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Chemical properties of Indonesian aloe vera and its hepatoprotective activity by targeting reactive oxygen species and TACE protein: comparison between gel and whole parts

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Abstract

This study evaluated the chemical properties of Indonesian aloe vera and compared the hepatoprotective activity of aloe gel (AO) and whole plant (WAO) against H_2O_2-induced toxicity in HepG2 cells. Chemical characterization was performed using ultraviolet-visible (UV-Vis), Fourier transform infrared (FTIR) spectroscopy, liquid chromatography–high-resolution mass spectrometry (LC-HRMS), and high-performance liquid chromatography analyses, and compound interactions with tumor necrosis factor (TNF)-α converting enzyme (TACE/ADAM17) were examined. AO contained a higher level of O-acetyl polysaccharides (10.36 ± 0.63% by mass [b/b]) than WAO (6.39 ± 0.16% b/b). Aloin A was rich in WAO (1,342.3 ± 23.94 ppm b/b) but absent in AO. FTIR spectra were similar for both samples, while LC-HRMS profiling revealed phenolic, flavonoid, and terpenoid compounds in AO and WAO. Various concentrations of AO effectively inhibited H_2O_2-induced toxicity in HepG2 cells, with 200 µg/mL showing the highest protective effect (22.44 ± 6.04%). In contrast, WAO reduced toxicity at lower concentrations but became cytotoxic at higher concentrations. Both extracts decreased intracellular reactive oxygen species (ROS) compared to H_2O_2 alone. At 200 µg/mL, AO and WAO reduced ROS level, compared to H_2O_2 alone, by 0.964±0.241 fold and 0.768±0.128 fold, respectively ($P < 0.05$). Docking studies showed squalene ($C_{30}H_{50}$) from

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AO exhibited the strongest affinity for TACE protein with a docking score of -1,16.6±2.54. Overall, AO extract may serve as a potent hepatoprotective agent.

Keywords: aloe vera; aloe gel; hepatoprotective; TNF-α converting enzyme (TACE); squalene

Introduction

Aloe vera is a native plant of southern and eastern Africa (Adlakha *et al.*, 2022). Nevertheless, it is easily cultivated globally, including in several regions of Indonesia. Different environments affect the plant metabolite composition (Quispe *et al.*, 2018). Aloe vera is a spiky cactus-like xerophyte belonging to the Liliaceae family. As a plant having a high content of water, aloe vera contains 0.5–1.0% of active compounds. Several polysaccharides, such as acemannan, arabinan, galactomannan, and glucomannan, have been identified in aloe vera gel (AO). Acemannan, the major polysaccharide in AO, ameliorates wound-healing of fibroblast cells. Minor amounts of various sterol, phenol, and flavonoid compounds, including kaempferol, naringenin, quercetin, and apigenin and its derivatives, are also determined in AO (Handayani *et al.*, 2021). Several foods, beverages, and cosmetics use aloe plants as their ingredient because of its bioactive properties that benefit our health (Chandra *et al.*, 2024). Polysaccharides and phenolic and flavonoid compounds contained in aloe plant have nutraceutical effects. These active compounds have prevention and protection attributes for inflammation, wounds, aging, and cancer proliferation as well as toward overproduction of free radicals (Añibarro-Ortega *et al.*, 2019; Babu and Noor, 2021; Handayani *et al.*, 2021, 2023; Quispe *et al.*, 2018).

Aloe vera is commonly used as a topical remedy to treat sunburns and heal wounds. Promisingly, aloe beverage also provides many health benefits, such as reduce cholesterol and triglycerides, mitigate oxidative stress, fortify the immune system, alleviate nausea, ameliorate adverse effects of radiation therapy, promote hair growth, diminish arthritis, relieve joint and muscle discomfort, lower blood glucose levels, improve digestive functioning, alleviate acid reflux symptoms, enhance blood oxygenation, address dental and gum diseases, and decelerate the aging process (Akbari *et al.*, 2022; Añibarro-Ortega *et al.*, 2019; Guo and Mei, 2016). Furthermore, promotion of healthier aloe-based beverages enhances the nation's socioeconomic status while also serving as a hepatoprotective agent. In Indonesia, despite numerous aloe vera products being produced, limited research is focused on the processing of aloe beverages, resulting in a lack of information on distinct aloin contained in AO and whole aloe plant (WAO), related to its toxicity level.

Aloe vera, particularly WAO leaves, contains high levels of aloin. Aloin is a major anthraquinone discovered in aloe perennial. Aloin has anticancer activity through the induction of cell death or apoptosis (Hu *et al.*, 2022). However, several studies reported that aloin also has toxicity and carcinogenic effects depending on its concentration (Guo and Mei, 2016; Yang *et al.*, 2022). The usage of aloe plants as a functional food ingredient requires a more comprehensive toxicity analysis, so that safe functional foods are produced. The EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS) and the US Food and Drug Administration (FDA) have set up 1.2–2.4 mg as maximum concentration of aloin for daily oral administration. The International Aloe Science Council (IASC) and the European Economic Community (EEC) established a quality standard of not more than 10 ppm (10 mg/kg) for aloin A and aloin B in all aloe vera products meant for oral consumption (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) *et al.*, 2018).

The liver is an important organ for detoxification of toxic substances. Therefore, this causes high potential damage to the liver. The mechanism of cell toxicity commonly involves reactive oxygen species (ROS), tumor necrosis factor (TNF)-α, and TNF-α converting enzyme (TACE). In normal conditions, the production of highly reactive ROS is an important feature of cellular systems, including fertilization, ovulation, arachidonic acid metabolism, and phagocytosis. However, the multiple times higher production of ROS indicates pathological complications (Chaudhary *et al.*, 2023). The production of ROS in the cells shows the level of oxidative stress (Handayani *et al.*, 2021). The liver is the main target of ROS under oxidative stress. Toxic substances induce hepatotoxicity by creating free radicals, including ROS (Handayani *et al.*, 2021). On the other hand, as a response to oxidative stress, cytokines, such as TNF-α, are released and trigger apoptosis and inflammation (Chaudhary *et al.*, 2023). Meanwhile, the over-expression of TNF-α in several inflammation cases was caused by the increasing levels of TNF-α converting enzyme (Lakshmi *et al.*, 2019). As the involvement of TNF-α and its converting enzyme is increasingly recognized in liver inflammation, natural agents such as aloe vera have gained interest for their potential to interfere in these pathways and offer hepatoprotective benefits.

Hepatoprotective agents prevent toxic compounds causing liver damage and protect the liver from diseases. Compounds that have anti-inflammation, antioxidant, and inhibition of cell death have a role in hepatoprotective activity. The synergistic hepatoprotective effect may be driven by several phytochemicals discovered in AO (Handayani *et al.*, 2021). Various studies revealed the protective effect of aloe vera on mice liver (Gupta *et al.*, 2019, 2023; Padmanabhan and Jangle, 2014). Active phytochemicals in aloe vera reveal their hepatoprotective effects through various mechanisms, including protection against ROS and TNF-α (Handayani *et al.*, 2021).

The *in silico* studies revealed the affinity of several compounds detected in aloe vera toward TNF-α receptor (Chinnadurai *et al.*, 2024; Kaloni *et al.*, 2019). Nevertheless, the *in silico* study on the affinity of different compounds detected in aloe vera toward the TACE enzyme is still limited. While numerous studies have demonstrated the general hepatoprotective effects of aloe vera, most have not distinguished between AO and WAO components related to specific molecular pathways. Moreover, limited research has addressed the role of aloe vera phytochemicals in modulating ROS and TACE activity under oxidative stress conditions. Zhou and Zhao (2021) stated in their study that H_2O_2 is one type of free radical production inducer that causes cell toxicity. Most of the superoxide anions produced by mitochondria are mutated into H_2O_2 (Chaudhary *et al.*, 2023). In addition, the HepG2 cell is used as a model for liver injuries, including hepatoprotective activity (Al-Sheddi *et al.*, 2024; Lee *et al.*, 2021; Sahoo *et al.*, 2023). Using this model, the present work aims to study the hepatoprotective activity of AO and WAO extract in H_2O_2-induced HepG2 cells, particularly in relation to ROS modulation and TACE enzyme involvement. To support this investigation, a foundational chemical characterization of aloe vera extracts was conducted to obtain prominent metabolites while minimizing aloin content. This was intended to determine the effective and safest functioning of aloe vera for potential therapeutic application.

Materials and Methods

Sample preparation

The aloe vera plant used in this study was obtained from the local plantation in Gunungkidul Region of Indonesia. This plant was authenticated as *Aloe vera* (L.) Burm.f. by Medicinal Plant and Traditional Medicine Research and Development Centre, Ministry of Health, Republic of Indonesia (No. KM.04.01/2/162/2021). WAO and AO from aloe leaves were sliced and dried at 50°C using an oven (Memmert, Germany). A part of AO was crushed using a blender to make AO juice (AJ). Then, WAO, AO, and AJ were separately extracted using 70% ethanol (Merck, Germany) to prepare ethanol extracts.

Soil analysis

The aloe plantation soil was collected from three locations. The soil from the same area that had not planted aloe vera was used as a control. Soil texture was analyzed at the Faculty of Geography, Universitas Gadjah Mada (No. 2/GLMB/V/2023). The soil components were analyzed using an Epsilon 4 X-ray Fluorescence Spectrometer (Malvern Panalytical, UK).

Chemical profiling

Fourier transform infrared (FTIR) spectroscopy measurement

The FTIR spectra were obtained by the attenuated total reflectance (ATR) sampling technique using an FTIR spectrophotometer (Bruker Vertex 80, Germany). The samples were put on ATR crystal and spectra acquisitions were measured in 4,000–6,000 cm^{-1} region (Lestari *et al.*, 2024).

Liquid chromatography–high-resolution mass spectrometry (LC-HRMS) measurement

Compound characterization of the extract was conducted utilizing liquid chromatography (Thermo Scientific™ Vanquish™ UHPLC Binary Pump), high-resolution mass spectrometry (Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ High Resolution Mass Spectrometer), and an autosampler apparatus. The samples were applied into a column (Accucore™ Phenyl-Hexyl 100 mm × 2.1 mm ID × 2.6 μm) and eluted gradiently (0.30 mL/min) with water (0.1% formic acid) and methanol (0.1% formic acid) as mobile phases for 25 min. A mass Orbitrap spectrometer was employed to collect the data. Untargeted metabolites in both positive and negative ionization polarities were collected using the whole MS/dd-MS2 acquisition method. The identified metabolites were scanned with 70,000 full MS and 17,500 dd-MS2 resolutions in the 66.7–1,000-m/z range. The Xcalibur software (Thermo Scientific, Bremen, Germany), version 4.4, was used to process the data (Artanti *et al.*, 2023; Suryani *et al.*, 2025).

High-Performance Liquid Chromatography (HPLC) of aloin

The aloin content was quantified by HPLC (Thermo Scientific™ Vanquish™ UHPLC Quaternary Pump, US) using UV-Vis/fluorescence detector and Aloin A (Supelco, US) as the reference standard according to Brown *et al.* (2014), with slight modification. A column of Hypersil GOLD™ C18 (250 × 4.6 mm, and particle size 5 μm) was used as a stationary phase. The HPLC-grade acetonitrile (Merck, US) containing (A) 0.1% acetic

acid (Merck, US) and (B) HPLC-grade water (Merck, US) containing 0.1% acetic acid in a ratio of 70:30 was used as a mobile phase. Aloin A was detected at a wavelength of 357 nm.

O-acetyl polysaccharides measurement

The O-acetyl polysaccharides measurement was performed according to Metcalfe (2019) using the spectrophotometry method at 540 nm (Thermo Scientific™ Multiscan GO, US). The quantification of o-acetyl polysaccharides of the sample was plotted on the acetylcholine standard curve.

Bioactivity testing

In vitro *hepatoprotective activity*

HepG2 cells (1×10^4 cells/well) were grown and treated with various concentrations of AJ, AO, and WAO ethanolic extracts (0, 12.5, 25, 50, 100, and 200 μg/mL), and incubated for 20 h. H_2O_2, 1.5 mM, was added to all wells, except the control cell and incubated for next 4 h. MTT reagent (Sigma-Aldrich, US) was added and incubated for 4 h. The reaction was stopped by adding 0.01-M HCl (containing 10% sodium dodecyl sulfate). The plate was incubated overnight in the dark and the absorbance measurement was performed at 595 nm by an ELISA reader (Bio-Rad, US).

The reactive oxygen species-level measurement

The ROS at intracellular level was monitored using a fluorogenic probe dichlorodihydrofluorescein diacetate (DCFDA) staining ROS-based assay. Cells were collected and resuspended in a supplemented buffer. DCFH-DA (10 mM) was added to the cells (5×10^5 cells/mL) and incubated at 37°C for 30 min. Cells were treated with AJ, AO, and WAO at various concentrations (0, 50, 100, and 200 μg/mL), then incubated for 2 h, followed by 0.5-mM H_2O_2 treatment for 1 h. Cells without any sample treatment were used as an untreated group. The DCF fluorescence of the cells was detected and analyzed using flow cytometry (BD Accuri C6 flow cytometer) with an excitation/emission wavelength of 488–525 nm.

In silico testing

Docking of metabolite compound of aloe gel against TACE protein

The MarvinSketch software (https://chemaxon.com/products/marvin) was used for visualization of two-dimensional (2D) and 3D structures of compounds. Docking protocol was validated by estimating Root Mean Square Deviation (RMSD) values between the native ligand from TACE receptor (PDB ID 2FV5) PDB file and its conformation of the docked ligand. The YASARA 20.8.23 software was used to calculate the RMSD value. Further steps were processed if the RMSD value of the docking protocol was <2.0 Å. The protein–ligand interaction was calculated by a standard molecular docking procedure using the Protein-Ligand Ant System (PLANTS) docking tool (Meiyanto *et al.*, 2014). The docking scores showed the energy of ligand when binding to the target protein. The more negative the docking score, the stronger the ligand binding affinity. Afterward, the Ligplot+ v.2.2 software was used for the 2D visualization of docking interaction between protein receptor and ligand.

Statistical analysis

Student's *t*-test and one-way ANOVA, followed by the Tukey's *post hoc* test (GraphPad Prism 10.13.01 statistical software), were used to analyze the data. In all, three replications (n = 3) were used for statistical analysis. Letter differences ($P < 0.05$) showed a statistically significant difference between entities.

Results and Discussion

Characteristics of soil

Many factors, including geographic and environmental conditions, influence the secondary metabolites contained in plants. Thus, a characteristic evaluation of the tested aloe plant and its environment was necessary before its bioactivity investigation. The aloe plant used in this study was collected from a local plantation situated in Nglipar, Gunungkidul Regency, Yogyakarta, Indonesia (7° 49' 11.1" S–7° 55' 2.6" S and 110° 35' 10" E–110° 41' 4.2" E), at an altitude of 236 m (BPS Kab.Gunungkidul, 2019). According to the physiography of Nglipar, altitude-wise this region is a foothill region (Nair *et al.*, 2018). In 2018–2019, the annual rainfall of Gunungkidul Regency was 238–262 mm (BPS Yogyakarta, 2020); and because the annual rainfall was <300 mm, the area was categorized as an arid region (Abdelhak, 2022).

The soil analysis was conducted to ensure that the aloe vera used in this study was grown in the usual environment for cultivation. Soil texture influences its properties. According to soil analysis, the soil texture of four sites around the Nglipar area showed a clay, silty clay, and loamy texture (Table 1). Aloe vera plants generally grow in this soil condition. In Bangladesh as well, aloe vera plants grow in clay, silty loam, and clay loam classes of soil texture (Chowdhury *et al.*, 2018; Sultana *et al.*, 2021).

Aloe vera plant shows good physical and chemical fertility in sandy soils, is chemically active in clay soils, and has good physical conditions in loamy soils

Table 1. Analysis of soil texture of aloe plantation in Nglipar, Indonesia.

No.	Analysis	Soil control	Soil Av-1	Soil Av-2	Soil Av-3
1.	Texture				
	(a) Sand (%)	4.58	7.98	29.77	7.18
	(b) Silt (%)	37.64	46.28	48.00	49.92
	(c) Clay (%)	57.78	45.74	22.23	42.90
2.	Class of texture	Clay	Silty clay	Loamy	Silty clay

Table 2. Analysis of soil elemental composition of aloe plantation in Nglipar, Indonesia.

Elements	Unit	Concentration			
		Control soil	Soil Av-1	Soil Av-2	Soil Av-3
Si	%	10.592	14.798	12.196	11.892
Al	%	5.181	7.033	5.468	5.983
Ca	%	1.193	1.617	1.818	1.315
Ti	%	0.867	1.109	1.134	1.046
P	%	0.208	0.248	0.217	0.222
Mg	%	0.102	0.129	0.165	0.109
Ag	ppm	841.8	897.8	955.3	859.1
Cl	ppm	459.7	509	479	476.5
S	ppm	197.6	327.6	193.1	0
I	ppm	24.7	23.7	22.9	25.6
Pd	ppm	1.9	1.9	1.7	2.7
Fe	ppm	1.3	1.6	1.6	1.6
Cd	ppm	0.3	2.2	0.2	0.1
Na	ppm	0	0	0	0
K	ppm	0	0	0	0
Mn	ppm	0	0.1	0	0.1
Co	ppm	0	0	0	0
Ni	ppm	0	0	0	0
Cu	ppm	0	0	0	0
Zn	ppm	0	0	0	0
As	ppm	0	0	0	0
Hg	ppm	0	0	0	0
Pb	ppm	0	0	0	0

(García *et al.*, 2023). According to García *et al.* (2023), aloe vera at Nglipar was probably abundant in chemically active compounds and was in good physical condition.

The other crucial characteristic of soil for plantation is heavy metal composition. Toxic heavy metals (i.e., mercury [Hg], arsenic [As], and lead [Pb]) contaminated in the soil are absorbed by aloe plants (Dobbins *et al.*, 2021; Shokri *et al.*, 2016). This contamination reduces the quantity and quality of the harvested aloe product (Dobbins *et al.*, 2021). As observed in Table 2, Hg, As, and Pb were not detected in all soils studied in this study. On the other hand, phosphorus (P) in the soil increases the productivity and quality of harvested plants, including aloe vera (Sultana *et al.*, 2021). In this study, the concentration of P in the soil was 0.208–0.248% (Table 2). Calcareous soil is recommended for aloe cultivation because this soil increases the leaf biomass yield of aloe (Chowdhury *et al.*, 2018). Nglipar's soil is classified as calcareous soil because of its high calcium (Ca) concentration (1.193–1.818%; Table 2).

FTIR spectra of aloe vera

The FTIR spectrometer generates a characteristic spectrum for the compounds known as "fingerprint region" (Kassem *et al.*, 2023). The FTIR spectra of aloe samples

are shown in Figure 1 and Table 3. The broad spectrum at 3,274 cm^{-1} detected for AO and WAO was indicated as a hydroxyl group (-OH), usually because of medicinal compounds, such as alcohols, phenols, and acids and their derivatives. The stretching vibrations of hydroxyl group are shown at the widest wave numbers of 2,900–3,600 cm^{-1} (Abbasi *et al.*, 2020). The sharp absorption peaks on AO spectra at 2,920 cm^{-1} and 2,854 cm^{-1} and on WAO spectra at 2,920 cm^{-1} and 2,850 cm^{-1} showed alkyl functional groups. Alkyl functional groups of compounds, such as alkanes and acetates, from aloe buds are observed at 2,855 cm^{-1} and 2,924 cm^{-1} (Abbasi *et al.*, 2020). The spectra at 1,728 cm^{-1} for AO and 1,716 cm^{-1} for WAO were related to the carbonyl group (C=O). The carbonyl group of acids, aldehydes, and ketones for aloe buds is detected at 1,711 cm^{-1} (Abbasi *et al.*, 2020). Sharp peaks at 1,562 cm^{-1} and 1,581 cm^{-1} on AO and WAO probably contributed to C=C group from aromatic compounds. According to Abbasi *et al.* (2020), C=C group from aromatic compounds is detected at 1,531 cm^{-1} and 1601 cm^{-1} for aloe buds. Absorption at 1,407 cm^{-1} and 1,396 cm^{-1} were related to carboxyl group (R-COOH).

Carboxyl group from WAO extract was detected at 1,402 cm^{-1} (Fardsadegh and Jafarizadeh-Malmiri 2019). The spectrum at 1,323 cm^{-1} for AO and 1,315 cm^{-1} for WAO matched the functional group, R-$COCH_3$ of the spectrum at 1,311 cm^{-1} as stated by Abbasi *et al.* (2020). Spectra at 1,242 cm^{-1} for AO and 1,245 cm^{-1} for WAO were similar to those at 1,232 cm^{-1} related to the ether group, R=C-O-C as shown by Abbasi *et al.* (2020). The C=C group is related to unsaturated five- or six-member ring compounds, which were probably detected at 1,018 cm^{-1} for AO and 1,022 cm^{-1} for WAO. The suggested compound group was detected at 1,058 cm^{-1} by Abbasi *et al.* (2020). Spectra at 806 cm^{-1} and 814 cm^{-1}, detected for AO and WAO, respectively, probably belonged to alkenes. According to Abbasi *et al.* (2020), alkenes were detected around 819 cm^{-1}. The spectrum at 764 cm^{-1} on AO and 771 cm^{-1} on WAO were similar to the peak of 769 cm^{-1} as shown by Abbasi *et al.* (2020), which matched the C-H peak belonging to five-member aromatics. Fingerprint region of functional groups is used to assess the quality of a material by comparing it to reference spectra of the same material from different sources (Kassem *et al.*, 2023). Through the fingerprint region of functional groups using FTIR data, we demonstrated that aloe vera in this study was of similar quality as that from other sources.

The LC-HRMS data of aloe vera

The LC-HRMS data detected several active compounds in Nglipar aloe, including polysaccharides, terpenoids, phenolics, and flavonoids (Table 4). Aloesin was detected in both AO and WAO of Nglipar aloe. Nevertheless, aloesin discovered in AO was less than that in WAO. This finding was similar to Añibarro-Ortega *et al.*'s (2019) results regarding aloesin in AO. The area under curve (AUC) of most terpenoid, phenolic, and flavonoid compounds was lower in AO than in WAO. As reported previously, kaempferol was detected in the peel of Chile aloe but not in its gel (Quispe *et al.*, 2018). Compared to Chile aloe, kaempferol was discovered in Nglipar AO. Glucomannan, galactomannan, and acemannan polysaccharides were also discovered in the Nglipar aloe. Interestingly, acemannan was higher in AO than in WAO. Acemannan is used as a specific marker for aloe polysaccharides (Liu *et al.*, 2019; Metcalfe, 2019).

Table 3. The FTIR spectral positions and its expected functional groups of Nglipar aloe gel (AO) and whole plant (WAO) (Abbasi *et al.*, 2020; Fardsadegh and Jafarizadeh-Malmiri, 2019).

FTIR spectral position (cm^{-1})			Expecting functional group
AO	WAO	References	
3,274	3,274	2,900–3,600	Hydroxyl group, OH
2,920	2,920	2,924	Alkyl compounds, such as alkanes, acetates, esters, acids, ethers, etc.
2,854	2,850	2,855	Alkyl compounds, such as alkanes, acetates, esters, acids, and ethers
1,728	1,716	1,711	Carbonyl group, C=O, belonged to acids, aldehydes, and ketones
1,562	1,581	1,531 and 1,601	C=C belonged to aromatic medicinal compounds
1,407	1,396	1,402	Carboxyl group, R-COOH
1,323	1,315	1,311	R-$COCH_3$ containing compounds and their derivatives
1,242	1,245	1,232	R=C-O-C belonged to ethers
1,018	1,022	1,058	C=C related to unsaturated five- or six-member ring compounds
806	814	819	Alkenes
764	771	769	C-H peak belonged to five-member aromatics

Note: FTIR: Fourier transform infrared spectroscopy.

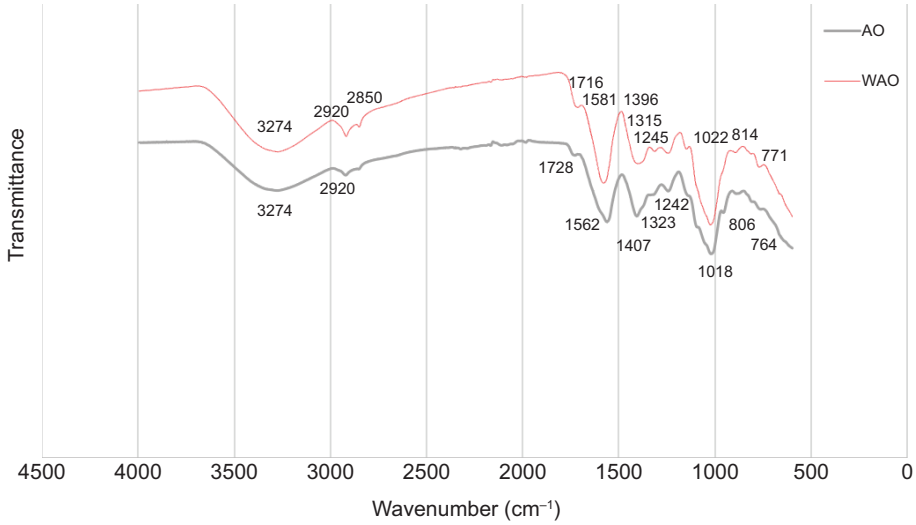

Figure 1. The FTIR spectra of Nglipar's aloe gel (AO) and whole plant (WAO).

Table 4. Compounds discovered in aloe gel (AO) and whole plant (WAO) leaves according to LC-HRMS data.

Compounds	Retention Time (min)	Area under curve (AUC, ×10⁶)	
		AO	WAO
Glucomannan	1.176	6.95	9.13
Galactomannan	1.182	13.63	33.18
Acemannan	1.202	28.85	25.4
Aloesin	5.871	0.67	3.82
Kaempferol	5.916	0.01	7.16
Gambiriin A1	7.985	0.02	7.52
Eupatorin	8.033	7.57	66.63
Curcumene	8.685	0.05	1.7
Aloin	8.856	–	372.49
Genistein	9.271	0.09	5.8
Dammarenediol	18.102	0.18	55.7
Sakuranin	18.102	1.3	8.36
Squalene ($C_{30}H_{50}$)	20.013	5.77	1.73

Note: LC-HRMS: liquid chromatography high-resolution mass spectrometry.

Aloin is supposed to inducing DNA damage and having carcinogenic effects. Removal of aloin reduces adverse effects and escalate the safety of oral products (Ding *et al.*, 2014; Kim *et al.*, 2023). The result shows high levels of aloin content in WAO but not in AO of Nglipar aloe (Table 4).

Quantification of aloin A by HPLC

Quantification of aloin A in AO and WAO using HPLC showed that aloin A was not discovered in Nglipar AO but was abundantly found in WAO (Figure 2A). This finding was similar to the LC-HRMS results of aloin (Table 4). Thus, according to the level of aloin in AO, it was considered safe for oral consumption.

Measurement of O-acetyl polysaccharides

Because acemannan can be used as a specific marker for aloe polysaccharides (Liu *et al.*, 2019; Metcalfe, 2019), this study calculated the concentration of acemannan by measuring the content of o-acetyl polysaccharides according to Metcalfe (2019). The content of o-acetyl polysaccharides in AO was higher than that in WAO (Figure 2B). This result was consistent with the AUC measurement of acemannan in LC-HRMS results (Table 4). Combining of polysaccharides and aloesin has been reported to possess hepatoprotective activity through antioxidant mechanisms (Yimam *et al.*, 2016). This study revealed that skin removal of aloe leaves drastically reduced the concentration of several active compounds, including aloesin (Table 4). Thus, the *in vitro* hepatoprotective evaluation was conducted to confirm whether this condition affected the hepatoprotective activity of AO, compared to WAO.

Cell viability

The viability of HepG2 cells was measured by MTT assay. Cell viability was used as an indicator of cytotoxicity prevention. Treatment with H_2O_2 was used to induce cytotoxicity in HepG2 cells. The result showed that 1.5 mM H_2O_2 reduced the viability of HepG2 cells by 35.74±1.75%. AJ was used for AO control without drying.

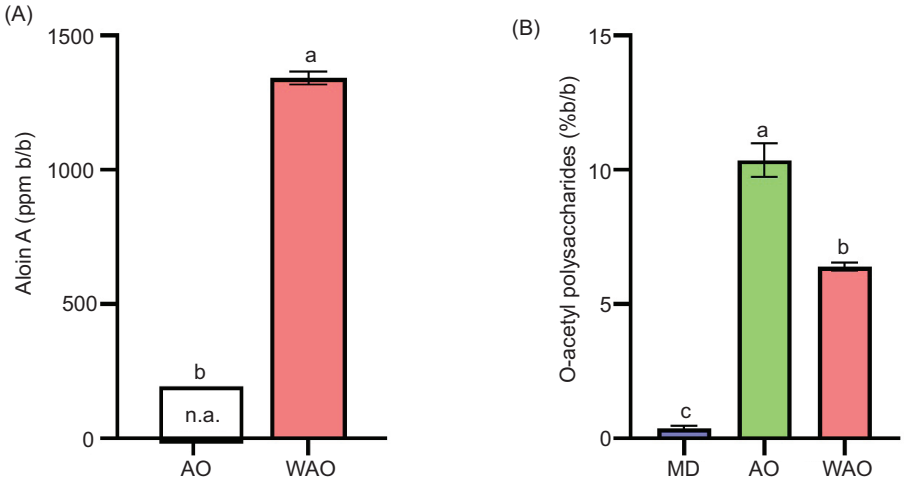

Figure 2. Concentration of (A) aloin A and (B) total o-acetyl polysaccharides content in aloe gel (AO) and whole plant (WAO) leaves. The concentration of aloin A was measured using HPLC. The concentration of o-acetyl polysaccharides was measured by UV-Vis spectrophotometer using maltodextrin (MD) as a negative control. Different alphabets show significant differences between samples (*P* < 0.05).

Different concentrations of AJ (25, 100, and 200 μg/mL) significantly prevented the cytotoxicity of HepG2 cells by 20.97±8.87%, 16.11±4.80%, and 18.57±3.50%, respectively, after H_2O_2 treatment (Figure 3A). Different concentrations of AO prevented the cytotoxicity of HepG2 cells after H_2O_2 treatment, with no significant differences. However, 200 μg/mL of AO showed the highest prevention of 22.44±6.04% (Figure 3B). The lower concentration of WAO (12.5 μg/mL) prevented the cytotoxicity of HepG2 cells by 17.20±2.70% after H_2O_2 treatment. Nevertheless, the higher concentration of WAO tended to induce cytotoxicity of HepG2 cells more than that by H_2O_2 treatment (Figure 3C). The result revealed that treatment with AO extract prevented H_2O_2-induced cytotoxicity in HepG2 cells, compared to WAO.

Measurement of reactive oxygen species level

Alterations in ROS at intracellular level was monitored using the DCFDA staining ROS-based assay. The ROS of cells was measured to show the level of oxidative stress after sample treatment. The result showed that H_2O_2 treatment increased ROS level by 2.4-fold, compared to untreated cells, while the ROS level of all aloe extract treatments was lower than that of H_2O_2 treatment. AJ, 200 μg/mL, and 100 and 200 μg/mL of AO showed no significant ROS level, compared to untreated cells (Figure 3D).

Docking of metabolite compound of aloe gel against TACE protein

As a main protein having a role in fibrogenesis and other liver injury models, high expression of TNF-α in inflammation cases was caused by the increased level of TACE. Thus, inhibition of TACE may decrease hepatic fibrosis through TNF-α signal pathway (Lakshmi *et al.*, 2019). Studies about the affinity of different compounds discovered in AO (Table 4) toward TACE enzyme were limited, especially for the *in silico* docking method. In this study, various compounds discovered in LC-HRMS data (Table 4) were tested for *in silico* docking test of TACE enzyme. Polysaccharides were excluded from this test because the software cannot process the high molecular weights of these compounds.

The docking protocol in this study was valid because the RMSD of TACE (2VF5) conforms to the required value (≤2 Å) after running with the docking protocol. Internal native ligand (IK682) and silibinin were used as reference standards (Mogadem *et al.*, 2022), and 13 compounds detected in aloe gel were tested for its binding affinity against TACE enzyme using this protocol. The docking score was measured as a parameter for protein–ligand interaction. The native ligand showed the lowest docking score (-124.5±5.05). The other 14 compounds (including positive TACE inhibitor silibinin) that showed interaction with TACE enzyme from the lowest to the highest docking scores were squalene ($C_{30}H_{50}$), gambiriin A1, silibinin, naringenin 4' methoxy 7 o-glucuronide, sakuranin, sakuranetin, curcumene, aloesin, 7 methyl ether 2' feruloylaloesin, isoaloeresin D, dammarenediol, genistein, kaempferol, and eupatorin (Table 5). Squalene and gambiriin A1 had a lower docking score than the reference standard silibinin, while other compounds showed higher docking scores.

Native inhibitor of TACE protein has active sites on Glu406, Gly349, and Leu348, while silimaryn/silybinin

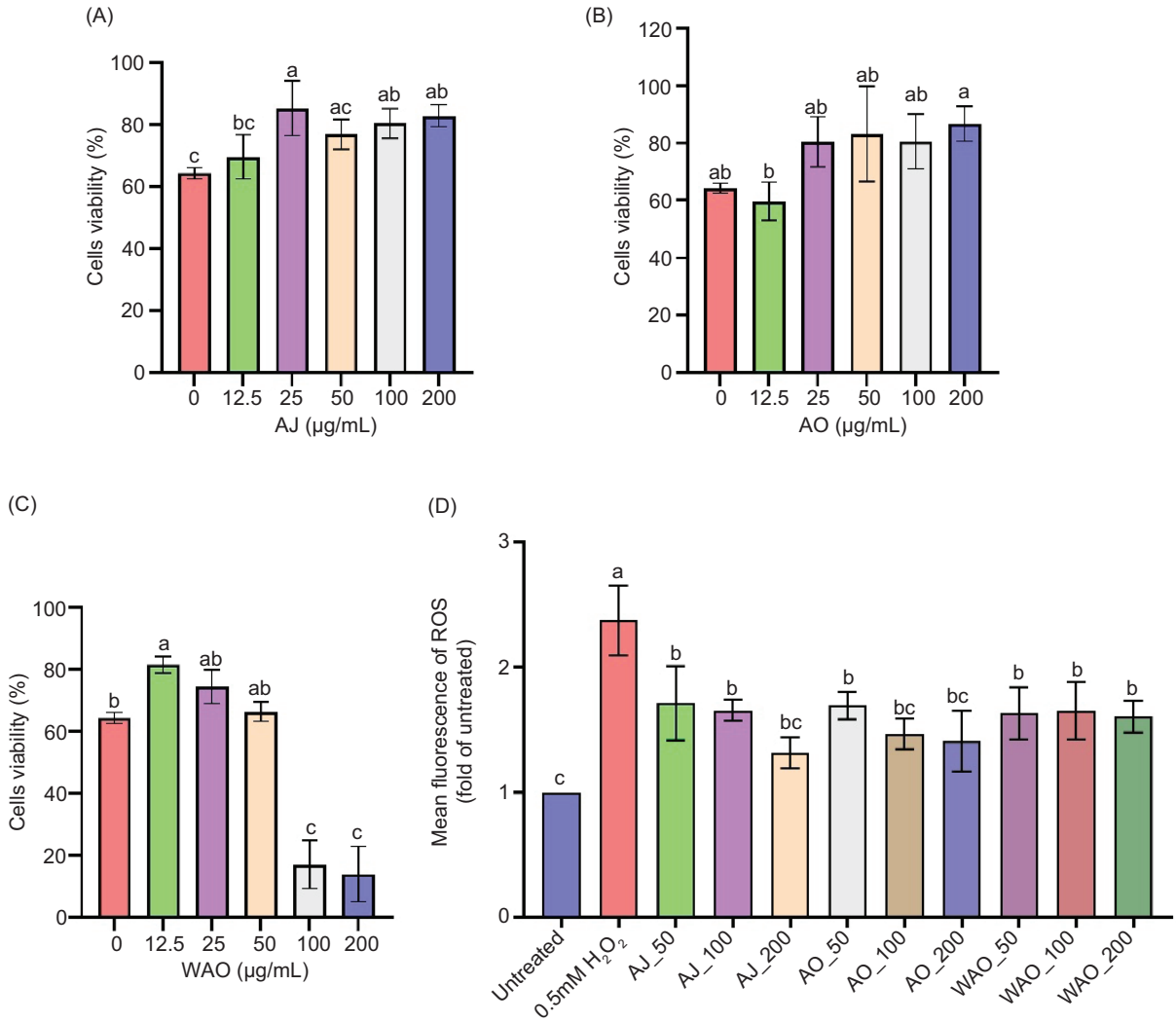

Figure 3. Hepatoprotective activity of Nglipar AJ, AO, and WAO. Cell viability by varying concentrations (µg/mL) of (A) AJ, (B) AO, and (C) WAO on HepG2 cells after H_2O_2 induction. (D) Intracellular ROS levels of HepG2 cells after extract treatment (µg/mL). All measurements are analyzed according to the methods. Different alphabets show significant differences among samples ($P < 0.05$).

has active sites on Glu406, Gly349, Glu398, Asn447, Tyr433, and Lys432 (Borah *et al.*, 2016). The TACE–ligand interaction in this study showed that the native ligand of TACE (IK682) had six hydrogen bonds and 13 hydrophobic interactions, including its interaction with active sites Gly349, Leu348, Asn447, and Tyr433. Silibinin had 10 hydrophobic interactions and three external bonds, including its interaction with active sites Glu406, Glu398, and Leu348. Squalene had 13 hydrophobic interactions and 12 external bonds, including its interaction with active sites Gly349, Leu348, and Tyr433. Meanwhile, gambiriin A1 had two hydrogen bonds, three hydrophobic interactions, and 15 external bonds, including its interaction with active site Glu406 (Table 5). Nevertheless, some other compounds also interacted with the active sites of TACE protein while the docking score was still higher than that of the reference ligand.

Aloesin and its derivatives have various beneficial bioactivities for health (Handayani *et al.*, 2021, 2023). In this study, aloesin was rich in WAO but less in AO (Table 4). On the other hand, AO contained higher o-acetyl polysaccharides than WAO (Figure 2B). Polysaccharides have a role in hepatoprotective effect (Qu *et al.*, 2020). Combining polysaccharides and aloesin increases hepatoprotective activity through an antioxidant mechanism (Yimam *et al.*, 2016). Interestingly, the data of viability cells revealed that AO inhibited the cytotoxicity of HepG2 cells after H_2O_2 treatment. Meanwhile, the higher concentration of WAO tended to induce cytotoxicity in HepG2 cells (Figures 3A–C). The cytotoxicity of WAO at high concentrations probably was due to the high presence of aloin (Figure 2A). WAO contained more aloin and other anthraquinones than AO. Administration of commercial AO beverages containing less aloin (3.43 ppm) for

Table 5. Protein–ligand interaction of TACE protein (2VF5) with expected compounds in aloe leaves.

No.	Compound	Docking score	H-bond		Hydrophobic bond	External bond
			Residue	Distance (Å)		
1.	IK682 (native ligand)	–124.5±5.05	Zn His405 His415 Gly349 Leu348	2.20; 2.37 3.34 3.13 3.18 2.86	Ala439; Asn447; Gly346; Leu350; Leu401; Pro437; Ser441; Thr347; Tyr433; Tyr436; Val402; Val434; Val440	–
2.	Silibinin (TACE inhibitor)	–99.2±1.25			Ala439; Ile438; Glu398; Glu406; Gly346; Leu348; Leu401; Met435; Pro437; Val402	His405 (2); Val434 (1)
3.	7 Methyl ether 2'-feruloylaloesin	–88.7±1.15			His405; His415; Ile438; Glu406; Gly349; Leu401; Met435; Pro437; Ser441; Tyr433; Tyr436; Val402	Ala439 (5); Glu398 (1); Val434 (4)
4.	Aloesin	–89.2±2.07	Zn	2.79	Ile438; Glu406; Gly349; Leu348; Met435; Pro437; Thr347; Tyr433; Val402; Val 434	Ala439 (1); His405 (1); Tyr436 (1)
5.	Isoaloeresin D	–88.8±2.11	His405	3.09	His415; Ile438; Glu398; Glu406; Gly349; Leu348; Leu350; Leu401; Met435; Ser441; Tyr436; Val402; Val440	Ala439 (3); Pro437 (1); Val434 (3)
6.	Naringenin 4'-Methoxy 7 o-glucuronide	–93.3±3.39	His405 Tyr436	2.89 2.37	Ala439; Ile438; Leu348; Leu401; Met435; Pro437; Tyr433; Val402	Glu406 (3); Gly349 (1); Val434 (1)
7.	Eupatorin	-80.4±1.83	Gly349 Leu348	2.36 2.18	–	–
8.	Curcumene	–89.8±0.39			His405; Glu406; Gly349; Leu348; Thr347; Tyr436; Val402; Val440	Ala439 (1); Ile438 (1)
9.	Dammarenediol	–88.9±0.59	His405	3.21	Ala439; Asn447; Ile438; Glu406; Gly346; Gly349; Gly442; Leu348; Leu401; Pro437; Ser441; Thr347; Tyr433; Val402; Val434	
10.	Gambiriin A1	–102.8±2.86	His409 Glu406	2.41 3.16	His415; Ile438; Val440	Ala439 (7); His405 (1); Ile438 (2); Leu348 (1); Leu401 (1); Pro437 (1); Val434 (2)
11.	Genistein	–86.8±0.64	Thr347	3.04	Ala439; Gly349; Leu348; Pro437; Tyr436; Val434	Thr347 (1)
12.	Kaempferol	–82.2±0.55			Ala439; Ile438; Glu406; Gly349; Leu348; Pro437; Thr347; Tyr436; Val402; Val440	
13.	Sakuranetin	–90.7±2.14			Ala439; His405; Ile438; Glu406; Gly349; Leu348; Pro437; Thr347; Tyr436; Val402; Val440	
14.	Sakuranin	–92.5±0.79	Zn His405 Thr347	2.57 2.33 2.06	Ile438; Glu398; Leu348; Lys448; Pro437; Val402; Val434	Ala439 (3); Asn447 (1); Leu401 (4)
15.	Squalene	–116.6±2.54			Gly346; Leu348; Leu350; Leu401; Met435; Pro437; Thr347; Thr403; Thr404; Tyr433; Tyr436; Val402; Val440	Ala439 (1); His405 (5); Ile438 (3); Gly349 (1); Val434 (2)

Note: TACE: tumor necrosis factor (TNF)-α converting enzyme.

90 days did not alter the histopathology of mice's organs (Hayes *et al.*, 2024). Aloin at a low concentration protects SH-SY5Y cells against peroxide-induced toxicity, but not at a higher concentration (Kaparakou *et al.*, 2021).

One of the hepatoprotective mechanisms of aloe is the prevention of ROS production (Handayani *et al.*, 2021). The measurement of ROS revealed that treatment with AO or WAO after H_2O_2 treatment inhibited ROS levels, compared to H_2O_2 treatment alone. AJ was used to ensure that the AO drying method did not affect AO extract's bioactivity. The result showed that the ROS level of AJ and AO did not differ significantly. Thus, the drying method in this study did not affect the bioactivity of AO extract. The insignificant ROS levels of 200 μg/mL for AJ, and 100 μg/mL and 200 μg/mL for AO, compared to untreated cells, revealed their prevention activity toward H_2O_2-induced ROS production (Figure 3D). Thus, the hepatoprotective mechanism of AO revealed in this study was due to the prevention of ROS production. Pro-inflammatory cytokine, TNF-α, induces ROS production (Wu and Pan 2019).

Aloe vera inhibited TNF-α and did not alter the histopathological pattern of mice liver (Gupta *et al.*, 2023). High expression of TNF-α in inflammation is caused by the increased TACE level. TACE, also known as metalloproteinase 17 (ADAM17), has an important role in the cleavage and release of TNF-α. Soluble TNF-α binds to its receptors and initiates downstream signaling pathways. Increasing TACE activity in oxidative stress induces insulin resistance and hepatitis. Thus, the strategies targeting TACE are considered effective in treating liver diseases via TNF-α signaling pathway (Lakshmi *et al.*, 2019; Srinivas *et al.*, 2024). The affinity of all compounds toward TACE receptor was less than that of IK682 (native ligand). Interestingly, the affinity of squalene and gambiriin A1 discovered in Nglipar aloe was higher than the reference compound silibinin (Table 5). Silymarin and silibinin are the known compounds that act as TACE inhibitors (Borah *et al.*, 2016). Squalene is generally discovered in sea fish. Nevertheless, squalene is also discovered in various plants. A high concentration of squalene can be extracted from tea leaves also (Sheng *et al.*, 2022). According to LC-HRMS data, squalene showed a bigger AUC in AO than WAO (Table 4). On the other hand, even though gambiriin A1 was first found in *Uncaria gambir* Roxb, this compound was also found in other plants (Mazlan *et al.*, 2018; Zhou *et al.*, 2022). Thus, the affinity of squalene and gambiriin A1 toward TACE enzyme probably acted as TACE inhibitor.

In addition, Van der Waals (VdW) forces are an essential parameter for the molecular recognition of a ligand by the target receptor-binding pocket (Bitencourt-Ferreira *et al.*, 2019). The ligplot software showed all external bonds, including VdW forces. In this study, silibinin, squalene, and gambiriin A1, and not other compounds, had external bonds with His405 and Val434 (Table 5). His405, His409, and His415 had catalytic interaction with Zn, followed by activation of TACE enzyme (Hermenean *et al.*, 2017). Squalene had five external bonds with His405, while gambiriin A showed hydrogen interaction with His409, a hydrophobic bond with His415, and one external bond with His405. These interactions could increase the affinity of squalene and gambiriin A1 toward TACE protein and break the catalytic interaction of amino acid residues of TACE with Zn. Thus, squalene and gambiriin A1 probably had a role in the hepatoprotective effects of AO through inhibition of TACE enzyme. The data support the hepatoprotective activity and the safety of AO, especially from Nglipar plantation in Indonesia.

Conclusion

This study of aloe vera plantation Nglipar in Indonesia demonstrated that AO provided a higher protection than WAO against H_2O_2-induced hepatotoxicity in HepG2 cells. Soil analyses from four sites around Nglipar revealed clay, silty clay, and loamy textures, with no detectable levels of heavy metals (Hg, As, or Pb), suggesting a favorable environment condition for growth of aloe vera. The functional groups of both AO and WAO were similar; however, aloin A was abundant in WAO but absent in AO extract. AO extract effectively inihibited H_2O_2-induced toxicity in HepG2 cells, whereas WAO offered protection only at lower concentrations and caused toxicity at higher concentrations. Both 100 μg/mL and 200 μg/mL of AO extract significantly reduced H_2O_2-induced ROS production. LC-HRMS analysis revealed that squalene was more concentrated in AO than in WAO, and docking studies revealed that squalene had the highest affinity for TACE protein. These findings confirm that AO extract has potential inhibitory activity against oxidative stress-induced damage in HepG2 cells, suggesting it can serve as a safe and strong hepatoprotective agent. However, this study has several limitations that must be addressed in the future research. First, the hepatoprotective effects of aloe extracts were investigated only in HepG2 cells. Additionally, H_2O_2 was the sole oxidative stress inducer used, which limits the assessment of protective effects. Therefore, the future studies must include animal models and multiple hepatotoxic agents to confirm comprehensively the hepatoprotective activity.

Data Availability

The data supporting the findings are available upon reasonable request.

Author Contributions

Sari Haryanti: formal analysis, investigation, data curation, writing – original draft, and writing – review and editing. Mujiyanto Mujiyanto: investigation, data curation, writing – original draft, and visualization. Nur Maulidah Rahmah: data curation, visualization, and writing – review & editing. Ade Erma Suryani, Santosh Chokkakulula, Khoirun Nisa, Balasubramani Ravindran, and Soon Woong Chang: formal analysis, and writing – review and editing. Sri Handayani and Ravishankar Ram Mani: conceptualization, methodology, investigation, resources, data curation, writing – review and editing, visualization, supervision, and project administration.

Conflicts of Interest

The authors declared no conflict of interest.

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