SHORT REPORT

A C G publications

Rec. Nat. Prod. 14:2 (2020) 139-143

records of natural products

Melanin Synthesis Inhibitors from *Olea europeae*

Amira Elkattan [1,2], Ahmed Gohar [1], Mohamed Amer [1], Zain M. Naeem [1], Ahmed Ashour [1,2] and Kuniyoshi Shimizu [2*]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt*

[2]*Department of Agro-environmental Sciences, Faculty of Agriculture, Kyushu University, 6-10-1 Nishi-ku, Fukuoka 819-0395, Japan*

(Received April 26 , 2019; Revised July 01, 2019; Accepted July 02, 2019)

Abstract: The aim of this study was to discover more candidates for development of novel anti melanogenesis compounds from leaves of *O. europeae***.** Seventeen compounds have been isolated from the leaves of *O. europeae*. The isolated compounds were identified as *α*, *β*-amyrin mixture (**1**), *β*-sitosterol (**2**), uvaol, erythrodiol mixture (**3**), oleanolic acid (**4**), maslinic acid (**5**), vomifoliol (**6**), *β*-sitosterol 3-*O*-*β*-D-glucoside (7), luteolin (**8**)**,** oleoside dimethylester (**9**), oleuropein (**10**), hydroxypinoresinol 1-*O*-*β*-D-glucoside (**11**), luteolin-7-*O*-*β*-D-glucoside (**12**), diosmetin 7-*O*-*β*-D-glucoside (**13**), verbascoside (**14**), oleoside 11-methylester (**15**), secoxyloganin (**16**) and hydroxytyrosol 8-*O*-*β*-D-glucoside, hydroxytyrosol 4`-*O*-*β*-D-glucoside mixture (**17**). This is the first report on the identification of vomifoliol (**6**) in the *oleaceae* family. Results showed that several compounds other than oleuropein exhibited inhibition of melanin synthesis and at the same time with low cytotoxicity.

Keywords *Olea europeae*; melanin; vomifoliol

1. Plant Source

Olive (*Olea europaea* L.) is one of the most ancient cultivated fruit trees and one of the most important oil-producing crop in the Mediterranean countries [1]. *O. europaea* extracts of different organs as well as the isolated components have shown a wide spectrum of pharmacological activities like antiallergic [2] anti-inflammatory, immunomodulatory [3], antioxidant [1], antimicrobial [4], antiviral [5], antidiabetic [6], anticancer [7], neuroprotective [8], antihypertensive [9], gastroprotective [10], and collagen production promoter [11].

2. Previous Studies

In a previous study, we investigated the anti-melanogenesis properties of ethanol extract of olive leaf as well as its major bioactive component, oleuropein. It was demonstrated that these leaves extract as well as the pure oleuropein suppressed the melanin synthesis [11]. However, we expected that there may be other bioactive compounds in the leaves of *O. europeae* that contribute to melanin synthesis inhibition. The aim of this study was thus to discover such compounds from the leaves of *O. europeae* as good candidate for development of novel anti-melanogenesis.

* Corresponding author: E-Mail: shimizu@agr.kyushu-u.ac.jp

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products March-April 2020 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.142.19.04.1264

3. Present Study

By using different chromatographic techniques, we isolated seventeen compounds (**1**-**17**) from the methanol extract of leaves of *O. europeae* (Figure 1). Identification of isolated compounds was achieved by several spectroscopic techniques as well as comparison of different spectroscopic data of the isolated compounds with those reported in literature.

In a previous study performed in our lab [11], it was shown that the ethanol extract of olive leaves (which cultivated in Kyushu Island, Japan) give the strongest inhibition of melanin synthesis however there was a little correlation between the activity strength and oleuropein content of the extract. So the authors suggested that there are existed some other effective compounds than oleuropein according to their conclusion. This encouraged us to examine the anti-melanogenesis effect of all of isolated compounds through this study. In present study, the anti-melanogenic activities of the isolated compounds from *O. europeae* leaves were evaluated by using B16 melanoma cells in order to evaluate the inhibition of melanin formation and cell viability at different concentration using arbutin as positive control. In Table 1, the inhibitory activities of these compounds on melanin formation in B16 melenoma cells was shown at various concentrations. Taking into consideration the cytotoxicity to cell lines, we have found several active compounds exhibiting inhibition of melanin synthesis (sometimes, better than positive control) and at the same time with low cytotoxicity.

Oleuroepin (**10**), oleoside dimethyl ester (**9**) and oleoside 11-methyl ester (**15**) showed good inhibition for melanin synthesis; however, oleoside dimethyl ester and oleoside 11-methyl ester were more active than oleuropein. The absence of the hydroxytyrosol moiety in the latter two compounds may be responsible for increasing their activities. Concerning the cell viability, oleuropein and oleoside dimethyl ester showed moderate cytotoxicity but oleoside 11-methyl ester didn't show any cytotoxic effect and this may be due to replacement of one methyl ester in oleoside dimethyl ester with acid moiety in oleoside 11-methyl ester. Also, secoxyloganin (**16**), which sharing the same structure with oleoside 11-methyl ester (**15**) except of the position of the double bond, didn't show any cytotoxic effect and showed good inhibition of melanin synthesis but lower than oleoside 11-methyl ester. This may be due to the shifting in the position of the double bond. Clearly, Further work is needed to better confirm the Structural Activity Relationships (SAR) of these compounds. The flavonoid compounds luteolin (**8**), luteolin7-*O*-*β*-D-glucoside (**12**), diosmetin 7-*O*-*β*-D-glucoside (**13**), all of them showed strong inhibition of melanin synthesis with low cytotoxicity; however, the flavone aglycone, luteolin, was more active than the other two flavone glycosides. Concerning the pentacyclic triterpenoid compounds, it found that oleanolic acid (**4**) and maslinic acid (**5**) showed moderate inhibition of the melanin synthesis at 25 µM and 50 µM. Although, oleanolic acid is not cytotoxic, maslinic acid showed moderate cytotoxicy at 50 µM. No effect on melanin inhibition was recorded concerning uvaol, erythrodiol mixture (**3**) or *α*, *β*-amyrin mixture (**1**). The other phenolic compounds, verbascoside (**14**), hydroxyl pinoresinol 1-*O*-*β*-D-glucoside (**11**) and hydroxyltyrosol 8-*O*-*β*-D-glucoside, hydroxyl tyrosol 4`-*O*-*β*-D-glucoside mixture (**17**), also showed good inhibition of the melanin synthesis with low cytotoxicity. Vomifoliol (**6**) showed cytotoxicity against B16 melenoma cells at concentration of 50 µM rather than inhibition of melanin formation; however, it showed very strong inhibition for melanin synthesis (~48% and 38%) at concentration of 25 and 12.5 µM, respectively. In conclusion, we have clarified that there are several compounds from olive leaves acting as promising and potential drugs for treating hyperpigmentation, as skin-whitening agents with less toxicity even than the standard drug arbutin. To the best of our knowledge, this is the first time to isolate vomifoliol from family oleaceae which proved to be a potential drug for treatment of patients with hyperpigmentation as well as clarification of compounds other than oleuropein which can be used as candidate for being a melanin inhibitor. Collectively, these findings could provide important clues for the improvement of skin treating disease from the leaves of *O. europeae*

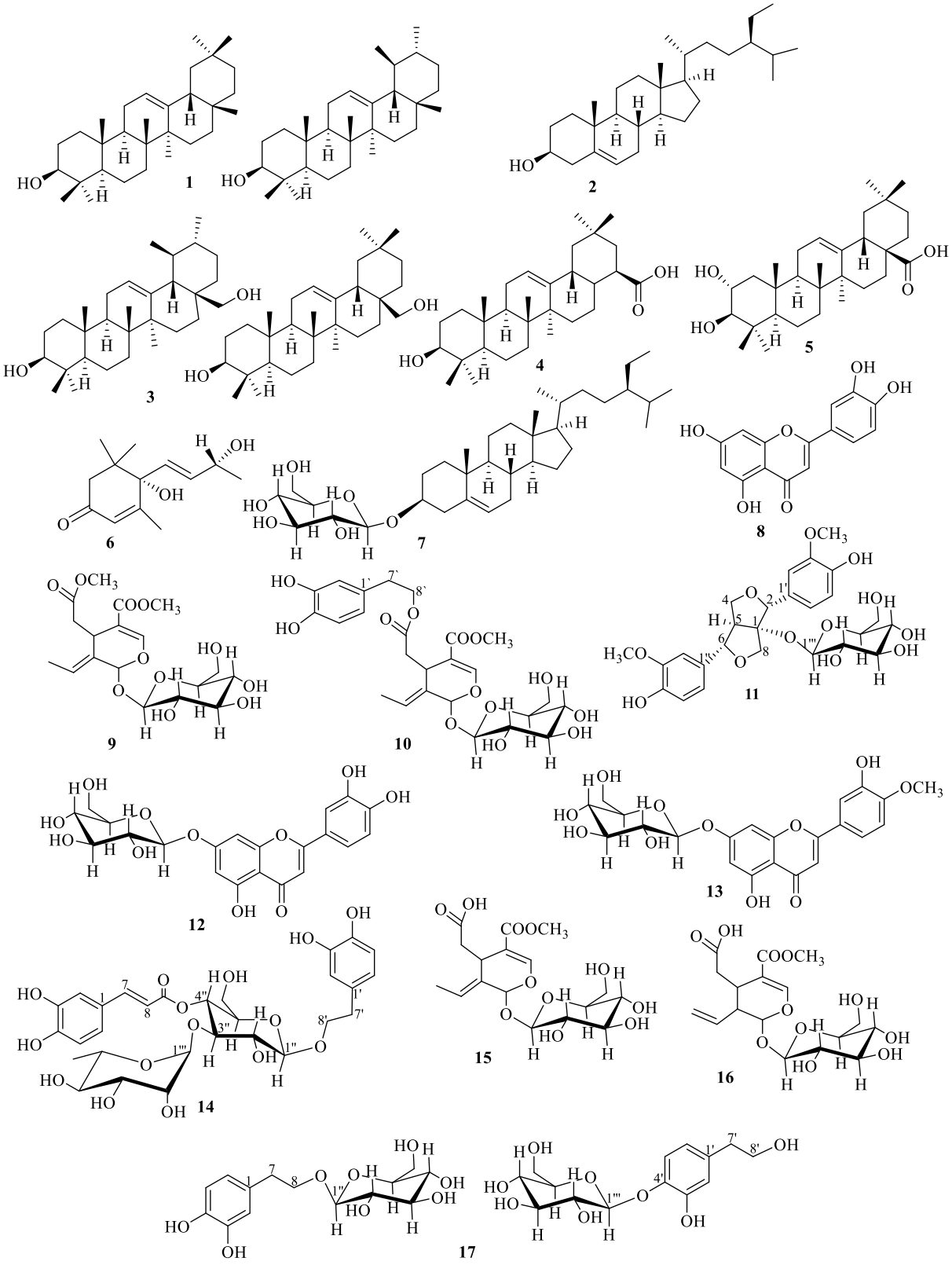

Figure 1. Structures of the isolated compounds (**1-17**)

Table 1. Effects of different compounds of *O. europeae* on melanin biosynthesis and cell proliferation of B16 melanoma cells

Compound	12.5 µM		25 µM		50 µM	
	MC (%)	CV (%)	MC (%)	CV (%)	MC (%)	CV (%)
1*	115.1±2.0	76.7±6.7	98.2±11.9	73.7±2.0	89.2±4.0	66.0±6.2
3*	114.2±3.5	68.8±6.8	116.9±4.8	66.5±3.4	103.5±3.5	67.4±2.8
4	91.0±6.3	114.8±5.1	72.3±6.8	118.8±10.4	60.7±3.5	120.9±3.9
5	92.8±6.1	104.1±5.6	76.3±2.7	95.5±3.8	61.6±4.0	67.2±1.7
6	62.5±4.7	103.0±3.1	52.6±7.4	90.0±4.8	10.2±5.0	69.0±4.8
8	7.1±2.3	105.3±6.0	0.8±2.6	93.4±10.1	0.5±7.6	84.1±7.6
9	33.9±4.0	88.3±10.1	28.1±0.7	81.6±3.1	5.3±3.8	76.5±7.2
10	63.8±3.3	75.8±2.8	46.4±3.3	72.5±2.5	33.9±15.4	73.7±1.7
11	48.6±4.8	105.1±5.5	27.2±10.4	127.2±11.1	26.7±12.1	136.7±1.2
12	30.3±8.0	94.1±3.4	10.2±8.5	83.4±6.0	10.7±5.0	77.4±0.6
13	25.4±4.3	121.6±3.5	17.8±5.8	124.8±9.4	4.9±7.8	94.6±1.0
14	35.2±4.6	103.7±5.4	21.8±4.6	99.7±8.2	9.3±0.7	104.8±6.4
15	25.8±8.1	107.2±0.4	22.3±14.0	135.5±4.0	16.0±10.2	165.8±1.6
16	51.3±7.0	115.3±5.4	44.1±6.8	106.9±3.0	38.3±7.8	110.2±6.3
17*	64.7±3.5	112.7±4.2	36.1±4.7	93.0±1.4	25.0±8.9	78.8±1.8

Data presented as means ±SD (n=3), MC, melanin content (%); CV, cell viability (%). Arbutin was used as a positive control at 20 µM, CV=91.3±2.6, MC=62.1±7.
*The tested compounds are in mixture (please see the text)

Acknowledgments

The Egyptian Government is acknowledged for the fellowship support to Amira Abdalla Elkattan.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Amira Elkattan : 0000-0002-1747-1197
Ahmed Gohar : 0000-0001-5906-1543
Mohamed Amer : 0000-0002-4092-6372
Zain M. naeem : 0000-0003-0343-5627
Ahmed Ashour : 0000-0003-4081-0369
Kuniyoshi Shimizu: 0000-0001-5960-1503

References

[1] L.Abaza, A. Taamalli, H. Nsir, and M. Zarrouk (2015). Olive tree (*Olea europeae* L.) leaves: Importance and advances in the analysis of phenolic compounds, *Antioxidant (Basel, Switzerland)*. **4**, 682-698.
[2] A. Kishikawa, Y. Amen and K. Shimizu (2017). Anti-allergic triterpenes isolated from olive milled waste, *Cytotechnology* **69**, 307–315.
[3] T. Vezza, F. Algieri, A. Rodriguez-Nogales, J. Garrido-Mesa, M. Utrilla, N. Talhaoui, A. Gomez-Caravaca, A. Seguro-Carretero, M. Rodriguez-Cabezas, G. Montelelone and J. Galvez (2017).

Immunomodulatory properties of *Olea europaea* leaf extract in intestinal inflammation, *Mol. Nutr. Food Res.* **61,** 1601066.

[4] M. Mukesi, B. C. Iweriebor, L. C. Obi, U. U. Nwodo, S. R. Moyo and A. I. Okoh (2019). The activity of commercial antimicrobials, and essential oils and ethanolic extracts of Olea europaea on *Streptococcus agalactiae* isolated from pregnant women, *BMC Complement. Altern. Med.* **19**, 1-9.

[5] A. K. Rania, E. D. S. H. Alaa, A. A. Mostafa, H. F. Rania and A. E. Nariman (2016). Anti-HSV type-1 activity of olive leaves extract crude form acting as a microemulsion dosage form, *Afr. J. Microbiol. Res.* **10**, 820–828.

[6] M. de Bock, J. G B Derralk, C. M Brennan, J. Biggs, P. E Morgan, S. C Hodgkinson, P. L Hofman and W. S Cutfield (2013). Olive (*Olea europaea* L.) Leaf polyphenols improve insulin sensitivity in middle-aged overweight men: a randomized, placebo-controlled, crossover trial, *PLoS One*. **8**, e57622.

[7] B. Tunca, G. Tezcan, G. Cecener, U. Egeli, S. Ak Aksoy, H. Malyer, G. Tumen and A. Bilir (2012). Olea europaea leaf extract alters microRNA expression in human glioblastoma cells, *J. Cancer Res. Clin. Oncol.* **138**, 1831–1844.

[8] M. Sarbishegi, E. A. Charkhat Gorgich, O. Khajavi, G. Komeili and S. Salimi (2018). The neuroprotective effects of hydro-alcoholic extract of olive (*Olea europaea* L.) leaf on rotenone-induced Parkinson's disease in rat, *Metab. Brain Dis*. **33**, 79–88.

[9] P. Efentakis, E. Iliodromitis, E. Mikros, A. Papachristodoulou, N. Dagres, A. Skaltsounis and I. Andreadou (2015). Effects of the olive tree leaf constituents on myocardial oxidative damage and atherosclerosis, *Planta Med.* **81**, 648–654.

[10] S. Al-Quraishy, M. S. Othman, M. A. Dkhil and A. E. Abdel Moneim (2017). Olive (*Olea europaea*) leaf methanolic extract prevents HCl/ethanol-induced gastritis in rats by attenuating inflammation and augmenting antioxidant enzyme activities, *Biomed. Pharmacother*. **91**, 338–349.

[11] A. Kishikawa, A. Ashour, Q. Zhu, M. Yasuda, H. Ishikawa and K. Shimizu (2015). Multiple biological effects of olive oil by-products such as leaves, stems, flowers, olive milled waste, fruit pulp, and seeds of the olive plant on skin, *Phyther. Res*. **29**, 877–886.