Journal of Food and Nutrition Research, 2015, Vol. 3, No. 9, 587-592
Available online at http://pubs.sciepub.com/jfnr/3/9/5

DOI:10.12691/jfnr-3-9-5

Free Radical-scavenging, Nitrite-scavenging, and N-nitrosamine Formation Inhibitory Activities of Extracts from Kunlun Compositae Tea (*Coreopsis tinctoria* Nutt.)

Xincheng Yao[1,2], Ting Zhang[1], Hui Tang[1,2,*], Heng Wang[1], Wen Chen[1,2]

[1]School of Pharmacy, Shihezi University, Shihezi, Xinjiang, P.R.China
[2]Key Laboratory of Xinjiang Endemic Phytomedicine Resources, Ministry of Education, Shihezi University, Shihezi, Xinjiang, P.R.China
*Corresponding author: th_pha@shzu.edu.cn

Abstract *Coreopsis tinctoria* Nutt., Asteraceae, is widely used as an ornamental plant and as a popular tea production in China. In this paper, we report about the free radical-scavenging, nitrite-scavenging and N-nitrosamine formation inhibitory activities of ethyl acetate extract (EAE) and n-butanol extract (BE) of *C. tinctoria* flowering tops (CTF). The results showed the BE contained higher total phenolic content (TPC) , total flavonoid content (TFC) and total proanthocyanidin (TPA) contents with DPPH, ABTS and *OH radical-scavenging activities (IC_{50}) of 25.79±2.753, 26.61±3.158 and 2349.8±0.672 μg/ml, respectively. However, EAE showed higher radical-scavenging activities, with IC_{50} ABTS and *OH values of 13.71±2.973 and 321.6±0.576 μg/ml, respectively. The nitrite-scavenging and N-nitrosamine inhibitory activities (IC_{50}) of EAE were 122.10±1.03 and 2362.86±11.26 μg/ml, respectively, whereas those of BE were 118.23±1.30 and 2182.79±10.74 μg/ml, respectively. Given these activities, we propose that CTF can be potentially used as a rich source of natural free radical and nitrite scavengers.

Keywords: *radical-scavenging activity, nitrite-scavenging activity, N-nitrosamine formation inhibitory activity, Coreopsis tinctoria Nutt*

Cite This Article: Xincheng Yao, Ting Zhang, Hui Tang, Heng Wang, and Wen Chen, "Free Radical-scavenging, Nitrite-scavenging, and N-nitrosamine Formation Inhibitory Activities of Extracts from Kunlun Compositae Tea (*Coreopsis tinctoria* Nutt.)." *Journal of Food and Nutrition Research*, vol. 3, no. 9 (2015): 587-592. doi: 10.12691/jfnr-3-9-5.

1. Introduction

Potentially reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet-}$, *OOH), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^{*}), and peroxyl (ROO^{*}) radicals, are generated continuously inside the human body as a consequence of exposure to exogenous chemicals in our environment and/or to endogenous metabolic processes. The ROS play an important role in the pathogenesis of various serious diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, and inflammation. [1] Antioxidants with free radical-scavenging activities (RSA) may have great significance in the protection and therapeutics of diseases involving free radicals [2].

Vegetables and fruits provide beneficial nutrients to the human body. However, vegetables and plants produce trace nitrite or imine during growth with nitrogenous fertilizer as its metabolite. In addition, some spoiled or pickled vegetables, meat, and other food have high nitrite contents. People are gradually paying attention to food safety related to these substances. Many studies have shown that nitrate- and amine-rich food intake may result in increased risk of endogenous formation of carcinogenic N-nitroso compounds.[3] Therefore, materials that can scavenge nitrites or prevent nitrosamine formation are of great interest.

According to several studies, compounds that can scavenge free radicals or inhibit electron transfer, such as ascorbic acid, phenolics, flavonoids, and volatile oil, can effectively block nitrite combined with secondary amine to generate N-nitrosamines. [4] Traditional folk herbs or plants for the treatment of some diseases demonstrate antioxidant or free RSA. Recent studies have focused on antioxidant compounds or free radical-scavenging substances, particularly those from natural plant sources [5,6].

Coreopsis tinctoria Nutt.. (also known as plains coreopsis) of the family Compositae is an annual forb that grows in disturbed areas, such as roadsides and cultivated fields. The plant is frequently called "calliopsis" by indigenous residents in different regions worldwide. [7] *C. tinctoria* infusion has been traditionally used for a long time in many countries, specifically in Portugal, to control hyperglycemia for treating the symptoms of diabetes.[8] However, *C. tinctoria* is widely used not only as an ornamental plant but also as a functional food in China. This plant has long been cultivated on a large scale at the

Kunlun Mountains (altitude: ~3000 m) in north-western China (Xinjiang Uygur Autonomous Region). It is often called “Kunlun snow chrysanthemum” or “high altitude aromatic chrysanthemum” by indigenous residents. *C. tinctoria* flowering tops (CTF) is rich in vitamins, amino acids, volatile oil, polyphenols, selenium, zinc, and other essential nutrients; CTF has been demonstrated to have many nutraceutical properties, such as helping reduce blood pressure and cholesterol levels, as well as regulate blood sugar levels.[9][10] In recent years, the CTF from the Kunlun Mountains have become a popular health product used as an herbal tea/beverage in China. Consumers of these CTF believe that the product has substantial biological activities, such as for scattering cold, cleaning heat, detoxification, brightening eyes, sedation, reducing blood pressure, increasing appetite and treating gastrointestinal discomfort. [11] Thus, the CTF from Kunlun Mountains has recently been the subject of considerable scientific and therapeutic studies.

In recent years, a great number of researches about the CTF have been reported. The CTF extracts have been shown to have excellent pharmacologic properties, including hypolipidemic, hypoglycaemic, antioxidant, and the reduction of blood pressure as well as vasorelaxant properties [12,13,14,15].

The present study aimed to prepare extracts of CTF from the Kunlun Mountains in China, namely, ethyl acetate extract (EAE) and n-butanol extract (BE). The free RSA of the extracts were evaluated through four in vitro models, namely, 2,2-diphenylpicrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), $^{*}OH$, and $^{*}O_2^{-}$. Moreover, the nitrite-scavenging and N-nitrosamine formation inhibitory activities of EAE and BE were investigated. This study may provide a foundation for broadening the applications of CTF in the preparation of food ingredients or raw materials.

2. Materials and Methods

2.1. Plant and Materials

C. tinctoria Nutt. (Compositae) flowering tops was collected in October 2013 in Hetian County (Xijiang Uygur Autonomous Region, China) and was identified by Vice Prof. Peng Li (School of Pharmacy, Shihezi University). A voucher specimen (No.CTF-006) was kept in the herbarium of the Key Laboratory of Xinjiang Endemic Phytomedicine Resources, Shihezi University.

2.2. Chemicals and Reagents

2,2’-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) was purchased from Fluka (Menlo Park, CA,USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma–Aldrich Co. (St. Louis, USA). Quercitrin, proanthocyanidin and gallic acid (>98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ascorbic acid, butylated hydroxyanisole (BHA), ethylene diamine tetraacetic acid (EDTA) and other chemicals were of analytical reagent grade. All reagents were obtained from Sinopharm chemical reagent Co., Ltd. (Shanghai, China).

2.3. Preparation of CTF Extracts

Dried CTF (200 g) were crushed into coarse powder and exhaustively extracted three times with 70% aqueous ethanol at 80 °C for 1 h by ultrasonic-assisted extraction. The solvent-seed ratio used was 23:1 (v/w). The crude alcoholic extract was concentrated under reduced pressure at 40 °C and obtained a viscous mass of 46 g. The extract was suspended in distilled H_2O (0.5 L) and defatted with petroleum ether (0.5L).The aqueous layer was successively extracted with ethyl acetate and n-butanol. The resulting extracts, EAE and BE, were collected. The solvents were evaporated in a rotary evaporator at 40 °C, and the residues were lyophilized.

2.4. Determination of Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and Total Proanthocyanidin (TPA) content

TFC was measured using the colorimetric-based method assay. [16] TFC was calculated from the calibration curve and expressed as milligram of quercitrin per gram dry extracts,. The standard curve regression equation for TFC was as follows: A = 0.0357 C + 0.0168 R^2 = 0.9994, where A is the absorption, C is the quercitrin concentration in μg/ml, and R^2 is the relative regression coefficient.

TPC and TPA content were determined using a previously described method. [17] TPC and TPA were calculated from the calibration curve and expressed as milligram of gallic acid and proanthocyanidin per gram dry extracts, respectively. The standard curve regression equation for TPC was as follows: A = 0.0872 C + 0.0291 R^2 = 0.9997, where A is the absorption, C is the gallic acid concentration in μg/ml. The standard curve regression equation for TPA was as follows: A = 0.00897C − 0.01109 R^2 = 0.9976, where A is the absorption, C is the proanthocyanidin concentration in μg/ml.

2.5. DPPH, ABTS, and $^{*}OH$ Radical-scavenging Assay

The DPPH, ABTS, and $^{*}OH$ radical-scavenging properties of the extracts with different concentrations (10–300 μg/ml for DPPH; 10–150 μg/ml for ABTS and $^{*}OH$) were determined using a previously described method. [18] Ascorbic acid and BHA at the same concentrations as the samples were both set as the positive controls.

2.6. Scavenging Activity of the Superoxide Anion (O_2^{*-}) Assay

Up to 0.5 ml of EAE or BE solution (150 μg/ml in methnol) was added to 4.5 ml of 50 mmol/L Tris–HCl buffer (pH 8.2). The mixture was incubated at 25 °C for 20 min, and then 10 μL of 45 mmol/L pyrogallol (25 °C) was added. The absorbance of the reaction mixture was measured at 325 nm every 30 s until the reaction time reached 6 min. The same concentration of EAE or BE without pyrogallol was used as the blank. The auto-oxidation rate constant (Kb) of pyrogallic acid was calculated from the curve of λ = 325 nm vs. time. The negative control did not contain any test sample. A low

Kb value suggests a high anti-autoxidation activity. Ascorbic acid and BHA at the same concentrations as the samples were set as the positive controls.

2.7. Nitrite-scavenging Activity

The nitrite-scavenging activity of the extracts was determined using a previously described method with slight modification. [19] In brief, EAE or BE solutions (200 μg/ml in methanol) measuring 0.05, 0.1, 0.2, 0.5, and 1.0 ml were placed in a 10.0 ml volumetric flask. Approximately 2.0 ml of citric acid–disodium hydrogen phosphate aqueous buffer (pH3.0) was added. After vigorous shaking, 1.0 ml of $NaNO_2$ (100 μg/ml) was added. The solution was placed in a 37 °C water bath for 1 h. Subsequently, 2.0 ml of p-aminobenzene sulfonic acid (0.4%, w/v) was added, and the solution was blended for 5 min. Approximately 1.0 ml of naphthalene ethylene-diamine hydrochloric acid solution (0.2%, w/v) was added, followed by the addition of distilled water to obtain a 10.0 ml solution. The solution was mixed, incubated for 15 min at room temperature, and scanned by a UV-2401PC spectrophotometer using quartz cuvettes (1.0 cm) (Shimadzu, Japan) at λ = 538 nm against the control. The control contained all the reagents except for the test sample. Ascorbic acid and BHA, at the same concentrations as the samples, were both set as the positive controls. The assay was performed in triplicate. The decrease in absorbance upon the addition of the test samples was used to calculate the nitrite-scavenging activity. The nitrite-scavenging activity was calculated using equation 1.

$$\text{Nitrite scavenging activity (\%)} = \left(1-\frac{\text{sample OD}_s\text{-sample OD}_0}{\text{Control OD}}\right)\times 100 \quad (1)$$

where control OD, sample OD_s, and sample OD_0 are the absorbances of the control (containing all reagents without test sample), the test sample, and the sample blank (containing test sample without $NaNO_2$ solution), respectively. Basing on the plot of concentration (log C) vs. nitrite scavenging activity (%), we conducted a linear regression analysis to determine the IC_{50} (50% concentration of inhibition for nitrite scavenging activity) values of each test sample.

2.8. Inhibition of N-nitrosamine Formation Assay

The inhibition of N-nitrosamine formation of the extracts was determined using a previously described method. [20] The inhibition of N-nitrosamine formation was calculated using the equation 1.

2.9. Statistical Analysis

Statistical analyses were performed using SPSS 10.0 software. Data are presented as mean ± standard deviation (SD). The total variation present in a set of data was estimated through one-way-ANOVA. Statistical significance was considered at $P < 0.05$.

3. Results and Discussion

3.1. Determination of TFC, TPC, and TPA Contents

Table 1 presents the TPC, TFC, and TPA contents of CTF extracts. EAE and BE had significantly higher TPC and TFC in dry extracts, but BE had the highest TFC and TPA. In addition, the TFC and TPA content of BE were significantly different ($P < 0.05$) from those of EAE. These results suggested that BE fraction contained a large amount of phenolics, flavonoids, and proanthocyanidin. However, these results were significantly different from a previous study by Woo et al. (2010) who reported that EAE fraction of CTF from Korea show the highest TPC and TFC. [21] These differences may be due to the geographical location, climatic and ecologic parameters, and harvesting period.

Table 1. Total Phenolic, Flavonoid, and Proanthocyanidin Contents of *C. tinctoria* Flowering Tops Extracts

Sample	Total phenolic content[A]	Total flavonoids content[B]	Total proanthocyanidin content[C]
EAE	549.62±3.03[a]	201.22±1.17[a]	14.16±0.50[a]
BE	588.63±6.16[a]	272.46±1.63[b]	20.77±0.072[b]

Each value is presented as mean ± SD (n = 3). [A]Expressed as milli-gram of gallic acid per gram dry extract. [B]Expressed as milligram of rutin per gram dry extract. [C]Expressed as milligram of proanthocyanidin per gram dry extract.
Means that do not share the same letter are significantly different (LSD) at the $P < 0.05$ level in each column. EAE, ethyl acetate extract; BE, n-butanol extract.

3.2. In Vitro Testing of Radical-scavenging Capacity of CTF Extracts

The DPPH RSA of EAE and BE are presented in Figure 1(A). The RSA values increased with increasing DPPH sample concentration. At 40–80 μg/ml, BE showed significantly higher RSAs than EAE and positive controls ascorbic acid and BHA.

In $ABTS^{\cdot+}$ assay, the RSA of EAE and BE increased in a dose-dependent manner (Figure 1(B)). At >10 μg/ml, EAE showed significantly higher RSA than the other samples. RSA decreased in the following order: EAE > ascorbic acid or BHA > BE.

In the assay for scavenging activity against $^{*}OH$, the RSA of EAE and BE exhibited a dose-dependent increase (Figure 1(C)). EAE showed excellent radical-scavenging activity. BE exhibited a certain extent of $^{\cdot}OH$ radical-scavenging activity similar to that of ascorbic acid and BHA.

In the assay for scavenging activity against O_2^{*-}, the auto-oxidation rate of pyrogallic acid was spectrophotometrically recorded for 6 min (Figure 1(D)). EAE and BE inhibited O_2^{*-}, but their activities were weaker than that of ascorbic acid. In this section, the Kb values of pyrogallic acid were calculated from the plot of OD values versus time (Table 2). The scavenging activity of BE against O_2^{*-} was similar to BHA ($P > 0.05$) but significantly more efficient than EAE ($P < 0.05$). EAE showed certain anti-

autoxidation activities compared with the control ($P < 0.05$), but weaker activities than ascorbic acid and BHA. These results suggested that EAE and BE could scavenge superoxide radicals and help prevent oxidative damage.

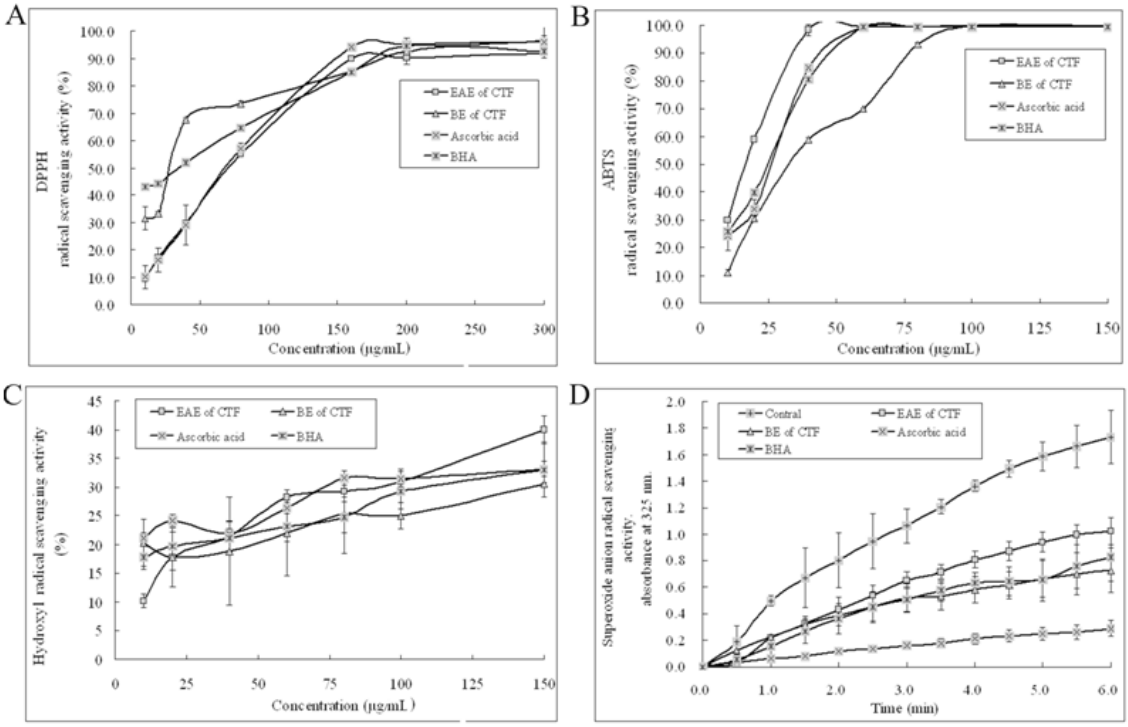

Figure 1. α,α-Diphenyl-β-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), superoxide anion ($^{*}O_2$), and hydroxyl radical ($^{*}OH$)-scavenging activity of extracts from *Coreopsis tinctoria* flowering tops. Data are presented as mean ± SD ($n = 3$). DPPH assay (A); ABTS assay (B); hydroxyl radicals assay (C); superoxide anion radical-scavenging assay (D). EAE, ethyl acetate extract; BE, n-butanol extract; BHA, butylated hydroxyanisole; CTF, *Coreopsis tinctoria* flowering tops

Table 2. Inhibition of Pyrogallic Acid Auto-oxidation by *C. tinctoria* Flowering Tops Extracts

Sample	Control	EAE	BE	Ascorbic acid†	BHA†
Kb value	0.285±0.026^{d}	0.180±0.019^{c}	0.114±0.016^{b}	0.0466±0.002^{a}	0.135±0.012^{b}

Values that do not sharing the same letter are significantly different (LSD) at the $P < 0.05$ level in each row. EAE, ethyl acetate extract; BE, n-butanol extract; and BHA, butylated hydroxyanisole.
†Reference compounds.

Table 3 lists the RSA of EAE, BE, and standard compound (ascorbic acid and BHA) needed to inhibit the three radicals, as indicated by IC_{50} (50% concentration of inhibition for RSA). In DPPH RSA, BE showed a significant difference ($P < 0.05$) from EAE and ascorbic acid, as well as higher inhibition than EAE. In the ABTS assay, EAE and BE showed significant differences ($P < 0.05$) from each other. The inhibitory effect of EAE on $ABTS^{\cdot+}$ was strongest than that of BE, ascorbic acid, or BHA. In addition, EAE showed the highest inhibition among BE, ascorbic acid, and BHA in the assays for the scavenging activity against $^{*}OH$.

Table 3. IC_{50} of *C. tinctoria* Flowering Tops Extracts for Various Antioxidant Systems (μg /ml)

Sample	DPPH radical scavenging assay (IC_{50})	$ABTS^{*-}$ radical scavenging assay (IC_{50})	$^{*}OH$ radical scavenging assay (IC_{50})
EAE	53.47 ± 3.644^{b}	13.71 ± 2.973^{a}	321.6 ± 0.576^{a}
BE	25.79 ± 2.753^{a}	26.61 ± 3.158^{c}	2349.8 ± 0.672^{b}
Ascorbic acid†	46.54 ± 1.741^{b}	18.02 ± 2.247^{b}	2458.4 ± 0.025^{b}
BHA†	22.91 ± 2.012^{a}	17.78 ± 3.061^{b}	2759.0 ± 0.056^{b}

Each value in the table is expressed as mean ± SD (n = 3). Means that do not sharing the same letter are significantly different (LSD) at the $P < 0.05$ level in each column. EAE, ethyl acetate extract; BE, n-butanol extract; and BHA, butylated hydroxyanisole.
† Reference compounds.

Several reports have conclusively shown a close relationship between TPC and antioxidative activity or radical-scavenging capacity of fruits and vegetables. [22] Zalaru et al (2014) reported that a good relationship between the TPC of CTF from Romania and its antioxidant capacity. [23] In our results, BE contained relatively high TPC, TFC, and TPA contents, but its RSA capacity was weaker than EAE except for DPPH assay. These results showed that TPC was not consistent with free radical-scavenging ability. Hwang et al. (2010) reported that EAE of CTF from Korea can significantly scavenge DPPH and $ABTS^{+}$ radicals, and EAE fraction effectively inhibits DPPH and $ABTS^{+}$ radicals in a dose-dependent manner. [24] These results were consistent with our results showing that EAE had higher inhibitory capacity for DPPH and $^{*}OH$.

3.3. Nitrite Scavenging and Inhibition of N-nitro Samine Formation Activities

The nitrite-scavenging and N-nitrosamine formation inhibitory activities of EAE and BE were investigated using a diazotization-coupling reaction system in vitro (Table 4). In the nitrite-scavenging activity assay, the IC_{50} values of EAE, BE, ascorbic acid, and BHA were 122.10±1.03, 118.23±1.30, 11.51±0.38, and 99.80±1.67 μg/ml, respectively. The IC_{50} values of EAE, BE, and BHA were significantly different ($P < 0.05$) from those of ascorbic acid. Nitrite-scavenging capacity decreased in the following order: ascorbic acid > BHA or BE or EAE (Figure 2(A)). The nitrite-scavenging activity of EAE or BE was comparable with that of BHA ($P > 0.05$) but weaker than that of ascorbic acid.

Table 4. IC_{50} of *C. tinctoria* Flowering Tops Extracts for Nitrite-scavenging and N-nitrosamine Formation Inhibitory Capacities (μg/ml)

sample	EAE	BE	ascorbic acid†	BHA†
Nitrite scavenging activity IC_{50}	122.10±1.03[b]	118.23±1.30[b]	11.51±0.38[a]	99.80±1.67[b]
N-nitrosamine inhibitory activity IC_{50}	2362.86±11.26[b]	2182.79±10.74[b]	4.64 ± 0.86[a]	2097.42 ± 13.21[b]

Values that do not sharing the same letter are significantly different (LSD) at the $P<0.05$ level in each row. EAE, ethyl acetate extract; BE, n-butanol extract; and BHA, butylated hydroxyanisole.
†Reference compounds.

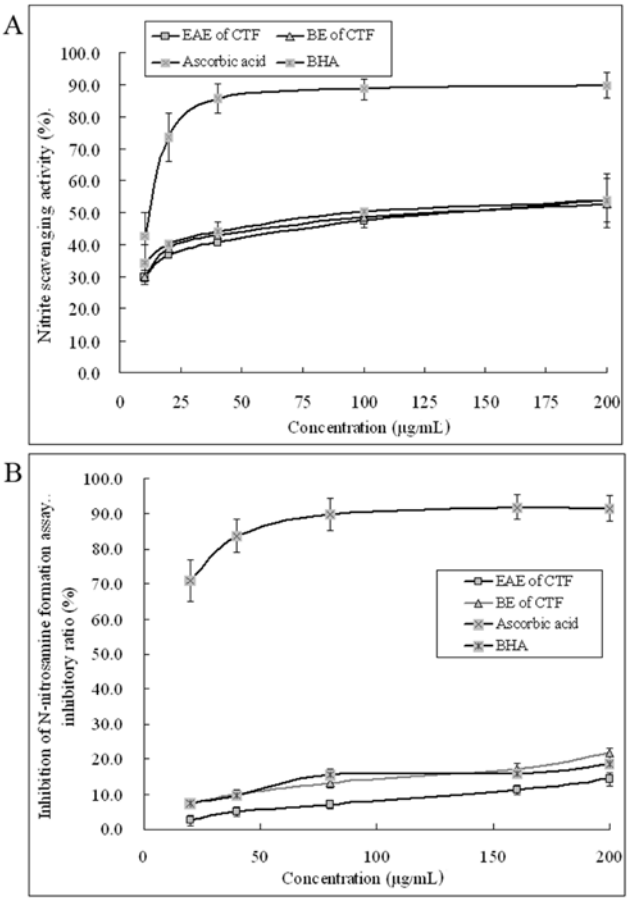

Figure 2. Nitrite-scavenging and N-nitrosamine formation inhibitory activities of extracts from *C. tinctoria* flowering tops. Data are presented as mean ± SD (n = 3). Nitrite scavenging assay (A); N-nitrosamine formation inhibitory activity assay (B). EAE, ethyl acetate extract; BE, n-butanol extract; BHA, butylated hydroxyanisole; CTF, *Coreopsis tinctoria* flowering tops

The IC_{50} values of inhibition of N-nitrosamine formation of EAE, BE, ascorbic acid, and BHA were 2362.86±11.26, 2182.79±10.74, 4.64±0.86, and 2097.42 ± 13.21 μg/ml, respectively. The IC_{50} values of EAE, BE, and BHA were significantly different ($P < 0.05$) from those of ascorbic acid. The N-nitrosamine formation inhibitory capacities decreased in the following order: ascorbic acid > BHA or BE or EAE (Figure 2(B)).

It has been reported that the antioxidant and nitrite-scavenging capacities of different solvent extracts from sugarcane tops are positively correlated with their TPC.[25] In our results, BE contained relatively high TPC content, but its nitrite-scavenging and N-nitrosamine formation inhibitory capacities were similar to EAE. These results showed that TPC was not consistent with nitrite-scavenging and N-nitrosamine formation inhibitory capacities.

The main chemical components of CTF are essential oils, flavonoids, organic acids, polyphenols, terpenoids, saponins, steroids and other chemical components. [26] There are a number of compounds have been identified from CTF, such as kaempferol-3-O-D-glycoside, quercetin-3-O-glycoside, quercetin-3-O-rutinoside, 3,4',5,6,7-pentahydroxyflavanone-O-hexoside, chlorogenic acid, flavanomarein, flavanokanin, quercetagitin-7-O-glucoside, 3,4',5,6,7-pentahydroxyflavanone, 3',5,5',7-tetrahydroxy-flavanone-O-hexoside, marein, 3',5,5',7-tetrahydroxy flavanone, okanin, dicaffeoylquinic acid, and coreopsin. These aglycones and glycosides of flavanone/chalcone are different types of flavonoids, and they are known for their antioxidant and free RSA.

4. Conclusions

The results presented in this study should be considered as the first report on the nitrite-scavenging, N-nitrosamine formation inhibitory activity of CTF extracts.

In vitro studies demonstrated that EAE and BE of CTF from Kunlun Mountains possessed significant free radical-scavenging properties, as well as certain nitrite-scavenging and N-nitrosamine formation inhibitory activities. Therefore, we propose that CTF can be used as a rich source of natural free radical and nitrite scavengers. Additional studies are required for the analyses of chemical compositions in these extracts.

Acknowledgements

This study was supported by the National Mega Project on Major Drug Development project for the construction of incubator-based innovative drugs from natural sources in Xinjiang (2011ZX09401-007), by the key areas of the science and technology research projects in Xinjiang Production and Construction Corps (2014BA031), and through the high-level talents of the scientific research foundation of Shihezi University (RCZX201328). The

authors are grateful to Vice Prof. Dr. Chengzhi Gu, School of chemistry and chemical engineering, Shihezi University, China for his help to assist our entire work.

Declaration of Interest

The authors report no declarations of interest.

References

[1] Kris-Etherton P, Lefevre M, Beecher G, Gross M, Keen C, Etherton T. Bioactive compounds in nutrition and health-research methodologies for establishing biological function: the antioxidant and antiinflammatory effects of flavonoids on atherosclerosis. *Annu Rev Nutr*, 2004 ; 24, 511-538.

[2] Gordon M. Significance of dietary antioxidants for health. *Int J Mol Sci*, 2011;13, 173-179.

[3] Lee S, Kim S, Jeong S, Park J. Effect of far-infrared irradiation on catechins and nitrite scavenging activity of green tea. *J Agr Food Chem*, 2006; 54(2), 399-403.

[4] Rundlöf T, Olsson E, Wiernik A, Back S, Aune M, Johansson L, Wahlberg I. Potential nitrite scavengers as inhibitors of the formation of N-nitrosamines in solution and tobacco matrix systems. *J Agr food Chem*, 2000; 48(9), 4381-4388.

[5] Ban XQ, Huang B, He JS, Chen YX, Zeng H, Han L, Wang YW. In vitro and in vivo antioxidant properties of extracts from *Coptis chinensis* Inflorescence. *Plant food hum nutr*, 2011; 66, 175-180.

[6] Kola OA, Janet JO, Ebenezer TB, Ogboma UJ. Antioxidant activities, total flavonoid and total phenolic contents of whole plant of *Kyllinga Erecta Shumach*. *J Food Nutr Res*, 2015; 3(8): 489-494.

[7] Smith E, Parker H. A biosystematic study of *Coreopsis tinctoria* and *Coreopsis cardaminefolia* (Compositae). *Brittonia*, 1971; 23, 161-170.

[8] Dias T, Mota-Filipe H, Liu B, Jones P, Houghton P, Paulo A. Recovery of oral glucose tolerance by Wistar rats after treatment with *Coreopsis tinctoria* infusion. *Phytother Res*, 2010; 24, 699–705.

[9] Li Y, Chen X, Xue J, Liu J, Chen X, Wulasihan M. Flavonoids furom *Coreopsis tinctoria* adjust lipid metabolism in hyperlipidemia animals by down-regulating adipose differentiation-related protein. *Lipids Health Dis*, 2014, 13, 193-200.

[10] Jiang BP, Le L, Wan WT, Zhai W, Hu KP, Xu LJ, Xiao PG. The flower tea *coreopsis tinctoria* increases insulin sensitivity and regulates hepatic metabolism in rats fed a high-fat diet. *Endocrinology*, 2015; 156(6), 2006-2018.

[11] Commissione Redactorum Florae Xinjingensis. Flora Xinjiangensis, Xinjiang Science & Technology & Hygiene Publishing House, Urumqi, Xinjiang. 1999, 93.

[12] Lan S, Lin J, Zheng N. Evaluation of the antioxidant activity of *Coreopsis Tinctoria* Nuff. and optimisation of isolation by response surface methodology. *Acta Pharm*, 2014; 64 369–378.

[13] Liang S., Ha M., Pang S., et al. Experimental study on hypertensive effects of extracts of Coreopsis basalis. *Lishizhen Med Mater Med Res*, 2010, 21: 1619-1621.

[14] Mao X., Han X., Lu W., et al. Effect of the ethyl acetate extract from Coreopsis tinctoria nutt. on fasting blood levels and blood lipids in diabetes mice. *Pharmacol Clin Chinese Mater Med*, 2014, 30(2): 78-82.

[15] Sun Y, Zhao J, Jin HT, Cao Y, Ming T, Zhang L. Vasorelaxant effects of the extracts and some flavonoids from the buds of *Coreopsis tinctoria. Pharm Biol*, 2013; 51, 1158-1164.

[16] Yao XC, Zhang T, Shi R，Wang B, Chen W. Study on the determination method of total flavonoids in *Coreopsis tinctoria* Nutt. from Xinjiang. *J Shihezi Univ (Natural Science)*, 2015; 33(2), 183-187.

[17] Yao XC, Gu CZ, Tian L, Wang X, Tang H. Comparative study on the antioxidant activities of extracts of *Coreopsis tinctoria* flowering tops from Kunlun Mountains, Xinjiang, north-western China. *Nat Prod Res*, 2015.

[18] Yao XC, Zhu L, Chen YX, Tian J, Wang YW. In vivo and in vitro antioxidant activity and α-glucosidase, α-amylase inhibitory effects of flavonoids from *Cichorium glandulosum* seeds. *Food Chem*, 2013; 139(1), 59-66.

[19] Choi S, Chung M, Lee S, Shin J, Sung N. N-nitrosamine inhibition by strawberry, garlic, kale, and the effects of nitrite-scavenging and N-nitrosamine formation by functional compounds in strawberry and garlic. *Food Control*, 2007; 18, 485-491.

[20] Choi S, Chung M, Sung N. Volatile N-nitrosamine inhibition after intake Korean green tea and *Maesil* (*Prunus mume* SIEB. et ZACC.) extracts with an amine-rich diet in subjects ingesting nitrate. *Food Chem Toxicol*, 2002; 40(7), 949-957.

[21] Woo J, Jeong H, Chang Y, Shin S, Lee C. Antioxidant activities of fractions obtained from flowers of *Coreopsis tinctoria* Nutt. *Korean J Hortic Sci*, 2010; 28, 115-119.

[22] Deighton N, Brennan R, Finn C, Davies H. Antioxidant properties of domesticated and wild Rubus species. *J Sci Food Agr*, 2000; 80(9), 1307-1313.

[23] Zalaru C, Crisan CC, Calinescu I, Moldovan Z, Tarcomnicu I, Litescu, S.C., Tatia, R., Moldovan, L., Boda, D.and Iovu, M. Polyphenols in *Coreopsis tinctoria* Nutt. fruits and the plant extracts antioxidant capacity evaluation. *Cent Eur J Chem*, 2014; 12(8), 858-867.

[24] Hwang I, Kim H, Shin S, Lee C, Lee J, Jang K, Jeong H. Biological activities of *Coreopsis tinctoria* Nutt. flower extracts. *Korean J Hortic Sci*, 2010; 28, 857-863.

[25] Sun J, He X, Zhao M, Li L, Li C, Dong Y. Antioxidant and Nitrite-Scavenging Capacities of Phenolic Compounds from sugarcane (*Saccharum officinarum* L.) tops. *Molecules*, 2014; 19(9), 13147-13160.

[26] Crisan CC, Buleandra M, Calinescu I., Zalaru C, David IG, Badea I A. Chemical Composition of the Aerial Part and Fruits of *Coreopsis tinctoria. Chem Nat Comp*, 2015; 51(3), 571-572.