-$. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.00- 2.20 (4H, m, H-2 and H-6), 4.13 (1H, m, H-5), 4.98 (1H, m, H-4), 5.48 (1H, m, H-3), 6.21 (1H, d, *J* = 16.0 Hz, H-8'); 6.26 (1H, d, *J* = 16.0 Hz, H-8"); 6.77 (2H, d, *J* = 8 Hz, H-5' and H-5"), 6.94 (1H, br d, *J* = 8 Hz, H-6"), 6.96 (1H, br d, *J* = 8 Hz, H-6'),7.05 (1H, br s, H-2"), 7.06 (1H, br s, H-2'), 7.47 (1H, d, *J* = 16 Hz, H-7'), 7.49 (1H, d, *J* = 16 Hz, H-7");[13] C-NMR (data from HSQC and HMBC spectra, DMSO-d6) δ (ppm): 35.9 (C-2), 40.0 (C-6), 64.6 (C-5), 68.3 (C-3), 72.8 (C-1), 73.2 (C-4), 114.0 (C-8"), 114.2 (C-8'), 114.7 (C-2'), 114.8 (C-2"), 115.8 (C- 5' and C-5″), 121.4 (C-6'), 121.5 (C-6"), 125.4 (C-1'), 125.5 (C-1"), 145.2 (C-7′), 145.4 (C-7″), 145.6 (C-3′ and C-3″), 148.4 (C-4' and C-4"), 165.9 (C-9' and C-9"), unobserved signal (C-7).

Compound 3 (4, 5- dicaffeoylquinic acid): brown powder. ESI-MS (m/z): 515.2 $[M-H]^-$, 1031.5 $[2M-H]^-$. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.00- 2.20 (4H, m, H-2 and H-6), 4.27 (1H, m, H-3), 5.04 (1H, m, H-4), 5.48 (1H, m, H-5), 6.18 (1H, d, *J* = 16.0 Hz, H-8"); 6.26 (1H, d, *J* = 16.0 Hz, H-8'); 6.78 (2H, d, *J* = 7.5 Hz, H-5' and H-5"), 6.95 (2H, ov, H-6' and H-6"), 7.06 (2H, br s, H-2' and H-2"), 7.47 (1H, d, *J* = 16.0 Hz, H-7"), 7.53 (1H, d, *J* = 16.0 Hz, H-7'); ^{13}C-NMR (data from HSQC and HMBC spectra, DMSO-d6) δ (ppm): 37.1 (C-2), 37.5 (C-6), 66.3 (C-3), 67.7 (C-5), 73.3 (C-4), 73.6 (C-1), 113.6 (C-8"), 113.9 (C-8'), 114.9 (C-2' and C-2"), 115.9 (C- 5' and C-5″), 121.5 (C- 6' and C-6″), 125.5 (C-1' and C-1"), 145.6 (C-7′ and C-7″), 145.6 (C-3′ and C-3″), 148.5 (C-4' and C-4"), 165.6 (C-9"), 166.1 (C-9'), 174.8 (C-7).

Discussion

The semi-preparative HPLC of fraction E (eluted by 60% methanol in water in a reversed-phase VLC) resulted in the isolation of three dicaffeoylquinic acids. The structures of isolated compounds were elucidated by ESIMS, ^{1}H- and 2D-NMR.

All compounds exhibited the same pseudo-molecular-ion peak at m/z 515.2 ($[M-H]^-$), in their ESI-MS, representing the molecular formula $C_{25}H_{24}O_{12}$, which was consistent with the presence of two attached caffeoyl moieties on quinic acid.

The ^{1}H NMR spectrum of all compounds showed the presence of two caffeoyl groups. The large coupling constants (J = 16.0 Hz) indicated *trans*-geometries of the double bonds in caffeoyl moieties. The linkage positions of caffeoyl groups were determined on the basis of the observed HMBC correlations between H-atoms of quinic acid (H-3, H-4, and H-5) and C-9' or C-9". The signals of the H-atoms (H-3, H-4, and H-5) in compound 1 were observed between 3.85 and 5.23 ppm, while the same H-atoms in compound 2 resonated between 4.13 and 5.48 ppm. The signals of the intended H-atoms in compound 3 were observed between 4.27 and 5.48 ppm. The up field protons were H-4 in compound 1, H-5 in compound 2, and H-3 in compound 3. An up field shift was observed for alcoholic carbons (C-4 in compound 1, C-5 in compound 2 and C-3 in compound 3) while γ effect resulted in a downfield shift for C-6 and C-2 in compounds 2 and 3 (in comparison with corresponding carbon atoms in compound 1), respectively. These facts, along with the comparison of the rest of spectroscopic data with those reported in literature[16 - 19], allowed identification of 1, 2 and 3 as 3,5-di-O-caffeoylquinic acid (3,5-di-CQA), 3,4-di-O-caffeoylquinic acid (3,4-di-CQA) and 4,5-di-O-caffeoylquinic acid (4,5-di-CQA), respectively. There is no previous report on the presence of three DCQ acid isomers (isochlorogenic acids A-C) in *A. ciniformis*, but these phytochemicals have been proven to be present in some other species of the genus like *A. pectinata*, *A. selengensis* and *A. annua*.[20-22] The compounds have shown valuable effects such as inhibition of natural protein tyrosine phosphatase 1B,[23] α-Amylase and α-glucosidase,[24] aldose reductase,[25] potent activity against HBV DNA replication,[26] and antioxidant activity[21,27] in different species of *Artemisia*. 3,4-DCQ is known as a strong inhibitor of the peroxidation process.[28] In comparison with chlorogenic acid, isochlorogenic acid derivatives like 3,4-DCQ and 3,5-DCQ demonstrated significantly higher DPPH radical-scavenging and inhibitory activities against cholesteryl ester hydroperoxide formation in copper ion-induced oxidation of diluted rat blood plasma.[29] Scavenging actions of the isomers for superoxide anion have been reported stronger than that of ascorbic acid.[30]

In the present study, three isochlorogenic acid isomers were isolated and identified from the active fraction in cell-free antioxidant assays[13] which corroborated previous reports on the biological effects of the these compounds and their distribution in the genus *Artemisia*.

Conclusion

The antioxidant activities reported from *A. ciniformis* are probably associated with the presence of various DCQ isomers, which can affect the bioactivity potential of hydroethanolic extract from this species.

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Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Miraldi E, Ferri S, Mostaghimi V. Botanical drugs and preparations in the traditional medicine of West Azerbaijan (Iran). J Ethnopharmacol. 2001;75(2-3):77-87. doi:10.1016/s0378-8741(00)00381-0
2. Ghorbani A. Studies on pharmaceutical ethnobotany in the region of Turkmen Sahra, north of Iran. J Ethnopharmacol. 2005;102(1):58-68. doi:10.1016/j.jep.2005.05.035
3. Bora KS, Sharma A. The genus *Artemisia*: a comprehensive review. Pharm Biol. 2011;49(1):101-

9. doi:10.3109/13880209.2010.497815
4. Mozaffarian V. A dictionary of Iranian plant names: Latin, English, Persian. Farhang Mo'aser; 1996.
5. Taghizadeh Rabe SZ, Mahmoudi M, Ahi A, Emami SA. Antiproliferative effects of extracts from Iranian *Artemisia* species on cancer cell lines. Pharm Biol. 2011;49(9):962-9. doi:10.3109/13880209.2011.559251
6. Emami SA, Taghizadeh Rabe SZ, Ahi A, Mahmoudi M. Study on toxic effects of Artemisia spp. fractions from Iran on human cancer cell lines. J Zanjan Univ Med Sci. 2010;18(70):58-67.
7. Tayarani-Najaran Z, Hajian Z, Mojarrab M, Emami SA. Cytotoxic and apoptotic effects of extracts of *Artemisia ciniformis* Krasch.& Popov ex Poljakov on K562 and HL-60 cell lines. Asian Pac J Cancer Prev. 2014;15(17):7055-9. doi:10.7314/APJCP.2014.15.17.7055
8. Emami SA, Zamanai TaghizadehRabe S, Ahi A, Mahmoudi M. Inhibitory Activity of Eleven *Artemisia* Species from Iran against *Leishmania major* Parasites. Iran J Basic Med Sci. 2012;15(2):807-11.
9. Mojarrab M, Naderi R, Heshmati Afshar F. Screening of different extracts from *Artemisia* species for their potential antimalarial activity. Iran J Pharm Res. 2015;14(2):603-8.
10. Taherkhani M. Chemical Constituents, Antimicrobial, Cytotoxicity, Mutagenic and Antimutagenic Effects of *Artemisia ciniformis*. Iran J Pharm Res. 2016;15(3):471-81.
11. Taherkhani M. Chemical Investigation and Protective Effects of Bioactive Phytochemicals from *Artemisia ciniformis*. Iran J Chem Chem Eng. 2016;35(2):15-26.
12. Mojarrab M, Jamshidi M, Ahmadi F, Alizadeh E, Hosseinzadeh L. Extracts of *Artemisia ciniformis* Protect cytotoxicity induced by hydrogen peroxide in H9c2 cardiac muscle Cells through the Inhibition of Reactive Oxygen Species. Adv Pharmacol Sci. 2013;2013:1-5. doi:10.1155/2013/141683
13. Mojarrab M, Nasseri S, Hosseinzadeh L, Farahani F. Evaluation of antioxidant and cytoprotective activities of *Artemisia ciniformis* extracts on PC12 cells. Iran J Basic Med Sci. 2016;19(4):430-8.
14. Rustaiyan A, Masoudi S, Kazemi M. Volatile oils constituents from different parts of *Artemisia ciniformis* Krasch. et M. Pop. Ex Poljak and *Artemisia incana* (L.) Druce. from Iran. J. Essent. Oil Res. 2007;19(6):548-51. doi:10.1080/10412905.2007.9699328
15. Firouzni A, Vahedi H, Sabbaghi F, Bigdeli M. Composition of the essential oil of *Artemisia ciniformis*, *A. kopetdaghensis*, and *A. khorasanicain* Iran. Chem Nat Compd. 2008;44(6):804-6. doi:10.1007/s10600-009-9212-9216
16. Guo W, Wang L, Gao Y, Zhao B, Wang D, Duan W, et al. Isolation of isochlorogenic acid isomers in flower buds of *Lonicera japonica* by high-speed counter-current chromatography and preparative high performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2015;981:27-32. doi:10.1016/j.jchromb.2014.12.020
17. Wang Y, Liu B. Preparative isolation and purification of dicaffeoylquinic acids from the *Ainsliaea fragrans* champ by high- speed counter- current chromatography. Phytochem Anal. 2007;18(5):436-40. doi:10.1002/pca.999
18. Timmermann BN, Hoffmann JJ, Jolad SD, Schram KH, Klenck RE, Bates RB. Constituents of *Chrysothamnus paniculatus* 3: 3,4,5-tricaffeoylquinic acid (a new shikimateprearomatic) and 3,4-, 3,5- and 4,5-dicaffeoylquinic acids. J Nat Prod. 1983;46(3):365-8. doi:10.1021/np50027a012
19. Puangpraphant S, Berhow MA, Vermillion K, Potts G, Gonzalez de Mejia E. Dicaffeoylquinic acids in Yerba mate (*Ilex paraguariensis* St. Hilaire) inhibit NF- κB nucleus translocation in macrophages and induce apoptosis by activating caspases- 8 and- 3 in human colon cancer cells. Mol Nutr Food Res. 2011;55(10):1509-22. doi:10.1002/mnfr.201100128
20. Ma CM, Hattori M, Chen HB, Cai SQ, Daneshtalab M. Profiling the phenolic compounds of *Artemisia pectinata* by HPLC- PAD- MSn. Phytochem Anal. 2008;19(4):294-300. doi:10.1002/pca.1045
21. Zhang L, Tu ZC, Wang H, Wen QH, Fu ZF, Xie X. Antioxidant Activity and Phenolic Acids Profiles of *Artemisia selengensis* Turcz Extracted with Various Methods by HPLC- QTOF- MS/MS. J Food Biochem. 2016;40(4):603-12. doi:10.1111/jfbc.12255
22. Carbonara T, Pascale R, Argentieri MP, Papadia P, Fanizzi FP, Villanova L, et al. Phytochemical analysis of a herbal tea from *Artemisia annua* L. J Pharm Biomed Anal. 2012;62:79-86. doi:10.1016/j.jpba.2012.01.015
23. Zhang J, Sasaki T, Li W, Nagata K, Higai K, Feng F, et al. Identification of caffeoylquinic acid derivatives as natural protein tyrosine phosphatase 1B inhibitors from *Artemisia princeps*. Bioorg Med Chem Lett. 2018;28(7):1194-7. doi:10.1016/j.bmcl.2018.02.052
24. Olennikov DN, Chirikova NK, Kashchenko NI, Nikolaev VM, Kim SW, Vennos C. Bioactive Phenolics of the Genus *Artemisia* (Asteraceae): HPLC-DAD-ESI-TQ-MS/MS Profile of the Siberian Species and Their Inhibitory Potential Against α-Amylase and α-Glucosidase. Front Pharmacol. 2018;9:756. doi:10.3389/fphar.2018.00756
25. Jung HA, Islam MN, Kwon YS, Jin SE, Son YK, Park JJ, et al. Extraction and identification of three major aldose reductase inhibitors from *Artemisia montana*. Food Chem Toxicol. 2011;49(2):376-84. doi:10.1016/j.fct.2010.11.012
26. Zhao Y, Geng CA, Ma YB, Huang XY, Chen H, Cao TW, et al. UFLC/MS-IT-TOF guided isolation of anti-HBV active chlorogenic acid analogues from *Artemisia capillaris* as a traditional Chinese herb for the treatment of hepatitis. J Ethnopharmacol. 2014;156:147-54. doi:10.1016/j.jep.2014.08.043
27. Dahmani- Hamzaoui N, Salido S, Linares- Palomino PJ, Baaliouamer A, Altarejos J. On- Line Radical

Scavenging Detection and Characterization of Antioxidants from *Artemisia herba-* alba. Helv Chim Acta. 2012;95(4):564-76. doi:10.1002/hlca.201100367

28. Sroka Z, Cisowski W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem Toxicol. 2003;41(6):753-8. doi:10.1016/s0278-6915(02)00329-0
29. Kim JY, Cho JY, Ma YK, Park KY, Lee SH, Ham KS, et al. Dicaffeoylquinic acid derivatives and flavonoid glucosides from glasswort (Salicornia herbacea L.) and their antioxidative activity. Food Chem. 2011;125(1):55-62. doi:10.1016/j.foodchem.2010.08.035
30. Aniya Y, Koyama T, Miyagi C, Miyahira M, Inomata C, Kinoshita S, et al. Free radical scavenging and hepatoprotective actions of the medicinal herb, *Crassocephalum crepidioides* from the Okinawa Islands. Biol Pharm Bull. 2005;28(1):19-23. doi:10.1248/bpb.28.19