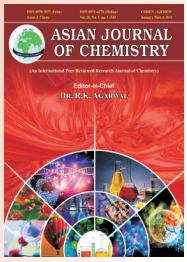
Asian Journal of Chemistry; Vol. 26, No. 11 (2014), 3435-3437

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17544



Chemical Constituents of *n*-Butanol Extract of *Capparis spinosa* L.

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Received: 24 February 2014; *Accepted*: 23 April 2014; *Published online*: 25 May 2014; AJC-15266

The aim of this study was to ascertain chemical constituents of *n*-butanol extract of *Capparis spinosa* L. fruits. The *n*-butanol extract of *Capparis spin*osa L. was effective at controlling S180 tumour growth in a murine model. Silica gel column chromatography, Sephadex LH-20 and re-crystallization methods were used to isolate and purify the *n*-butanol extract. Chemical structures were elucidated by spectroscopic analyses and compared with literature. As results, nine known compounds were isolated and identified as: *bis*-(5-fomylfurfuryl) ether (**1**), 5-(hydroxymethyl)furfural (**2**), uracil (**3**), adenosine (**4**), 2-hydroxy-6-methoxy-1*H*-indol-3-ethanone (**5**), rutin (**6**), 2-methyl-6-(1',2',3',4'-tetrahydroxybutyl)pyrazine (**7**), *p*-hydroxy-benzoic acid (**8**) and stachydrine (**9**). There in, the compound **7** was identified in *Capparis spinosa* L. for the first time.

Keywords: *Capparis spinosa* L., *n*-Butanol extract, Antitumor activity, Chemical constituents, Spectroscopic analyses.

INTRODUCTION

Capparis spinosa L. is a perennate shrub belonging to the family Capparidaceae. It is widely grown on dry regions in west or central Asia and Mediterranean basin[1]. In China, *Capparis spinosa* L. is mainly distributed in the Xinjiang Autonomy Region. Different parts of this plant, such as the flowers buds, fruits, seeds, shoots and bark of roots, have been used as food or folk medicines. *Capparis spinosa* L. has various pharmacological activities and is used in phytomedicine around the world for its antifungal ability[2], antiinflammatory[3] and antihepatotoxic effects[4], *etc*.

In our previous studies, the *n*-butanol extract of *Capparis spinosa* L. was confirmed for its antitumor effect against SGC-7901 and HepG-2 cells *in vitro* test. The present study was carried out to answer the question whether *n*-butanol extract of *Capparis spinosa* L. is also capable of inhibiting tumors *in vivo* or not. Evaluation was made in the sarcoma 180 mice. Then other doubt arises: which compound in *n*-butanol extract of *Capparis spinosa* L takes on the antitumor effect? So we carry a study on chemical constituents of *n*-butanol extract of *Capparis spinosa* L. fruits to find the answer.

EXPERIMENTAL

The dry mature fruits of *Capparis spinosa* L. were collected from Xinjinag Urumqi and identified by Prof. Q.H. Wang from institute for drug control of Hei longjiang, Harbin, China. Petroleum ether (60-90 ºC), chloroform, ethyl acetate, methanol, *n*-butanol, acetone and all chemicals and reagents used were analytically pure. NMR spectra in DMSO-d_6 were recorded on a Bruker-600MHz (600 MHz for ^{1}H; 100 MHz, ^{13}C). Mass spectra were obtained in a MATLCQ electro-spray mass spectrometry. Analytically pure solvents were used in all experiments.

Extraction: The fruits of *Capparis spinosa* L. were extracted with ethanol (95 %).The extracts were concentrated under vacuum. Then the resulting residue was dissolved in water and extracted with petroleum ether (60-90 ºC), ethyl acetate, *n*-butanol, respectively[5].

Assay of antitumor activity *in vivo*: Sarcoma 180 ascites tumor cells (1×10^7 cell/mL) were implanted subcutaneously into the right anterior armpit of the mice. After 24 h inoculation, *n*-butanol extract of *Capparis spinosa* L.was dissolved in physiological saline and administered by gavage for 7 d in mice transplanted with Sarcoma 180 tumor. Cyclophosphamide was used as a positive control. The negative control was administrated by gavage with physiological saline. On day 8, the mice were sacrificed. Tumors were extirpated and weighed. The inhibition ratio (%) was calculated by the following formula: inhibition ratio (%) = $[(A-B)/A] \times 100$, where A is the tumor weight average of the negative control and B is that of the treated group. Data are presented as mean

± SD. The differences between experimental groups were compared by ANOVA followed by least significant difference (LSD) test. Differences are considered significant when $p \leq 0.05$.

Isolation: The dried fruits of *Capparis spinosa* L (10 kg) were extracted with 95 % EtOH. Evaporation of the solvent under reduced pressure gave the dry extract, which was partitioned successively with light petroleum, ethyl acetate and *n*-butanol. The *n*-butanol extract (108 g) was then subjected to further separation using repeated normal-phase silica gel column, Sephadex LH-20, ODS silica gel and HPLC to give nine compounds (Compound 1-9).

RESULTS AND DISCUSSION

Antitumor activity *in vivo* of *n*-butanol extract of *Capparis spinosa* L.: The effect of *n*-butanol extract of *Capparis spinosa* L. on the antitumor activity in sarcoma 180 bearing rats is shown in Table-1. The mean tumor weights at high, middle and low dose in the treated groups were compared to those in the untreated control. *n*-butanol extract of *Capparis spinosa* L. treated tumors were significantly smaller compared to control tumors on 200, 400, 800 mg kg^{-1} d^{-1}. It was demonstrated that *n*-butanol extract of *Capparis spinosa* L. could significantly restrict further growth of Sarcoma 180.

TABLE-1
INHIBITION EFFECTS OF *n*-BUTANOL EXTRACT OF *Capparis spinosa* L. ON S180 TUMOR IN MICE ($\bar{x}$ ± SD)

Groups	Dose (mg kg^{-1} d^{-1})	N	Tumor weight (g)	Inhibition rate (%)
Control	–	12	1.121±0.158	–
n-Butanol extract of *Capparis spinosa* L.	200	12	0.906±0.131**	19.16
	400	12	0.754±0.116**	32.76
	800	12	0.604±0.107**	46.14
CTX	20	12	0.493±0.092**	55.17

**Compared with control $p < 0.001$.

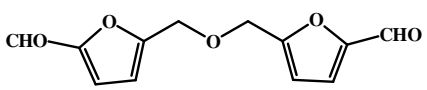
Identification of compounds of *n*-butanol extract of *Capparis spinosa* L.: As a result, 9 compounds have been isolated and identified from *n*-butanol extract of *Capparis spinosa* L. and described as following (Fig. 1):

Compound 1: *bis*-(5-Fomylfurfuryl) ether[6], colourless needles, melting point: 113.5-115.5 ºC. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.57 (2H, s), 6.73 (2H, d, J = 3.5, H-3, 3'), 7.50 (2H, d, J = 3.5, H-4,4'), 4.57 (2H, s, H-6,6'). ^{13}C NMR (100 MHz, DMSO) δ: 178.5 (CHO), 157.9(C, C-2, C-2'), 153.4(C, C-5, C-5'), 122.3(CH, C-4, C-4'), 112.1(CH, C-3, C-3'), 65.2 (CH_2, C, C-6, C-6').

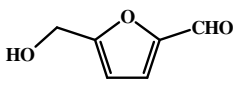
Compound 2: 5-(Hydroxymethyl)furfural[7], colourless oil. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.54 (1H, s), 7.48(1H, d, J = 3.5 Hz, H-3), 6.59 (1H, d, J = 3.5 Hz, H-4), 4.50 (2H, s). ^{13}C NMR (100 MHz, DMSO) δ: 178.1 (CHO), 161.2(C, C-5), 152.9 (C, C-2), 123.9 (CH, C-3), 111.2 (CH, C-4).

Compound 3: Uracil[7], white powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 7.38 (1H, d, J = 7.4 Hz, H-6), 5.37(1H, d, J = 7.4 Hz, H-5), 3.52 (1H, brs, 1,3-NH_3). ^{13}C NMR (100 MHz, DMSO) δ: 164.4(C-4), 151.6(C-2), 142.2(C-6), 100.3(C-5).

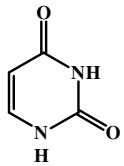
Compound 4: Adenosine[8], white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.34 (H, s, H-8), 8.13(1H, s, H-2), 7.33(2H, s, NH_2), 5.87(1H, d, J = 6.2 Hz, H-1'), 4.61(1H, d, J = 3.1 Hz, H-2'), 4.14 (1H, d, J = 3.1 Hz, H-3'), 3.96(1H, d, J = 3.1 Hz, H-4'), 3.67 (1H, dt, J = 11.9, 4.0 Hz, H-5'a), 3.58 (1H, dd, J = 11.9, 4.0 Hz, H-5'b), 5.49-5.37 (2H, m, OH -2',5'), 5.16(1H, d, J = 4.1 Hz, OH-3'). ^{13}C NMR (100 MHz, DMSO) δ: 156.3 (C-6), 152.7(C-2), 149.8 (C-4), 140.3 (C-8), 119.6 (C-5), 88.3 (C-1'), 73.8 (C-2'), 70.9 (C-3'), 86.2 (C-4'), 61.9 (C-5').



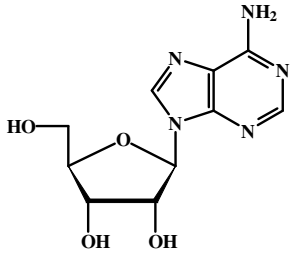
Bis-(5-fomylfurfuryl)ether (**1**)



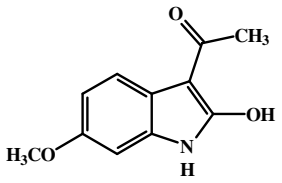
5-(Hydroxymethyl)furfural (**2**)



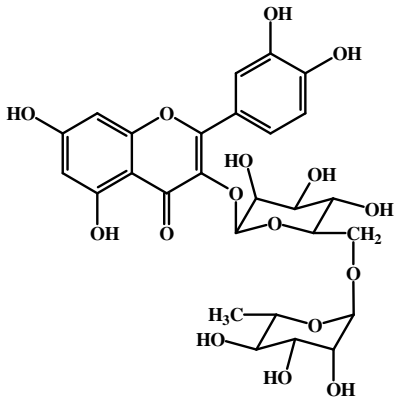
Uracil (**3**)



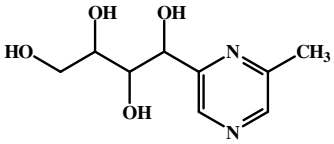
Adenosine (**4**)



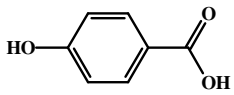
2-Hydroxy-6-methoxy-1*H*-indol-3-ethanone (**5**)



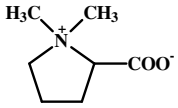
Rutin (**6**)



2-Methyl-6-(1',2',3',4'-tetrahydroxybutyl)pyrazine (**7**)



p-Hydroxy-benzonic acid (**8**)



Stachydrine (**9**)

Fig. 1. Chemical structures of compounds **1-9**

Compound 5: 2-Hydroxy-6-methoxy-1*H*-indol-3-ethanone[9], yellow crystal, ^{1}H NMR (600 MHz, DMSO-d_6) δ: 7.85 (1H, d, J = 8.5 Hz, H-4), 7.00 (1H, dd, J = 8.5, 2.6 Hz, H-5), 7.11 (d, J = 2.6 Hz, H-7), 3.69 (3H, s, H-11), 2.61(3H, s, H-12).

Compound 6: Rutin[10], pale yellow crystal. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 12.59 (1H, s, OH-5), 10.84(1H, s, OH-7), 9.67(1H, s, OH-3'), 9.17(1H, s, OH-4'), 6.19(1H, s, H-6), 6.39 (1H, d, *J* = 2.5 Hz, H-8), 7.54 (1H, s, H-2'), 6.84 (1H, d, *J* = 8.2 Hz, H-5'), 7.53 (1H, s, H-6'), 5.34 (1H, d, *J* = 6.9 Hz, H-2''), 4.33 (1H, brs, H-2'''), 0.99 (3H, d, *J* = 6.1 Hz, H-6'''), 3.70-3.07 (10H, m). ^{13}C NMR (100 MHz, DMSO) δ: 177.4(C-4), 164.1(C-7), 161.2(C-5), 156.4(C-2), 133.3(C-3), 100.8(C-6), 98.7(C-8), 148.4(C-3'), 144.8(C-4'), 121.6(C-1'), 121.2(C-6'), 116.3(C-5'), 115.2(C-2'), 93.6(C-1''), 75.9(C-5''), 74.1(C-2''), 71.9(C-3''), 67.00(C-6''), 70.0(C-4''), 104.0(C-1'''), 76.5(C-4'''), 70.6(C-2'''), 70.4(C-3'''), 68.3(C-5'''), 17.7(C-6''').

Compound 7: 2-Methyl-6-(1',2',3',4'-tetrahydroxybutyl)-pyrazine[11], colourless needles.^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.41(1H, s, H-5), 8.59(1H, s, H-3), 2.46(3H, s, H-2), 5.29(1H, d, *J*=6.4Hz, OH), 4.37(1H, d, *J* = 5.7 Hz, OH), 4.63 (1H, br, OH), 4.35(1H, br, OH), 3.5-3.61(3H, m, H-2'-4'). ^{13}C NMR (100 MHz, DMSO) δ: 155.6 (C-2), 141.9 (C-3), 140.4 (C-5), 71.3 (C-1'), 73.8 (C-2'), 71.3 (C-3'), 63.6 (C-4'), 21.0 (CH_3).

Compound 8: *p*-Hydroxy-benzoic acid[12], colourless needles. ^{1}H NMR (600 MHz, CD_3OD) δ: 7.87(2H, d, *J* = 7.87 Hz, H-2, 6), 6.82 (2H, d, *J* = 7.87 Hz, H-3,5). ^{13}C NMR (100 MHz, CD_3OD) δ: 122.8 (C-1), 130.0 (C-2), 116.0 (C-3), 163.3 (C-4), 116.0 (C-5), 133.0 (C-6), 170.3 (COOH).

Compound 9: Stachydrine, colourless oil. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 3.77(1H, t, *J* = 9.6 Hz, H-2), 2.09, 2.17(1H, m, H-3), 1.92 (2H, m, H-4), 3.00(3H, s, H-1'), 3.22(3H, s, H-2'). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 76.0 (C-2), 24.5(C-3), 18.4(C-4), 65.7(C-5), 44.7(C-1'), 51.3(C-2'), 166.2(COOH).

Conclusion

The antitumor activity of *n*-butanol extract of *Capparis spinosa* L. was confirmed and nine compounds were isolated from this extract. They are *bis*-(5-fomylfurfuryl)ether (**1**), 5-(hydroxymethyl)furfural (**2**), uracil (**3**), adenosine (**4**), 2-hydroxy-6-methoxy-1*H*-indol-3-ethanone (**5**), rutin (**6**), 2-methyl-6-(1',2',3',4'-tetrahydroxybutyl)pyrazine (**7**), *p*-hydroxy-benzoic acid (**8**), stachydrine (**9**). More importantly, the compound (**7**) as a known alkaloid is extracted from *Capparis spinosa* L. for the first time.

ACKNOWLEDGEMENTS

This work was supported financially by National Natural Science Foundation, China (30902013), Ministry of Education Doctoral Program of Higher Specialized Research Foundation, China (20092332120001), Natural Science Foundation for Young Scientists of Heilongjiang Province, China (QC2010020), Open Research Program for Key Laboratory of College of Heilongjiang Province, China (CPAT-2012003), Natural Science Item of Department of Education of Heilongjiang Province, China.

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