Flavonoids and Terpenoids Present in Sarcotestae of *Ginkgo biloba* and Their Seasonal Changes

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Abstract : In order to utilize sarcotestae of *Ginkgo biloba* for medical purposes, their flavonoids and terpenoids were qualitatively and quantitatively analyzed. Flavonols such as kaempferol, quercetin and isorhamnetin present in leaves could not be detected in sarcotestae while anthocyanidins of delphinidin and cyanidin were found. Sarcotestae were noted to contain small amounts of ginkgolides A, B and C as terpenoids, as did also the leaves. The composition of ginkgolides in sarcotestae were varied during maturation whereas quantity of anthocyanidins was independent on harvest season.

Key words : *ginkgo biloba*, flavonoid, terpenoid, anthocyanidin, ginkgolide

1 Introduction

Since leaf extracts from *Ginkgo biloba* have specific physiological functions such as antitumor activity, antioxidant and neurotropic function etc., they are available as medicine and functional foods^(1,2). The *Ginkgo biloba* extracts are a complex mixture of flavonoids and several other natural products such as terpenoids, steroids and organic acids. In particular, flavonoids and terpenoids are major classes which contribute to pharmacological and therapeutic actions. Nuts of *Ginkgo biloba* are also used as food and medicine so that their nutritional and antinutritional components have been investigated³⁾. However, chemical compositions except for alkyl phenols (e.g. anacardic acids) which cause contact dermatitis⁴⁾ and inhibit some dehydrogenase activities^{5,6)}, especially flavonoids and terpenoids which seem to be contained in sarcotestae of *Ginkgo biloba* have not been studied yet since they are usually abolished after harvesting nuts.

In this study, flavonoids and terpenoids were qualitatively and quantitatively analyzed for sarcotestae of *Ginkgo biloba* in order to utilize it as medical materials. Moreover, seasonal variation in flavonoids and terpenoids present in sarcotestae was also investigated.

2 Experimental

2.1 Materials

Sarcotestae of *Ginkgo biloba* (variety : Kyuji) were harvested in Sobue-cho, Aichi, once a month from August to November, 1997, and then they were stored at -30°C until analysis.

Cyanidin, delphinidin, kaempferol, quercetin, and isorhamnetin were purchased as standard flavonoids (HPLC grade) from Extrasynthese S.A. Ginkgolides A, B, and C, and bilobalide were purchased as standard terpenoids (HPLC grade) from Sigma Chemical Co.

2.2 Analytical Methods

Flavonoids were extracted as aglycons by refluxing 1 g of each freeze-dried sarcotestae with 20 ml of 3% HCl-methanol solution for 1 h⁷⁾. The mixture was filtrated with a glass filter and then

concentrated by a rotary evaporator. The concentrates were prepared in 25 ml of 0.01% HCl-methanol solution. Flavonoid aglycons were qualified by TLC and HPLC. For the TLC analysis⁷⁾, a HPTLC cellulose plate and phenol/water (73:27, vol) as mobile phase were used. Flavonoid aglycons were detected by spraying 1% AlCl_3 -ethanol solution. For the HPLC analysis⁸⁾, two Shodex ODSpak F-511 columns (4.6×250 mm, Shoko Co. Ltd., Tokyo) were used and kept at 40°C . The mobile phase was a mixture of aqueous 10% acetic acid and acetonitrile (85:15, vol) and the flow rate was 0.8 mL/min. Flavonoid aglycons, viz. anthocyanidins, were monitored with a photodiode array detector and determined from the peak area at 530 nm using the calibration curve of standard anthocyanidins.

Terpenoids were extracted according to the method of Tallevi and Kurz⁹⁾. Briefly, 4 g of freeze-dried sarcotestae in 80 ml water were boiled for 20 min, followed by filtration through Whatman No.1 paper and celite (Hyflo Supercel). Activated charcoal was added to the filtrate and allowed to stir for 12 h at room temperature. After the mixture was centrifuged, the residual charcoal resuspended in acetone and then purified with Waters Sep-Pak Plus NH_2 cartridge column jointed with Sep-Pak Plus C 18 cartridge column according to the method of Van Beek *et al.*¹⁰⁾ prior to HPLC analysis. An Inertsil ODS-2 column (4.6×250 mm, GL Science Inc., Tokyo) was used as a HPLC column and was kept at 50°C . The mobile phase was a mixture of water and methanol (67:33, vol) and the flow rate was 0.8 mL/min. Terpenoids were monitored with a photodiode array detector and determined from the peak area at 220 nm using the calibration curve of standard terpenoids.

3 Results and Discussion

It is well known that leaves of *Ginkgo biloba*

contain flavonoids, mainly flavonols at level of 0.4 ~ 0.7 w%¹¹⁾. Major flavonols are kaempferol, quercetin and isorhamnetin. However, no flavonols were found in tested sarcotestae on the TLC plate. In the spectrophotometric analysis, HCl-methanol extracts from sarcotestae had an absorption maximum at 530 nm. This observation suggested the presence of anthocyanin (or anthocyanidin) in sarcotestas. Thus, anthocyanidins, aglycons of anthocyanins, were qualified by HPLC equipped with a photodiode array detector. Two major peaks were detected at retention time (Rt.) of 11.8 min and 15.7 min on the HPLC chromatogram at 530 nm. The compound eluted at Rt.11.8 min had an absorption maximum at 535 nm and its absorption spectrum was completely consistent with that of delphinidin (Fig.1). Moreover, it showed a molecular ion peak of 303 on ESI-MS. An another compound at Rt.15.7 min had an absorption maximum at 527 nm and its absorption spectrum was the same as that of cyanidin (Fig.1). It showed a molecular ion peak of 287 on ESI-MS. Delphinidin and cyanidin were quantified by their area on the HPLC chromato-

Fig.1 Structures of Anthocyanidins.

Table 1 Anthocyanidin Contents (mg/100 g freeze-dried) in Sarcotestae of *Ginkgo biloba*.

	Delphinidin	Cyanidin	Total
August	54.5 ± 2.7	32.4 ± 1.6	86.9 ± 3.5
September	39.4 ± 1.2	18.7 ± 0.6	58.1 ± 1.6
October	50.4 ± 1.5	28.1 ± 0.8	78.5 ± 2.4
November	46.6 ± 1.4	24.8 ± 0.7	71.4 ± 1.4

Data means average $\pm$ standard deviation (n=5)

gram at 530 nm. As shown in **Table 1**, total amounts of delphinidin and cyanidin were ranged from 58 to 87 mg/100 g (dry basis) in sarcotestae and they were almost similar to those in fruits such as cranberry and red raspberry. The ratio of delphinidin and cyanidin was almost 2:1 and was not affected by harvest season. On the other hand, these anthocyanidins were not found in leaf extracts of *Ginkgo biloba*.

Terpenoids extracted from four sarcotestae were supplied to HPLC analysis. Three major terpenoids corresponding to ginkgolides A, B and C (**Fig.2**) which could act as antagonists of platelet activating factors^(1,2) were found at Rt.18.2, 20.2 and 14.9 min, respectively, on the HPLC chromatogram at 220 nm. Their UV spectra and retention time were consistent with those of standard ginkgolides. Moreover, these ginkgolides A, B, and C were colored blue (Rf.0.52), yellow (0.47), and pale orange (0.28), respectively by UV-irradiation on a TLC plate developed with ethyl acetate/toluene/acetone/hexane (40:30:20:10, vol). On the other hand, bilobalide, major terpenoids contained in leaves, and ginkgolide J

were not found in any tested sarcotestae on HPLC and TLC analyses. Ginkgolides A, B, and C were quantified by their area on the HPLC chromatogram at 220 nm. **Table 2** shows amounts of terpenoids in four sarcotestae. They were varied by harvest season. Immature sarcotestae harvested on August and September contained 12 mg/100 g of ginkgolides while mature ones on October and November contained higher amounts of ginkgolides (27 mg/100 g). Total amounts of terpenoids in sarcotestae were higher than those in nuts (1.5 mg/100 g) but were 1/10 of those in leaves. Moreover, the composition of ginkgolides in sarcotestae depended on harvest season. Only ginkgolides A and B were present in an immature sarcotesta harvested on August although ginkgolide C was also found in ones on September, October and November as well as ginkgolides A and B. Amounts of ginkgolide A were not varied during maturation. Ginkgolide B was increased until October but decreased on November while ginkgolide C increased with maturation. These observations suggest that ginkgolide C may be synthesized from ginkgolide B.

Flavonoids and terpenoids were analyzed for sarcotestae of *Ginkgo biloba*. As a result, flavonoids present in sarcotestae are quite different from those in leaves. Considerable amounts of anthocyanidins were contained in sarcotestae although flavonols were not found in them. We found trace amounts of tocopherols and carotenoids in sarcotestae (data not shown). Thus, these anthocyanidins may protect sarcotesta cells from oxidative damage as natural antioxidants instead of tocopherols and flavonols. Amounts and compositions of terpenoids present in sarcotestae are also different from those in leaves and nuts. Unfortunately, sarcotestae could not substitute with leaves as of sources of flavonols and terpenoids. However, anthocyanins extracted

Ginkgolide A : R₁ = OH, R₂ = R₃ = H

Ginkgolide B : R₁ = R₂ = OH, R₃ = H

Ginkgolide C : R₁ = R₂ = R₃ = OH

Fig.2 Structures of Terpenoids.

Table 2 Terpenoid Contents (mg/100 g freeze-dried) in Sarcotestae of *Ginkgo biloba*.

	Ginkgolide A	Ginkgolide B	Ginkgolide C	Total
August	7.7±0.2	4.0±0.1	0	11.7±0.3
September	7.6±0.2	4.4±0.1	1.0±0.1	12.7±0.3
October	10.6±0.3	11.4±0.3	5.3±0.2	27.3±0.6
November	10.0±0.3	6.1±0.2	11.3±0.3	27.4±0.6

Data means average±standard deviation (n=5)

from sarcotestae might be available as medical materials and coloring agents.

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[報文]

N-ラウロイルグルタミン酸塩の中和 による性質の変化

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N-ラウロイルグルタミン酸塩の中和の度合いによる性質の変化を調べた。起泡力は中和度が 1.6 で極大を示し、乳化力は中和度に伴い減少した。¹³C-NMR の結果より、 α 位のカルボン酸から優先的に中和され、引き続き γ 位のカルボン酸が中和度 1.6 以降で中和されることがわかった。さらに蛍光プローブ法から、中和度 1.6 付近でミセルの微視的極性および会合数が急激に増加することがわかった。これらの結果から、 α 位のカルボン酸が分子間の相互作用に寄与していることが示唆された。

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[ノート]

イチョウ果皮に存在するフラボノイドと テルペノイドおよびその季節変動

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廃棄物であるイチョウ果皮を医薬品として有効利用するため、イチョウ果皮に含まれるフラボノイドとテルペノイドの定性・定量分析を行った。イチョウの葉に存在するケンフェロールやケルセチン、イソラムネチンのようなフラボノールは果皮では検出されなかったが、デルフィニジンやシアニジンのアントシアニジンが存在していた。また果皮には、葉と同様にテルペノイドとしてギンコライドを少量含んでいた。果皮のアントシアニジン量は収穫時期に依存しなかったが、ギンコライドの組成は成熟中に変化した。

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