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by Miksusanti Salbi

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Derivatization of Inseparable Ursolic and Oleanolic Acids of *Fagraea fragrans* Fruits to Enhance Their Anticancer Activity

Dasril Basir^{1*}, Miksusanti¹, Susilawati²

¹Department of Chemistry, Faculty of Sciences, University of Sriwijaya, Inderalaya 30662, Indonesia

²Department of Parasitology, Faculty of Medicine, University of Sriwijaya, Palembang, Indonesia.

*Corresponding Author email : debasril_chem@yahoo.com

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ABSTRACT. Inseparable ursolic acid and its isomeric oleanolic acid are the major compounds in *Fagraea fragrans* fruits. The white solid crystals, 3.1 % of these triterpenic acids are easily isolated from alcoholic extracts of these dried fruits. They are well known in both cosmetic and medicinal industries. Therefore the objective of this work is to derivative those to be the inseparable ethyl ursolate 3-ethyl ether [its isomeric ethyl oleanolate 3-ethyl ether], and to evaluate their anticancer activity against P-388 murine leukemia cells. The results are 71% of the derivatives have been successfully made from the inseparable ursolic [its isomeric oleanolic] acids by *in situ* reaction between those triterpenic acids with thionyl chloride and ethanol in benzene. The anticancer activity of ethyl ursolate 3-ethyl ether [its isomeric ethyl oleanolate 3-ethyl ether] against P-388 murine leukemia cells with IC₅₀ value of 31.36 µg/mL is twofold (1.7) more potent than their mother compounds, the inseparable ursolic [its isomeric oleanolic] acids with IC₅₀ value of 53.5 µg/mL.

Keywords: Anticancer, ethyl oleanolate 3-ethyl ether, ethyl ursolate 3-ethyl ether, *F. fragrans*.

INTRODUCTION

Such as anticancer drugs, cosmetic compound candidates had also to be evaluated their anti-neoplastic activity potentiality before they were applied as new therapeutic agents with the cancer chemopreventive effect on the cosmetic uses thereof because the primary neoplastic diseases of the skin was common. Ursolic [oleanolic] acids as a mixture or inseparable white solid crystals were the well known triterpenic acids for skin cares because of their pharmacological properties; antiinflammatory (Vasconcelos et al., 2006), antiallergic, antiparasitic, antibacterial (Catteau et al., 2017) antiviral, hepatoprotective, antiulcer, cardioprotective, and antitumor activities (Furtado et al., 2008; Meng et al., Song et al., 2010; Sultana, 2011). The synergistic activity of the acid mixture on skin cancer models was also reported as well as the application these natural products as dermatocosmetology or various other therapeutic preparations (Soica et al., 2014; Wójciak-Kosior et al., 2011), including as a protective effect against colon carcinogenesis (Furtado et al., 2008). They are also highly synergistic with β-lactams (ampicillin and oxacillin) at sub-MIC concentrations (Catteau et al., 2017). The recent progress of ursolic [oleanolic] acids was that they were having potential effects for treatment of type 2 diabetes acting as hypoglycemic and anti-obesity agents (Silva et al., 2016), including OA (oleanolic acid) and its isomer

UA (ursolic acid) are also potent anti-cancer agents to cause apoptosis in human liver cancer HepG2, Hep3B, Huh7 and HA22T cell lines at OA or UA at 2, 4, 8 µmol/L (Mlala et al., 2019; Yan et al., 2010). Those triterpenic acids having lower toxicity and attractive many pharmacological effects were not only found in apple peels (*Malus X domestica* Borkh) but also in other species such as *Crataegus pinnatifida* var. *Psilosa* fruits, *Miconia fallax*, *Galium tortumense* (Guenalp et al., 2006), and *Eriope blanchetti* (De L.e. Silva et al., 2012), but also in *Fagraea fragrans* fruits. These were harvested two times a year in May and September as ripe months of the fruits (Basir & Julinar, 2012). In short these triterpenic acids, especially modified ones could be used in both topical cosmetic, and oral drug (Zacchigna et al., 2014) or food supplement candidates (Silva et al., 2016), including as praprotect compounds for making their other derivatives with potent anticancer efficacy (Basir et al., 2014; Sultana, 2011).

This paper preferably describes the increase of P-388 murine leukemia anticancer activity of ursolic acid [oleanolic acid] derivatives, namely ethyl ursolate 3-ethyl ether [its isomeric ethyl oleanolate 3-ethyl ether]. Those were made by means of alkoxylation at C-3 and esterification at C-28 between ursolic [oleanolic] acids of *Fagraea fragrans* fruits, thionyl chloride, and ethanol in benzene as *in situ* reaction, see Figure 1. Evethough, it had more than hundreds

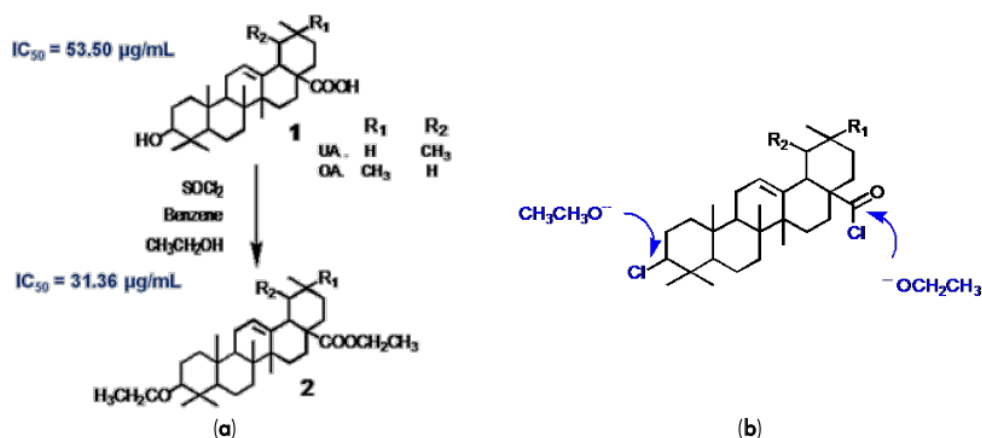


Figure 1. Derivatization reaction (a) and plausible reaction mechanism (b) of 1 to be 2

of scientific articles published recently dealing with ursolic acid⁶ and oleanolic acid including their derivatives (Chen et al., 2015; Gnoatto et al., 2008; Shanmugam et al., 2014; Sun et al., 2006). We are firstly reporting the transformation reaction of ursolic [oleanolic] acids to be the ethyl ursolate 3-ethyl ether [its isomeric ethyl oleanolate 3-ethyl ether] as new derivatives (Wang et al., 2014), and their antiproliferation activity in inhibiting P388 leukemia cell growth (Chu et al., 2014; De L.e. Silva et al., 2012; Nelson et al., 2015). As a result, these modified compounds are expected to be the potential ingredients for topical cosmetic rather than their mother compounds due to potent antitumor and no itching effects in topical delivery (Zacchigna et al., 2014). In short, the *Fagraea fragrans* fruits as the raw material which are rich with 3.1% of ursolic acid and its isomeric oleanolic acids (relatively 50% : 50%). They are similar in polarity and biological activities; they are only difference in position of methyl group in ring-E so that they can be potential starting material for both cosmetic and medicinal compounds in the future. In addition both of these inseparable triterpenic acids can easily explored just by means of recrystallization from the concentrated methanol extracts of *F. fragrans* fruits. It seems to be profitable to make these anticancer derivatives from those fruits.

EXPERIMENTAL SECTION

Materials

Technical methanol, activated carbon, Whatmann paper, HCl (Unilab), Silica gel Plate G60 F254, Silica gel G60 (70-230 mesh), Na₂SO₄ anhydrous, ethyl acetate, n-hexane aquadest, thionyl chloride, ethyl (p.a), and acetone, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), fetal bovine serum (FBS), penicillin, streptomycin, P388 murine leukemia cell culture supplied by Department of Chemistry (ITB), dimethyl sulfoxide (DMSO, phosphor

buffer solution (PBS), artonin E, sodium dodecyl sulphate.

Instrumentation

Melting point was determined using Fisher Johns 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. ¹H (CD₃OD) and ¹³C-NMR (CD₃OD) were determined on JEOL ECA500 spectrometer operating at 500 MHz (¹H), and 125 MHz (¹³C), respectively. HITACHI L6200 LC-MS ESI positive ion: Injection volume (5 µl), low (1 mL / min), column C-8 (15 mm x 6 mm), and eluent (MeOH).

Fruits Collection and Recovery of The Isomeric Triterpenic Acids

The fruits of *Fagraea fragrans*, Loganiaceae as raw materials of this work were collected in Inderalaya swamp forest, South Sumatra, Indonesia at the first week of November 2020, dried under sun light for a week and at room temperature for a week, milled to be powder, and then extracted with methanol for preparing the starting materials for this works. The recovery methods of ursolic [oleanolic] acids were done according to our previous work (Basir & Julinar, 2012; Basir et al., 2014).

Derivatization of 1 to be 2

Compound 1 (700 mg, 1.5 mmol) in benzene (20 mL) was stirred slowly for one hour at room temperature, and then thionyl chloride (0.5 mL, 7.5 mmol) was carefully dropped to the stirred benzene solutions. The temperature of reflux was raised to be 60° C and stirring process was continuing for five hours. After that ethanol (0.2 mL, 3.0 mmol) was added to the thionyl chloride solutions while it was stirring for another three hours. Alkoxylation and esterification products of the *in situ* reactions were tested by TLC in 15% ethyl acetate in n-hexane (R_f=0.2), and reaction will soon be stopped. Ethyl

acetate (200 mL) was added to this later products, washed with water (3 x 75 mL), shaken in separating funnel and then water solution was separated from ethyl acetate layers. The later organic solutions were dried with Na₂SO₄ anhydrous (5 g), filtered with Whatmann paper and evaporated by reduced pressure rotary evaporator.

Residue was preadsorbed to silica gel G60 (8 g) and subjected to silica gel G60 column (45 g) with increasing solvent polarity from 10 to 20% ethyl acetate in hexane. Compound 1 (497 mg) or 71% was collected from 38 to 91 vials (5 mL/vial), tested for both spectroscopy (LCMS positive ion, H and C NMR) including melting point measurement, and evaluated its P388 leukemia cell anticancer.

Anticancer Test

P-388 murine leukemia cancer cell cultures (3 x 10³ cell/mL) were suspended into RPMI 1640 media having contained FBS (Fetal Bovine Serum), penicillin, and streptomycin. Cells were inoculated in microplate 96 well plate and incubated in CO₂ incubator for one day. At the second day, the DMSO (dimethylsulfoxide) solution of the crystals of 1[2], 3[4], and 5[6] was diluted with PBS (phosphoric buffer solution, pH = 7.30-7.65) for variation concentrations of 100, 30, 10, 3, 1, 0.3, and 0.1 µg/mL media and then was dropped into those cells respectively. These last cells in microplate were incubated again in CO₂ incubator. The DMSO was used as negative control and artonin E (IC₅₀ = 0.7 µg/mL) as positive control.

After 48 h the incubation process, MTT reagent was added into the cells, incubated during 4 h, and SDS (Sodium Dodecyl Sulphate) was then added, shaken as well, and continuously incubated for other 24 h. The color change of MTT in viable mitochondria cells from yellow to purple could be quantified with spectrophotometer at λ = 550 nm. The values of OD and concentration (µg/mL) of tested compounds were reported as the mean three of replicates. The IC₅₀ value was noted from antilog graphics based on the correlation of tested compound concentrations (µg/mL) and color intensity of cell viable solution (Basir & Julinar, 2012; Basir et al., 2014).

RESULTS AND DISCUSSION

Compound 1 consisted of ursolic [oleanolic] acids; it had IC₅₀ value of 5.78 µg/mL and IC₅₀ value of 53.5 µg/mL against L-1210 murine leukemia cells and P-388 murine leukemia cells respectively, including its graphics of the concentration versus the

absorption (OD) of P388 leukemia cells, tabel of IC₅₀ and absorbance (optical density) at a wave length of 550 nm, spectroscopy data and purification method from the dried *Fagraea Fragrans* fruits (Basir & Julinar, 2012; Basir et al., 2014).

Compound 2 consisted of ethyl ursolate 3-ethyl ether [its isomeric ethyl oleanolate 3-ethyl ether]; it had m.p. = 144-145 °C with retention time (rt) = 0.2 in 15% ethyl acetate in hexane. LC-MS ESI pos ion with eluent MeOH gave respectively a single peak at retention time (r.t.) = 6.12 min, see Figure 3B; and main peaks at m/z = 1025.5 [2M + H]⁺ as protonated dimeric ions, 519.3 [M + Li]⁺ as pseudomolecular ions, 484.2 [M - CH₂ = CH₂]⁺ as fragmentation molecular ions, and 467.0 [M - OCH₂CH₃]⁺, see Figure 4a. Therefore, molecular ion was M⁺ = 512 with C₃₄H₅₆O₃ as empirical formula, m/z = 277.7 [base peak of 2]⁺, while the base peak of 1 is at m/z = 248 so that m/z = 247 was come from the [base peak of 2 (m/z = 277.7) - CH₂ = CH₂ (m/z = 28) - 2H (m/z = 2)]⁺ ion, see Figure 4b (Basir & Julinar, 2012).

The main fragment reaction of 2 as retro Diels Alder for its base peak at m/z = 277.7 (experiment) and 278 (calculated) was given in Figure 2. IR gave peaks (cm⁻¹) at 2924, 2854 (-CH), 1697 (-C=O), 1458, 1381 (-C = CH-), 1273, 1165, 1010, 887, 825, 579, 532. ¹H NMR and ¹³C NMR of 2 were showed in Table 1 including DEPT technique. In addition, HMBC spectral of 2 clearly had some circles countour coming from the ethylene proton (δ = 4.07, H_{1'}) of the ester part to its carbon carbonyl of -C₂₈OO-CH₂-C (δ = 179.6) at C-28 position and methylene proton (δ = 2.91, H_{3'}) of the ethoxyde part to the carbon of -C₃-O-CH₂-C (δ = 69.5) at C-3 position connectivities. DEPT technique of 2 also indicated that the peaks at (δ) 84.5 (OA), 69.5 (UA) for CH at C-3, 123.7 (OA), 126.8 (UA) for CH at C-12, and 48.2 (OA), 47.2 (UA) for CH₂ at -C₃-O-CH₂-C were isomeric peaks of ethyl ursolate 3-ethyl ether [its isomeric ethyl oleanolate 3-ethyl ether]. Such as pentacyclic triterpene saponins, namely chikusetsusaponin IVa isolated from *Altermenthera philoxeroides* (Mart.) Griseb, compound 2 with two simetrical ethoxyde (CH₃CH₂O-) substituents at C-3 and C-28 of inseparable mixture of the ethyl ursolate 3-ethyl ether [its isomeric ethyl oleanolate 3-ethyl ether] could be be also considered to be tested for antiviral activities (Wang et al., 2014).

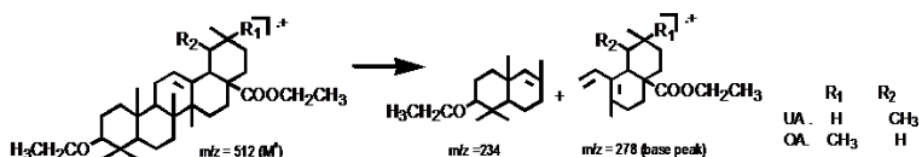


Figure 2. The main fragment reactions of 2.

Moreover, A number of different reagents and methods had separately made methyl ursolate 3-methyl ether and methyl oleanolate 3-methyl ether each other as the NO production inhibition activities on LPS-induced RAW264.7 cells (Kwon et al., 2009). As a summary, all of the aboved chemical and spectral data confirmed that the compound 2 was formed accurately as it is shown in **Figure 1a**, mechanism reaction is likely to be true as it is given

in **Figure 1b**, and their fragmentation reaction was also synchronizing to the target compound 2 as it is shown in **Figure 2**. Kinetically, another organic chemistry work is still necessary to evaluate the *in situ* transforming reactivity of the secondary alcohol via secondary alkyl chloride at C-3 and carboxyl at C-28 via acid chloride in ursolic [oleanolic] acids to be ethyl ursolate 3-ethyl ether [its isomeric ethyl oleanolate 3-ethyl ether].

Table 1. ^1H and ^{13}C NMR chemical shifts of 2 including DEPT technique

No	Compound 2			DEPT
	C	δC	δH	
1		38.1		CH ₂
2		28.8	1.41 (2H, <i>m</i>)	CH ₂
3		84.5 (OA), 69.5 (UA)	3.61 (1H, <i>dd</i> , $J = 10.1/4.5$ Hz)	CH
4		40.8	-	C-
5		54.5		CH
6		19.6		CH ₂
7		33.8		CH ₂
8		40.6		C-
9		42.8		CH
10		39.3		C-
11		24.1	1.52 (2H, <i>dd</i>)	CH ₂
12		123.7 (OA), 126.8 (UA)	5.26 (1H, <i>t</i> , $J = 3.5$ Hz)	CH
13		145.2	-	C-
14		31.6		C-
15		24.6		CH ₂
16		24.0		CH ₂
17		33.7		C-
18		40.4	1.63 (1H, <i>dd</i> , $J = 12.1/4.1$ Hz)	CH
19		33.8		CH ₂ (OA), CH (UA)
20		39.3		C- (OA), CH (UA)
21		33.9		CH ₂
22		34.9		CH ₂
23		29.4	0.94 (3H, <i>s br</i>)	CH ₃
24		17.5	0.76 (3H, <i>s br</i>)	CH ₃
25		14.6	0.81 (3H, <i>s br</i>)	CH ₃
26		17.8	1.01 (3H, <i>s br</i>)	CH ₃
27		26.5	1.23 (3H, <i>s br</i>)	CH ₃
28		179.6	-	C-
29		24.0	0.94 (3H, <i>s br</i>) OA [0.86 (3H, <i>s br</i>)	CH ₃
30		21.7	0.79 (3H, <i>s br</i>) OA [0.91 (3H, <i>s br</i>),	CH ₃
1'		61.6	4.07 (<i>m</i>), -COO-CH ₂ -C	CH ₂
2'		40.4	1.93 (<i>m</i>), -COO-C-CH ₃	CH ₃
3'		48.2 (OA), 47.2 (UA)	2.91 (<i>m</i>), -O-CH ₂ -C	CH ₂
4'		33.6	1.68 (<i>m</i>), -O-C-CH ₃	CH ₃

UA = ursolic acid, OA = oleanolic acid; Solvent CD₃OD

Anticancer Activity of Compound 2

Compound 2, ethyl ursolate 3-ethyl ether [its isomeric ethyl oleanolate 3-ethyl ether] making in solvent condition by reacting ursolic acid [its isomer oleanolic acid], thionyl chloride, ethanol, and benzene as solvent clearly gave IC_{50} values of the P-388 murine leukemia cell anticancer 31.36 $\mu\text{g}/\text{mL}$. The results of this *in vitro* test of compound 2 were given in **Table 2** and compound 1 were reported in our previous work (Basir et al., 2014). Artonin E and DMSO were used as positive and negative controls, while the absorbance (OD) was read at a wavelength of 550 nm. The IC_{50} values of compound 2 was determined from the graphics of the tested compound concentration versus the P388 leukemia cell absorption, see semilog graphics of 31.3598315 (x-axis) as concentration ($\mu\text{g}/\text{mL}$) and 0.24630058 (y-axis) as absorbance or optical density (OD), see **Figure 3A**, while the graphics of the tested compound concentration of mother compound 1 versus the P388 leukemia cell absorption with semilog graphics is 53.3818 (x-axis) as concentration ($\mu\text{g}/\text{mL}$) and 0.2937 (y-axis) as absorbance or optical density (OD),

see also our previous work (Basir et al., 2014). As a result, the effect of $-\text{OCH}_2\text{CH}_3$ group at C-3 and $-\text{COOCH}_2\text{CH}_3$ group at C-28 in compound 2 with the P-388 murine leukemia cell anticancer activity (31.36 $\mu\text{g}/\text{mL}$) significantly increased almost twofold (1.7) more potent than compound 1 with the P388 leukemia cell anticancer activity (53,5 $\mu\text{g}/\text{mL}$) having $-\text{OH}$ group at C-3 and $-\text{C}_6\text{OH}$ group at C-28. However in our previous work, *N*-butyl-urs-2,12-dien-28-amide [7]-butyl-olean-2,12-dien-28-amide], and *N*-phenyl-urs-2,12-dien-28-amide [N-phenyl-olean-2,12-dien-28-amide] making in free solvent condition by reacting between ursolic [oleanolic] acids, thionyl chloride, *n*-butylamine, and phenylamine respectively gave the IC_{50} value of 81, 4 $\mu\text{g}/\text{mL}$ and 83.6 $\mu\text{g}/\text{mL}$. It was 1.5 times lower than the IC_{50} value of mother compounds (53,5 $\mu\text{g}/\text{mL}$) because the $-\text{OH}$ group at C-3 was converted to be $-\text{C}=\text{C}-$ group (Basir et al., 2018). It means that ethoxy group at C-3 is very important for anticancer activity rather than hydroxyl group, and hydroxyl group at C-3 is much better than unsaturated carbons ($-\text{C}=\text{C}-$) at C-2 and C-3 positions (Ovesná et al., 2006).

Table 2. IC_{50} and absorbance (optical density) of 2 at a wavelength of 550 nm.

No	Compound 2		
	Concentration ($\mu\text{g}/\text{mL}$)	Optical density (OD)	
1	100	-0.02266	
2	30	0.25573	
3	10	0.30200	
4	3	0.33501	31.36
5	1	0.34000	
6	0.3	0.40333	
7	0.1	0.38767	

OD of positive blank for 1 = 0.2943 and 2 = 0.2463

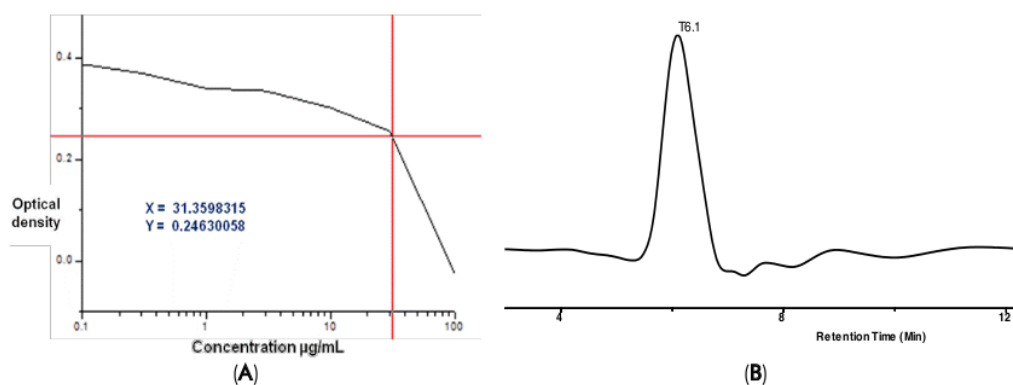


Figure 3. (A) = Graphics of the concentration of 2 vs the absorption (OD) of P388 leukemia cells with $IC_{50} = 31.36 \mu\text{g}/\text{mL}$ and (B) = LC chromatogram of 2 with retention time = 6.12 minutes.

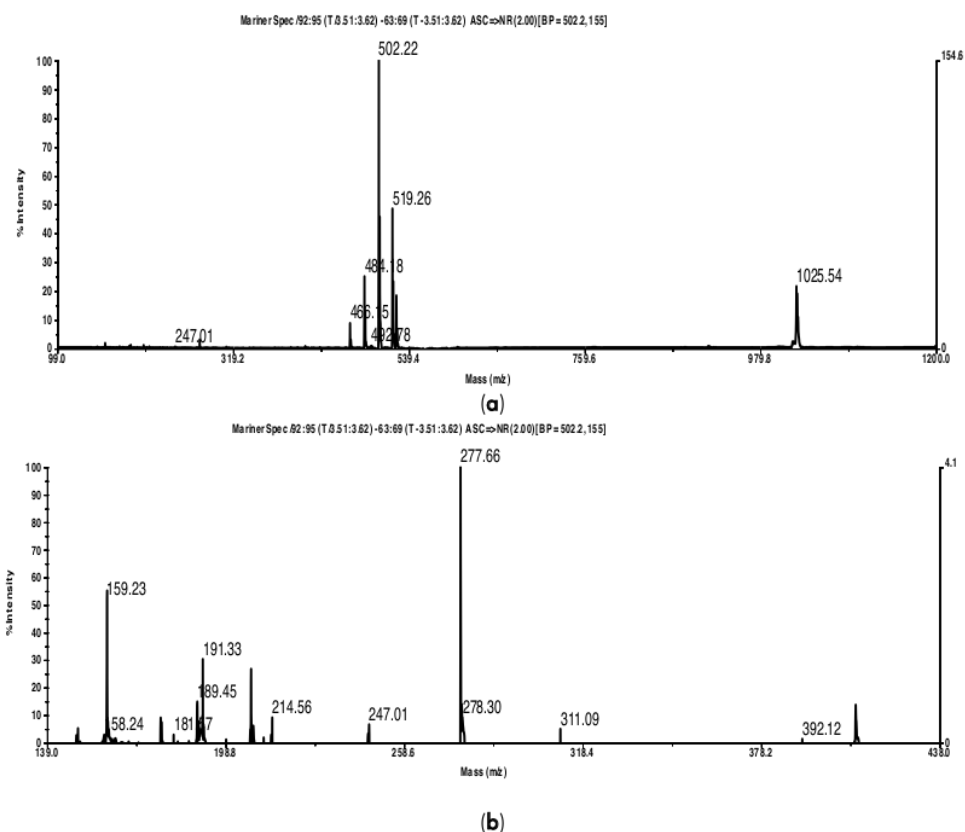


Figure 4. LC-MS ESI pos ion of **2** : (a) = pseudomolecular ion peak (519.26 [M+Li]⁺), protonated dimeric ion peak (1025.54 [2M+H]⁺), and (b) = its base peak (277.66 [M-234]⁺).

Table 3. The effectiveness of **1** and **2** against Leukemic cells based on their IC₅₀

No.	Leukemic Cells	IC ₅₀ of Compounds	
		1 (cosmetic use)	2 (drug use)
1	P-388	53.5 µg/mL	31.4 µg/mL*
2	L-1210	5.8 µg/mL	3.1 µg/mL**

Standard value for cancer drug is 40 µg/mL for P-388 leukemic cell (*) and 4.0 µg/mL for L-1210 leukemic cell (**)

As a result not only P-388 but also L-1210 murine leukemia cells seem to be especially sensitive to both of these triterpenic acids, ursolic acid [its isomeric oleanolic acid] (Ayeleso et al., 2017; Shanmugam et al., 2014; Sun et al., 2006; Yan et al., 2010), but also ethyl ursolate 3-ethyl ether [its isomeric ethyl oleanolate 3-ethyl ether], the C-3 and C-28 modified triterpenic acids indicated more potent anticancer activity. compound **2** is a promising one to be use in topical cosmetic with no itching effects but needs molecular mechanism of mutagenesis test, especially in sperm quality whenever it is used in oral application

(Ovesná et al., 2006; Wójciak-Kosior et al., 2011), see **Table 3**.

CONCLUSIONS

It is clear, this work was improved that both of secondary alcohol at C-3 and carboxyl at C-28 of ursolic [oleanolic] acids were successfully converted to secondary alkyl chloride and acid chloride respectively, and then *in situ* transformed to be ethyl ursolate 3-ethyl ether [its isomeric ethyl oleanolate 3-ethyl ether]. They could become safe and promising molecules for both topical cosmetics and medicines

rather than their ursolic [oleanolic] acids form because their anticancer efficacy is stronger than their mother triterpenic acids.

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DECLARATIONS

The authors declare that this is an original work and there is wrong spelling in our previous publications dealing with latin name; it was written *Fagraea fragrans* Roxb fruits, but it must be *Fagraea fragrans* Roxb fruits. The last botanical name is the correct one.

REFERENCES

- Ayeleso, T. B., Matumba, M. G., & Mukwevho, E. (2017). Oleanolic acid and its derivatives: Biological activities and therapeutic potential in chronic diseases. *Molecules*, 22(11). <https://doi.org/10.3390/molecules22111915>
- Basir, D., Hanafi, M., Julinar, Saputra, A., and Wati, T., (2018). Free solvent amidation of ursolic and oleanolic acids of *Fagraea fragrans* fruits. *Journal of Physics: Conference Series* 1095 012006. <https://doi.org/10.1088/1742-6596/1095/1/012006>
- Basir, D., Harmida, & Julinar. (2020). Secondary metabolite profile of *Fagraea fragrans* fruits identified with LCMS/MS: The fruits for herbal cosmetic. *AIP Conference Proceedings*, 2243(1), 20004. American Institute of Physics Inc. <https://doi.org/10.1063/5.0001088>
- Basir, D., & Julinar. (2012). The restorative cosmetic constituents of *Fagraea fragrans* fruits. *Indonesian Journal of Chemistry*, 12(1), 84–88. <https://doi.org/10.22146/ijc.21376>
- Basir, D., Julinar, Agustriana, E., & Untari, B. (2014). Oxidation and acetylation of ursolic and oleanolic acids isolated from *Fagraea fragrans* fruits: Antiproliferation of p388 leukemia cells. *Indonesian Journal of Chemistry*, 14(3), 269–276. <https://doi.org/10.22146/ijc.21238>
- Catteau, L., Reichmann, N. T., Olson, J., Pinho, M. G., Nizet, V., Van Bambeke, F., & Quetin-Leclercq, J. (2017). Synergy between ursolic and oleanolic acids from *Vitellaria paradoxa* leaf extract and β -lactams against methicillin-resistant *Staphylococcus aureus*: In vitro and in vivo activity and underlying mechanisms. *Molecules*, 22(12). <https://doi.org/10.3390/molecules22122245>
- Chen, H., Gao, Y., Wang, A., Zhou, X., Zheng, Y., & Zhou, J. (2015). Evolution in medicinal chemistry of ursolic acid derivatives as anticancer agents. *European Journal of Medicinal Chemistry*, 92, 648–655. <https://doi.org/10.1016/j.ejmech.2015.01.031>
- Chu, F., Xu, X., Li, G., Gu, S., Xu, K., Gong, Y., ... Lei, H. (2014). Amino acid derivatives of ligustrazine-oleanolic acid as new cytotoxic agents. *Molecules*, 19(11), 18215–18231. <https://doi.org/10.3390/molecules191118215>
- De L.e. Silva, M., David, J. P., Silva, L. C. R. C., Santos, R. A. F., David, J. M., Lima, L. S., ... Fontana, R. (2012). Bioactive oleanane, lupane and ursane triterpene acid derivatives. *Molecules*, 17(10), 12197–12205. <https://doi.org/10.3390/molecules171012197>
- Furtado, R. A., Rodrigues, É. P., Araújo, F. R. R., Oliveira, W. L., Furtado, M. A., Castro, M. B., ... Tavares, D. C. (2008). Ursolic acid and oleanolic acid suppress preneoplastic lesions induced by 1,2-dimethylhydrazine in rat colon. *Toxicologic Pathology*, 36(4), 576–580. <https://doi.org/10.1177/0192623308317423>
- Gnoatto, S. C. B., Vechia, L. D., Lencina, C. L., Dassonville-Klimpt, A., Da Nascimento, S., Mossalayi, D., ... Sonnet, P. (2008). Synthesis and preliminary evaluation of new ursolic and oleanolic acids derivatives as antileishmanial agents. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 23(5), 604–610. <https://doi.org/10.1080/14756360802204870>
- Güvenalp, Z., Kilig, N., Kazaz, C., Kaya, Y., & Demirezer, O. L. (2006). Chemical constituents of *Galium tortumense*. *Turkey Journal of Chemistry*, 30, 515–523.
- Kwon, T. H., Lee, B., Chung, S. H., Kim, D. H., & Lee, Y. S. (2009). Synthesis and NO production inhibitory activities of ursolic acid and oleanolic acid derivatives. *Bulletin of the Korean Chemical Society*, 30(1), 119–123. <https://doi.org/10.5012/bkcs.2009.30.1.119>
- Meng, Y., Song, Y., Yan, Z., & Xia, Y. (2010). Synthesis and in vitro cytotoxicity of novel ursolic acid derivatives. *Molecules*, 15(6), 4033–4040. <https://doi.org/10.3390/molecules15064033>
- Mlala, S., Oyedeji, A. O., Gondwe, M., & Oyedeji, O. O. (2019). Ursolic acid and its derivatives as bioactive agents. *Molecules (Basel, Switzerland)*, 24(2751), 1-25 <https://doi.org/10.3390/molecules24152751>
- Nelson, A. T., Camelio, A. M., Claussen, K. R., Cho, J., Tremmel, L., Digiovanni, J., & Siegel, D. (2015). Synthesis of oxygenated oleanolic and ursolic acid derivatives with anti-inflammatory properties. *Bioorganic and Medicinal Chemistry Letters*, 25(19), 4342–4346. <https://doi.org/10.1016/j.bmcl.2015.07.029>

- Ovesná, Z., Kozics, K., & Slameňová, D. (2006). Protective effects of ursolic acid and oleanolic acid in leukemic cells. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, 600(1–2), 131–137. <https://doi.org/10.1016/j.mrfmmm.2006.03.008>
- Shanmugam, M. K., Dai, X., Kumar, A. P., Tan, B. K. H., Sethi, G., & Bishayee, A. (2014). Oleanolic acid and its synthetic derivatives for the prevention and therapy of cancer: Preclinical and clinical evidence. *Cancer Letters*, 346(2), 206–216. <https://doi.org/10.1016/j.canlet.2014.01.016>
- Silva, F. S. G., Oliveira, P. J., & Duarte, M. F. (2016). Oleanolic, ursolic, and betulinic acids as food supplements or pharmaceutical agents for type 2 diabetes: Promise or illusion? *Journal of Agricultural and Food Chemistry*, 64(15), 2991–3008. <https://doi.org/10.1021/acs.jafc.5b06021>
- Soica, C., Oprean, C., Borcan, F., Danciu, C., Trandafirescu, C., Coricovac, D., ... Dehelean, M. M. (2014). The synergistic biologic activity of oleanolic and ursolic acids in complex with hydroxypropyl- γ -cyclodextrin. *Molecules*, 19(4), 4924–4940. <https://doi.org/10.3390/molecules19044924>
- Sultana, N. (2011). Clinically useful anticancer, antitumor, and antiwrinkle agent, ursolic acid and related derivatives as medicinally important natural product. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 26(5), 616–642. <https://doi.org/10.3109/14756366.2010.546793>
- Sun, H., Fang, W.-S., Wang, W.-Z., & Hu, C. (2006). Structure-activity relationships of oleanane-and ursane-type triterpenoids. *Botanical Studies*, 47, 339–368.
- Vasconcelos, M. A. L., Royo, V. A., Ferreira, D. S., Miller Crotti, A. E., Andrade E Silva, M. L., Carvalho, J. C. T., ... Cunha, W. R. (2006). In vivo analgesic and anti-inflammatory activities of ursolic acid and oleanolic acid from *Miconia albicans* (Melastomataceae). *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*, 61(7–8), 477–482. <https://doi.org/10.1515/znc-2006-7-803>
- Wang, C., Lu, L., Na, H., Li, X., Wang, Q., Jiang, X., ... Liu, K. (2014). Conjugation of a nonspecific antiviral sapogenin with a specific HIV fusion inhibitor: A promising strategy for discovering new antiviral therapeutics. *Journal of Medicinal Chemistry*, 57(17), 7342–7354. <https://doi.org/10.1021/jm500763m>
- Wójciak-Kosior, M., Paduch, R., Matysik-Woźniak, A., Niedziela, P., & Donica, H. (2011). The effect of ursolic and oleanolic acids on human skin fibroblast cells. *Folia Histochemica et Cytobiologica*, 49(4), 664–669. <https://doi.org/10.5603/FHC.2011.0050>
- Yan, S. lei, Huang, C. yin, Wu, S. tzy, & Yin, M. chin. (2010). Oleanolic acid and ursolic acid induce apoptosis in four human liver cancer cell lines. *Toxicology in Vitro*, 24(3), 842–848. <https://doi.org/10.1016/j.tiv.2009.12.008>
- Zacchigna, M., Cateni, F., Drioli, S., Procida, G., & Altieri, T. (2014). PEG-Ursolic acid conjugate: Synthesis and in vitro release studies. *Scientia Pharmaceutica*, 82(2), 411–421. <https://doi.org/10.3797/scipharm.1309-17>

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