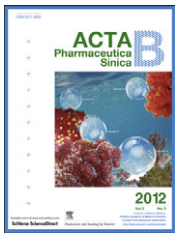
Acta Pharmaceutica Sinica B 2012;2(3):246–249

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

Free radical scavenging activity of Eagle tea and their flavonoids

Qiong Meng[a,b], Zhengming Qian[a,b], Xiaoxiang Li[c], Deqiang Li[a,b], Weihua Huang[a,b], Jing Zhao[a,b,*], Shaoping Li[a,b,*]

[a]*State Key Laboratory of Quality Research in Chinese Medicine, University of Macau, Macao SAR, China*
[b]*Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China*
[c]*Anhui New Star Pharmaceutical Development Co., Ltd., Hefei 230000 China*

Received 19 December 2011; revised 20 January 2012; accepted 23 February 2012

KEY WORDS

ABTS;
Eagle tea;
Flavonoids;
Free radical scavenging;
HPLC

Abstract In this study, an online HPLC-DAD-MS coupled with 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) assay was employed for evaluating free radical scavenging activity of Eagle tea and their active components. Twenty-three chromatographic peaks were detected, and nineteen components had free radical scavenging activity. Among them, eight compounds were identified as flavonoids (hyperin, isoquercitrin, quercitrin, quercetin, kaempferol), catechins, chlorogenic acid and epicatechin based on MS data and standard chromatographic characters.

*Corresponding authors. Tel.: +853 8397 4692; fax: +853 2884 1358.
E-mail addresses: JINGZHAO@UMAC.MO (Jing Zhao), SPLI@UMAC.MO (Shaoping Li).

Peer review under the responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.
http://dx.doi.org/10.1016/j.apsb.2012.02.012

1. Introduction

Eagle tea, derived from the leaves of *Litsea coreana* Levl. var. *lanuginosa* (Migo) Yang et. P. H. Huang (family Lauraceae), has been used for relief from heatstroke, detoxification and detumescence for hundreds years[1]. Previous studies showed that Eagle tea contained various kinds of compounds such as flavonoids, polyphenols, essential oil, saponins, amino acid, multi-vitamins and multi-minerals[2–4]. Flavonoids, which possess multiple pharmacological activities such as anticancer[5], preventing adjuvant-induced arthritis[6] and hepatic steatosis[7], anti-inflammation[8], immuno-regulation[9], and antioxidation[10], are believed to be the major bioactive compounds in Eagle tea. Actually, most pharmacological activities of Eagle tea are related to its scavenging oxygen free radicals or reactive oxygen species[11], which indicates that antioxidant activity of Eagle tea is crucial to its health benefits. Therefore, investigation of free radical scavenging activity of Eagle tea and their flavonoids is very important for its quality control. Unfortunately, the antioxidant activities of most flavonoids in Eagle tea were unknown, though three flavonoids such as astragalin (kaempferol-3-*O*-*β*-*D*-glucopyranoside), isoquercitrin (quercetin-3-*O*-*β*-*D*-glucopyranoside) and kaempferol were investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay[12].

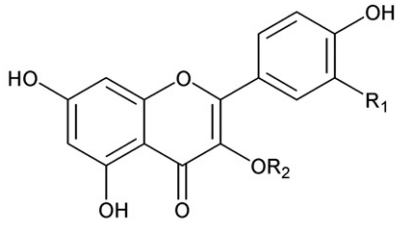
In present study, the antioxidant activities of Eagle tea was investigated by using HPLC coupled with ABTS assay, and the active flavonoids were also elucidated.

2. Materials and methods

2.1. Chemicals, reagents and materials

HPLC grade methanol and ethanol were purchased from Merck (Darmstadt, Germany). ABTS was purchased from International Laboratory (San Bruno, CA). Potassium persulfate was from Fluka (Seelze, Germany). Deionized water was prepared using a Millipore Milli Q-Plus system (Millipore, Billerica, MA).

Quercetin, kaempferol, chlorogenic acid and epicatechin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); hyperin, isoquercitrin, kaempferol-3-*O*-*β*-*D*-galactopyranoside, quercitrin and astragalin were isolated from Eagle tea in our laboratory. All purities were more than 95% (determined by HPLC), and their structures (Fig. 1) were confirmed by comparing their UV, MS, ^{1}H NMR and ^{13}C NMR data with literatures[13–19]. Sample of Eagle tea was collected from Ningguo (S1) of Anhui Province, China.



R_1 = -OH, R_2 = -*β*-*D*-gal: Hyperin
R_1 = -OH, R_2 = -*β*-*D*-glc: Isoquercitrin
R_1 = -H, R_2 = -*β*-*D*-gal: Kaempferol-3-*O*-*β*-*D*-galactopyranoside
R_1 = -OH, R_2 = -*α*-*L*-rha: Quercitrin
R_1 = -H, R_2 = -*β*-*D*-glc: Astragalin
R_1 = -OH, R_2 = -H: Quercetin
R_1 = -H, R_2 = -H: Kaempferol
gal = galactopyranoside, glc = glucose, rha = rhamnose

Figure 1 Chemical structures of seven flavonoids.

2.2. Sample preparation

Sample preparation was performed using PLE on a Dionex ASE 200 system (Dionex, Sunnyvale, CA, USA) under optimized conditions. In brief, dried powder of Eagle tea (1.0 g) was mixed with diatomaceous earth in the ratio of 1:1, and placed into an 11 mL stainless steel extraction cell, respectively. The extraction cell was extracted under the optimum conditions: solvent, 80% methanol aqueous solution; particle size, 0.15–0.20 mm; temperature, 120 °C; static extraction time, 5 min; pressure 1,500 psi; flush volume, 40%; static cycle, 1, and number of extraction, 1. Then the extract was transferred into a 50 mL volumetric flask which was made up to its volume with extraction solvent and filtered through a 0.22 μm nylon membrane filter (Whatman, Maidstone, UK) prior to injection into the HPLC system.

2.3. Free radical scavenging activity of investigated flavonoids

HPLC and ABTS-based assay was used to evaluate the free radical scavenging activity of Eagle tea and their flavonoids according to our previous reports[19,20]. In brief, the separation was achieved on a Zorbax SB C18 column (250 mm × 4.6 mm i.d., 5 μm) with gradient elution of water (A) and methanol (B) at a flow rate of 1.0 mL/min: 0.0–10.0 min, 20% B; 10.0–20.0 min, 20–35% B; 20.0–40.0 min, 35% B; 40.0–50.0 min, 35–45% B; 50.0–60.0 min, 45% B; 60.0–75.0 min, 45–60% B; 75.0–80.0 min, 60% B. The detection wavelength was set at 270 nm and injection volume was 10 μL. Mass spectrometry was carried out in the positive scan mode from *m*/*z* 50–1400. ESI-MS conditions were as follows: drying gas, N_2, 7 L/min; temperature, 350 °C; nebulizer pressure, 35 psi. ESI-MS/MS conditions: isolation width 4, fragment amplification 1.5. The flow rate of $ABTS^+$ solution was set at 0.5 mL/min, and any bleaching of the initial color was detected as a negative peak at 750 nm.

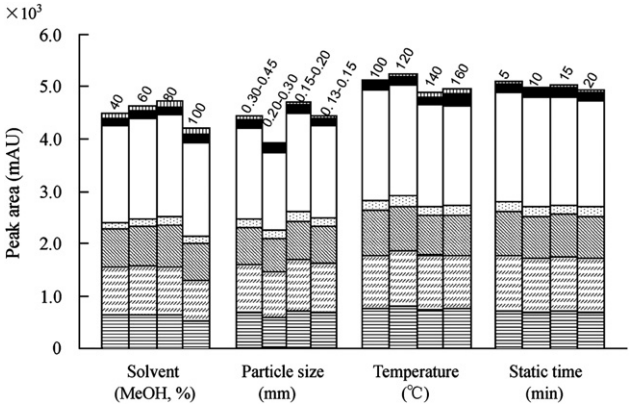


Figure 2 Effects of (A) solvent type, (B) particle size, (C) temperature and (D) static time on PLE extraction efficiency of investigated flavonoids from Eagle tea collected from Ningguo of Anhui Province, China. To determine one of the parameters, the others were set at the default (temperature, 100 °C; static extraction time, 5 min; solvent, 100% methanol; particle size, 0.30–0.45 mm). ☰ hyperin; ▨ isoquercitrin; ▨ kaempferol-3-*O*-*β*-*D*-galactopyranoside; ⁝⁝ quercitrin; □ astragalin; ■ quercetin; ||| kaempferol.

3. Results and discussion

3.1. Optimization of PLE

For the extraction of flavonoids from Eagle tea by PLE, the parameters such as solvent (40%, 60%, 80%, 100% methanol aqueous solution), particle size (0.3–0.45, 0.20–0.30, 0.15–0.20 and 0.13–0.15 mm), extraction temperature (100, 120, 140 and 160 °C), and static time (5, 10, 15 and 20 min) were studied by using univariate approach while other conditions were kept default (pressure, 1,500 psi; flush volume, 40% and one extraction cycle). The seven investigated compounds were used as the markers for evaluation of extraction efficiency based on sample S1. The results of optimization were shown in Fig. 2. The exhausted extraction for the PLE procedure was determined by performing consecutive pressurized liquid extractions on the same sample under the optimized PLE conditions, until no investigated compounds were detected by the analysis. The exhausted extraction was calculated based on the total extracted amount of the investigated components during the consecutive extractions, and the rate of the first time extraction was 99.3%. Finally, the optimum conditions for PLE were obtained (Section 2.2).

3.2. Validation of method

The repeatability of the developed method was evaluated at three levels (0.8, 1.0 and 1.2 g) of the sample S1. The samples of each level were extracted and analyzed in triplicates as mentioned above. The repeatability present as RSD ($n=3$), which was less than 4.39%, 1.46% and 3.84% for the three levels (low, medium and high) of test sample, respectively.

3.3. Evaluation of free radical scavenging activity of Eagle tea and investigated flavonoids

HPLC-DAD-MS and ABTS assays were employed to investigate and evaluate the free radical scavenging activity of seven flavonoids and other potential compounds with antioxidation in Eagle

Table 1 MS and UV data of identified compounds in Eagle tea.

Peak No.	MS (m/z)	UV λ_{max} (nm)	Identification
1	465 $[M+H]^+$, 303 $[M+H\text{-gal}]^+$	255, 355	Hyperin
2	465 $[M+H]^+$, 303 $[M+H\text{-glc}]^+$	255, 355	Isoquercitrin
3	449 $[M+H]^+$, 287 $[M+H\text{-gal}]^+$	265, 350	Kaempferol-3-*O*-*β*-*D*-galactopyranoside
4	449 $[M+H]^+$, 303 $[M+H\text{-rha}]^+$	255, 355	Quercitrin
5	449 $[M+H]^+$, 287 $[M+H\text{-glc}]^+$	265, 350	Astragalin
6	303 $[M+H]^+$	255, 355	Quercetin
7	287 $[M+H]^+$	265, 365	Kaempferol
12	291 $[M+H]^+$	270	Catechins
14	355 $[M+H]^+$	240, 330	Chlorogenic acid
16	291 $[M+H]^+$	280	Epicatechin

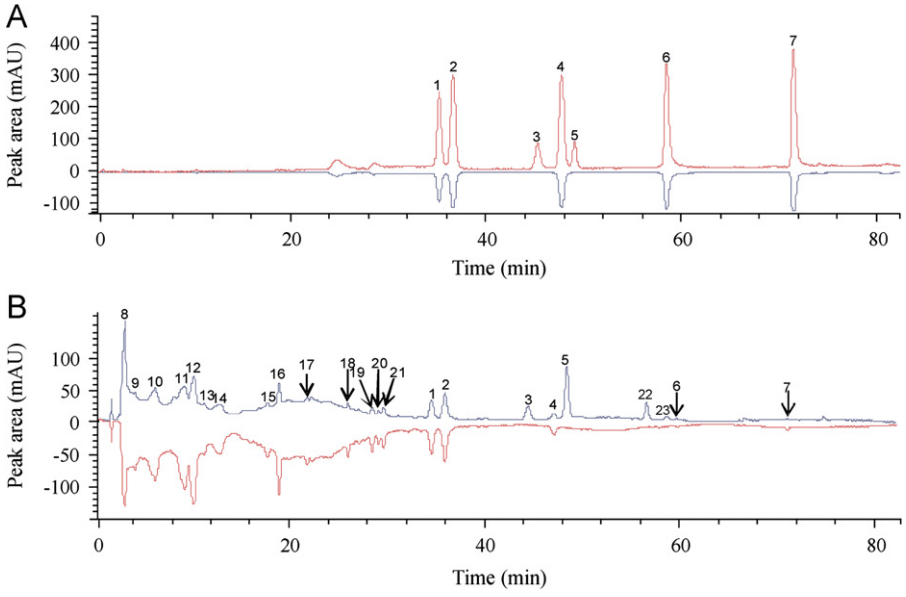


Figure 3 Typical UV-chromatograms and ABTS assay profiles (free radicals scavenger detected as negative) of (A) mixed standards and (B) Eagle tea collected from Ningguo, Anhui, China. 1, hyperin; 2, isoquercitrin; 3, kaempferol-3-*O*-*β*-*D*-galactopyranoside; 4, quercitrin; 5, astragalin; 6, quercetin; 7, kaempferol; 12, catechin; 14, chlorogenic acid; 16, epicatechin. Peaks **8**, **9**, **10**, **11**, **13**, **15**, **17**, **18**, **19**, **20**, **21**, **22** and **23**, unknown.

tea. In order to identify more components, the detect wavelength was set at 270 nm. As a result, twenty-three peaks were detected in Eagle tea (S1), and ten of them were identified as hyperin (**1**), isoquercitrin (**2**), kaempferol-3-*O*-*β*-*D*-galactopyranoside (**3**), quercitrin (**4**), astragalin (**5**), quercetin (**6**) and kaempferol (**7**), catechin (**12**), chlorogenic acid (**14**), epicatechin (**16**) by comparing their MS and UV data (Table 1) with standard compounds and/or references[13–19,21]. ABTS assay showed that 5 out of 7 investigated flavonoids had free radical scavenging activity (Fig. 3). But two compounds of kaempferol-3-glycoside (**3** and **5**) had no free radical scavenging action due to their 3-glycoside rather than 3-OH[22]. It is also considered that quercetin-3-glycoside has higher antioxidant activity than kaempferol-3-glycoside, which attributes to 3′,4′-catechol structure in the B-ring[23,24]. In addition, catechin (**12**), chlorogenic acid (**14**), epicatechin (**16**) and other compounds of 11 peaks also processed the antioxidant activity, but further study is necessary to elucidate these components.

In previous study, three components (kaempferol, isoquercitrin and astragalin) were proven as antioxidant in Eagle tea[15]. In present study, nineteen peaks were found to possess antioxidant activity in Eagle tea and eight of them were identified as hyperin (**1**), isoquercitrin (**2**), quercitrin (**4**), quercetin (**6**). kaempferol (**7**), catechin (**12**), chlorogenic acid (**14**) and epicatechin (**16**). This research is helpful to elucidate the antioxidant active components in Eagle tea.

4. Conclusions

Free radical scavenging activity of Eagle tea was investigated and 5 flavonoids, including hyperin, isoquercitrin, quercitrin, quercetin and kaempferol, were found having free radical scavenging activity. Besides, a lot of other compounds, such as catechin, chlorogenic acid, epicatechin, in Eagle tea also contributed to its antioxidant activity, which should be further studied.

Acknowledgments

The research was supported by a grant from University of Macau (UL015/09-Y3 to Shaoping Li).

References

1. Li J, Zhang J. Advances in studies of *Listea coreana* Levl. *Var*. *J Sichuan Agric Univ* 2005;**23**:247–52.
2. Yu JP, Gu LQ. The chemical constituents of Laoying tea from Guizhou. *J Plant Res Environ* 2001;**10**:61–2.
3. Yu JP, Gu LQ, Ren SX. The essential oil of Laoying tea from Guizhou. *Food Sci* 2001;**22**:63–4.
4. Lu WL, Li J, Zhou WC, Cheng WM, Tang WJ. The optimization of the extracting method of total saponins from *Litsea coreana* through orthogonal test. *Lishizhen Med Mater Med Res* 2009;**20**:1154–5.
5. Zhao X, Shao LN, Zheng YF. Antimutagenic and *in vitro* anticancer effect of *Litsea coreana* Levl var. *lanuginose*. *Food Eng* 2008;**4**:29–31.
6. Wang TY, Li J, Ge JF, Li CY, Jin R, Lv XW, et al. Preliminary study of total flavonoids from *Litsea coreana* Levl. on experimental adjuvant-induced arthritis in rats. *Am J Chin Med* 2008;**36**:899–912.
7. Wang JQ, Li J, Zou YH, Cheng WM, Lu C, Zhang L, et al. Preventive effects of total flavonoids of *Litsea coreana* leve on hepatic steatosis in rats fed with high fat diet. *J Ethnopharmacol* 2009;**121**:54–60.
8. Chen L, Cheng WM, Hu CM, Jing Y, Li R, Li J. Study on anti-inflammatory effects of total flavonoids of *Litsea coreana* Levl *Var*. *Acta Univ Med Anhui* 2004;**39**:439–42.
9. Hu CM, Chen L, Li R, Cheng WM, Li J. Immunomodulatory property of total flavonoids from *Litsea coreana leveille* on immunosuppressive mice. *Chin Pharmacol Bull* 2007;**23**:804–8.
10. Ye H, Yu JP. The preliminary studies on antioxidation of three kinds of flavonoids from *Litsea coreana*. *J Chin Med Mater* 2004;**27**:113–6.
11. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007;**39**:44–84.
12. Ye H, Yu JP, Wang JG. The study of antioxidation extractives from *Litsea coreana*. *Lishizhen Med Mater Med Res* 2004;**15**:198–9.
13. Chen YP, Cheng WM, Li J. Analyse on the chemical constituents from flavonoids of *Litsea coreana* L. *Acta Univ Med Anhui* 2008;**43**:65–7.
14. Kim SY, Gao JJ, Kang HK. Two flavonoids from the leaves of *Morus alba* induce differentiation of the human promyelocytic leukemia (HL-60) cell line. *Biol Pharm Bull* 2000;**23**:451–5.
15. Liao LP, Li P. Compounds from leaf of *Ilex purpurea Hassk*. *J China Pharm Univ* 2004;**35**:205–6.
16. Li Y, Guo SX, Wang CL, Yang JS, Xiao PG. Studies on flavonoids of *Saussurea involucrata*. *Chin Pharm J* 2007;**142**:575–7.
17. Yu JP, Ye H, Gu LQ. The flavoniods of *Litsea coreana*. *Acta Sci Nat Univ Sunyatseni* 2001;**40**:110–1.
18. Li B, Zhu JF, Zou ZJ, Yin YQ, Shen ZB. Studies on the chemical constituents of *Dryopteris fragrans*. *J Chin Med Mater* 2009;**32**:1232–3.
19. Qian ZM, Guan J, Yang FQ, Li SP. Identification and quantification of free radical scavengers in Pu-erh tea by HPLC-DAD-MS coupled online with 2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid) diammonium salt assay. *J Agric Food Chem* 2008;**56**:11187–91.
20. Li SY, Yu Y, Li SP. Identification of antioxidants in essential oil of radix *Angelicae sinensis* using HPLC coupled with DAD-MS and ABTS-based assay. *J Agric Food Chem* 2007;**55**:3358–62.
21. Xu HT, Xu YJ. Simultaneous determination of three phenolic acid in Pugongying Granules by high performance liquid chromatography-mass spectrometry. *Chemistry* 2008;**71**:415–9.
22. Lu X, Wang L, Wei H, Yang ZQ, Wang W. Structure–activity relationship of flavonoids in antioxidant activity. *Food Sci* 2006;**27**:233–7.
23. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. *J Nutr Biochem* 2002;**13**:572–84.
24. Bansal P, Paul P, Nayak PG, Pannakal ST, Zou JH, Laatsch H, et al. Phenolic compounds isolated from *Pilea microphylla* prevent radiation-induced cellular DNA damage. *Acta Pharm Sin B* 2011;**1**:226–35.