Research Article Open Access

Hydroxypterocarpans with estrogenic activity in Aguaje, the fruit of *Mauritia flexuosa* (Peruvian moriche palm)

Hiroshi Shimoda[1*], Shogo Takeda[1], Toru Takarada[1], Yurina Kato[1], Norihito Shimizu[1], Kazuya Toda[1], Masafumi Nakamura[2], Hiroshi Handa[2], Seikou Nakamura[3], Hisashi Matsuda[3]

[1]Oryza Oil & Fat Chemical Co., Ltd.; [2]Hiyoshi Co.; [3]Kyoto Pharmaceutical University; Phytoestrogens in moriche palm fruit

[*]**Corresponding author:** Hiroshi Shimoda PhD, Executive of R&D in Oryza Oil & Fat Chemical Co. Ltd. Numata 1, Kitagata-cho, Ichinomiya, Aichi, 493-8001 Japan.

Submission date: November 21st, 2018**, Acceptance Date:** April 28th, 2019, **Publication Date:** April 30th, 2019

Citation: Shimoda H., Takeda S., Takarada T., Kato Y., Shimizu N., Toda K., Nakamura M., Handa H., Nakamura S., Matsuda H. Hydroxypterocarpans with estrogenic activity in Aguaje, the fruit of *Mauritia flexuosa* (Peruvian moriche palm). *Bioactive Compounds in Health and Disease* 2019; 2(4): 64-77. DOI: https://doi.org/10.31989/bchd.v2i4.585

ABSTRACT

Background: Phytoestrogens in edible plants, including soybean isoflavones and pomegranates, have been used for alleviation of premenopausal/postmenopausal syndromes and osteoporosis. *Mauritia flexuosa* (moriche palm) is grown in Peru and Brazil for its edible fruit that is said to contain phytoestrogens, but the relevant estrogenic compounds have not been identified. We investigated the constituents of moriche palm fruit extract and evaluated their estrogenic ability.

Objective: The hydrophobic fraction of the moriche palm fruit was purified to search for estrogenic compounds and two hydroxypterocarpans were identified with estrogenic activity. Pterocarpans belong to the flavonoids and are structurally similar to isoflavonoids known as typical

phytoestrogens. The study is to investigate the properties of estrogenic effects of these hydroxypterocarpans.

Materials and methods: Ethyl acetate fraction of dried moriche palm was purified by column chromatography and HPLC chromatography. To evaluate estrogenic activity, we performed [1] simulation of binding to the human estrogen receptor (ER), [2] investigation of the proliferative effect on MCF-7 cells, and [3] the estrogen-chemically activated luciferase gene expression (E-CALUX) assay.

Results: Two hydroxypterocarpans were isolated including lespeflorin G_8 (LF) and 8-hydroxyhomopterocarpan (8-HHP). The binding affinity of LF to ER was higher than that of 8-HHP, with inhibition constants of 81.9 nM and 1.99 M, respectively. However, LF and 8-HHP exhibited a similar proliferative effect on MCF-7 cells at 10 M. The E-CALUX assay demonstrated that LF is a full ER agonist and 8-HHP is a partial agonist.

Conclusion: LF was identified as a major estrogenic compound in the fruit of *Mauritia flexuosa*.

Keywords: *Mauritia flexuosa*, moriche palm, lespeflorin G_8, homopterocarpan, estrogenic activity, MCF-7, E-CALUX

BACKGROUND

Complementary herbal therapies have traditionally been used for alleviation of nonspecific complaints [1] including black cohosh [2], red clover [3], wild yam [4], evening primrose [5], and *Angelica sinensis* [6]. While pomegranate juice is said to prevent breast cancer [7, 8] without concrete evidence of its active ingredients and anti-cancer mechanism. Additionally, soybean isoflavones are used to treat estrogen deficiency syndromes, including osteoporosis [9], endometrial disorders [10], vaginal atrophy [11], and menopausal symptoms [12]. The key ingredients in these edible plants are so-called phytoestrogens and such complementary therapy is defined as phytoestrogen therapy.

Mauritia flexuosa (moriche palm or aguaje) is cultivated in the Amazon River territory of Peru and Brazil for its edible fruit [13]. Oil from the fruit is used for cooking [14] and the pulp is processed to make juice and confectionaries [15]. The biological activities of moriche palm fruit such as cholesterol-lowering effect [16], anti-thrombotic effect [17], and antioxidant effect [18, 19]

have been reported. Fruits include fatty acids [20, 21] and polysaccharides [22]. Peruvian moriche palm fruit is also said to contain phytoestrogens that induce breast augmentation but its active ingredients have not been identified.

MATERIALS AND METHODS

Isolation of estrogenic compounds from Peruvian moriche palm fruit

Dried moriche palm fruit pulp was obtained from Peru through Ajuto Inc. (Tokyo, Japan). The powdered pulp (2 kg) was defatted 3 times with *n*-hexane (2 L) at 40°C, after which the residue was extracted with ethanol (3 L) for 2 hr at 60°C and the solvent was evaporated. Then *n*-hexane (2 L) was added to remove residual oily substances. Finally, an ethanol extract (124.8 g) was obtained with a yield of 6.2%. This extract was partitioned twice with ethyl acetate (0.5 L) to obtain the ethyl acetate portion (12 g), which was separated by silica gel column chromatography (100 g, YMC gel, YMC Co. Ltd., Kyoto, Japan) with ethyl acetate (0.3 L) to obtain the ethyl acetate fraction. Next, this fraction was repeatedly purified by HPLC (RPAQUEOUS, Nomura Chemical Co. Ltd., Seto, Japan, 20 ϕ ×250 mm) with 85% methanol to obtain compound **1** (28.7 mg) and compound **2** (6.7 mg). These compounds were identified as 8-hydroxy-3,9-dimethoxy-10-isoprenylpterocarpan (**1**, lespeflorin G_8) and 8-hydroxyhomopterocarpan (**2**) by comparing the H^1-NMR spectra with reference data [23] (Fig. 1).

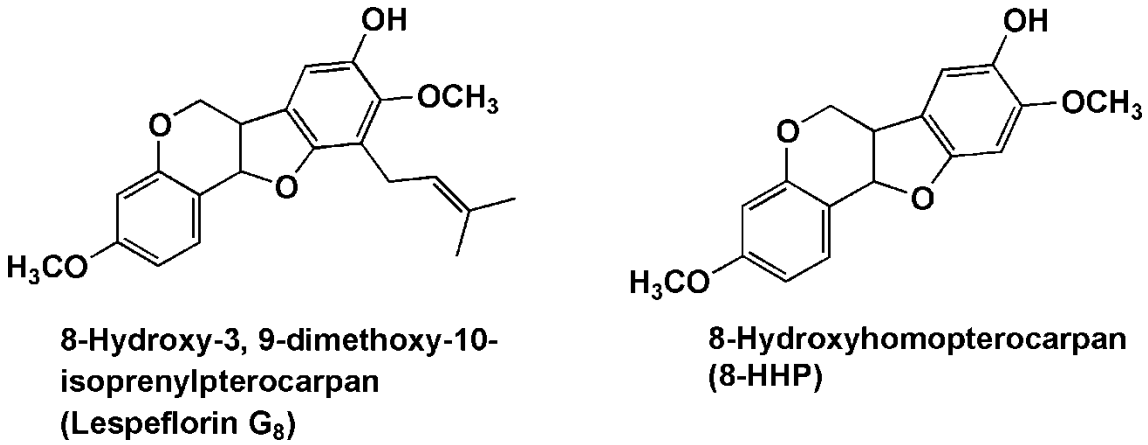

Fig. 1. Hydroxypterocarpans isolated from moriche palm fruit

Reagents

Dulbecco's modified Eagle's medium (D-MEM), phenol red-free D-MEM, penicillin (100 units/ml) and streptomycin (100 μg/ml) mixture, daidzein, and genistein were obtained from FUJIFILM Wako Pure Chemical Co. Ltd. (Osaka, Japan). Fetal bovine serum (FBS) was obtained

from Biosera (Boussense, France). Charcoal/dextran-treated FBS was sourced from GE Healthcare Japan, (Tokyo, Japan). (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) was purchased from Dojindo Laboratories (Kumamoto, Japan). For the E-CALUX assay, RPMI 1640 medium with L-glutamine was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). D-MEM without L-glutamine and phenol red was purchased from MP Biomedicals Inc. (California, USA), while UCDA qualified FBS was from Mediatech Inc. (Virginia, USA). 17β-estradiol was sourced from Sigma-Aldrich Japan, (Tokyo, Japan). The luciferase assay system was purchased from Promega (Wisconsin, USA).

Cell culture

The human metastatic breast cancer cell line MCF-7 (JCRB0134) was purchased from the JCRB Cell Bank (Japan). The cells were subcultured in D-MEM supplemented with 10% FBS and penicillin (100 units/ml) and streptomycin (100 μg/ml) mixture at 37°C under a 5% CO_2 atmosphere. The human BG1Luc4E2 cell line was obtained from XDSI Inc. (North Carolina, USA). The cells were maintained in RPMI-1640 containing 1% penicillin and streptomycin and 8% FBS. After reaching 80% confluence, the medium was changed to phenol red-free D-MEM containing 4.5% charcoal-stripped FBS, 1.9% L-glutamine, and 0.9% penicillin and streptomycin for the E-CALUX assay.

Simulation of receptor binding

The 3D structures of human ERα (PDB ID: 1G50) and ERβ (PDB ID: 3OLS) were obtained from the RCSB Protein Data Bank. ERα and ERβ consist of three and two identical chains, respectively. To obtain the Apo state structure of ERα and ERβ, two identical chains of ERα and one chain of ERβ were removed by using AutoDock software (ver. 4.2, The Scripps Research Institute). Additionally, all H_2O molecules and the bound ligand (17β-estradiol) were removed from the structures. 3D structures of LF and 8-HHP were obtained from the PubChem database. Energy minimization and optimization of the molecular geometry of these compounds were carried out with Arguslab software (ver. 4.0.1). Molecular docking studies were performed with AutoDock software, using the Lamarckian Genetic Algorithm [24]. Blind docking was performed to identify the most favorable binding model. The input grid box size was set at 70×70×70 points, with the active site residues at the center of the box. For simulation of docking, the conformation ranking

was based on the score calculated with an energy scoring function. After docking simulation, the most favorable docking poses and hydrogen bond interactions were visualized and analyzed with PyMOL software (Schrodinger).

MCF-7 proliferation assay

According to previously reported methods [25], subcultured MCF-7 cells (2,000/100 μL) were suspended in phenol red-free D-MEM containing 5% CS-FCS, seeded into a 96-well culture plate, and incubated for 24 hr. Then a test solution dissolved in DMSO and diluted in medium (11 μL) was added to the wells and the cells were cultured for 4 days. Subsequently, MTT solution (5 mg/mL in PBS) was added (10 μL) and incubation continued for 4 hr. After removing the medium, 40 mM HCl in isopropanol (100 μL) was added to dissolve the formazan product and absorbance of the formazan solution was measured with at 570 nm referenced at 660 nm.

E-CALUX assay

This experiment was performed according to the OECD guideline [26]. Subcultured BG1Luc4E2 cells (2.0×10^5 /200 μL) were seeded into a 96-well culture plate and incubated overnight. Then the medium was replaced with D-MEM containing 1% penicillin and streptomycin, 10% charcoal-stripped FBS, and 2% glutamine. A test solution (190 μL) dissolved in DMSO (final concentration of 1.0%) was added and culture was done for 24 hr at 37°C under a 5% CO_2 atmosphere, after which luminescence was measured in the range of 300 to 650 nm by using a Luciferase assay system and a luminometer.

Statistics

Results are presented as the mean±SE in Fig. 3 and as the mean±SD in Fig. 4. For statistical analysis, one-way analysis of variance (ANOVA) was performed, followed by Dunnett's test, and $p<0.05$ or $p<0.01$ was considered to indicate significance.

RESULTS

Hydroxypterocarpans in moriche palm fruit

The compounds found in the oily components (ethyl acetate fraction) mainly consisted of free oleanolic acids, as well as oleanolic acid diglycerides and triglycerides. However, both lespeflorin G_8 (LF) and 8-hydroxyhomopterocarpan (8-HHP) were clearly distinguished as orange spots by

performing RP-18 thin-layer chromatography using 80% MeOH, followed by spraying with 10% H_2SO_4 and heating at 180°C (Fig. 2).

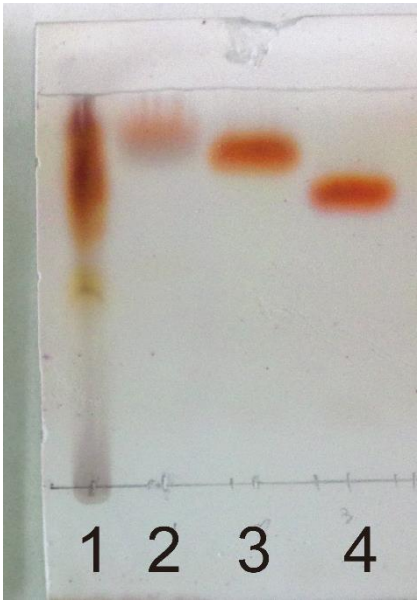

Fig. 2. A thin-layer chromatogram of ethyl acetate fraction of moriche palm, LF and 8-HHP.

Lane 1: ethyl acetate fraction of moriche palm, lane 2: unknown substances, lane 3: LF, Lane 4: 8HHP. All lanes were developed by 80% MeOH on a RP-18 plate. The spots were visualized by spraying 10% H_2SO_4 and heating at 180°C

***Simulation of binding to the human estrogen receptor* (ER)**

Molecular docking studies were performed to investigate binding between LF or 8-HHP and ER receptors. The most favorable docking model was identified by using the energy scoring function of Autodock software (Fig. 3). The binding parameters for LF and 8-HHP to ER receptors are listed in Table 1. The estimated binding free energy of LF and 8-HHP to ERα was calculated to be -9.67 and -7.78 kcal/mol, respectively. The LF-ERα complex demonstrated lower values of intermolecular and van der Waals desolation energy compared to the 8-HHP-ERα complex. The best docked poses of LF and 8-HHP with ERα are shown in Fig. 2. LF forms two hydrogen bonds with Arg394 and Leu525 of ERα, while 8-HHP forms one hydrogen bond with Arg394. These residues are known to contribute to stable hydrogen bonding with 17β-estradiol. Consequently, the inhibition constant of LF and 8-HHP for ERα was 81.9 nM and 1.99 μM, respectively. The results indicated that LF has much higher binding affinity for ERα than 8-HHP. On the other hand, the inhibition constant of LF and 8-HHP for binding to ERβ was 2.46 μM and 33.97 μM, respectively, being larger than for binding with ERα. From these data, LF and 8-HHP are suggested to show high binding affinity and selectivity for ERα, but not ERβ.

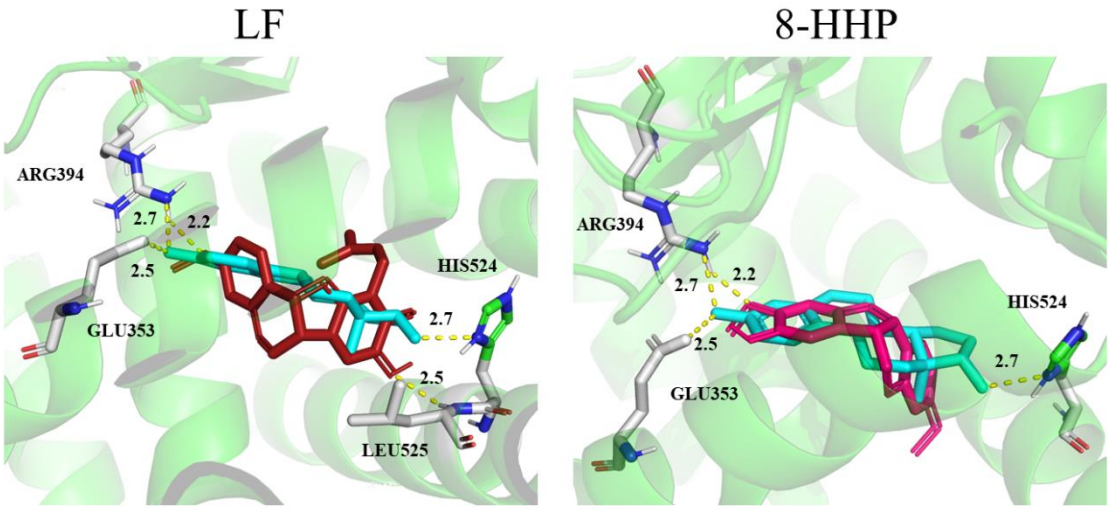

Fig. 3. Superimposed binding modes of 17β-estradiol (cyan) and LF (red) or 8-HHP (magenta) at the active site of ERα. Hydrogen bonds are represented as yellow colored dashed lines. The key residues involved in hydrogen bonds are also shown.

Table 1. Binding parameters for ERα and ERβ

Auto dock parameter	LF-ERα	8-HHP-ERα
Binding energy (kcal/mol)	-9.67	-7.78
Inhibition constant	81.9 nM	1.99 μM
Intermolecular energy	-11.16	-8.67
Van der Waals H-bond desolvation energy	-11.05	-8.45
Electrostatic energy (kcal/mol)	-0.11	-0.22
No. of hydrogen bonds	2	1
Residue involved in hydrogen bonding	ARG394, LEU525	ARG394
	LF-ERβ	8-HHP-ERβ
Binding energy (kcal/mol)	-7.65	-6.10
Inhibition constant	2.46 μM	33.97 μM
Intermolecular energy	-9.14	-6.99
Van der Waals H-bond desolvation energy	-9.11	-6.86
Electrostatic energy (kcal/mol)	-0.03	-0.13
No. of hydrogen bonds	3	1
Residue involved in hydrogen bonding	GLY472, HIS475, LEV476	ARG345

Estrogen-dependent cell proliferation

After treatment with LF or 8-HHP at 10 μM, MCF-7 cells demonstrated a significant increase of proliferation by approximately 25% compared to the control (Fig. 4). Daidzein derived from soy beans (0.3 to 10 μM) also significantly activated the proliferation of MCF-7 cells and genistein (0.1 to 10 μM) had an even stronger effect on MCF-7 cell proliferation.

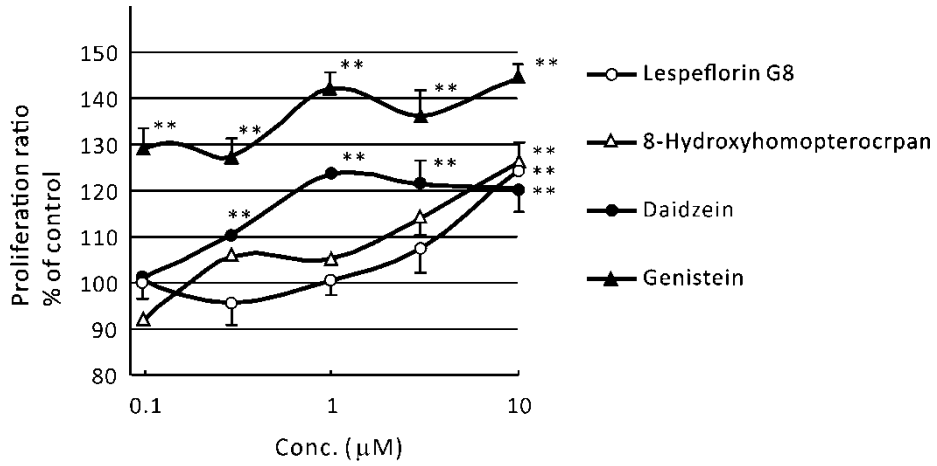

Fig. 4. Estrogenic proliferation of MCF-7 cells in response to LF and 8-HHP.Cells were treated with LF or 8-HHP for 4 days. Each point represents the mean and SE (n=8). **: significant difference from the control at $p<0.01$.

E-CALUX assay

Figure 5 displays binding curves for LF and 8-HHP. LF showed ER binding from 1.56 to 12.5 μM, with an EC_{50} value of 4.4 μM (Table 2), and its maximum relative luminescence was similar to that of 17β-estradiol. 8-HHP showed ER binding from 3.13 to 25 μM, with an EC_{50} value of 9.6 μM, and its maximum relative luminescence was only half that of 17β-estradiol. The shape of the inhibition curve of LF is more similar to 17β-estradiol than that of 8-HHP. Based on these findings, LF and 8-HHP seemed to be full and partial agonists, respectively.

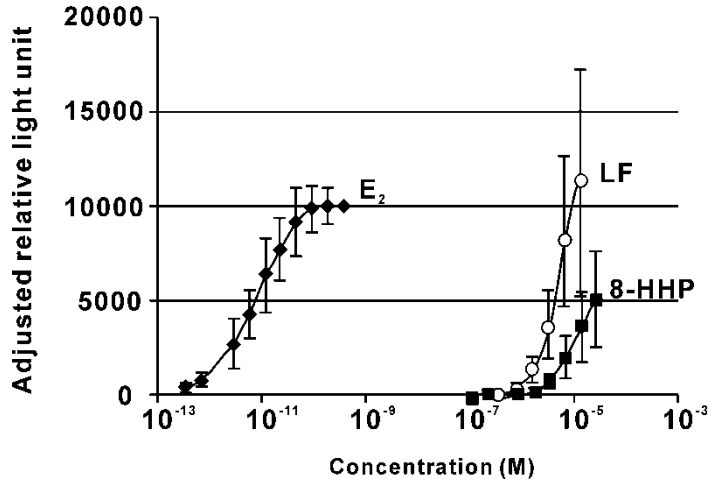

Fig. 5. ER agonistic effects of LF and 8-HHP in BG1Luc4E2 cells. E_2: 17β−estradiol. Cells were treated

with LF or 8-HHP for 20 hr. Each point represents the mean and SD (n=4).

Table 2. EC_{50} values and relative activity of LF and 8-HHP vs.17β-estradiol in the E-CALUX assay

	EC_{50} (μM)	Relative activity
17β-Estradiol	0.00000783	1
Lespeflorin G_8	4.40	1 / 562,578
8-Hydroxyhomopterocarpan	9.64	1 / 1,231,336

DISCUSSION

In this study, we isolated candidate estrogenic compounds from moriche palm oil. Ptercarpans present in Legminosase plants [soybean leaves (27) and Japanese clover (23)] and Fabaceae plants [Babch (28) and *Sophora tonkinensis* (29)]. Its structure is similar to that of isoflavons, and coumestans, which are well known phytoestrogens. However, as there have not been any studies reporting the existence of pterocarpans in fruits of Arecaceae plants rich in oil substances, such as palms, our finding is the first report to find estrogenic compounds in Arecaceae plants. On the other hand, several pterocarpans have been reported to exhibit estrogenic activities. Glyceollin I, which accumulates in stressed soy bean, stimulates both ERα and ERβ [30]. Medicarpin isolated from legumes strongly activates osteoblasts by stimulating ERβ and suppresses bone loss in rats [31]. Thus, we speculated that hydroxypterocarpans isolated from moriche palm might exhibit estrogenic activities.

According to the results of the binding simulation, LF was suggested to bind strongly to ERα with 30-fold higher affinity than for ERβ. On the other hand, the binding affinity of 8-HHP for ERα was approximately 1/24 of that shown by LF and its affinity for ERβ was quite low. The results of the binding simulation suggested that LF would show estrogenic activity. Accordingly, we evaluated the estrogenic effects of LF and 8HHP on proliferation of MCF-7 cells expressing the ERα . We found that LF and 8-HHP both showed slight estrogenic activity, which is weaker than that of soybean isoflavones. In addition, LF and 8-HHP both exhibited agonistic activity in the E-CALUX assay, with EC_{50} values of 4.4 and 9.6 μM, respectively. BG1Luc4E2 cells show predominant expression of ERα over ERβ. Since LF had a higher affinity for ERα than 8-HHP, it is reasonable for LF to display stronger agonistic activity than 8-HHP. According to the OECD

guideline, the EC_{50} values of daidzein and genistein are 0.795 and 0.271 μM, respectively [26]. Thus, the activity of LF is 5.5-fold lower than that of daidzein and 16.2-fold lower than that of genistein.

Recently, several phytoestrogens in *Pueraria candollei var. mirifica* [32] which has potent estrogenic activity, have been reported to show adverse effects in Japanese female including atypical genital bleeding. These estrogenic compounds are miroestrol and isomiroesterol, which exhibit strong estrogenic activities similar to estradiol [33-35]. With this background of phytoestrogens in mind, mild phytoestrogens with estrogenic activity milder than soy isoflavons are favorable. LF and 8-HHP, which are mild phytoestrogens, will satisfy the demand of functional foods applicable for female symptoms caused by estrogen deficiency.

CONCLUSION

LF was identified as a major estrogenic compound in moriche palm fruit and was found to be a full ERα agonist. 8-HHP was a partial agonist bound to ER. This report is a first to have found estrogenic compounds in the oil fraction of palms.

Conflict of interests: There is no conflict of interest to be declared by the authors.

Abbreviations: ER - estrogen receptor; E-CALUX - estrogen-chemically activated luciferase gene expression; LF - lespeflorin G_8; 8-HHP - 8-hydroxyhomopterocarpan; D-MEM – Dulbecco's modified Eagle's medium; FBS - fetal bovine serum; MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS - phosphate-buffered saline

Competing Interest: The authors declare no conflicts of interest associated with this manuscript.

Authors' Contributions: Dr. Shimoda isolated the compounds, tested MCF-7 cells, and wrote the manuscript. Mr. Takeda identified the chemical structures. Professor Matsuda and Dr. S. Nakamura provided support for structural identification. Dr. Takarada performed docking simulation. Mr. Shimizu extracted moriche palm extract and Miss Kato purified the chemical compounds. Dr. M. Nakamura and Dr. Handa performed the E-CALUX assay.

Acknowledgment and funding: This study was funded by Oryza Oil & Fat Chemical Co. Ltd.

REFERENCES

1. Phylp HA: Hot flash - A review of the literature on alternative and complementary treatment approach. Alternative Medicine Review 2003, 8:284-302.
2. Ross SM: Menopause: a standardized isopropanolic black cohosh extract (Remifemin) is found to be safe and effective for menopausal symptoms. Holist Nurs Pract 2012, 26:58-61.
3. Shakeri F, Taavoni S, Goushegir A, Haghani H: Effectiveness of red clover in alleviating menopausal symptoms: a 12-week randomized, controlled trial. Climacteric 2015, 18:568-573.
4. Wu WH, Liu LY, Chung CJ, Jou HJ, Wang TA: Estrogenic effect of yam ingestion in healthy postmenopausal women. J Am Coll Nutr 2005, 24:235-243.
5. Farzaneh F, Fatehi S, Sohrabi MR, Alizadeh K: The effect of oral evening primrose oil on menopausal hot flashes: a randomized clinical trial. Arch Gynecol Obstet 2013, 288:1075-1079.
6. Kupfersztain C, Rotem C, Fagot R, Kaplan B: The immediate effect of natural plant extract, *Angelica sinensis* and *Matricaria chamomilla* (Climex) for the treatment of hot flushes during menopause. A preliminary report. Clin Exp Obstet Gynecol 2003, 30:203-206.
7. Sturgeon SR, Ronnenberg AG: Pomegranate and breast cancer: possible mechanisms of prevention. Nutr Rev 2010, 68:122-128.
8. Kapoor R, Ronnenberg A, Puleo E, Chatterton RT Jr, Dorgan JF, Seeram NP, Sturgeon SR: Effects of pomegranate juice on hormonal biomarkers of breast cancer risk. Nutr Cancer 2015, 67: 1113-1119.
9. Pawlowski JW, Martin BR, McCabe GP, McCabe L, Jackson GS, Peacock M, Barnes S, et al.: Impact of equol-producing capacity and soy-isoflavone profiles of supplements on bone calcium retention in postmenopausal women: a randomized crossover trial. Am J Clin Nutr 2015, 102:695-703.
10. Quaas AM, Kono N, Mack WJ, Hodis HN, Felix JC, Paulson RJ, Shoupe D: Effect of isoflavone soy protein supplementation on endometrial thickness, hyperplasia, and endometrial cancer risk in postmenopausal women: a randomized controlled trial.

Menopause 2013, 20:840-844.

11. Lima SM, Yamada SS, Reis BF, Postigo S, Galvão da Silva MA, Aoki T: Effective treatment of vaginal atrophy with isoflavone vaginal gel. Maturitas 2013, 74:252-25.
12. Steinberg FM, Murray MJ, Lewis RD, Cramer MA, Amato P, Young RL, Barnes S, et al.: Clinical outcomes of a 2-y soy isoflavone supplementation in menopausal women. Am J Clin Nutr 2011, 93, 356-367.
13. Gurgel-Gonçalves R, Palma AR, Menezes MN, Leite RN, Cuba CA: Sampling *Rhodnius neglectus* in *Mauritia flexuosa* palm trees: a field study in the Brazilian savanna. Med Vet Entomol 2003, 17: 347-350.
14. Zanatta CF, de Faria Sato AM, de Camargo FB Jr, Campos PM, Rocha-Filho PA: Rheological behavior, zeta potential, and accelerated stability tests of Buriti oil (*Mauritia flexuosa*) emulsions containing lyotropic liquid crystals. Drug Dev Ind Pharm 2010, 36:93-101.
15. Peters CM, Balick MJ, Kahn F, Anderson AB: Oligarchic forests of economic plants in Amazonia: utilization and conservation of an important tropical resource. Conserv Biol 1989, 3:341-349.
16. Aquino JS, Soares JKB, Magnani M, Stamford TCM, Mascarenhas RJ, Tavares RL, Stamford TLM: Effects of dietary Brazilian palm oil (*Mauritia flexuosa* L.) on cholesterol profile and vitamin A and E status of rats. Molecules 2015, 20:9054-9070.
17. Fuentes E, Rodríguez-Pérez W, Guzmán L, Alarcón M, Navarrete S, Forero-Doria O, Palomo I: *Mauritia flexuosa* presents *in vitro* and *in vivo* antiplatelet and antithrombotic activities. Evid Based Complement Alternat Med 2013: 653257.
18. Dos Santos M de FG, Mamede RVS, Rufino M do SM, de Brito ES, Alves RE: Amazonian native palm fruits as sources of antioxidant bioactive compounds. Antioxidants 2015, 4: 591-602.
19. Da Rocha Romero AB, De Carvalho E Martins Mdo C, Moreira Nunes PH, Ravenna Trindade Ferreira N, da Silva Brito AK, Marinho da Cunha PF, et al.: *In vitro* and *in vivo* antioxidant activity of buriti fruit (*Mauritia Flexosa* L.F.). Nutr Hosp 2015, 32: 2153-2161.
20. Ferreira BS, De Almeida CG, Faza LP, De Almeida A, Diniz CG, da Silva VL, Grazul

RM, et al.: Comparative properties of Amazonian oils obtained by different extraction methods. Molecules 2011, 16:5875-5885.

21. Cordeiro LM, de Almeida CP, Iacomini M: Unusual linear polysaccharides: (1→5)-α-*L*-arabinan, (1→3)-(1→4)-α-D-glucan and (1→4)-β-D-xylan from pulp of buriti (*Mauritia flexuosa*), an edible palm fruit from the Amazon region. Food Chem 2015, 173:141-146.
22. Cantu-Jungles TM, Almeida CP, Iacomini M, Cipriani TR, Cordeiro LM: Arabinan-rich pectic polysaccharides from buriti (*Mauritia flexuosa*): an Amazonian edible palm fruit. Carbohydr Polym 2015, 122:276-281.
23. Mori-Hongo M, Takimoto H, Katagiri T, Kimura M, Ikeda Y, Miyase T: Melanin synthesis inhibitors from *Lespedeza floribunda*. J Nat Prod 2009, 72:194-203.
24. Garrett M, David G, Robert H, Ruth H, William H, Richard B, Arthur O: Automated Docking Using a Lamarckian Genetic Algorithm and Empirical Binding Free Energy Function. J Comp Chem 1998, 19:1639-1662.
25. Matsuda H, Shimoda H, Morikawa T, Yoshikawa M: Phytoestrogens from the roots of *Polygonum cuspidatum* (Polygonaceae): Structure-requirement of hydroxyanthraquinones for estrogenic activity. *Bioorg. Med. Chem. Lett*., **11**, 1839—42 (2001).
26. OECD/OCDE BG1Luc estrogen receptor transactivation test method for identifying estrogen receptor agonists and antagonist. OECD guideline for the testing of chemicals/OECD October 2, 2012.
27. Ryu R, Jeong TS, Kim YJ, Choi JY, Cho SJ, Kwon EY, Jung UJ, Ji HS, et al.: Beneficial effects of pterocarpan-high soybean leaf extract on metabolic syndrome in overweight and obese Korean subjects: Randomized controlled trial. Nutrients 2016, 8: pii: E73.
28. He J, Dong Z, Hu Z, Kuang Y, Fan J, Qiao X, Ye M: Regio-specific prenylation of pterocarpans by a membrane-bound prenyltransferase from *Psoralea corylifolia*. Org Biomol Chem 2018, 16: 6760-6766.
29. Lee JW, Lee JH, Lee C, Jin Q, Lee D, Kim Y, Hong JT, et al.: Inhibitory constituents of *Sophora tonkinensis* on nitric oxide production in RAW 264.7 macrophages. Bioorg Med Chem Lett 2015, 25: 960-962.

30. Payton-Stewart F, Khupse RS, Boue SM, Elliott S, Zimmermann MC, Skripnikova EV, Ashe H, et al.: Glyceollin I enantiomers distinctly regulate ER-mediated gene expression. Steroids 2010, 75: 870-878.
31. Bhargavan B, Singh D, Gautam AK, Mishra JS, Kumar A, Goel A, Dixit M, et al.: Medicarpin, a legume phytoalexin, stimulates osteoblast differentiation and promotes peak bone mass achievement in rats: evidence for estrogen receptor β-mediated osteogenic action of medicarpin. J Nutr Biochem 2012, 23: 27-38.
32. Kongkaew C, Scholfield NC, Dhippayom T, Dilokthornsakul P, Saokaew S, Chaiyakunapruk N: Efficacy and safety of *Pueraria candollei var. mirifica* (Airy Shaw & Suvat.) Niyomdham for menopausal women: A systematic review of clinical trials and the way forward. J Ethnopharmacol. 2018, 216: 162-174.
33. Jearapong N, Chatuphonprasert W, Jarukamjorn K: Miroestrol, a phytoestrogen from *Pueraria mirifica*, improves the antioxidation state in the livers and uteri of β-naphthoflavone-treated mice. J Nat Med 2014, 68: 173-180.
34. Cain JC: Miroestrol: an oestrogen from the plant *Pueraria mirifica*. Nature. 1960, 188: 774-777.
35. Chansakaow S, Ishikawa T, Seki H, Sekine (née Yoshizawa) K, Okada M, Chaichantipyuth C: Identification of deoxymiroestrol as the actual rejuvenating principle of "Kwao Keur", *Pueraria mirifica*. The known miroestrol may be an artifact. J Nat Prod. 2000, 63: 173-175.