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Simultaneous determination of phytochemical constituents in *Paeonia lactiflora* extracts using the HPLC-UV method

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Abstract Qantitative analysis of six compounds: (+)-catechin, benzoic acid, gallic acid methyl ester, paeonol, paeoniflorin, and albiflorin from *Paeonia lactiflora* extracts was performed using high-performance liquid chromatography and an ultraviolet (UV) detector, following different extraction methods. A reverse-phase column was used in a gradient elution system, and UV detection was performed at 280 nm. The results showed that the quantity of paeoniflorin was the highest in ethanol and water extracts (73.89 and 57.87 mg/g, respectively) among the six compounds. This study contributes a good analysis method for the contents of *P. lactiflora* and would be propitious for developing medicines and functional foods.

Keywords Albiflorin · Benzoic acid · (+)-Catechin · Gallic acid methyl ester · *Paeonia lactiflora* · Paeoniflorin ·Paeonol · Quantitative analysis

Introduction

Paeonia lactiflora (PL), belonging to the family Paeoniaceae, is a species of herbaceous perennial flowering plants, and its origin is in central and eastern Asia, from eastern Tibet, across northern China to eastern Siberia. In particular, the roots of PL were traditionally consumed as oriental medicine [1]. PL has been used to treat dysmenorrhea, amenorrhea, and spasm [2,3]. In addition, PL is well known for its vasodilatory [4], anti-hyperlipidemic [5], anti-oxidant, and anti-bacterial effects [6].

PL has a variety of bioactive components, such as monoterpenes [7,8], triterpenes [9,10], volatile oils [11], tannins [12], stilbenes [13], flavonoids [14], and polyphenols [15,16]. Among them, paeoniflorin and albiflorin are the primary components of PL [17]. Paeoniflorin has been reported to have anti-inflammatory, immunomodulatory [18], spasmolytic [19], and hypoglycemic activities [20]. Albiflorin is effective for the treatment of inflammation [21], neuropathic pain [22], osteoporosis [23], and depression [24].

In this study, we aimed to quantify the phytochemical constituents in PL using a high-performance liquid chromatography (HPLC)-ultraviolet (UV) detector and compare the quantity of each compound in the extract after different extraction methods.

Materials and Methods

Plant materials

The ethanolic (EtOH) (3-19-0091) and water (3-19-0048) extracts of PL were provided by Korea Institute of Oriental Medicine, Daejeon, Korea.

Chemicals and apparatus

Chromatographic analysis was performed using HPLC system (Agilent technology 1290 Infinity II, Santa Clara, CA, USA) equipped with a pump, an auto-sampler, and a UV detector. Solvents used for HPLC (water and acetonitrile) were HPLC grade and purchased from J. T. Baker (Avantor, Radnor, PA, USA). Acetic acid (99.7%) was purchased from Samchun Pure Chemicals (Pyeongtaek, Korea). Six compounds: (+)-catechin, benzoic acid, gallic acid methyl ester, paeonol, paeoniflorin, and

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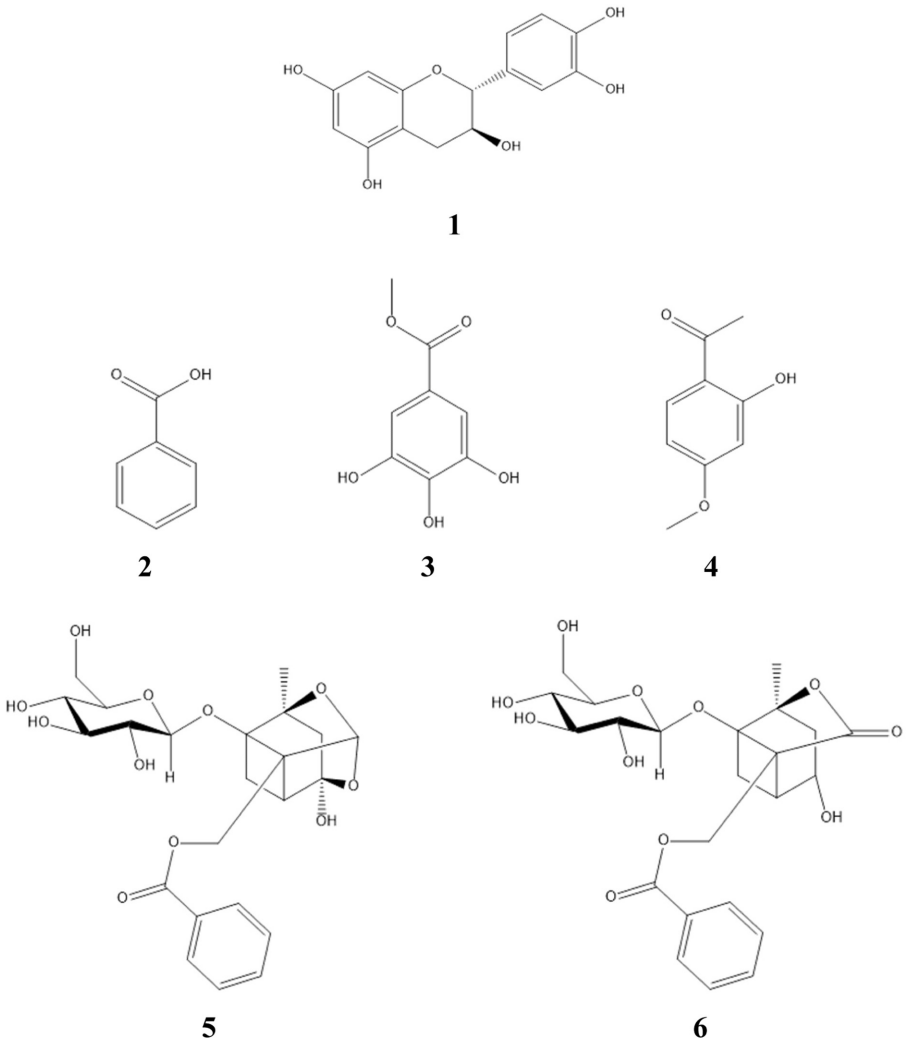


Fig. 1 Chemical structures of the six compounds [(+)-catechin (**1**), benzoic acid (**2**), gallic acid methyl ester (**3**), paeonol (**4**), paeoniflorin (**5**), and albiflorin (**6**)] in PL

albiflorin (Fig. 1) were obtained from Natural Product Institute of Science and Technology (www.nist.re.kr), Anseong, Korea.

Sample extraction methods

Ethanolic [70% EtOH (4 L)] extraction of dried and crushed PL (1 kg) was performed under sonication for 1 h; the extraction process was run two times. Subsequently, the samples were filtered, evaporated at 37 °C, freeze-dried, and homogenized using a 600-μm sieve to obtain EtOH extract of PL (EEP). Water (4 L) extraction of dried and crushed PL (1 kg) was performed for 3 h under reflux conditions (100 °C). After extraction, the samples were filtered using a 53-μm sieve, evaporated at 37 °C, and homogenized using a 600-μm sieve to obtain water extract of PL (WEP). The homogenized powders were stored in a tight-sealed bottle and kept in a refrigerator away from light until analysis.

Preparation of samples for HPLC

EEP and WEP (1 mg each) were dissolved separately in methanol (MeOH) under sonication for 20 min and filtered using a PVDF Membrane filter of 0.45-μm pore size. These were used as the experimental stock solutions. One milligram of each of the six compounds was dissolved seperately in MeOH under sonication for 20 min and filtered using a 0.45-μm PVDF membrane filter. These were used as the standard solutions.

HPLC conditions

Quantitative analyses were performed using a reverse-phase HPLC system with an INNO C18 column (250 mm×4.6 mm, 5 μm). UV detection was at 280 nm, and the temperature of the column was maintained at room temperature. The injection volume was 10 μL, and the flow rate was set to 1 mL/min. The

Table 1 Data from the calibration curves of the six compounds [(+)-catechin (**1**), benzoic acid (**2**), gallic acid methyl ester (**3**), paeonol (**4**), paeoniflorin (**5**), and albiflorin (**6**)]

Compound	t_R	Calibration equation[a]	Correlation coefficient, r^2 [b]
1	16.313	Y =3.2366X+39.55	0.9989
3	16.525	Y =37.871X+27.792	0.9992
6	20.473	Y =0.6301X+44.929	0.9987
5	22.099	Y =0.8803X+8.6333	0.9999
2	30.719	Y =4.8757X+105.49	0.9996
4	36.643	Y =64.79X+3.4631	0.9998

[a] Y = peak area, X = concentration of the standards (mg/mL)
[b] r^2 = correlation coefficient based on five data points in the calibration curves

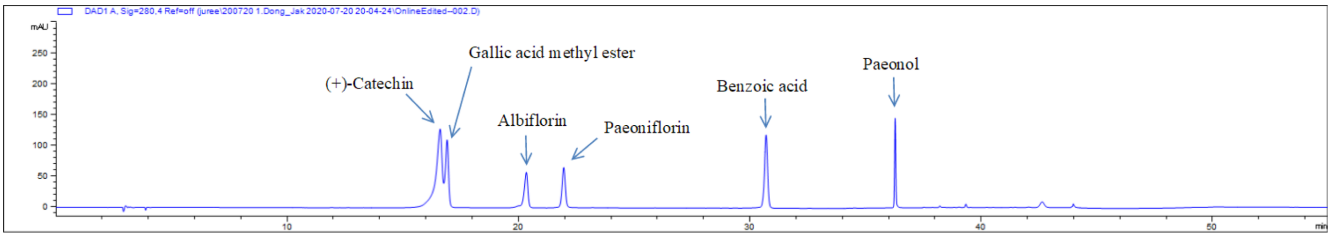


Fig. 2 HPLC chromatogram of the six compounds

mobile phase of the gradient elution system consisted of 0.5% acetic acid in water (A) and acetonitrile (B). The composition of the gradient elution system was as follows: 95% A at 0 min, 75% A at 25 min, 60% A at 30 min, 100% B at 35 min, 100% B at 40 min, 95% A at 45 min, and 95% A at 55 min.

Calibration curves

The standard stock solutions were prepared by dissolving the compounds in MeOH (1 mg/mL). The working solutions, which were prepared by serially diluting the stock solutions, were used to construct the calibration curves. The calibration functions of the standards were calculated using the peak area (Y), concentration (X, mg/mL), and mean ± standard deviation (n =3).

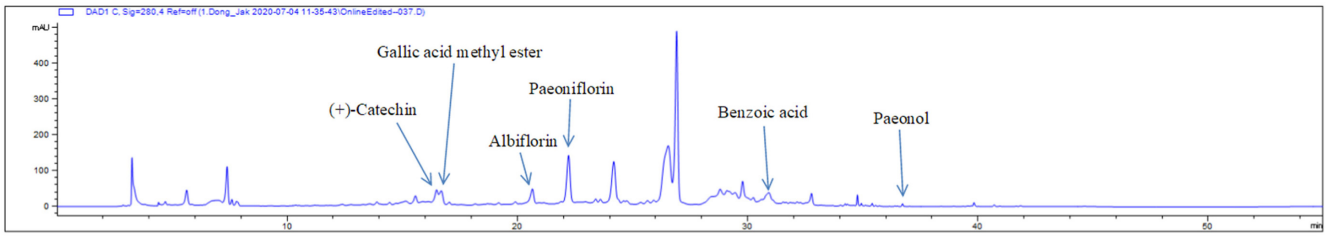
Results and Discussion

PL has anti-oxidant [25], anti-diabetic [26], anti-microbial [27], and anti-hyperlipidemic effects [28]. A recent study revealed the skin-depigmenting potential of PL in hyperpigmentation disorders [29]. It has a variety of bioactive components, such as paeoniflorin, albiflorin, benzoic acid, oleanolic acid, hederagenin, and oxypaeoniflorin [16]. Among them, paeoniflorin and albiflorin are the primary constituents of PL [17].

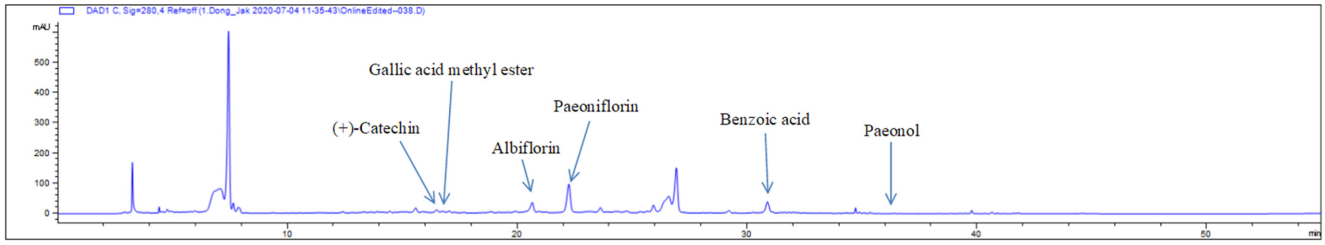
Quantitative analysis of the six representative compounds in EEP and WEP was performed using HPLC with a reverse phase column and gradient elution of solvents A and B in the mobile phase. The HPLC method showed good separation, and a wavelength of 280 nm was found to be effective for detection. The data from the calibration curves of the standards are shown in Table 1. The calibration curves were constructed by linearly plotting the peak area against the prepared concentrations of the standard solutions and were analyzed using linear regression. The linear regression coefficients (r^2) for the standards were between 0.9987 and 0.9999.

Chromatograms of the standard solutions of the six compounds are shown in Fig. 2. Chromatographic peaks of (+)-catechin, gallic acid methyl ester, albiflorin, paeoniflorin, benzoic acid, and paeonol showed good separation in EEP and WEP (Fig. 3). Table 2 shows the quantity of the six compounds in EEP and WEP. The results showed that the quantity of the six compounds was generally higher in EEP than in WEP. However, considering the dry weights, the quantity of albiflorin, paeoniflorin, and benzoic acid was higher in WEP than in EEP. This seems to be due to the variation in yield according to the extraction method. The extraction yields of EEP and WEP were 16.63% (w/w) and 27.64% (w/w), respectively, which shows the extraction efficiency of water extraction method. Among the six compounds in PL, the quantity of paeoniflorin was remarkably high, followed by albiflorin, in both EEP and WEP.

Choung et al. found suitable conditions for analysis of paeoniflorin using different extraction methods and times [30]. They reported that reflux extraction produced sufficient yield in 1 h, whereas sonication extraction for 1 to 2 h yielded less quantity than reflux extraction. Additionally, there was no marked difference in yield even after 3 to 4 h of sonication extraction. Moreover, Kim et al. reported that, extraction using 70% EtOH was effective when extracting from dried powder of PL [31]. Another study showed that paeoniflorin was efficiently extracted with 70% MeOH or water [32], although not with pure EtOH or



(A)



(B)

Fig. 3 HPLC chromatograms of EEP (A) and WEP (B)

Table 2 Quantity of the six compounds [(+)-catechin (**1**), benzoic acid (**2**), gallic acid methyl ester (**3**), paeonol (**4**), paeoniflorin (**5**), and albiflorin (**6**)] in the EEP and WEP

Compound	EEP		WEP	
	(mg/g ext.)	(mg/g DW)	(mg/g ext.)	(mg/g DW)
1	1.58±0.01	0.26±0.00	0.37±0.05	0.10±0.01
3	0.21±0.01	0.03±0.00	trace	trace
6	30.03±0.21	4.98±0.03	23.14±0.17	6.39±0.05
5	73.89±0.76	12.27±0.13	57.87±0.20	15.97±0.06
2	3.45±0.10	0.57±0.02	2.96±0.02	0.82±0.01
4	0.02±0.00	trace	trace	trace

MeOH [33]. In this study, the quantity of paeoniflorin was higher in EEP than in WEP, which shows that the extraction using 70% EtOH is more suitable. Additionally, if reflux extraction had been used for the ethanolic extracion of PL, the extraction yield would have increased.

Paeoniflorin has anti-hyperlipidemic [28], anti-wrinkle [34], and anti-depressant effects [35]. Albiflorin has anti-inflammatory [36], hematopoietic [37], and anti-depressant [38] effects. We quantified a total of six phytochemical constituents of PL, including albiflorin and paeoniflorin, the major components of PL, using different extraction methods. In conclusion, this study provides a good analysis method for determining the contents of PL. These results would be beneficial in developing medicines and functional foods.

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References

1. Lee TB (1985) Colored Flora of Korea I. Hyangmoon Press, Seoul
2. Zhang W, Dai S (2012) Mechanisms involved in the therapeutic effects of *Paeonia lactiflora* Pallas in rheumatoid arthritis. Int Immunopharmacol 14: 27–31
3. Wu SH, Wu DG, Chen YW (2010) Chemical constituents and bioactivities of plants from the genus *Paeonia*. Chem Biodivers 7: 90–104
4. Goto H, Shimada Y, Akechi Y, Kohta K, Hattori M, Terasawa K (1996) Endothelium-dependent vasodilator effect of extract prepared from the roots of *Paeonia lactiflora* on isolated rat aorta. Planta Med 62: 436-439
5. Li J, Chen CX, Shen YH (2011) Effects of total glucosides from paeony (*Paeonia lactiflora* Pall) roots on experimental atherosclerosis in rats. J Ethnopharmacol 135: 469-475
6. Zhou JX, Braun MS, Wetterauer P, Wetterauer B, Wink M (2019) Antioxidant, cytotoxic, and antimicrobial activities of *Glycyrrhiza glabra* L., *Paeonia lactiflora* Pall., and *Eriobotrya japonica* (Thunb.) Lindl. extracts. Medicines (Basel) 6: 43
7. Braca A, Kiem PV, Yen PH, Nhiem NX, Quang TH, Cuong NX, Minh CV (2008) New monoterpene glycosides from *Paeonia lactiflora*. Fitoterapia 79: 117–120

8. Wang HB, Gu WF, Chu WJ, Zhang S, Tang XC, Qin GW (2009) Monoterpene glucosides from *Paeonia lactiflora*. J Nat Prod 72: 1321–1324
9. Kamiya K. Yoshioka K, Saiki Y, Ikuta A, Satake T (1997) Triterpenoids and flavonoids from *Paeonia lactiflora*. Phytochemistry 44: 141–144
10. Ikuta A, Kamiya K, Satake T, Saiki Y (1995) Triterpenoids from callus tissue cultures of *Paeonia* species. Phytochemistry 38: 1203–1207
11. Kumar N, Motto MG (1985) Volatile constituents of peony flowers. Phytochemistry 25: 250–253
12. Tanaka T, Fukumori M, Ochi T, Kouno I (2003) Paeonianins A−E, new dimeric and monomeric ellagitannins from the fruits of *Paeonia lactiflora*. J Nat Prod 66: 759–763
13. Kim HJ, Chang EJ, Bae SJ, Shim SM, Park HD, Rhee CH, Park JH, Choi SW (2002) Cytotoxic and antimutagenic stilbenes from seeds of *Paeonia lactiflora*. Arch Pharm Res 25: 293–299
14. Jia N, Shu QY, Wang LS, Du H, Xu YJ, Liu ZA (2008) Analysis of petal anthocyanins to investigate coloration mechanism in herbaceous peony cultivars. Sci Hortic 117: 167–173
15. Guo D, Ye G, Guo H (2006) A new phenolic glycoside from *Paeonia lactiflora*. Fitoterapia 77: 613–614
16. Kang SS, Kim JS, Yun-Choi HS, Han BH (1993) Phytochemical studies on Paeonia radix. Kor J Pharmacogn 24: 247–250
17. Choung MG, Kang KH (1997) Isolation and determination of paeoniflorin and albiflorin in Korea peony (*Paeonia lactiflora* Pall) root. Korean J Medicinal Crop Sci 5: 249–254
18. He DY, Dai SM (2011) Anti-inflammatory and immunomodulatory effects of *Paeonia lactiflora* Pall., a traditional Chinese herbal medicine. Front Pharmacol 2: 10
19. Abdel-Hafez AA, Meselhy MR, Nakamura N, Hattori M, Watanabe H, Murakami Y, El-Gendy MA, Mahfouz NM, Mohamed TA (1999) Anticonvulsant activity of paeonimetabolin-I adducts obtained by incubation of paeoniflorin and thiol compounds with *Lactobacillus brevis*. Biol Pharm Bull 22: 491–197
20. Hsu FL, Lai CW, Cheng JT (1997) Antihyperglycemic effects of paeoniflorin and 8-debenzoylpaeoniflorin, glucosides from the root of *Paeonia lactiflora*. Planta Med 63: 323–325
21. Wang QS, Gao T, Cui YL, Gao LN, Jiang HL (2014) Comparative studies of paeoniflorin and albiflorin from *Paeonia lactiflora* on anti-inflammatory activities. Pharm Biol 52: 1189–1195
22. Zhou J, Wang L, Wang J, Wang C, Yang Z, Wang C, Zhu Y, Zhang J (2016) Paeoniflorin and albiflorin attenuate neuropathic pain via MAPK pathway in chronic constriction injury rats. Evid Based Complement Alternat Med 2016: Article ID 8082753 (11 pages)
23. Suh KS, Choi EM, Lee YS, Kim YS (2013) Protective effect of albiflorin against oxidative-stress-mediated toxicity in osteoblast-like MC3T3-E1 cells. Fitoterapia 89: 33–41
24. Wang YL, Wang JX, Hu XX, Chen L, Qiu ZK, Zhao N, Yu ZD, Sun SZ, Xu YY, Guo Y, Liu C, Zhang YZ, Li YF, Yu CX (2016) Antidepressant-like effects of albiflorin extracted from Radix Paeoniae Alba. J Ethnopharmacol 179: 9–15
25. Heo JI, Kim JH, Lee JM, Kim SC, Park JB, Kim J, Lee JY (2013) Antioxidant activity and its mechanism of *Paeonia lactiflora* Pall extract. Nat Prod Sci 19: 49–53
26. Lee S, Ji S (2004) Effect of *Paeonia lactiflora* extracts on α-glucosidase. Nat Prod Sci 10: 223–227
27. Park KD, Cho SH (2010) Antimicrobial characteristics of *Paeonia lactiflora* Pall. extract tested against food-putrefactive microorganisms. Korean J Food Preserv 17: 706–711
28. Yang HO, Ko WK, Kim JY, Ro HS (2004) Paeoniflorin: an antihyperlipidemic agent from *Paeonia lactiflora*. Fitoterapia 75: 45–49
29. Qiu J, Chen M, Liu J, Huang X, Chen J, Zhou L, Ma J, Sextius P, Pena AM, Cai Z, Jeulin S (2016) The skin-depigmenting potential of *Paeonia lactiflora* root extract and paeoniflorin: in vitro evaluation using reconstructed pigmented human epidermis. Int J Cosmet Sci 38: 444–451
30. Choung MG, Kang KH (1994) Extraction methods and HPLC analysis conditions of paeoniflorin in peony, *Paeonia lactiflora* Pall. Korean J Crop Sci 39: 542–547
31. Kim TK, Kim KJ, Joo GJ, Rhee IK (1997) Changes of paeoniflorin content in peony roots by heat-treatmnt. Korean J Food Preserv 4: 69–75
32. Kim TK, Joo GJ, Chung JD, Rhee IK (1996) Analysis of the content of paeoniflorin in peony roots cultivated on kyeongbuk area. Agric Res Bull Kyungpook Natl Univ 14: 15–28
33. Asakawa N, Hattori T, Ueyama M, Shinoda A, Miyake Y (1979) Determination of paeoniflorin in Paeony extract by high performance liquid chromatography. Yakugaku Zasshi 99: 598–601
34. Cho WG, Kyung KY, Yu SM (2009) Stability of paeoniflorin used as anti-wrinkle agents in emulsions. J Korean Oil Chem Soc 26: 191–198
35. Qiu F, Zhong X, Mao Q, Huang Z (2013) The antidepressant-like effects of paeoniflorin in mouse models. Exp Ther Med 5: 1113–1116
36. Xu X, Liu H, Pan Y, Liu Z, Chen D, Zhang L, Yuang H (2020) Albiflorin attenuates inflammation and apoptosis by upregulating AMPK-mediated expression of CDX2 in a mouse model of ulcerative colitis. Trop J Pharm Res 19: 995–999
37. Zhu Y, Zhou J, Wang L, Yang Z, Zhang J (2015) Hematopoietic effects of paeoniflorin and albiflorin on radiotherapy and chemotherapy-induced anemia mice. Planta Med 81: 928
38. Wang J, Wan Y, Zheng N (2020) Albiflorin ameliorates depressive-like behaviors in mice induced by chronic unpredictable mild stress. Curr Topics Nutraceut Res 18: 102–107