THE RESTORATIVE COSMETIC CONSTITUENTS OF *Fragraea fragrans* FRUITS

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ABSTRACT

This paper describes 3-hydroxyurs-12-en-28-oic acid and its structural isomer 3-hydroxyolean-12-en-28-oic acid isolated from Fragraea fragrans fruits and their biological activities; anti-tumor, anti-inflammation, anti-microbial, and anti-fungal included their ultra violet photo-protective effect after exposed under sunlight radiation. They are useful for cosmetic ingredient. The above triterpenes are very promoting compounds for leukemia L1210 anti-tumor due to limited reports dealing with this type triterpenoid anti-tumor test. They significantly gave IC_{50} value of 5.78 µg/mL against leukemia L1210 cells, relatively closed to IC_{50} value of drugs, 4.0 µg/mL [12-13,17].

Keywords: *Isomeric; ursolic acid; oleanolic acid; Fragraea fragrans; cosmetic*

ABSTRAK

Artikel ini bertujuan untuk menjelaskan asam 3-hidroksiurs-12-en-28-oat dan pasangan isomer strukturnya, asam 3-hidroksiolean-12-en-28-oat yang berasal dari buah Fragraea fragrans dan aktifitas biologoisnya; yakni anti-tumor, anti-infalamasi, anti-bakteri, dan anti-fungi termasuk efek fotoprotektifnya setelah dijemur terhadap sinar ultraviolet yang berasal dari matahari. Oleh karena itu asam 3-hidroksiurs-12-en-28-oat dan asam 3-hidroksiolean-12-en-28-oat merupakan material yang bermanfaat untuk ingredien kosmetika. Selanjutnya kedua pasangan triterpenoid ini lebih dipromosikan untuk antitumor leukemia L1210 karena disamping terbatasnya laporan tentang uji antitumor untuk jenis ini. Triterpenoid tersebut juga memiliki nilai IC_{50} sebesar 5,78 µg/mL dalam menghambat pertumbuhan sel kanker leukemia L1210, nilai IC_{50} yang mendekati obat antitumor leukemia L1210 dengan IC_{50} sebesar 4,0 µg/mL [12-13, 17].

Kata Kunci: *Isomerik; asam ursolat; asam oleanat; Fragraea fragrans; kosmetika*

INTRODUCTION

It is releasing that the main handicap in producing biologically active compounds for pharmaceutical and cosmetic industries in Indonesia is due to the insufficient compounds in large scale because of the long separation process and limited resources. Ursolic acid [3-hydroxyurs-12-en-28-oic acid] and its isomer oleanolic acid [3-hydroxyolean-12-en-28-oic acid] isolated from *Fragraea fragrans* fruits could be the very promising compounds. In many countries, ursolic acid has been used as cosmetic ingredients because they had some benefit biological activities and their toxicity is low [7,9]. A number of plant species producing ursolic acid were *Ocimum sanctum, Rosmarinus officinalis, Mentha piperita, Lavandula augustifolia, Thymusvulgaris*, *Vaccinum myrtillus*, *Origanum vulgare, Harpagophytum procumbens*, *Sambucus nigra*, *Vinca minor*, *Crataegus laevigata*, *Prunus laurocerasus*, *Prunella vulgaris*, *Vaccinum macrocarpon*, *Actossaphylos uva-ursi*, *Malus spp,* and *Pyrus spp* [6,9]. *Australian solanaceae*, *Duboisia hydrids*, *Eriobotrya japonica*. In this paper we are reporting an alternative resources of ursolic acid (1), dan its isomer oleanolic acid (2) from the fruits of *fragraea fragrans* (Loganiaceae). This plant is locally named as *tembesu* plant, a genuine and potential plant in South Sumatra, Indonesia and its wood is domestically used for furniture and building construction. This plant is about 40 m tall and 80 cm in diameter, and its stalk has 8 to 15 fruits of the size of corn grains, fruiting every year on May to August or November to January [10].

Our phytochemistry work on the fruits of this plant by means of crystallization method, a solid crystal containing a mixture of 3-hydroxyurs-12-en-28-oic acid and its isomer of 3-hydroxyolean-12-en-28-oic acid (making up to 3.03% of the dry weight of the fruits) have easily been taken from methanol extract of the dried powder of those fruits, see Fig. 1, and now we are going to report their anti-cancer, anti-microbial, anti-inflammatory, anti-fungal properties, thus making them potential as an alternative of topical cosmetics.

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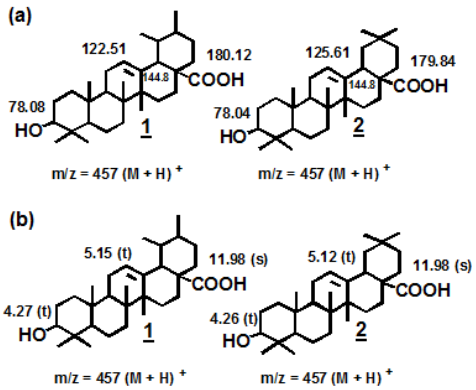

Fig 1. An inseparable mixture of 3-hydroxyurs-12-en-28-oic acid (1) and its isomer of 3-hydroxyolean-12-en-28-oic acid (2) with retention time = 5.82 min: (**a**) = the ^{13}C-NMR and (**b**) ^{1}H-NMR.significant signals observed

EXPERIMENTAL SECTION

Materials

Technical methanol, activated carbon, carrageenin, hydrocortisone acetate, sabouraud dextrose agar, sabouraud dextrose broth, Leukemia L1210 RIKEN Japan, Eagle's NEM medium, DMSO, deuterium pyridine.

Instrumentation

Melting point was determined using Fisher John apparatus. UV (in absolute ethanol) and IR (in KBr form) spectra were recorded on a Beck DU-7500 UV and Shimadzu 8400 FTIR spectrometers, respectively. ^{1}H (DMSO) and ^{13}C NMR (pyridine) were determined on JEOL ECA500 spectrometer operating at 500 MHz (^{1}H), and 125 MHz (^{13}C), respectively: included the ^{1}H-^{1}H COSY NMR. MS spectra were obtained with a JOEL JMS-LX 1000 spectrometer using FAB mode.

Plant Collection

The fruits of *fragraea fragrans (14.5 kg)* were collected in Inderalaya swamp forest, South Sumatra, Indonesia on October 2009, dried at room temperature for six weeks to give the dried fruits (6.1 kg), and milled to give dried powder (5.6 kg).

Procedure

Extraction and Crystallization

The dried fruits powder (3.0 kg) was extracted with methanol (15 L) at room temperature. The methanol extract was then evaporated under reduced pressure to small volume (ca 400 mL) and the precipitate (130.5 g) formed were filtered after 24 h. The precipitate was then dissolved in hot methanol, and activated carbons (15 g) were added and filtered to give a hot methanol filtrate. White crystals (91.0 g, 3.03% of the dried powder), m.p. 284-286 °C, was obtained after crystallization from aqueous methanol. The structural identification of the crystal based on spectroscopic data.

Antimicrobial test

This test was conducted according to procedures in references of 5, 15, and 16. The liquid cultures (1 mL) of *Shigella dysentriae*, *Escherichia coli*, *Bacillus substilis*, and *Staphylococus aureus* were respectively inoculated in petri dish containing NA media (12 mL) at 37 °C for 24 h and the bacteria were spread out by using *drigalski*. Paper disk with 6 mm diameter immersed into 20, 21, and 25 mg/mL tested crystals were placed on inoculated *Shigella dysentriae*, *Escherichia coli*, *Bacillus substilis*, and *Staphylococus aureus* cultures in petri dish. Four paper disks were put in each bacteria cultures i.e. control (1) and tested compounds of 1 and 2. The antimicrobial activity of the crystals was indicated by growth inhabitation of clear zone (cm^2) formed around paper disk, and MIC is noted as the least concentration (mg/mL) those were still inhibited bacterial growth. This method was modified and used for antifungal tests; *Candida albican* and *Micsporum sp* [5,15-16].

Anti-tumor test

Anti tumor test [17] has been conducted in multiwell plate tissue culture. Leukemia L1210 cells, supplied by BATAN Jakarta, were put into the hole of multiwell plate tissue culture (1 mL cells/hole) and the methanol solution of the crystals of 1 and 2 with concentrations of 2, 4, 6, 8, and 10 µg/mL media was dropped into these cells respectively. The cells were incubated in CO_2 incubator at 37 °C for 24 h and afterward cells were counted. In this test methanol has been used as control and inhibitory degree was expressed by IC_{50}. The same procedure was also used to test the crude extracts of the fruits at concentration 5, 10, 20, and 30 µg/mL [11-13].

Anti-inflammatory test

This test was topically carried out on the inflammation induced with 2% carrageenin [2-3]. Hydrocortisone acetate and Vaseline lava were used as comparative agent and carrier, respectively, while the concentrations of tested crystals of 1 and 2 or crude extract were 0.5, 1, 2.5, 5, and 10% (w/w). The cream of tested compounds (200 mg) was swept on webstar male mice skin induced by air (5 mL) and 2% caragenan in oleum sesami (0.5 mL). The treatment was repeated daily for four days. Exudates volume was determined at the fifth day.

Table 1. The growth inhibition of leukemia L1210 cells by 1 and 2

Doses (µg/mL)	Cell amount (x 10^4 cell/mL)				Inhibition (%)	IC_{-50} µg/mL
	I	II	III	Average		
10	17	18	15	16.67	83.38	
8	29	31	32	30.67	69.43	
6	46	49	48	47.67	52.49	5.78
4	69	70	72	70.33	29.90	
2	91	90	89	90.00	10.30	
0 (MeOH)	101	102	98	100.33		

Table 2. The growth inhibition of leukemia L1210 cells by crude residue coming from methanol extracts of *Fragraea fragran* fruits

Doses (µg/mL)	Cell amount (x 10^4 cell/mL)				Inhibition (%)	IC-50 µg/mL
	I	II	III	Average		
30	26	24	30	26.67	72.69	
20	51	47	49	30.67	49.83	
10	89	84	84	84.37	13.66	20.07
5	91	96	99	95.33	2.09	
0 (MeOH)	102	96	95	97.67		

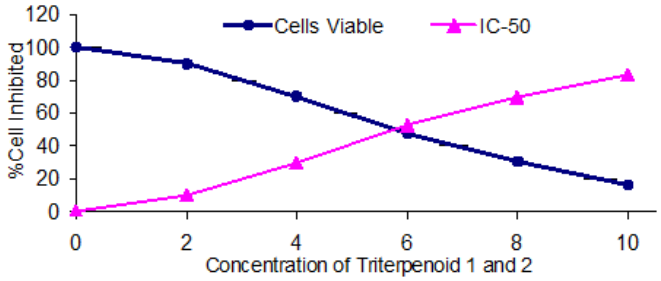

Fig 2. IC_{50} value of 5.78 µg/mL for 1 and 2 at concentration 10 µg/mL isolated from *Fragraea fragrans* fruits

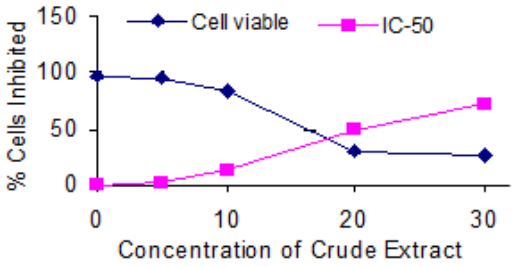

Fig 3. IC_{50} value of 20.07 µg/mL for crude residue at concentration 30 µg/mL coming from methanol extracts of *Fragraea fragrans* fruits

Photo-protective test

This test was conducted according Maier H. et al. [8] procedure. The solid crystals of 1 and 2 (100 mg) was dissolved in MeOH (25 mL) and then dropped into a circle dish with diameter of 8.4 cm. The MeOH was evaporated over 48 h at room temperature until it gave dried material with 1.5 cm in thickness. This dried material was then kept overnight in a desicator and exposed under sunlight over 60 (MD-1), 120 (MD-2), and 180 (MD-3) hours. The UV spectral of the exposed triterpenes 1 and 2 (3 mg/mL) was obtained in order to see the effect of sunlight exposure process, see Fig. 5.

RESULT AND DISCUSSION

Structural determination

A white solid crystal containing a mixture of ursolic acid (1) and oleanolic acid (2), accounted for 3.03% of the dry weight of the fruits, has been isolated from the methanol extract of dried powder of *F. fragrans* fruits by means of crystallization. This white crystal of 1 and 2 gave m.p. 284-286 °C with a single GC peak at 5.82 min due to their similar in polarity. These acids were also similar in pharmacological activity and in nature ursolic acid (1) rarely occurred without its isomer oleanolic acid (2) [7]. The FAB mass spectrum of the crystal showed $[M+H]^+$ at *m/z* 457, corresponds to a molecular formula $C_{30}H_{48}O_3$ (7 DBE consisting of 5 rings). The mass spectrum also indicated fragments at *m/z* 438 (M^+ -H_2O), 411 (M^+ -COOH), and 248 (base peak, M^+ -208), 203 (248 – CO_2 -H). The fragment reaction was given in Fig. 4. The IR spectrum of the crystal showed absorptions for hydroxyl (3447 cm^{-1}), aliphatic C-H (2943 cm^{-1}), carbonyl (1734 and 1716 cm^{-1}), and C=C (1686, 1624, 1541, and 1508 cm^{-1}) groups. The UV spectrum of the crystals gave maxima at 242 (*abs.=0.91*) and 336 (*abs.=0.03*) nm. The ^1H NMR spectrum in DMSO gave signals at δ_H 11.98 (s, 1H, -COOH), 5.15 (t, 1H, -CH=C- of 1, J=3.5 Hz), 5.12 (t, 1H, -CH=C- of 2, J=3.5 Hz), 4.27 (*t*, 1H, H-3α of 1), 4.26 (t, 1H, H-3α of 2), 2.98 ppm (m, 1H, H-2 in 1 and in 2), 2.72 (k, 1H, in H-18 in 1 and in 2), and 2.2 to 0.8 for the rest of methyl, methylene, and methine protons. In addition to ^1H NMR, the ^1H-^1H COSY NMR of 1 and 2 significantly showed the off-diagonal contours

Table 3. ^{13}C NMR data of 1 and 2

Carbon Number	1A	1B	2
1	38.28	38.81	38.82
2	23.21	23.57	23.57
3	80.86	78.08	78.04
4	37.85	37.32	37.32
5	55.27	55.76	55.76
6	18.15	18.74	18.74
7	32.94	33.21	33.13
8	39.43	39.71	39.71
9	47.41	46.61	46.63
10	37.85	37.31	37.31
11	23.59	23.65	23.65
12	125.37	122.51	125.61
13	138.11	144.78	144.79
14	41.99	41.95	41.95
15	29.57	28.73	28.26
16	24.13	23.77	23.71
17	47.79	48.07	47.99
18	52.74	53.49	53.49
19	39.01	39.32	39.32
20	38.82	38.89	38.89
21	30.58	30.91	30.44
22	36.74	34.16	34.16
23	28.02	28.04	28.04
24	16.82	16.49	16.49
25	15.4	15.51	15.51
26	16.77	15.62	15.62
27	25.74	26.12	26.12
28	180.05	180.12	179.84
29	16.91	17.46	17.38
30	21.04	21.37	21.37

A. 1 in *Maprounea guianensis*, *Quim. Nova* (2004), 27, 1, 62–65, $CDCl_3$ [4]

B. 1 in *Fragraea fragrans* fruits as isolated compound, pyridine

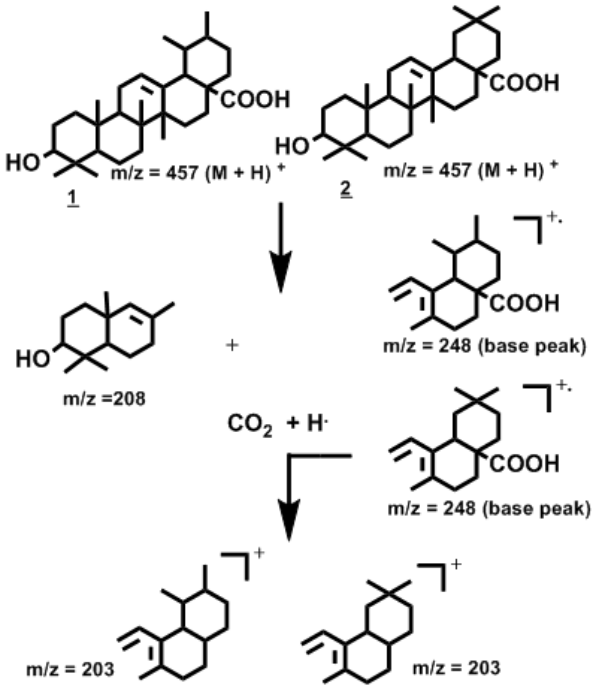

Fig 4. Fragmentation reaction of 1 and 2

representing ^{1}H-^{1}H spin-couplings between H-12 and H-11, H-3 and H-2, H-2 and H-1, H-18 (δ_H = 2.72) and H-19 (δ_H = 1.6) in 1, H-18 (δ_H = 2.72) and H-19 (δ_H = 1.1) in 2. The main proton signals for 1 and 2 observed were given in Fig. 1b. The ^{13}C NMR in pyridine showed signals at δc 179.8 (C-28 in 1), 181.1 (C-28 in 2), 144.7 (C-13 in 1 and 2), 122.5 (C-12 in 1), 125.6 (C-12 in 2), 78.07 (C-3 in 1), and 78.03 (C-3 in 2), see Table 3 and Fig. 1a [4]. These data suggested that the crystal was a mixture of compounds 1 and 2 as structural isomers. Treatment of compounds 1 and 2 with 5% KOH/methanolic gave the salt with *m/z* 533 (M^+ + 2K) (FAB). This salt was also prepared for cosmetic emulsifying agent.

Anti-tumor

The solid crystals of compounds 1 and 2 showed that it significantly inhibited 83.4% of leukemia L1210 cell growth at concentration 10 µg/mL with IC_{50} value of 5.8 µg/mL, see Fig. 2, and The growth inhibition data of leukemia L1210 cells by 1 and 2 was given in Table 1. While the crude extract inhibited 72.7% of the cells at concentration 30 µg/mL with IC_{50} value of 20.07 µg/mL, see Fig. 3 and the growth inhibition data of leukemia L1210 cells by those was given in Table 2 [11-13,17].

Antimicrobial

The results of this showed that the solid crystals of compounds 1 and 2 gave stronger growth inhibition zone (cm^2 in diameter) for *Shigella dysentriae* (1.06) *Escherichia coli* (1.16), *Bacillus substilis* (0.85), and *Staphylococus aureus* (1.00) at concentration of 25 mg/mL, and those did not gave any growth inhibition clear zone at concentration 20 mg/mL. Therefore compounds 1 and 2 had minimum inhibition concentration (MIC) range in (mg/mL): 21 (16.4 mm^2), 21 (24.3 mm^2), 21 (12.3 mm^2), and 22 (10.5 mm^2) respectively [5,15-16].

Antifungal

The isomeric pair of these acids also showed fine clear zone with the growth inhibition concentration on 25-35 mg/mL for *Candida albican* and *Micsporum sp*. Therefore, the solid crystals were potential constituents of the fruit for topical cosmetics as well as their antibacterial activity [1,15].

Anti-inflammatory

The inseparable mixture of 1 and its isomeric structure of 2 indicated that it could effectively reduce 40% of carrageenin induced inflammation, while the

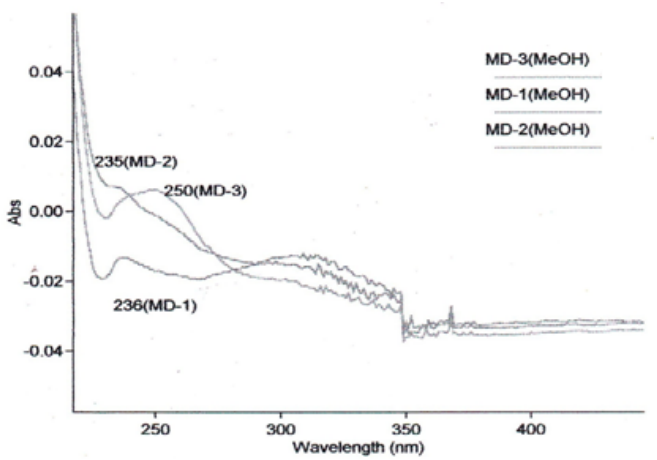

Fig 5. UV spectral of 1 and 2 in MeOH after sunlight exposure

crude extract was 49%. The mixture compounds at concentration 2.5% gave the same anti-inflammatory effect as those of hydrocortisone acetate at concentration 1%, while the crude extract at the concentration 5% corresponded to hydrocortisone acetate effect at concentration 2.5% [2-3].

Photo-protective

The objection of this work was only to evaluate the stability of 1 and 2 under sunlight exposure by observing their UV spectral pattern. The UV spectra of 1 and 2 after 60, 120, and 180 h exposure compared to unexposed one relatively did not change λ-max of C=C and C=O chromospheres in range of 242 (*abs.=0.91*) and 336 (*abs.=0.03*) nm. The UV spectral of this exposed triterpenes in methanol was given in Fig. 5. The UV maxima of C=C for exposure triterpenes was 236, 235, and 250 nm respectively [8]. As a result, the triterpenes 1 and 2 were wisely recommended for topical cosmetic ingredients.

CONCLUSION

This paper reported the chemical and biological investigation of the fruits of *F. fragrans*, a traditional medicinal plant of Sumatera. The fruit contained a large amount of solid crystals consisted of a mixture of isomeric compounds 1 and 2. On biological evaluation the inseparable triterpenes showed significant anti-tumor, anti-bacterial, anti-fungal, and anti-inflammatory effects; included photo-protective property. Therefore, the solid crystals were potential constituents of the fruit for topical cosmetic.

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REFERENCES

1. Abad, M.J., Ansuategui, M., and Bermejo, P., 2007, Active antifungal substances from natural sources, *ARKIVOC,* vii, 116–145.
2. Baricevic, D., Sosa, S., and Simonovska, B., 2001, *J. Ethnopharmacol*., 75, 2-3, 125–132.
3. Cao, B.J., Meng, Q.Y., and Ji, N., 1992, *Planta Med.*, 58, 496-498.
4. David, J.P., Meira, M., David, J.M., and Guedes, M.L.S., 2004, *Quim. Nova*, 27, 1, 62–65.
5. Gohari, A.R., Saeidnia, S., Shahverdi, A.R., Yassa, N., Malmir, M., Mollazade, K., and Naghinejad, A.R., 2009, *EurAsia J. BioSci.*, 3, 64–68.
6. Patočka, J., 2003, *J. Appl. Biomed.*, 1, 7–12.
7. Liu, J., 1995, *J. Ethnopharmacol.,* 49. 57–68.
8. Maier, H., Schauberger, G., Martincigh, B.S., Brunnhofer, K., and H. Honingsmann, 2005, *Photodermatol. Photoimmunol. Photomed.*, 21, 2, 84–92.
9. Majeed, M., and Nujoma, Y (*Copyright* 2000), Ursolic acid; its importance in skin and hair beautification and protection, http://www.sabinsa.com/products/ursolic-paper.htm.
10. Martawijaya, A., 1989, *Atlas kayu Indonesia*, vol. II, Departemen Kehutanan Badan Penelitian dan Pengembangan Kehutanan, Bogor.
11. Meng, Y., Song, Y., Yan, Z., and Xia, Y., 2010, *Molecules*, 15, 4033–4040.
12. Nagumo A., Takanashi, K., Hojo, H., and Suzuki, Y., 1991, *Toxicol Lett.,* 58, 3, 309–313.
13. Novotny L., Vachalkova, A., and Biggs, D., 2001, *Neoplasma*, 48, 4, 241–246.
14. Pillai, S., Oresajo, C., and Hayward, J., 2005, *Int. J. Cosmet. Sci.,* 27, 1, 17–34.
15. Rasyid, A., and Adachi, K., 2007, *Indo. J. Chem.,* 7, 3, 350–353.
16. Saleh, M., Kamel, A., Li, X., and Swaray, J., 1999, *Pharm. Biol.,* 37, 1, 63–66.
17. Sumatra, M., 1998, Bioassay in Vitro with L1210 Leukemia Cells, A screening method of anticancer constituents from natural products, *Proceeding of Indonesian marine biotechnology seminar,* Jakarta, 14-15 October, 183-188.