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Original Article

Anticancer activity and HPLC analysis of bioactive compounds in *Dipterocarpus alatus* Roxb. ex G.Don oleo-resin and its biodiesel byproducts

[Actividad anticancerígena y análisis por HPLC de compuestos bioactivos en la oleorresina de *Dipterocarpus alatus* y sus subproductos de biodiésel]

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Abstract

Context: *Dipterocarpus alatus* oleo-resin has been used as a folk medicine and energy source. During biodiesel production, massive amounts of waste are generated. To explore the reuse of biodiesel waste, this research investigates the health-promoting activities of biodiesel waste.

Aims: To compare biological activity, anticancer and antioxidant activities, and phytochemical contents of *D. alatus* oleo-resin (OR) and its byproducts from the degumming (DG) and distillation (DT) processes employed during biodiesel production.

Methods: All samples were evaluated for the cytotoxicity activity against SK-MEL-2, HCT 116, SiHa, SK-LU-1, U937 cancer cells, and the Vero cell line. Antioxidant activity was tested using DPPH, ABTS, and FRAP assays. The selected bioactive compounds, betulonic acid (**1**), dipterocarpol (**2**), and α-gurjunene (**3**) were quantified by HPLC analysis.

Results: The result showed that DT and DG have a potent cytotoxicity higher than OR in HCT 116, SK-LU-1, and SK-MEL-2, particularly in U937, which corresponded to the number of terpene components, especially DG and DT showed high dipterocarpol (**2**) content. Among all samples, OR showed a good source for α-gurjunene and total phenolic contain (TPC), which showed good cytotoxicity against SiHa cancer cells.

Conclusions: The biodiesel waste product from the distillation and degumming process of *D. alatus* elevates the dipterocalpol concentration, contributing to its anticancer activity. As a result, this waste material shows promise as a potential source for new anticancer drug development.

Keywords: betulonic acid; cytotoxicity; dipterocarpaceae; dipterocarpol; α-gurjunene; principal component analysis.

Resumen

Contexto: La oleorresina de *Dipterocarpus alatus* se ha utilizado como medicina popular y fuente de energía. Durante la producción de biodiésel se generan grandes cantidades de residuos. Para explorar la reutilización de los residuos de biodiésel, esta investigación estudia las actividades beneficiosas para la salud de los residuos de biodiésel.

Objetivos: Comparar la actividad biológica, anticancerígena y antioxidante, y el contenido fitoquímico de la oleorresina (OR) de *D. alatus* y sus subproductos procedentes de los procesos de desgomado (DG) y destilación (DT) empleados durante la producción de biodiésel.

Métodos: Se evaluó la actividad citotóxica de todas las muestras frente a las células cancerosas SK-MEL-2, HCT 116, SiHa, SK-LU-1, U937 y la línea celular Vero. La actividad antioxidante se comprobó mediante los ensayos DPPH, ABTS y FRAP. Los compuestos bioactivos seleccionados, ácido betulónico (1), dipterocarpol (2) y α-gurjuneno (3) se cuantificaron mediante análisis HPLC.

Resultados: El resultado mostró que DT y DG tienen una potente citotoxicidad mayor que OR en HCT 116, SK-LU-1, y SK-MEL-2, particularmente en U937, que correspondía al número de componentes terpénicos, especialmente DG y DT mostraron un alto contenido de dipterocarpol (2). Entre todas las muestras, OR mostró una buena fuente de α-gurjuneno y contenido fenólico total (TPC), que mostraron una buena citotoxicidad contra las células cancerosas SiHa.

Conclusiones: El producto de desecho biodiésel procedente del proceso de destilación y desgomado de *D. alatus* eleva la concentración de dipterocalpol, contribuyendo a su actividad anticancerígena. En consecuencia, este material de desecho resulta prometedor como fuente potencial para el desarrollo de nuevos fármacos anticancerígenos.

Palabras Clave: ácido betulónico; análisis de componentes principales; citotoxicidad; dipterocarpáceas; dipterocarpol; α-gurjuneno.

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INTRODUCTION

Nowadays, fossil fuels have been used extensively, contributing to increased environmental pollution such as smoke, particulate matter (PM), high levels of carbon monoxide (CO), nitrogen oxides (NO), and hydrocarbon (HC). These factors are major causes of global warming (Abomohra et al., 2016; Bhikuning et al., 2020). In contrast, biofuels are non-toxic and produce lower levels of smoke, PM, HC, and CO (Bhikuning et al., 2020). Therefore, biofuels are a viable alternative for engines. Additionally, biofuels offer a promising route for producing fuels with a similar carbon number to diesel oil (Elgharbawy et al., 2021).

Dipterocarpus alatus Roxb. ex G.Don (family *Dipterocarpaceae*) oleo-resin is used as an alternative biodiesel source with vacuum distillation methods used to obtain biodiesel (60-70 vol%) with a hard resin residual (30-40 vol%). This hard resin residual can be converted by the pyrolysis method to produce diesel-like fuel (DLF) and gasoline-like fuel (GLF) (Suiuay et al., 2019). During the biodiesel production from *D. alatus*, the oleo-resin is degummed with sodium hydroxide (NaOH) to remove phospholipids and waxes before the esterification (Costa et al., 2018). Katekaew et al. (2021) succeeded in producing biodiesel from *D. alatus* oleo-resin. This pre-treatment process produces the oil used in the biodiesel process, leaving the resin as a byproduct, including byproducts from the degumming (DG) and distillation (DT). The volume of solid waste generated also increases with industrial demand. To address this issue, our project explored the utilization of byproducts from the biodiesel process of *D. alatus* oleo-resin for health-promoting activities.

Previous reports have revealed that oleo-resin from *D. alatus* is used externally to heal wounds, and treat gonorrhea, menorrhagia, and various skin diseases (Appanah and Turnbull, 1998; Prasad, 2011; Wiart, 2006). In addition, the oleo-resin has demonstrated potent cytotoxicity against the U937 and SiHa cells. An analysis of the phytochemical contents of *D. alatus* oleo-resin showed a high content of terpene compounds, but low total phenolic content (Yongram et al., 2019). Glycerol is well known as the main byproduct of biodiesel production (Nitayavardhana and Khanal, 2011; Yang et al., 2012). It was carried out in the food and pharmaceutical sectors (Plácido and Capareda, 2016). Consequently, this research hypothesizes that the waste products from biodiesel production of *D. alatus* oleo-resins should contain bioactive compounds that contribute to their biological activity. Therefore, this study aims to evaluate the antioxidant, anticancer activity, and phytochemical content of *D. alatus* oleo-resins and their byproducts.

MATERIAL AND METHODS

Chemicals

All organic solvents, HCL, and DMSO, used for extraction and HPLC analysis were sourced from RCI Labscan (Samut Sakhon, Thailand). Folin–Ciocalteu reagent and 1,1-diphenyl-2-picrylhydrazyl (DPPH) purchased from Fluka Chemika (Buchs, Switzerland). 6-hydroxy-2,5,7,8-tetramethylchroman carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), trifluoroacetic acid, potassium persulfate, neutral red and gallic acid purchased from Sigma-Aldrich (MO, USA). iron(III)chloride ($FeCl_3$) and iron (II) sulfate ($FeSO_4$) were purchased from Ajax Finechem Pty Ltd. (Auckland, New Zealand). Sodium carbonate and sodium acetate trihydrate were purchased from RANKEM (Maharashtra, India). Sodium hydroxide was purchased from Q RëCTM (Chonburi, Thailand). Dulbecco's modified Eagle's medium (DMEM), RPMI Media 1640, 0.25% trypsin-EDTA (1×), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from GIBCO® (Invitrogen, Grand Island, NY, USA). Cisplatin was purchased from Boryung (Ansan, Korea).

Plant material

The oleo-resin (OR) of *D. alatus* was obtained from Phitsanulok province, Thailand, in June 2022 (17.096006, 100.841902). The *D. alatus* voucher specimen, No. PSKKF03683 was kept in the Department of Pharmacognosy and Toxicology Laboratory and was identified according to the botanical characteristics of *D. alatus* from Pooma (1996) by Assoc. Prof. Suppachai Tiyaworanant, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

Sample preparation

During the biodiesel production process, OR was treated with NaOH (in a ratio of 1:4) in the degumming process generated biodiesel, and a byproduct, namely DG, was obtained. During vacuum distillation, the OR was heated at 150°C under -760 mmHg in an oil distiller to obtain biodiesel and its byproduct, namely DT (Katekaew et al., 2021).

Cytotoxicity activity

The normal African green monkey kidney (Vero) and human colon cancer (HCT 116), human lung adenocarcinoma (SK-LU-1), melanoma (SK-MEL-2),

cervix adenocarcinoma (SiHa), and human myeloid leukemia (U937) cell lines were purchased from American Type Culture Collection (Manassas, VA, USA). All cell lines were cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum. U937 cells were cultured in 10% fetal bovine serum with RPMI 1640 medium. All media and cells were added 1% of penicillin/streptomycin, then cultured under 5% atmospheric CO_2 and 95% humidity at 37°C. Cytotoxicity was analyzed using the neutral red (NR) uptake assay. Samples in final concentrations of 10-500 µg/mL were added to each well. After 24 hours of incubation, tested cells were washed with PBS, added NR solution (50 µg/mL), and incubated for 2 hours. Then, cells were washed with PBS and 0.33% HCl in isopropanol. The absorbance was measured under a microplate reader (SunriseTM, Grödig, Austria) at 537 nm. Cisplatin served as the reference standard in this study. The IC_{50} (µg/mL) was calculated by plotting the % cytotoxicity and sample concentration (Srisongkram et al., 2019). The selectivity index (SI) was calculated by dividing the IC_{50} value of normal cells (Vero) by the IC_{50} value of extract on cancerous cells (Céspedes et al., 2023; Yahuafai et al., 2023).

Antioxidant capacity

The antioxidant capacity of the samples was assessed using the DPPH, ABTS, and FRAP assays. Three independent replicates of the experiment were conducted. Trolox was utilized as a positive control. The methodologies employed adhered to our earlier published procedures (Yongram et al., 2019), which were followed precisely as outlined in our previous work.

DPPH radical scavenging assay

The procedure involved combining equal volumes (100 µL each) of 200 µM DPPH solution and sample solution, with the latter prepared at various concentrations ranging from 10 to 500 µg/mL (Yongram et al., 2019). This mixture was then left to incubate for half an hour at ambient temperature, protected from light exposure. Following incubation, the absorbance of the solution was measured at a wavelength of 490 nm using a SunriseTM microplate reader (manufactured in Grödig, Austria). Trolox was used as a reference standard for antioxidant activity. To determine the effectiveness of the samples in scavenging DPPH radicals, the half-maximal inhibitory concentration (IC_{50}) was computed. This was achieved by constructing a graph that plotted the percentage of DPPH radical scavenging activity against the corresponding extract concentrations. The IC_{50} value was expressed in µg/mL.

ABTS radical scavenging assay

To evaluate antioxidant activity using the ABTS assay, the following procedure was employed (Yongram et al., 2019). A solution was created by combining 28 mM ABTS with 2.45 mM potassium persulfate in purified water and allowed to react for a period of 12-16 h. 150 µL of the prepared ABTS radical cation solution was combined with 50 µL of sample solution at final concentration ranging from 10-500 µg/mL (50 µL). The resulting mixture was incubated for 10 minutes at room temperature and shielded from light. Following incubation, the absorbance was measured at 415 nm using a microplate reader. The IC_{50} (µg/mL) was determined by graphing the percentage of ABTS radical scavenging activity against the corresponding extract concentrations.

Ferric reducing antioxidant power (FRAP) assay

The FRAP reagent was prepared by mixing 300 mM acetate buffer pH 3.6, 20 mM $FeCl_3$ solution, and 10 mM TPTZ in 40 mM HCl in a ratio of 10:1:1 (Yongram et al., 2019). A 10 µL sample solution (500 µg/mL) was mixed with 300 µL of FRAP reagent and 30 µL of ultrapure water, then incubated for 4 min. Absorbance was measured using a microplate reader at 595 nm. FRAP values are reported in mM Fe^{2+}/100 g extract units. The calibration curve for $FeSO_4$ was given by the equation $y = 20.596x + 0.021$, $R^2 = 0.9993$.

Total phenolic content

The total phenolic content (TPC) of *D. alatus* was determined using the Folin-Ciocalteu method (Puthongking et al., 2023). 16 µL of sample solution (500 µg/mL) was mixed with 117 µL of 10% Folin-Ciocalteu reagent and left for 5 min at room temperature. Then 117 µL of sodium carbonate (60 g/L) was added to complete the reaction. After incubation for 90 min at room temperature, the absorbance of the sample was read at 700 nm in a microplate reader. TPC was expressed in gallic acid equivalents (GAE) per gram extract (Tamprasit et al., 2019). The calibration curve for gallic acid is described by the equation $y = 0.058x + 0.0159$ with an R^2 value of 0.9994.

HPLC analysis of bioactive compounds

The HPLC analyses of betulonic acid **(1)**, dipterocarpol **(2)**, and α-gurjunene **(3)** were performed using an Agilent 1100 Series HPLC (Agilent Technologies, Santa Clara, CA, USA). The column used was Luna 5 µm C18, 100 Å, 150 × 3.9 mm with a security guard column (Phenomenex). The mobile phase was the mixture of A (acetonitrile) and B (0.05% TFA) in the ratio A:B (80:20). The isocratic system from 0-70 min with flow rate 0.8 mL/min, column temperature at 25°C, and 20 µL volume injection was used as the

HPLC condition. These compounds were detected at 210 nm (Yongram et al., 2021). The compounds were detected by comparison with a retention time of standard betulonic acid **(1)**, dipterocarpol **(2)**, and α-gurjunene **(3)**, and their content was calculated from the standard calibration curve of betulonic acid ($y = 328.52x + 5.9058$, $R^2 = 0.9996$), dipterocarpol ($y = 8424.9x - 21.917$, $R^2 = 0.9993$), and α-gurjunene ($y = 35051x + 598.98$, $R^2 = 0.9984$).

Statistical analysis

The data is shown as mean ± standard deviation. The experimental procedure was carried out in triplicate. The statical values were analyzed by one-way ANOVA and Tukey's HSD with SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) and agglomerative hierarchical cluster analysis (AHC) using the XLSTAT software were used to categorize the relationship between OR and its byproducts to bioactive compounds and anticancer activities. The dataset consisted of the content of TPC, betulonic acid, dipterocarpol, and α-gurjunene and the inhibition of cytotoxicity effects against HCT 116, SK-MEL-2, SK-LU-1, SiHa, and U937 cells at the concentration of 50 µg/mL. The AHC was calculated using Euclidean distance, and clustering used the Ward's hierarchical agglomerative clustering method.

RESULTS AND DISCUSSION

Cytotoxic activity

Previous findings from our study indicated that OR, DG, and DT exerted cytotoxicity against hepatocellular carcinoma (HepG2), cervical adenocarcinoma (HeLa), and T cell leukemia (Jurkat) cell lines (Puthongking et al., 2022). To explore the anticancer activity of *D. alatus* OR and its byproducts, additional cancer cell lines, including human colon cancer (HCT 116), human lung adenocarcinoma (SK-LU-1), melanoma (SK-MEL-2), cervix adenocarcinoma (SiHa), and human myeloid leukemia (U937) were investigated in this study. The cytotoxic activities of all tested samples are shown in Fig. 1. The result displayed that all tested samples showed higher cytotoxic activity against U937 and SiHa cancer cell lines than the standard drug, cisplatin. Interestingly, byproduct DG and DT showed 3-fold cytotoxicity against U937 compared with OR and 6-7-fold higher cytotoxicity than cisplatin. Particularly, DG and DT showed the most potent cytotoxicity against HCT 116, SK-LU-1, and U937 and demonstrated the highest overall cytotoxicity with an IC_{50} value of 23.6 ± 1.5 and 26.8 ± 1.4 µg/mL in U937, respectively. Both byproducts exerted significantly stronger cytotoxicity than the original OR (63.3 ± 2.1 µg/mL) and positive control, cisplatin (170.9 ± 15.7 µg/mL). Besides, while DG displayed the potential cytotoxicity among all tested simples, its toxicity to normal Vero cell lines was also reported (50.3 ± 0.9 µg/mL), whereas DT showed no toxicity to normal Vero cell lines (105.7 ± 3.4 µg/mL). Moreover, the selective index (SI) values in Table 1, DT, showed high selective cytotoxicity against the U937 cell line with an SI value of 3.9. Meanwhile, DG (SI = 2.1) and OR (SI = 1.4) showed moderate selectivity in the U937 cell line. SI value higher than 3 was classified as high selectivity for cancer cells (Bézivin et al., 2003). It was suggested that DT may be a candidate source for developing anticancer treatments, particularly those cytotoxic against human myeloid leukemia (U937 cell lines).

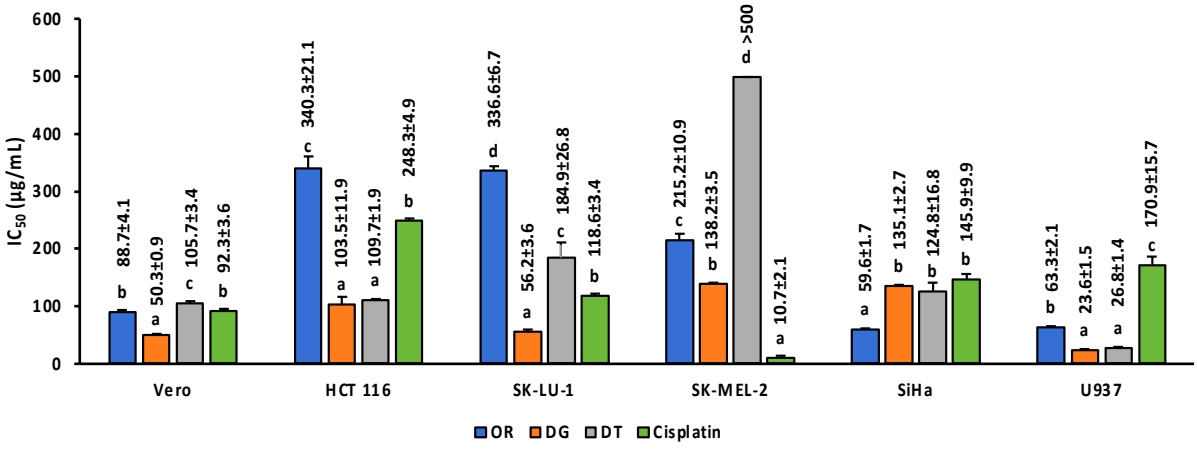


Figure 1. Cytotoxic activities of *D. alatus* oleo-resin and its byproducts, as well as cisplatin, are against normal Vero cells and cancer cell lines.

Data are shown as mean ± standard deviation (n = 3). The letters indicate significant differences at $p<0.05$ between samples in the same cell line. OR: oleo-resin; DG: byproducts from the degumming; DT: byproducts from the distillation.

Table 1. Selective index of *D. alatus* oleo-resin and its byproducts against several cancer cell lines.

Sample	HCT 116	SK-LU-1	SK-MEL-1	SiHa	U937
OR[#]	0.3	0.3	0.4	1.5	1.4
DG	0.5	0.9	0.4	0.4	2.1
DT	1.0	0.6	0.2	0.8	3.9
Cisplatin	0.4	0.8	8.6	0.6	0.5

[#]Results from our previous report (Yongram et al., 2019). OR: oleo-resin; DG: byproducts from the degumming; DT: byproducts from the distillation.

Table 2. Antioxidant capacities and the total phenolic content (TPC) of *D. alatus* oleo-resin and its byproducts.

Sample	Antioxidant capacity			TPC (mg GAE/g extract)
	DPPH assay IC_{50} (µg/mL)	ABTS assay IC_{50} (µg/mL)	FRAP value (mM Fe^{2+} /100 g extract)	
OR[#]	>1000[b]	>1000[b]	21.0 ± 0.2[b]	15.1 ± 0.6[a]
DG	>1000[b]	>1000[b]	14.3 ± 0.7[b]	2.4 ± 0.2[b]
DT	>1000[b]	>1000[b]	11.2 ± 1.0[b]	3.7 ± 0.6[b]
Trolox	3.9 ± 0.0[a]	10.2 ± 0.1[a]	771.7 ± 11.4[a]	-

[#]Results from our previous report (Yongram et al., 2019). Data are shown as mean ± standard deviation (n = 3). Different letters indicate significant differences at $p<0.05$ between rows in the same column. OR: oleo-resin; DG: byproducts from the degumming; DT: byproducts from the distillation.

Antioxidant capacity and the total phenolic content (TPC)

Antioxidant capacity revealed that OR, DG, and DT showed weak antioxidant activity in DPPH, ABTS, and FRAP assays (Table 2). This conforms to our previous report, in which *D. alatus* OR had a low antioxidant capacity as well as a low level of TPC and TFC (Yongram et al., 2019). Moreover, byproducts from biodiesel production, DG and DT, reduced the content of TPC and TFC. This suggests that OR and biodiesel byproducts are not good natural antioxidant sources. In contrast, other parts of *D. alatus,* including leaves, bark, and twigs, possess a good antioxidant activity that correlates to the content of phenolic compounds (Chatuphonprasert et al., 2023; Puthongking et al., 2023). Despite our findings indicating that OR, DG, and DT exhibited weak antioxidant properties and low levels of total phenolic and flavonoid content, these substances have shown promising anticancer potential. Notably, OR is recognized as a rich source of terpenes, especially dipterocarpol, which has been linked to anticancer effects (Yongram et al., 2019). Consequently, our study focused on conducting a quantitative analysis of terpene concentrations in both the *D. alatus* OR and the byproducts from its biodiesel production.

HPLC analysis of bioactive compounds

According to the cytotoxic activity results shown in Fig. 1, the DG and DT byproducts showed more selective cytotoxicity against cancer cell lines than their *D. alatus* OR precursor. This might result from changes to the phytochemical contents. The phytochemical composition of OR, DG and DT was therefore analyzed by HPLC. The major components of *D. alatus* OR have previously been reported to be α-gurjunene (30.31%) and dipterocarpol (37%) (Smirnova et al., 2012; Yongram et al., 2019). In addition, betulonic acid is found in *D. alatus* wood (0.009%) (Khiev et al., 2012). Therefore, betulonic acid (**1**), dipterocarpol (**2**), and α-gurjunene (**3**) were quantified in OR, DG, and DT using HPLC. HPLC chromatograms of all samples indicating compounds **1**, **2,** and **3** are shown in Fig. 2. HPLC results confirmed that the biodiesel production processes affected the chemical contents. α-Gurjunene was found in the highest amounts in OR and correlated to the cytotoxicity of OR against the SiHa cell line. The production of biodiesel byproducts, DG and DT, resulted in a dramatic decrease in α-gurjunene concentration, reducing it by approximately 11-17 times compared to the original OR (Fig. 3). Conversely, the concentration of dipterocarpol increased 2-4 times during this process. However, the biodiesel production processes cannot predict trends in the amount of compound betulonic acid**.** Interestingly, DT showed a significantly higher concentration compared to both OR and DG. Notably, DG and DT had potent cytotoxicity and exhibited high SI values for the U937 cell line, which might be correlated to the content of dipterocarpol. Dipterocarpol has previously been reported to show cytotoxicity against several cancer cell lines by inducing the apoptosis cell death pathway (Bézivin et al., 2003; Huong et al., 2013).

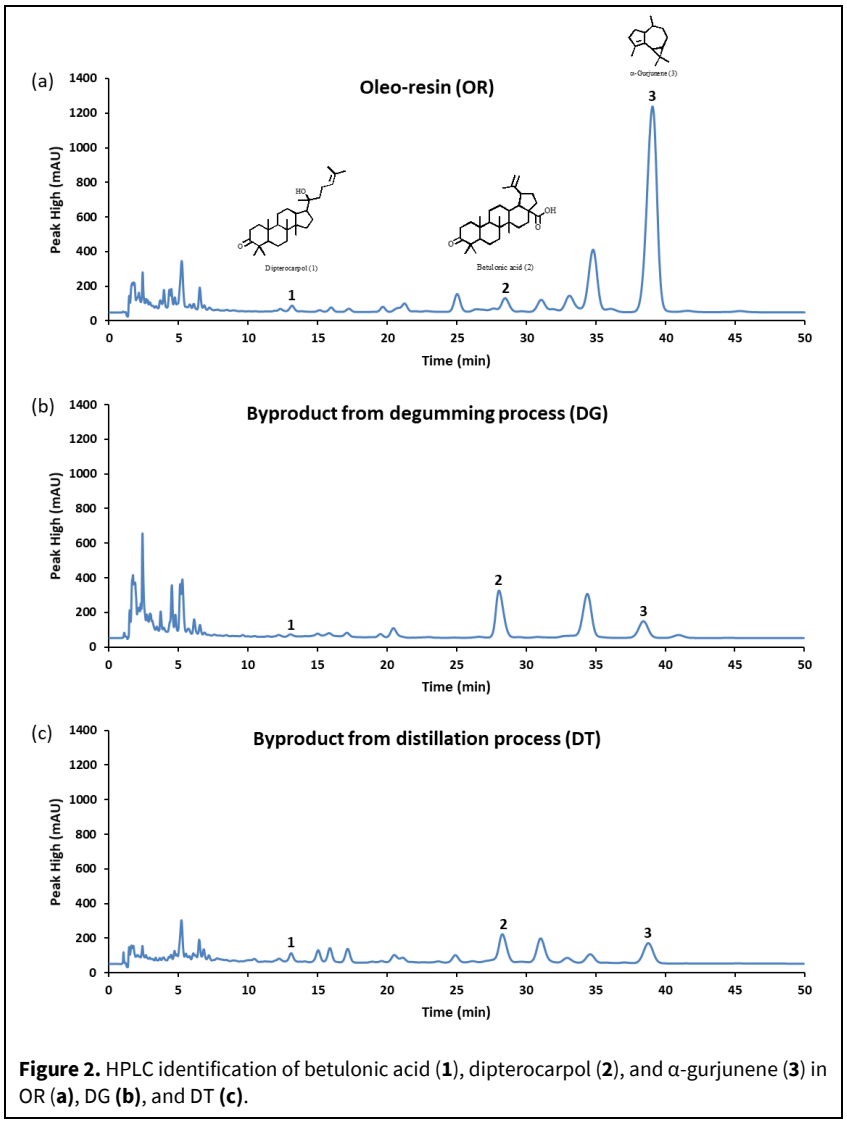


Figure 2. HPLC identification of betulonic acid (**1**), dipterocarpol (**2**), and α-gurjunene (**3**) in OR (**a**), DG **(b)**, and DT **(c)**.

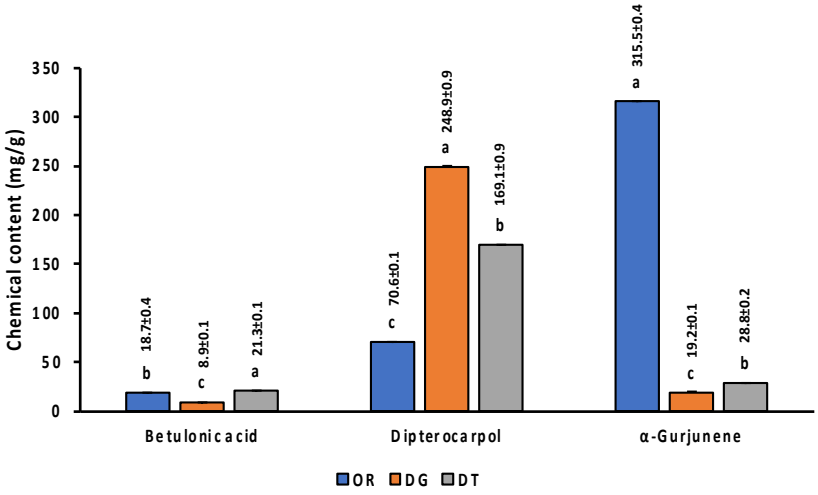


Figure 3. The chemical content in *D. alatus* oleo-resin and its byproducts.

Data are shown as mean ± standard deviation (n = 3). OR: oleo-resin; DG: byproducts from the degumming; DT: byproducts from the distillation.

Table 3. Pearson's correlation coefficient (*r*) of *D. alatus* oleo-resin and its byproducts.

Variables	HCT 116	SK-LU-1	SK-MEL-2	SiHa	U937	TPC	BA	Dip	α-Gur
HCT 116	1.000*								
SK-LU-1	0.754	1.000*							
SK-MEL-2	-0.601	0.072	1.000*						
SiHa	-0.581	-0.973	-0.301	1.000*					
U937	0.997*	0.798	-0.543	-0.637	1.000*				
TPC	-1.000*	-0.751	0.604	0.578	-0.997*	1.000*			
Betulonic acid	-0.408	-0.907	-0.484	0.980	-0.472	0.405	1.000*		
Dipterocarpol	0.934	0.939.	-0.275	-0.834	0.957	-0.932	-0.708	1.000*	
α-Gurjunene	-0.998*	-0.707	0.654	0.524	-0.990	0.998	0.344	-0.907	1.000*

*The superscript letter is different from 0 with a significant level (p=0.05). TPC: total phenolic content; BA: betulonic acid; Dip: dipterocarpol; α-Gur: α-gurjunene.

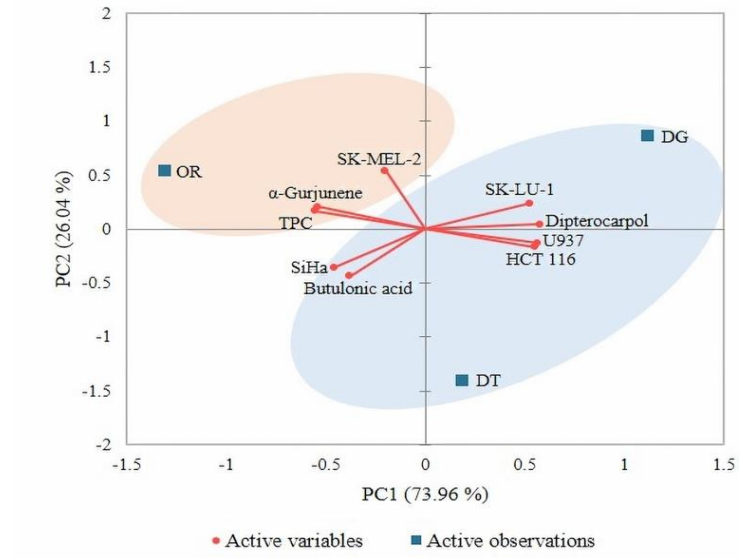


Figure 4. PCA biplot and clustering of *D. alatus* oleo-resin and its byproducts.
The difference in the color of oval areas was obtained from the agglomerative hierarchical cluster analysis (AHC).

Pearson's correlation and Principal Component Analysis (PCA)

Correlations between the phytochemical contents and anticancer activities of *D. alatus* OR and their biodiesel byproducts were examined according to Pearson's correlation. Dipterocapol (Dip) content showed a strong correlation with cytotoxic activity against U937 (r = 0.957), SK-LU-1 (r = 0.939), and HCT 16 (r = 0.934), whereas the correlations between dipterocapol and the other compounds were negative, as listed in Table 3. Betulonic acid demonstrated a strong positive correlation with cytotoxic activity against SiHa cells (r = 0.980), whereas the other cancer cell lines showed a weak negative correlation (r = -0.484 to -0.408). Additionally, TPC showed a positive correlation to cytotoxic activity against SK-MEL-2 (r = 0.604) and SiHa (r = 0.578) cells.

The PCA reduced the variables into two principal components (PCs) that explained 100% of the data variance with the eigenvalue higher than 1. PC1 included the dipterocapol content and the cytotoxic effects on the U937, HCT 116, and SK-LU-1 cell lines, with 73.96% of the variance. While PC2 described 26.04% with the main variance of cytotoxic activity against SK-MEL-2 cell and the total phenolic (TPC) and α-gurjunene contents (Fig. 4). Based on agglomerative hierarchical cluster analysis (AHC), the dataset was divided into 2 clusters. Cluster I included *D. alatus* OR with a high content of α-gurjunene and moderate cytotoxicity against SK-MEL-2 cell. Cluster II was its byproducts, which DG contained a high

dipterocarpol content and exhibited a strong anti-cancer against SK-LU-1, U937, and HCT 116 cells, and DT exhibited a high betulonic acid with a good cytotoxicity of SiHa cell.

CONCLUSION

The *D. alatus* oleo-resin (OR) and its byproducts (DG and DT) showed cytotoxicity against all tested cancer cells but especially against the cancer U937 cell, which was inhibited by DG and DT more than by OR. The biodiesel byproducts had a much higher dipterocarpol content than the parent OR, and the concentration of betulonic acid was higher in DT than in OR and DG. Based on the PCA results, OR is recommended as a good source of α-gurjunene while the biodiesel byproducts, DG and DT, are a good source of dipterocarpol, and DT is a source of betulonic acid, which showed good cytotoxicity against cancer cell lines. The results suggest that the byproducts generated during biodiesel production from *D. alatus* OR could serve as a valuable alternative source for drug discovery, especially in the field of cancer therapeutics.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Yongram C	Ratha J	Siriparu P	Datham S	Katekaew S	Thapphasaraphong S	Weerapreeyakul N	Puthongking P
Concepts or ideas	x	x	x	x	x	x	x	x
Design	x	x	x	x	x	x	x	x
Definition of intellectual content								x
Literature search	x	x	x	x				x
Experimental studies	x	x	x	x			x	x
Data acquisition	x							x
Data analysis	x	x	x	x				x
Statistical analysis	x	x	x	x				x
Manuscript preparation	x	x	x	x				x
Manuscript editing	x	x						x
Manuscript review	x	x	x	x	x	x	x	x

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