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A catechol-type lignan and neolignans are specifically present in the seed coat of tung trees

Toshisada Suzuki*, Yuma Onishi, Ayako Katagi, Tae-Wook Kim and Takeshi Katayama

Abstract

The tung tree produces large seeds with approximately 40–50% of their composition consisting of non-edible oil. *α*-Eleostearic acid makes up about 80% of tung oil, while linoleic acid and oleic acid make up 7 and 6%, respectively. The oil is readily oxidized due to the three, conjugated double bonds in *α*-eleostearic acid; however, it is stable inside the tung seed, which is expected to contain strong antioxidative compounds that protect the oil from oxidation. Previously, we isolated and identified a catechol-type lignan (3,3′-bisdemethylpinoresinol) and neolignans (isoamericanol A and americanol A) from the seeds of tung tree fruits that exhibited strong antioxidative activity, similar to that of Trolox. In this study, we show that the catechol-type lignan and neolignans are specifically present in the seed coat of the tung tree fruit seeds. The lignans were not detected in any other tissue, such as the pericarp, bark, leaves, or wood of the tree. The lignan content was determined in each developmental stage of the fruit, and the amount of the three lignans in the tung seeds was the highest in the green mature stage. This was likely because the amount of tung oil was also at its highest during this stage, and the oil composition was stable. Our results indicate that the catechol-type lignan and neolignans are located within the seed coat of the tung tree fruit seeds, where they protect the oil in the endosperm from autoxidation and polymerization processes.

Keywords: Catechol, C-lignin, Lignan, Neolignan, 9*c*, 11*t*, 13*t*-octadecatrienoic acid, *Vernicia fordii*

Introduction

The tung tree (*Vernicia fordii*), a deciduous tree of the Euphorbiaceae family, is native to southern China and was introduced to Japan in the nineteenth century. Approximately 40–50% of oil found in the seeds is not edible, because it causes diarrhoea [1]. Approximately 80% of tung oil is *α*-eleostearic acid (9*c*, 11*t*, 13*t*-octadecatrienoic acid), 7% is linoleic acid, and 6% is oleic acid [2, 3]. The oil is readily oxidized due to the three conjugated double bonds in *α*-eleostearic acid. The drying oil has many applications in the lacquer industry and is used as a raw material for linoleum, resins, and artificial leather [4].

Tung oil is easy to dry by autoxidizing the oil components; however, it is stable inside the tung seed. Therefore, we expect that the tung seed contains strong antioxidative compounds to protect the oil. Previously, we isolated and identified a catechol-type lignan and six neolignans from the seeds of tung trees that exhibited strong antioxidative activity like that of Trolox [5, 6]. The lignans, which may have been generated from the oxidative coupling of caffeyl alcohol or caffeic acid, have shown interesting physiological activities. Isoamericanol A has been reported to show neurotrophic activity on rat cells [7], antimelanogenic activity on Melan-a cells [8], anticancer activity on human breast cancer cell lines [9–11], as well as plant growth inhibitory activity [12]. Furthermore, isoamericanin A was isolated as a prostaglandin I_2 inducer [13], and (+)-3,3′-bisdemethylpinoresinol has been reported as a metabolite of (+)-sesamin in rat liver homogenate [14, 15].

Antioxidative compounds can protect oil found in seeds and fruit from autoxidation; therefore, the tissues in seeds

*Correspondence: suzuki.toshisdada@kagawa-u.ac.jp
Faculty of Agriculture, Kagawa University, Ikenobe 2393, Miki-cho, Kagawa 761-0795, Japan

and fruit containing antioxidative compounds have been investigated in detail. It was found that soybean seeds predominantly accumulate tocopherols in the axis and cotyledon [16]. Palm fruit mesocarp contains palm oil and high amounts of carotenoids [17]. In barley grain, tocopherols are preferentially localized in the germ, whereas tocotrienols are found in the pericarp and endosperm [18]. In sesame seeds, sesamin and sesamolin, which contribute to the stability of sesame oil, are the abundant lignans found in the endosperm and cotyledons, and small amounts are present in the seed coat [19].

The residues generated from plants during oil production contain bioactive compounds, such as antioxidants and colouring matters [20]. Tocopherols from soybeans and rapeseeds are widely applied antioxidants that stabilize edible fats and oils [21]. Carotene from palm oil is used as a colouring matter for margarine and butter [22]. Extracting the bioactive compounds from these residues contributes to the added value of these wastes. Using the antioxidants obtained from tung oil residues to stabilize edible fats and oil products may be difficult; however, the catechol-type lignans isolated from tung seeds could stabilize biodiesel [6]. To promote the utilization of the antioxidants in tung seeds, it is important to thoroughly analyse their amount present during the different developmental stages.

In this study, the seeds of tung trees were separated into the seed coat and endosperm. The antioxidative lignan and neolignans were quantitatively analysed within these plant tissues, and the catechol-type lignan and neolignans were analysed during the stages of the fruit development.

Materials and methods

Plant materials

Tung tree fruits in each of the four developmental stages (Fig. 1) were harvested from a farm in Sanuki City, Kagawa Prefecture, in September and October of 2018. The fruits contain pericarp and four or five seeds, which consist of a hard seed coat and an oily endosperm (Fig. 2). The seed coat and endosperm were separated from tung seeds in the fourth development stage and used to quantify the lignans in the tissues.

Fig. 1 The developmental stages of tung tree fruits. From left, stage 1 (immature green): green fruits about 4.5 cm in diameter, 4 months after anthesis. Stage 2 (mature green): green fruits about 6 cm in diameter, 5 months after anthesis. Stage 3 (turning): red brown fruits about 6 cm in diameter, 5 months after anthesis. Stage 4 (over ripe): brown fruit about 6 cm in diameter, 5 months after anthesis

Synthesis of isoamericanol A

Isoamericanol A, as a standard compound, was synthesized from the radical coupling reaction of caffeyl alcohol [6]. Caffeyl alcohol (892 mg, 5.37 mmol) was dissolved in an acetone–toluene mixture (50 mL, 1:2, v/v) and Ag_2CO_3 (1.427 g) was added to the solution at room temperature. After stirring for 20 h, the solid was filtered off and the solvent was concentrated under reduced pressure. The product was purified by silica gel column chromatography and a Sep-Pak C_{18} cartridge (Waters), followed by recrystallization, to obtain isoamericanol A (21.8 mg) as the 7′-8′ *trans*-isomer.

Isoamericanol A. colourless solid. LC–ESI–MS *m/z*: 329 $[M-H]^-$. ^{1}H NMR (CD_3OD): δ 3.47 (1H, dd, $J=12.0$, 4.6 Hz, H-9b), 3.67 (1H, dd, $J=12.0$, 2.3 Hz, H-9a), 3.99 (1H, ddd, $J=7.4$, 4.6, 2.3 Hz, H-8), 4.18 (2H, dd, $J=6.3$, 1.7 Hz, H-9'), 4.80 (1H, d, $J=8.0$ Hz, H-7), 6.20 (1H, dt, $J=16.0$, 5.7 Hz, H-8'), 6.49 (1H, d, $J=16.0$ Hz, H-7'), 6.76 (1H, dd, $J=8.0$, 2.3 Hz, H-6), 6.80 (1H, d, $J=8.0$ Hz, H-5), 6.85 (1H, d, $J=2.3$ Hz, H-2), 6.89 (1H, d, $J=8.0$ Hz, H-5'), 6.92 (1H, dd, $J=8.6$, 1.7 Hz, H-6'), 6.96 (1H, d, $J=1.7$ Hz, H-2'). ^{13}C NMR (CD_3OD): δ 61.9 (C-9), 63.6 (C-9'), 77.4 (C-7), 79.9 (C-8), 115.4 (C-2), 115.3 (C-2'), 116.1 (C-5), 117.8 (C-5'), 120.6 (C-6), 120.2 (C-6'), 129.3 (C-1), 131.2 (C-7'), 127.9 (C-8'), 131.8 (C-1'), 144.4 (C-3'), 145.1 (C-4'), 146.5 (C-3), 147.0 (C-4).

Preparation of samples

Each sample (ca. 8 g) of whole seed, seed coat, and endosperm were ground with a mortar and pestle, defatted with *n*-hexane (30 mL × 3), and extracted with methanol (30 mL × 3). The methanol extracts were concentrated under reduced pressure, and the oily extracts were purified on a Sep-Pak silica cartridge, eluting with 50% ethyl acetate/hexane (30 mL) and then 100% ethyl acetate (30 mL). The 100% ethyl acetate fraction was concentrated to dryness, and the concentrate was dissolved in 10 mL of MeOH, filtered through a 0.45-μm membrane filter, and then analysed by HPLC.

Fig. 2 The seed, seed coat, and endosperm of the tung tree from left

Quantitative HPLC analysis of catechol lignans

Quantitative analysis was performed using a JASCO LC-2000 plus system equipped with JASCO UV-2075 plus, a UV–VIS detector (254 nm), and Chromato Pro software (ver. 5.0). Separation was performed on a reversed-phase column (ϕ 4.6 × 250 mm, COSMOSIL, 5C_{18}-MS-II) with a guard column. The mobile phase consisted of 20% MeOH (containing 0.1% acetic acid) that was increased to 70% MeOH (containing 0.1% acetic acid) over 40 min using a linear gradient, followed by isocratic elution for 10 min. The flow rate was adjusted to 1.0 mL/min, and the column temperature was maintained at 40 °C. The injection volume of the samples and standards was 10 µL each. Standards of isoamericanol A, americanol A, and (+)-3,3′-bisdemethylpinoresinol were obtained from defatted tung tree seeds [5]. A calibration curve was constructed by plotting the peak area versus the concentration of the synthesized isoamericanol A (0.02–0.4 µg/µL). The limit of detection of isoamericanol A was found to be 0.4 ng/µL, tenfold the signal-to-noise ratio. The amounts of catechol lignan and neolignans were expressed as isoamericanol equivalents.

Oil content and fatty acid composition

Endosperm tissues (ca. 3 g) collected at the different developmental stages were milled into a fine powder. The powder was extracted with diethyl ether (120 mL) using a Soxhlet extractor. The extract was washed with saturated NaCl solution, evaporated in vacuo, and weighed. The fatty acid composition was determined using the methanolysis method [3]. The oil was added to KOH and methanol and heated at 60 °C for 2 h. The reaction mixture was cooled, and the ester layer was separated using a funnel, washed with water, and then run through a silica gel column. The fatty acid methyl esters (FAMEs) were analysed by GC–MS (Shimadzu, GCMS-QP2010 SE) equipped with a capillary column (GL Sciences, InertCap 225, 0.25 mm I.D. × 30 m, df 0.25 µm) using helium as the carrier gas. The oven temperature was set to 180 °C, and increased to 230 °C at a rate of 1 °C/min. The mass spectrometer was operated at an ionization energy of 70 eV and scanned from *m/z* 50 to 600. The FAMEs were identified by comparison with the standards and Wiley library data.

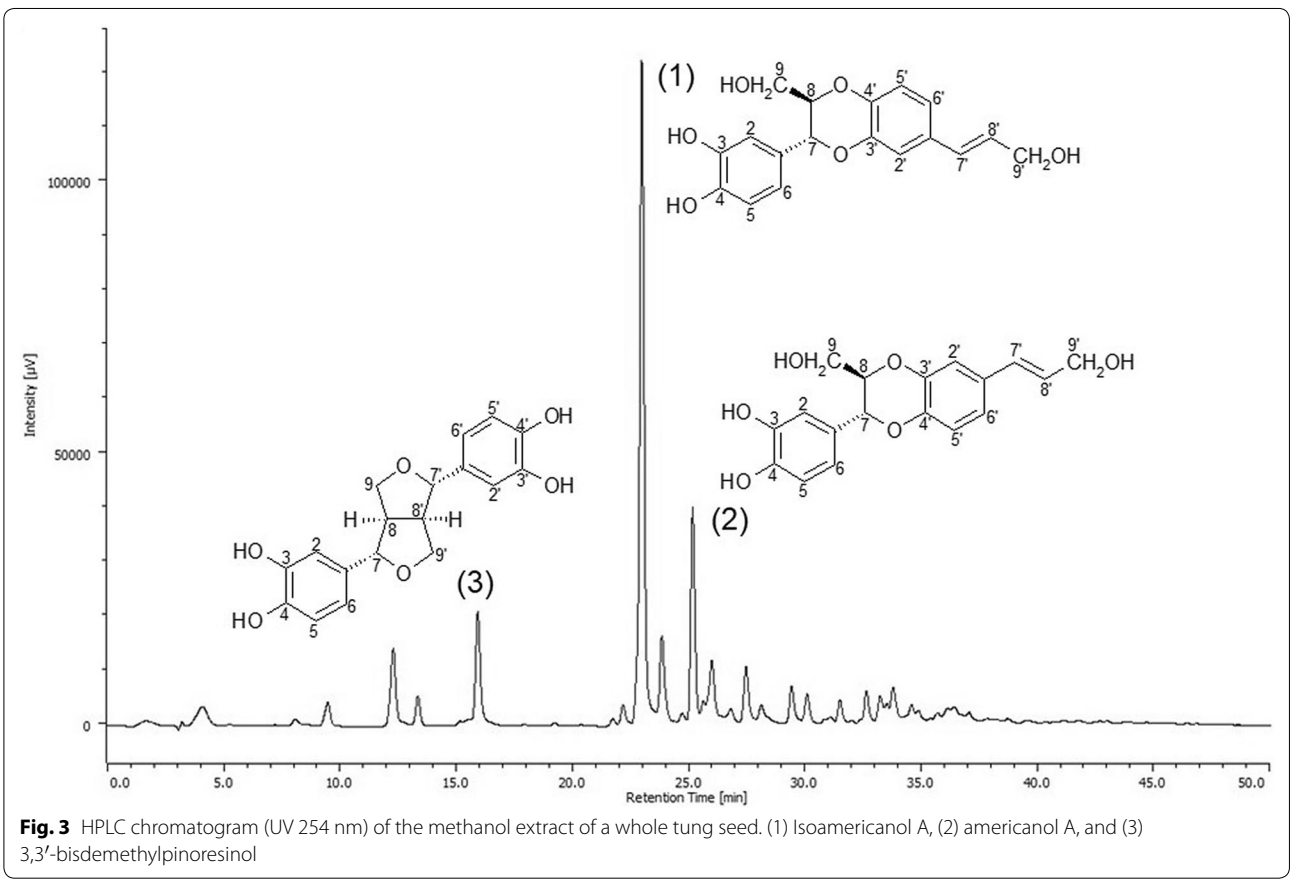

Fig. 3 HPLC chromatogram (UV 254 nm) of the methanol extract of a whole tung seed. (1) Isoamericanol A, (2) americanol A, and (3) 3,3′-bisdemethylpinoresinol

Table 1 Content of three catechol-type lignans in seed coat and endosperm of tung seed (μg/g FW)

	Isoamericanol A	Americanol A	3,3′-Bisdemethylpinoresinol
Seed coat	527.9 ± 12.23	180.1 ± 13.53	38.08 ± 3.72
Endosperm	ND	ND	ND

The data are the mean ± SE ($n = 4$)

ND, not detected

Results and discussion

Figure 3 shows an HPLC chromatogram of the methanol extract of a whole tung seed. The highest peak in the chromatogram was isoamericanol A (Rt. 23 min), followed by americanol A (Rt. 25 min) and then 3,3′-bisdemethylpinoresinol (Rt. 16 min). The three catechol-type lignans were quantitatively analysed, and 312 μg of isoamericanol A, 139 μg of americanol A, and 18 μg of 3,3′-bisdemethylpinoresinol were obtained per 1 g of tung seed (fresh weight). Minor catechol-type lignans (lignan derivatives and sesquineolignans) were detected after 26 min, but not quantified in this study.

The seeds of the tung tree were separated into the seed coats and endosperm, and the lignan and neolignans were quantitatively analysed in the tissues. Table 1 shows the content of the catechol-type lignan and neolignans found in the tissues. The catechol-type lignan and neolignans were only present in the seed coat of the tung seeds, and the lignans were not detected in any other tissue, such as pericarp, bark, leaves, or wood of the tree. Previously, we showed that the catechol-type lignans isolated from tung trees exhibited moderate-to-strong antioxidative activity, compared to that of the known antioxidant Trolox. These results indicate that the catechol-type lignan and neolignans are specifically present in the seed coat of tung seeds where they protect the oil in the endosperm from autoxidation and polymerization.

The lignan content in the seed coat was measured in the different developmental stages (Fig. 4). The amount of lignans was the highest in the mature green stage (stage 2) and decreased as the seed matured. The most abundant compound observed in each stage was isoamericanol A, followed by americanol A and then 3,3′-bisdemethylpinoresinol. During the synthesis of isoamericanol A, using Ag_2CO_3, the desired product was selectively obtained and the presence of americanol A and 3,3′-bisdemethylpinoresinol could not be confirmed. These results indicate that the in vivo polymerization reaction with peroxidase and/or laccase in the seed coat of tung tree seeds can be different from the in vitro polymerization with Ag_2CO_3 in terms of the coupling product preference.

Figure 5 shows the oil and fatty acid composition observed in each developmental stage. The oil content observed in endosperm tissue in stages 1 to 3 were almost identical and were seen to decrease in stage 4. Similarly, the fatty acid compositions were almost equivalent in the same stages, as about 70 to 80% of tung oil consists of α-eleostearic acid. Cui et al. also reported oil content and fatty acid composition in tung seeds [3]. In their study, tung oil synthesis began about 3 months after anthesis, and quickly accelerated until 5 months after anthesis, where the synthesis rate became constant as the mature period was reached. The composition of α-eleostearic acid increased rapidly until 4 months after anthesis, and then remained stable in the mature period. This data suggested that the level of the three lignans in tung seeds was the highest in the green mature stage, when amount of tung oil was also at its highest and the oil composition was stable. The amount of oil content in the endosperm was not fully synchronized with the amount of the catechol-type lignans in the seed coat; however, the catechol-type lignans with high antioxidative activity should play a role in protecting the oil. The quantity of lignans decreased after the green mature stage, probably due to their metabolization into their derivatives, sesquilignans [6], or C-lignin.

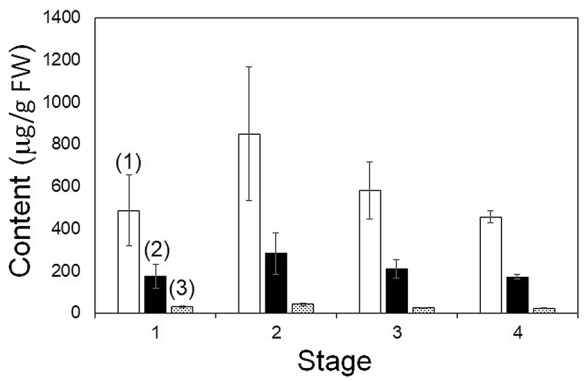

Fig. 4 Content of three catechol-type lignans found in the seed coat at the different developmental stages. (1) Isoamericanol A, (2) americanol A, and (3) 3, 3′-bisdemethylpinoresinol. The data represent the mean of four independent measurements (mean ± SE). Stage 1 (immature green): green fruits about 4.5 cm in diameter, 4 months after anthesis. Stage 2 (mature green): green fruits about 6 cm in diameter, 5 months after anthesis. Stage 3 (turning): red-brown fruits about 6 cm in diameter, 5 months after anthesis. Stage 4 (over ripe): brown fruit about 6 cm in diameter, 5 months after anthesis

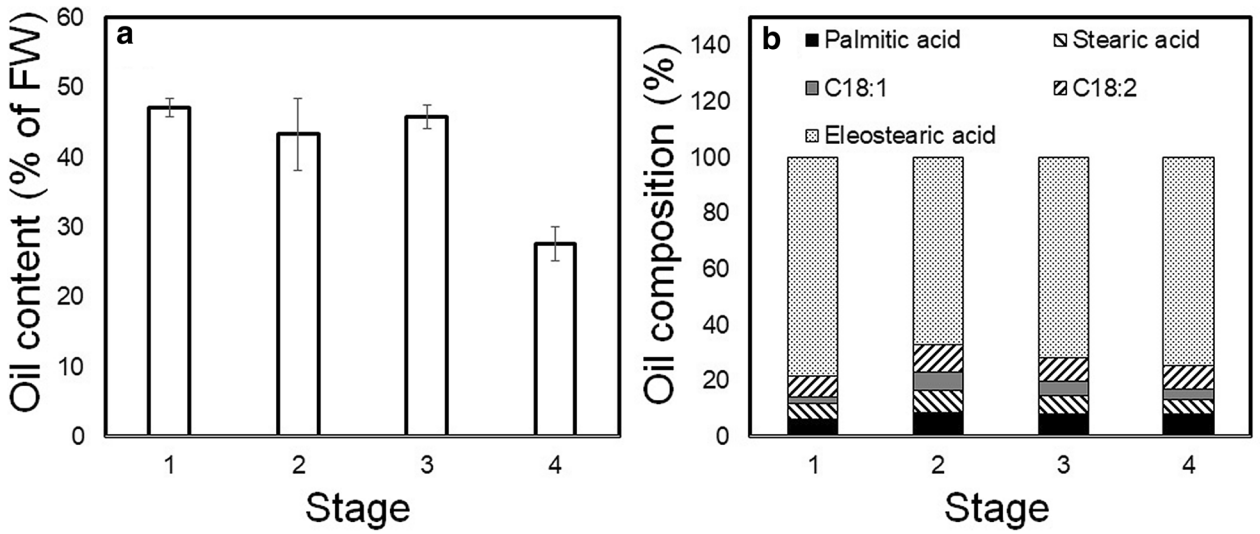

Fig. 5 Oil content (**a**) and oil composition (**b**) observed in the endosperm in the different developmental stages. The data represent the mean of four independent measurements (mean ± SE). Stage 1 (immature green): green fruits about 4.5 cm in diameter, 4 months after anthesis. Stage 2 (mature green): green fruits about 6 cm in diameter, 5 months after anthesis. Stage 3 (turning): red-brown fruits about 6 cm in diameter, 5 months after anthesis. Stage 4 (over ripe): brown fruit about 6 cm in diameter, 5 months after anthesis

C-lignin was recently discovered in the seed coat of vanilla orchids, Cactaceae species, and Euphorbiaceae plants [22]. The amount of C-lignin in the seed coat of the tung tree is the most abundant amount reported in a plant to date [23]. C-lignin is a unique homopolymer that is biosynthesized from the oxidative coupling of caffeyl alcohol and almost linked through benzodioxane linkages, which is the same 8-*O*-4′-type linkage in isoamericanol A. Generally, lignin would be biosynthesized in the cell wall and lignans would be biosynthesized in the vacuole, and their biosyntheses are thought to be spatially and temporally distinct [24]. However, the catechol-type lignans analysed in this study, at least partially, could be oligomeric products derived from the caffeyl alcohol polymerization that forms C-lignin. Further investigation is needed to unravel any existing relationship between the accumulation of the catechol-type lignans and C-lignin polymers during seed coat development in tung trees.

Conclusions

In this study, we demonstrated that the catechol-type lignan and neolignans are specifically present in the seed coat of tung trees and are absent in the oil-rich endosperm. The amount of the three lignans in tung seeds was the highest in the green mature stage, when amount of tung oil was also at its highest and the oil composition was stable. These results indicate that the catechol-type lignan and neolignans in the seed coat protect the oil in the endosperm of tung trees from autoxidation and polymerization. A continuous examination of the catechol-type lignan and neolignans would strengthen this proposition.

Abbreviations

GC–MS: Gas chromatography–mass spectrometry; HPLC: High-performance liquid chromatography; UV–VIS: Ultra-violet–visible.

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Authors' contributions

TS designed this study and wrote the manuscript. YO quantitatively analysed the lignans. AK and TW-K synthesized the standard compound. TK discussed the results and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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References

1. Lin TJ, Hsu CI, Lee KH, Shiu LL, Deng JF (1996) Two outbreaks of acute tung nut (*Aleurites fordii*) poisoning. J Toxicol Clin Toxicol 34:87–92

2. Cao H, Shockey JM (2012) Comparison of TaqMan and SYBR Green qPCR methods for quantitative gene expression in Tung tree tissues. J Agric Food Chem 60:12296–12303
3. Cui P, Lin Q, Fang D, Zhang L, Li R, Cheng J, Gao F, Shochey J, Hu S, Lü S (2018) Tung tree (*Vernicia fordii*, Hemsl.) genome and transcriptome sequencing reveals co-ordinate up-regulation of fatty acid β-oxidation and triacylglycerol biosynthesis pathways during eleostearic acid accumulation in seeds. Plant Cell Physiol 59:1990–2003
4. Kumar S, Dhillon MK, Singh M, Rathi RS, Misra AK, Rana JC (2017) Fatty and amino acid compositions of *Vernicia fordii*: a source of *α*-eleostearic acid and methionine. Indian J Exp Biol 55:734–739
5. Suzuki T, Kato T, Katagi A, Katayama T (2018) Antioxidative catechol lignans/neolignans isolated from defatted seed of Tung tree. J Forest Biomass Util Soc 13:47–51
6. Suzuki T, Eto K, Kubota Y, Katayama T, Pankasemsuk T (2016) Anitioxidative catechol lignans/neolignans isolated from defatted kernel of *Jatropha curcas*. J Wood Sci 62:339–348
7. Fukuyama Y, Hasegawa T, Toda M, Kodama M, Okazaki H (1992) Structures of americanol A and isoamericanol A having a neurotrophic property from the seeds of *Phytolacca americana*. Chem Pharm Bull 40:252–254
8. Kim KH, Moon E, Kim SY, Lee KR (2010) Lignans from the tuber-barks of *Colocasia antiquorum* var. *esculenta* and their antimelanogenic activity. J Agric Food Chem 58:4779–4785
9. Katagi A, Sui L, Kamitori K, Suzuki T, Katayama T, Hossain A, Noguchi C, Dong Y, Yamaguchi F, Tokuda M (2015) Inhibitory effect of isoamericanol A from *Jatropha curcas* seeds on the growth of MCF-7 human breast cancer cell line by G2/M cell cycle arrest. Heliyon 2:e00055
10. Katagi A, Sui L, Kamitori K, Suzuki T, Katayama T, Hossain A, Noguchi C, Dong Y, Yamaguchi F, Tokuda M (2017) High anticancer properties of defatted *Jatropha curcas* seed residue and its active compound, isoamericanol A. Nat Prod Commum 12:1881–1884
11. Katagi A, Sui L, Kamitori K, Suzuki T, Katayama T, Dong Y, Nakagawa T, Yube K, Hossain A, Yamaguchi F, Tokuda M (2019) Monopolar spindle induced by isoamericanol A suppresses human breast cancer cell (MCF-7) growth. Asian J Cell Bio 14:1–6
12. Chaipon S, Boonmee S, Iwasaki A, Suenaga K, Kato-Noguchi H (2019) (+)-Isoamericanol A: a plant growth inhibitor from *Heliotropium indicum*. Nat Prod Commum 14:1–3
13. Hasegawa T, Fukuyama Y, Koshino K, Nakagawa K, Tori M, Asakawa Y (1987) Structure of isoamericanin A, a prostaglandin I_2 inducer, isolated from the seeds of *Phytolacca americana* L. Chem Lett 16:329–332
14. Nakai M, Harada M, Nakahara K, Akimoto K, Shibata H, Miki W, Kiso Y (2003) Novel antioxidative metabolites in rat liver with ingested sesamin. J Agric Food Chem 51:1666–1670
15. Miyake Y, Fukumoto S, Okada M, Sakaida K, Nakamura Y, Osawa T (2005) Antioxidative catechol lignans converted from sesamin and sesaminol triglucoside by culturing with *Aspergillus*. J Agri Food Chem 53:22–27
16. Yoshida H, Kanei S, Tomiyama Y, Mizushina Y (2006) Regional distribution in the fatty acids of triacylglycerols and phospholipids within soybean seeds (*Glycine max* L.). Eur J Lipid Sci Technol 108:149–158
17. Sundram K, Sambanthamurthi R, Tan YA (2003) Palm fruit chemistry and nutrition. Asia Pacific J Clin Nutr 12:355–362
18. Falk J, Krahnstöver A, Kooji TAW, Schlensog M, Krupinska K (2004) Tocopherol and tocotrienol accumulation during development of caryopses from barley (*Hordeum vulgare* L.). Phytochem 65:2977–2985
19. Tashiro T (2015) Cultivation environment and chemical component of sesame. In: Namiki M (ed) Sesame function and science. Asakura Publishing Co, Shinjuku, pp 47–51 **(Japanese)**
20. Fang T, Goto M, Wang X, Ding X, Geng J, Sasaki M, Hirose T (2007) Separation of natural tocopherols from soybean oil byproduct with supercritical carbon dioxide. J Supercritical Fluids 40:50–58
21. Ooi CK, Choo YM, Yap SC, Baslron Y, Ong ASH (1994) Recovery of carotenoids from palm oil. J Am Oil Chem Soc 71:423–426
22. Chen F, Tobimatsu Y, Havkin-Frenkel D, Dixon RA, Ralph J (2011) A polymer of caffeyl alcohol in plant seeds. PNAS 109:1772–1777
23. Tobimatsu Y, Chen F, Nakashima J, Escamilla-Treviño LL, Jackson L, Dixon RA, Ralph J (2013) Coexistence but independent biosynthesis of catechyl and guaiacyl/syringyl lignin polymers in seed coats. Plant Cell 25:2587–2600
24. Suzuki S, Umezawa T (2007) Biosyntesis of lignans and norlignans. J Wood Sci 53:273–284

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