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ORIGINAL ARTICLE

Bioactivity-Guided Fractionation of *Cymbopogon caesius* (Ness) Stapf. Revealed Isoorientin as an Antihypertensive Agent

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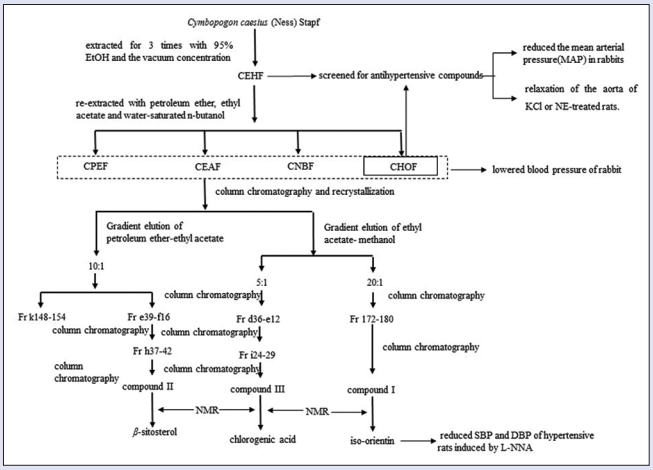
ABSTRACT

Background: *Cymbopogon caesius* (Ness) Stapf. is a traditional Uyghur medicinal material that is used for the treatment of pain, rheumatism, cold, indigestion, and injury. **Objective:** The aim of this study was to isolate the antihypertensive activity compounds from *C. caesius* (Ness) Stapf. **Materials and Methods:** *Cymbopogon caesius* (Ness) Stapf. was reflux extracted by EtOH, and the obtained extracts were screened for antihypertensive compounds via the hypertension model of rabbits and rats. The hypertensive extracts were separated via column chromatography and recrystallization and screened for antihypertensive compounds by the hypertension model of rats induced by L-NG-nitroarginine (L-NNA). Their structures were elucidated by nuclear magnetic resonance (NMR) spectroscopy and melt point. **Results:** Three compounds, namely, isoorientin (Compound I), β-sitosterol (Compound II), and chlorogenic acid (Compound III), were isolated from the dried aerial part of *C. caesius* (Ness) Stapf. via bioactivity-guided fractionation. Their structures were elucidated by NMR spectroscopy and melt point. Isoorientin may reduce the rats' blood pressure, which was elucidated through the diastolic blood pressure and systolic blood pressure of L-NNA-induced hypertensive rat model experiment. **Conclusion:** Isoorientin is one of the novel compounds of *C. caesius* (Ness) Stapf. for the treatment of hypertension diseases.

Key words: Antihypertensive activities, bioactivity-guided fractionation, compound, *Cymbopogon caesius* (Ness) Stapf., isolation, screen

SUMMARY

- *C. caesius* (Ness) Stapf. is a traditional Uyghur medicinal material that is used for the treatment of pain, rheumatism, cold, indigestion and injury. In Cuba, *C. caesius* (Ness) Stapf. is used as an antihypertensive and anti-inflammatory medication. However, the antihypertensive compounds of *C. caesius* (Ness) Stapf. remain unclear. *C. caesius* (Ness) Stapf. was reflux extracted by EtOH, and the obtained extracts were screened for antihypertensive compounds via the hypertension model of rabbits and rats. *Cymbopogon caesius* (Ness) Stapf. 95% EtOH extract fractions (CEHF) significant inhibited mean arterial pressure in rabbits, and relaxed of the aorta of KCl or NE-treated rats. *Cymbopogon caesius* (Ness) Stapf. petroleum ether fractions, CEAF, *Cymbopogon caesius* (Ness) Stapf. n-butanol fractions and CHOF were obtained after CEHF reextracted by organic solvents with proper polarity. CEAF was the compound of antihypertensive. The hypertensive extracts were separated via column chromatography and recrystallization, and screened for antihypertensive compounds using hypertension rat model induced by L-NG-nitroarginine (L-NNA). Compound I, compound II, and compound III of *C. caesius* (Ness) Stapf. were identified by nuclear magnetic resonance spectroscopy. Isoorientin (compound I), *β*-sitosterol (compound II), and chlorogenic acid (Compound III) were isolated from the dried aerial part of *C. caesius* (Ness) Stapf. via bioactivity-guided fractionation. Isoorientin may reduce the rats' blood pressure, which was elucidated through the diastolic blood pressure and systolic blood pressure of L-NNA-induced hypertensive rat model experiment. Isoorientin is one of the novel compounds of *C. caesius* (Ness) Stapf. for the treatment of hypertension diseases.



Abbreviations used: DBP: Diastolic blood pressure; SBP: Systolic blood pressure; MAP: Mean arterial pressure; NO: Nitric oxide; L-NNA: L-NG-nitroarginine; CEHF: *Cymbopogon caesius* (Ness) Stapf. 95% EtOH extract fractions; CPEF: *Cymbopogon caesius* (Ness) Stapf. petroleum ether fractions; CEAF: *Cymbopogon caesius* (Ness) Stapf. ethyl acetate fractions; CNBF: *Cymbopogon caesius* (Ness) Stapf. n-butanol fractions; CHOF: *Cymbopogon caesius* (Ness) Stapf. H_2O fractions; NMR: Nuclear magnetic resonance.

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INTRODUCTION

Hypertension is the most common disease in the world. It affects more than one in three adults and leads to more than nine million deaths worldwide every year. In addition, hypertension is the leading risk factor for the development of cardiovascular diseases, such as stroke, heart failure, and chronic kidney disease.[1] Over the past decades, plentiful research has enhanced our understanding of the mechanisms of hypertension.

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However, the exact pathophysiological mechanisms in hypertension continue to remain incompletely understood. Nitric oxide (NO) plays a crucial role in the regulation of hypertension development.[2] NO production is reduced in hypertension with elevation of blood pressure.[3] NO synthesis occurs with both the canonical and noncanonical pathways. The NO synthase (NOS)-independent pathway involving the reduction of nitrite to NO is the noncanonical pathways and considered as a complementary pathway to the canonical NOS-dependent pathway.[4] NO is synthesized from L-arginine by endothelial, NOS, which is the most important isoform for NO formation in hypertension.[5] Uyghur medicine is an important part of traditional Chinese medicine. Uyghur medicine formulas are composed of two or more Uyghur medicinal materials and play a critical role in China's Xinjiang region thousands of years of disease control and health protection.[6] Moreover, they have shown excellent preventive and therapeutic effects in both clinical trials and animal experiments. For example, catechin is responsible for antihypertensive activity *in vitro* via inhibiting angiotensin-converting enzyme.[7] The whole plant of *Cymbopogon caesius* (Ness) Stapf., also called citronella in traditional Uyghur medicine, is used for the treatment of pain, rheumatism, cold, indigestion, and injury.[8] The leaf extract of *C. caesius* (Ness) Stapf. exhibited spasmolytic activity and may be mediated by NO.[9] In Cuba, *C. caesius* (Ness) Stapf. is used as an antihypertensive and anti-inflammatory medication.[10] However, the antihypertensive compound of *C. caesius* (Ness) Stapf. is rarely reported. Thus, this research on the antihypertensive property of *C. caesius* (Ness) Stapf. may provide scientific and theoretical basis for the clinical application and antihypertensive drug discovery.

MATERIALS AND METHODS

Plant materials

The dried aerial part of *C. caesius* (Ness) Stapf. was identified by Professor Sheng Ping, College of Traditional Chinese Medicine, Xinjiang Medical University. It was purchased from Xinjiang Uygur Pharmaceutical Co., Ltd.

Animals

Male Wistar rats (150–180 g) and rabbits (2.0–2.5 kg) were purchased from the Animal Center of the Xinjiang Medical University. These animals were provided free access to food and water. They were maintained at a temperature of 22°C ± 2°C and a relative humidity of 50% ±10% before the experiment. The animal experiment program was conformed to the National Guide for the Use of Laboratory Animal Care and Research. All experiments were conducted with the permission of the Chinese government.

Chemicals

Captopril was purchased from Shanxi Jinhua Huixing Pharmaceutical Co., Ltd. L-NG-nitroarginine (L-NNA) was purchased from Shanghai Shifeng Biotechnology Co., Ltd. Column chromatography silica gel (200–300 mesh) and Thin Layer Chromatography (TLC) silica gel GF-254 were both purchased from Qingdao Ocean Chemical Plant. Carboxymethyl Cellulose (CMC)-Na was purchased from Tianjin Fuchen Chemical Reagent Factory. Urethane, heparin sodium, and berberine were provided by the National Drug Screening Center, and all organic solvents were chemically pure and purchased from Tianjin Yongsheng Fine Chemical Co., Ltd.

Extraction and isolation

As shown in Figure 1, 8.8 kg dried aerial part of *C. caesius* (Ness) Stapf. was reflux extracted for 3 times by 95% EtOH at 80°C with 1.5 h for each extraction. Then, the extracting solutions were combined together. To afford *C. caesius* (Ness) Stapf. 95% EtOH extract fractions (CEHF), the obtained extracts were concentrated to dryness under vacuum. The CEHF was suspended in H_2O and reextracted with petroleum ether, ethyl acetate, and water-saturated *n*-butanol to obtain the *C. caesius* (Ness) Stapf. fractions of petroleum ether (CPEF, 110.8 g), ethyl acetate (CEAF, 88.6 g), *n*-butanol (CNBF, 143.0 g), and H_2O (CHOF, 102.8 g), respectively. The CEAF (88.0 g) was separated continuously via column chromatography over silica gel and recrystallization to obtain compound I (70.2 mg, yield: 0.797%), compound II (10.3 mg, yield: 0.117%), and compound 3 (4.2 mg, yield: 0.048%). Column chromatography separation of 88.0 g CEAF was administrated by silica gel column. Gradient elution of petroleum ether–ethyl acetate (petroleum ether 50:1 30:1 20:1 10:1 5:1 3:1 ethyl acetate (Volume/Volume)) and ethyl acetate–methanol (ethyl acetate 50:1 30:1 20:1 10:1 7:1 3:1 2:1 1:1 methanol (V/V)) was adopted. A total of 606 fractions were collected. After silica gel column chromatography and recrystallization for many times, Compound II (10.3 mg) was obtained from Fr. E39-f16. After silica gel column was eluted with ethyl acetate and methanol repeatedly, Compound I (70.2 mg) was obtained from Fr. 172–180. After silica gel column chromatography for many times, Compound III (4.2 mg) was obtained from Fr. D37–e12. The separation process is shown in Figure 1.

Measuring the effect of isoorientin on hypertension rat model induced by L-NG-nitroarginine

A total of thirty male Wistar rats with barely difference in basal blood pressure were selected. Six rats were selected randomly as the control group and were administered 1 ml distilled water daily for 4 weeks. The other 24 Wistar rats were intraperitoneally injected with L-NNA, a NOS inhibitor, at 15 mg/kg/d dose for 4 weeks to prepare the hypertensive rat model according to the method of Li *et al.*[11] A blood pressure instrument was used to measure and record the rat blood pressure. The hypertensive rats induced by L-NNA were randomly divided into the model group, captopril group (3.75 mg/kg), high-dose isoorientin (25 mg/kg), and low-dose isoorientin (6.25 mg/·kg) group. The blood pressure changes in rats were monitored using a physiological recorder.[12]

Measuring the effect of *Cymbopogon caesius* (Ness) Stapf. extracts on rabbit's blood pressure

The thirty rabbits were randomly divided into six groups (n = 5): CPEF group (3.35 mg/kg), CEAF group (2.67 mg/kg), CNBF group (4.34 mg/kg), CHOF group (3.11 mg/kg), captopril group (0.45 mg/kg), and model group. The model group was administrated the same volume of distilled water which contained 0.52% tween 80. Each group received *C. caesius* extracts or captopril via ear margin veins, respectively. The blood pressure of all the rabbits were measured and recorded before and after injection as Burke *et al.* described.[13]

Testing of the effect of *Cymbopogon caesius* (Ness) Stapf. extracts and isoorientin on vasoconstriction of the aorta induced by KCl or NE

The preparation of the vessel rings was performed according to Zhang *et al.*[14] Briefly, the rats were sacrificed via decapitation. The thoracic aorta was quickly removed and placed in ice-cold freshly prepared K-H buffer filled with a mixture of 95% O_2 and 5% CO_2. The blood stains were removed and the connective tissue around the blood vessels was carefully peeled off. About 2–3 mm vascular rings were cut out. Two triangular stainless steel wires were carefully placed into the bath with 10 ml K-H buffer (pH 7.4). The upper end was connected to the tension transducer and the lower end was fixed on the stainless steel wire hook at the bottom of the bath to record the change in blood vessel tension.

The bath temperature was 37°C. The mixture of 95% O_2 and 5% CO_2 was continuously injected and the K-H buffer was renewed every 20 min. The resting tension of the vessel ring was 1.2 g and balancing time was 1–1.5 h. The vascular smooth muscle was depolarized with high K + concentration, and the responsiveness of the specimens was tested after maximal contraction. The specimens were washed repeatedly for 30 min until baseline rebalancing. Vasoconstriction was induced by 60 mol/L KCl or 10^{-3} mol/LNE. After maximum contraction, the final specimens at 1, 5, 10, 25, and 50 μg/ml concentrations were added to observe the vasodilation effect of each sample. The vasodilation rate of each sample was higher than 50% and the IC_{50} was calculated as follows:

$$\text{Vasodilation rate} = \frac{F_{\text{systolic value}} - F_{\text{adminstration value}}}{F_{\text{systolic value}} - F_{\text{Basic value}}} \times 100\%$$

Statistical analysis

Statistical analysis was processed by SPSS 17.0(IBM, Armonk, USA). The data were expressed as mean ± standard deviation $\left(\overline{\chi} \pm s\right)$. One-way analysis of variance (ANOVA) was conducted to analyze the statistical results, and the Student's *t*-test was performed for comparison among groups.

RESULTS

Treatment with *Cymbopogon caesius* (Ness) Stapf. H_2O fractions and *Cymbopogon caesius* (Ness) Stapf. 95% EtOH extract fractions reduced the mean arterial pressure (mean arterial pressure) in rabbits

Antihypertensive activities of *C. caesius* (Ness) Stapf. extract were explored with rabbit and rat after the dried aerial part of this plant was reflux extracted. Treatment with distilled water via ear margin veins had no effect on mean arterial pressure (MAP) in rabbits but explored with CEHF (100 mg/kg) via ear margin veins significant lowered MAP in rabbits. In rabbits, CHOF (3.11 mg/kg) also had effect on MAP level [Table 1].

Cymbopogon caesius (Ness) Stapf. H_2O fractions and *Cymbopogon caesius* (Ness) Stapf. 95% EtOH extract fractions treatment increased relaxation of the aorta of KCl or NE-treated rats

Positive drug (Berberine) reduced the efficacy of vasoconstrictions induced by KCl or NE with isolated aortic rings from rats in organ chamber. CEHF (1–50 μg/ml) increased relaxation of isolated aortic rings induced by KCl, isolated aortic rings induced by NE showed closely similar results. Tables 2 and 3 showed that the Vasodilation rates of CEHF were 112.66% and 26.61%, respectively. CHOF (1–50 μg/ml) relaxed isolated aortic rings induced by KCl and had no effect on vascular tone in the presence of NE in organ chambers [Tables 4 and 5]. CEHF was the main antihypertensive compound.

Table 1: Effects of *Cymbopogon caesius* (Ness) Stapf. H_2O fractions and *Cymbopogon caesius* (Ness) Stapf. 95% EtOH extract fractions on the rabbit mean arterial pressure ($\bar{x}$±s, *n*=5)

Group	Concentration (mg/kg)	MAP (mmHg)	
		Before treatment	After treatment
Distilled water	-	107.60±6.88	108.60±6.58
CHOF	3.11	104.40±5.41	95.00±5.20[a]
CEHF	100	109.40±5.32	95.80±5.26[b]

[a]CHOF compared with distilled water group, $P<0.05$, [b]CEHF compared with distilled water group, $P<0.05$. MAP: Mean arterial pressure; CHOF: *Cymbopogon caesius* (Ness) Stapf. H_2O fractions; CEHF: *Cymbopogon caesius* (Ness) Stapf. 95% EtOH extract fractions

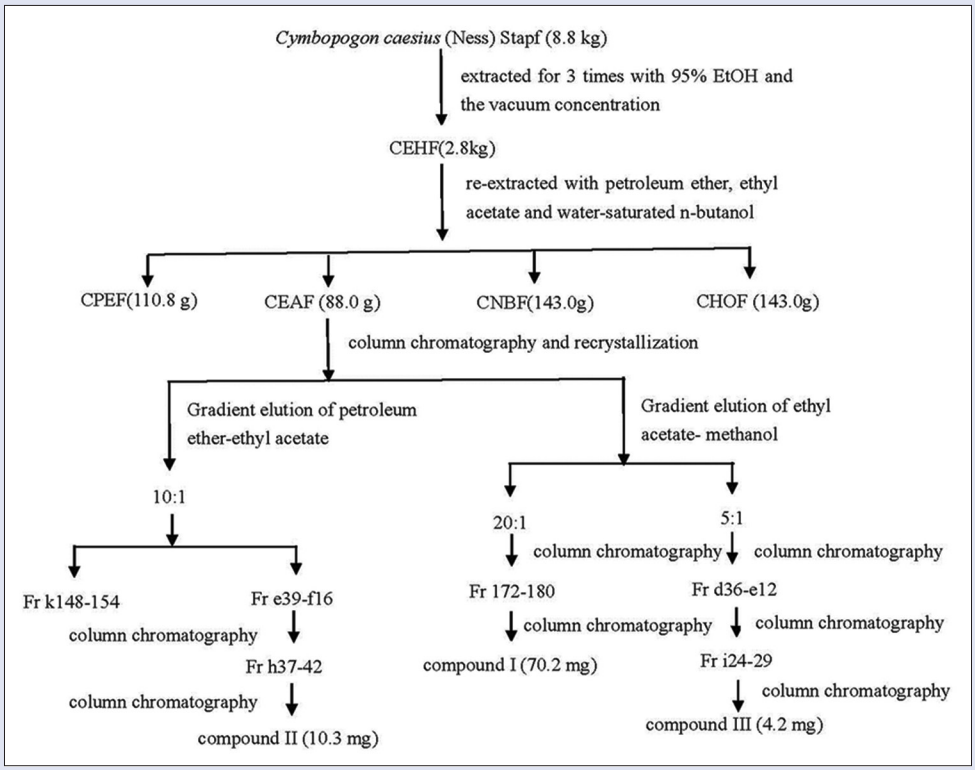


Figure 1: The extraction process of *Cymbopogon caesius* (Ness) Stapf

Table 2: Effect of *Cymbopogon caesius* (Ness) Stapf. H_2O fractions and *Cymbopogon caesius* (Ness) Stapf. 95% EtOH extract fractions on vasodilation of the aorta of rats induced by KCl

Serial number	Sample	$F_{Basic\ Value}$	$F_{Systolic\ Value}$	$F_{Injection\ Value}$	Vasodilation rate (%)
DF7996	CHOF	1.22	2.52	2.15	28.46
DF7997	CEHF	1.23	2.02	1.13	112.66
Positive	Berberine	1.27	2.31	1.78	50.90

CHOF: *Cymbopogon caesius* (Ness) Stapf. H_2O fractions; CEHF: *Cymbopogon caesius* (Ness) Stapf. 95% EtOH extract fractions

Table 3: Effect of *Cymbopogon caesius* (Ness) Stapf. H_2O fractions and *Cymbopogon caesius* (Ness) Stapf. 95% EtOH extract fractions on vasodilation of the aorta of rats induced by NE

Serial number	Sample	$F_{Basic\ Value}$	$F_{Systolic\ Value}$	$F_{Injection\ Value}$	Vasodilation rate (%)
DF7996	CHOF	1.23	2.26	2.32	−5.83
DF7997	CEHF	1.22	2.46	2.13	26.61
Positive	Berberine	1.18	3.11	2.09	52.80

NE: Norepinephrine; CHOF: *Cymbopogon caesius* (Ness) Stapf. H_2O fractions; CEHF: *Cymbopogon caesius* (Ness) Stapf. 95% EtOH extract fractions

Table 4: ^{1}H-nuclear magnetic resonance and ^{13}C-nuclear magnetic resonance data of compound I

^{1}H-NMR	δ_H (measurement)[a]	δ_H (literature)[b]	^{13}C-NMR	δ_c (measurement)[a]	δ_c (literature)[b]
2	-	-	2	164.8	164.1
3	6.55 (1H, s)	6.69 (1H, s)	3	103.9	103.3
4	-	-	4	184.0	182.3
5	-	-	5	162.0	161.2
6	-	-	6	109.2	109.3
7	-	-	7	162.0	163.7
8	6.49 (1H, s)	6.49 (1H, s)	8	95.1	94.0
9	-	-	9	158.6	156.7
10	-	-	10	105.1	103.9
1’	-	-	1’	120.5	121.9
2’	7.37-7.39 (2H, t, *J*=10.8Hz)	7.50-7.41 (2H, m)	2’	114.1	113.8
3’	-	-	3’	147.0	146.3
4’	-	-	4’	151.0	150.2
5’	6.90 (1H, d, *J*=8.4Hz)	6.90 (1H, d, *J*=8.5Hz)	5’	116.7	116.6
6’	7.37-7.39 (2H, t, *J*=10.8Hz)	7.50-7.41 (2H, m)	6’	120.3	119.5
1”	4.90 (1H, d, *J*=9.6Hz)	4.59 (1H, d, *J*=10.0Hz)	1”	72.6	73.6
2”	4.17-3.10 (m)	4.14-3.10 (m)	2”	71.7	71.2
3”	4.17-3.10 (m)	4.14-3.10 (m)	3”	80.1	79.5
4”	4.17-3.10 (m)	4.14-3.10 (m)	4”	71.7	70.8
5”	4.17-3.10 (m)	4.14-3.10 (m)	5”	82.6	82.1
6”	4.17-3.10 (m)	4.14-3.10 (m)	6”	62.8	62.1

[a]Data were recorded in CD_3OD at 600MHz for ^{1}H-NMR and 150MHz for ^{13}C-NMR, [b]Data were recorded in DMSO-d_6 at 500MHz for ^{1}H-NMR and 125MHz for ^{13}C-NMR. NMR: Nuclear magnetic resonance

Cymbopogon caesius (Ness) Stapf. ethyl acetate fractions treatment lowered blood pressure of rabbit after one-time injection, but not with *Cymbopogon caesius* (Ness) Stapf. petroleum ether fractions, *Cymbopogon caesius* (Ness) Stapf. n-butanol fractions and *Cymbopogon caesius* (Ness) Stapf. H_2O fractions

The CEHF were re-extracted with petroleum ether, ethyl acetate and water-saturated n-butanol, respectively. CPEF, CEAF, CNBF and CEHF were obtained from The CEHF by extraction with reagents. Compared to the rabbit Diastolic blood pressure (DBP) and MAP before intravenous injection via ear margin, the CEAF and positive drug (captopril) lowered DBP and MAP in rabbits after one-time injection, respectively, but CPEF, CNBF and CHOF had not significant effect on DBP and MAP of rabbit [Table 6]. The antihypertensive compound was CEAF based on the data above.

Structure identification

Three compounds were obtained from CEAF extracted and separated continuously via column chromatography and recrystallization. The compound structure was characterized and confirmed by nuclear magnetic resonance spectrometry (NMR). The compounds were isoorientin, β-sitoterol and chlorogenic acid, respectively [Table 7]. The results were published in Journal of Xinjiang Medical University (in Chinese), 2017.[15]

Compound I was a pale yellow powder and is soluble in methanol and water. However, compound I was insoluble in petroleum ether and chloroform and its m.p. was 260.0°C–262.0°C. The magnesium hydrochloride powder and aluminum trichloride reactions were positive. After the compounds were separated, they were identified by using three different development systems. The spots were all single and the m.p. did not decrease, thus indicating that the compound was a monomer. The ^{1}H-NMR and ^{13}C-NMR data in Table 4 were consistent with the report in reference.[16] Therefore, Compound I was identified to be isoorientin which isolated from *C. caesius* (Ness) Stapf. for the first.

Table 5: ^{1}H-nuclear magnetic resonance and ^{13}C-nuclear magnetic resonance data of compound II

^{1}H-NMR	δ_H (measurement)[a]	δ_H (literature)[b]	^{13}C-NMR	δ_C (measurement)[a]	δ_C (literature)[b]
1	-	-	1	37.2	37.2
2	-	-	2	31.6	31.6
3	-	-	3	71.8	71.8
4	-	-	4	42.3	42.3
5	-	-	5	140.7	140.7
6	5.31 (1H, brs)	5.35 (1H, brs)	6	121.7	121.7
7	-	-	7	31.9	31.9
8	-	-	8	31.9	31.9
9	-	-	9	50.1	50.1
10	-	-	10	36.5	36.5
11	-	-	11	21.1	21.1
12	-	-	12	39.8	39.8
13	-	-	13	42.3	42.3
14	-	-	14	56.8	56.8
15	-	-	15	24.3	24.3
16	-	-	16	28.2	28.2
17	-	-	17	56.0	56.0
18	1.01 (3H, m)	1.01 (3H, m)	18	11.8	11.8
19	0.60 (3H, m)	0.62 (3H, m)	19	19.4	19.4
20	-	-	20	36.1	36.1
21	0.89 (3H, m)	0.89 (3H, m)	21	18.8	18.8
22	-	-	22	33.9	33.9
23	-	-	23	26.0	26.0
24	-	-	24	45.8	45.9
25	-	-	25	29.1	29.1
26	0.86 (3H, m)	0.84 (3H, m)	26	19.8	19.9
27	0.87 (3H, m)	0.86 (3H, m)	27	19.0	19.0
28	-	-	28	23.1	23.0
29	0.80 (3H, m)	0.80 (3H, m)	29	12.0	12.0

[a]Data were recorded in $CDCl_3$ at 600MHz for ^{1}H-NMR and 150MHz for ^{13}C-NMR, [b]Data were recorded in $CDCl_3$ at 400MHz for ^{1}H-NMR and 100MHz for ^{13}C-NMR. NMR: Nuclear magnetic resonance

Table 6: Effects of different extracts on the rabbit diastolic blood pressure and mean blood pressure ($\bar{x}$±s, n=4)

Group	Concentration (mg/kg)	Before/after injection (mmHg)	
		DBP	MBP
Distilled water	-	101.25±13.15/98.25±10.28	109.75±6.08/104.25±6.85
Captopril	3.75	97.00±10.46/69.25±17.23*	102.00±7.02/89.75±13.67*
CPEF	3.35	92.25±7.13/90.00±7.07	101.75±5.74/99.75±6.85
CEAF	2.67	91.25±6.55/68.00±17.41*	96.50±5.07/79.25±14.03*
CNBF	4.34	94.00±8.60/91.25±3.30	99.25±6.13/95.00±3.56
CHOF	3.11	95.25±12.09/94.25±8.96	102.00±7.62/100.50±6.81

*CEAF compared with the distilled water group P<0.05, Captopril compared with distilled water group P<0.05. CPEF: *Cymbopogon caesius* (Ness) Stapf.petroleum ether fractions; CEAF: *Cymbopogon caesius* (Ness) Stapf. ethyl acetate fractions; CNBF: *Cymbopogon caesius* (Ness) Stapf. n-butanol fractions; CHOF: *Cymbopogon caesius* (Ness) Stapf. H_2O fractions; DBP: Diastolic blood pressure; MBP: Mean blood pressure

Compound II was white crystalline and soluble in chloroform and ethyl acetate but was insoluble in methanol and water. Its m.p. was 139.1°C–140.4°C. The Molisch reaction and acetic anhydride-concentrated sulfuric acid reaction were positive. The compound was sprayed with 10% H_2SO_4 ethanol solution and heated at 105°C for 5 min; afterwards, the spot turned purple-red. The compound was identified using three kinds of development systems (i.e., petroleum ether-ethyl acetate = 4:1, petroleum ether-ethyl acetate-methanol = 5:2:1 and petroleum ether-ethyl acetate-acetone-formic acid = 5:3:1:1). Each spot was single and its m.p. did not decrease. Thus, the compound was a monomer. The ^{1}H-NMR and^{13}C-NMR data are shown in Table 3. All the data in Table 5 were consistent with the article in reference.[17] Therefore, compound II was identified to be β-sitoterol which isolated from *C. caesius* (Ness) Stapf. for the first.

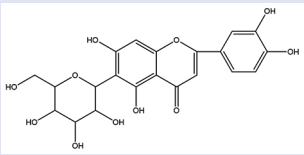
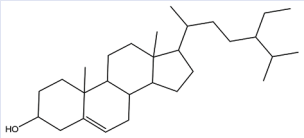
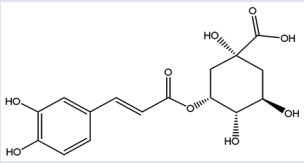
Compound III was white crystalline and was soluble in MeOH, EtOH and acetone. However, Compound II was insoluble in chloroform and water. Its m.p. was 139.1°C to 140.4°C. The Molisch reaction and acetic anhydride-concentrated sulfuric acid reaction were positive. The compound was sprayed with 10% H_2SO_4 ethanol solution and heated at 105°C for 5 min. The spot turned purple red. The compound was identified using three different development systems (e.g., petroleum ether-ethyl acetate = 4:1, petroleum ether-ethyl acetate-methanol = 5:2:1 and petroleum ether-ethyl acetate-acetone-formic acid = 5:3:1:1). Each spot was single and the m.p. did not decrease, suggesting that the compound was a monomer. The ^{1}H-NMR and ^{13}C-NMR data in Table 8 were consistent with the reference.[18] Therefore, compound III was identified to be chlorogenic acid which isolated from this herb for the first.

Effects of isoorientin on systolic blood pressure and diastolic blood pressure of hypertension rats induced by L-NG-nitroarginine

Compared to the DBP and MAP of rats before administration of drug, the number of rats DBP and MAP were increased induced by L-NNA

via intraperitoneal injection for 2 weeks and 4 weeks, respectively. To examine whether isoorientin reduces blood pressure, hypertension rats induced by L-NNA were treated with the indicated concentrations of isoorientin via rat tail vein and the systolic blood pressure (SBP) and DBP was determined. Treatment with isoorientin caused decreased in SBP and DBP in a concentration-dependent manner [Table 9].

Table 7: Compound structure

Number	Name	Structure	Identifying methods
Compound I	isoorientin		m.p. ^{1}H-NMR ^{13}C-NMR
Compound II	β-sitosterol		m.p. ^{1}H-NMR ^{13}C-NMR
Compound III	Chlorogenic acid		m.p. ^{1}H-NMR ^{13}C-NMR

NMR: Nuclear magnetic resonance

DISCUSSION

One of the arms of this study was that antihypertensive active compounds were obtained from *C. caesius* (Ness) Stapf. via bioactivity-guided fractionation. The present study revealed CHOF can partly reduce the blood pressure of rabbits, but CEHF had better activity than CHOF. The CEHF were re-extracted with different reagents, after we figured out that CEHF was the antihypertensive fraction. CEAF, CNBF and CEHF were obtained from CEHF. CEAF was the antihypertensive fraction re-extracted from CEHF after bioactivity-based screening.

Three compounds, namely, isoorientin, β-sitosterol and chlorogenic acid, were isolated from CEAF. β-Sitosterol which isolated from traditional Chinese herbs has been applied in treating many diseases because of the anti-cholinesterase, antioxidant, anti-inflammatory, anti-proliferative and anticancer effects.[19-22] Chlorogenic acid which found in many herbs possess antihypertensive, anti-oxidant, anti-inflammatory, antidiabetic, anticancer, antilipidemic and antineurodegenerative activities.[23]

N-Nitro-L-Arginine (L–NNA), a potent inhibitor of NO synthetase, can induced hypertension in rats by inhibited endothelium NO synthetase.[24] The DBP and SBP of L-NNA-induced hypertensive rats were significantly decreased in the high-dose and low-dose group of isoorientin, which indicated that isoorientin may reduce the blood pressure in hypertensive rats induce by L-NNA in concentration manner.[25]

Table 8: ^{1}H-nuclear magnetic resonance and ^{13}C-nuclear magnetic resonance data of compound III

^{1}H-NMR	δ_H (measurement)[a]	δ_H (literature)[b]	^{13}C-NMR	δ_C (measurement)[a]	δ_C (literature)[b]
1	-	-	1	77.0	76.8
2	2.25/2.04 (each 1H, m)	2.20/2.06 (each 1H, m)	2	39.1	38.3
3	5.33 (1H, m)	5.37 (1H, m)	3	74.3	73.9
4	3.72 (1H, m)	3.74 (1H, m)	4	72.8	72.0
5	4.18 (1H, m)	4.18 (1H, m)	5	72.1	71.8
6	2.25/2.04 (each 1H, m)	2.20/2.06 (each 1H, m)	6	39.6	39.3
7	-	-	7	177.9	178.6
1’	-	-	1’	128.7	127.6
2’	7.06 (1H, brs)	7.06 (1H, brs)	2’	116.1	115.2
3’	-	-	3’	147.7	146.4
4’	-	-	4’	150.4	149.4
5’	6.79 (1H, d, J=7.8Hz)	6.79 (1H, d, J=8.1Hz)	5’	117.3	116.5
6’	6.95 (1H, brd, J=7.8Hz)	6.95 (1H, brd, J=8.1Hz)	6’	123.8	123.0
7’	7.55 (1H, d, J=16.2Hz)	7.57 (1H, d, J=15.9Hz)	7’	147.9	147.0
8’	6.25 (1H, d, J=16.2Hz)	6.29 (1H, d, J=15.9Hz)	8’	116.0	115.4
9’	-	-	9’	169.5	168.7

[a]Data were recorded in CD_3 OD at 600MHz for ^{1}H-NMR and 150MHz for ^{13}C-NMR, [b]Data were recorded in CD_3 OD at 600MHz for ^{1}H-NMR and 150MHz for ^{13}C-NMR. NMR: Nuclear magnetic resonance

Table 9: Effects of isoorientin on systolic blood pressure and diastolic blood pressure of hypertensive rats induced by L- L-NG-nitroarginine (mmHg, $\bar{x}\pm s$, n=6)

Group	Project	Establishment of model			After injection
		−1 week	2 weeks	4 weeks	
Blank	SBP	96.26±3.11	96.58±3.99	95.91±2.99	95.85±4.09
	DBP	81.16±6.24	80.78±1.54	78.77±1.51	79.43±0.70
Model	SBP	94.76±1.95	124.15±2.13☆☆	133.82±2.75☆☆	132.05±1.53
	DBP	77.64±2.29	109.20±1.75☆☆	117.09±2.12☆☆	114.60±2.75
25 mg/kg	SBP	96.19±3.93	126.38±2.80☆☆	134.23±3.17☆☆	97.26±2.36**,##
	DBP	78.44±3.40	109.81±3.08☆☆	116.77±4.24☆☆	82.74±3.53**,##
6.25 mg/kg	SBP	97.42±2.78	126.29±3.07☆☆	134.08±2.15☆☆	126.24±5.88#
	DBP	80.04±2.31	110.52±3.23☆☆	115.60±2.76☆☆	106.98±6.24#
Captopril	SBP	95.09±1.82	126.29±1.08☆☆	134.73±2.65☆☆	93.05±1.72**,##
	DBP	79.79±1.22	109.25±1.84☆☆	113.98±5.48☆☆	80.02±3.92**,##

Compared with blank control group, ☆☆P<0.01; compared with model group, **P<0.01; compared with before injection, #P<0.05 and ##P<0.01. DBP: Diastolic blood pressure; SBP: Systolic blood pressure

CONCLUSION

Isoorientin is one of the novel compounds of *C. caesius* (Ness) Stapf. for the treatment of hypertension diseases. However, the action mechanism remains to be fully explored.

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Conflicts of interest

There are no conflicts of interest.

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