Research Article

Phytochemical Profiling and Antioxidant Evaluation of *Ananas comosus* Extract: An Integrated *In Vitro* and *In Silico* Approach Targeting KEAP1 and SIRT1

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Abstract | Pineapple (*Ananas comosus* (L.) Merr.) fruit contains diverse phytochemicals with antioxidant potential and may play an important role in oxidative stress-related pathways. This study evaluated the antioxidant activity of pineapple fruit extract using the DPPH assay, characterized its phytochemical composition by liquid chromatography–high-resolution mass spectrometry (LC–HRMS) and Fourier transform infrared spectroscopy (FTIR), and predicted the interactions of selected metabolites with Kelch-like ECH-associated protein 1 (KEAP1) and sirtuin 1 (SIRT1) through molecular docking. Antioxidant activity was quantified using a four-parameter logistic (4PL) regression model to provide a robust estimation of the IC_{50} value. The extract exhibited measurable antioxidant activity with an IC_{50} value of 221.53 μg mL^{-1} and excellent model fitting (R^2 > 0.999). LC–HRMS tentatively identified 33 metabolites, including organic acids, phenolics, sugars, and unsaturated fatty acids that may collectively contribute to the observed antioxidant activity. Among the identified compounds, α-linolenic acid demonstrated the strongest predicted binding affinity toward both KEAP1 and SIRT1, whereas 4-coumaric acid also exhibited promising binding affinity toward KEAP1. Although these molecular interactions remain predictive and require experimental validation, the integrated in vitro and in silico findings highlight the potential of *A. comosus* as a natural antioxidant source. These findings provide a scientific basis for future translational research, including preclinical and clinical validation and the development of functional pineapple-derived products such as functional confectionery and effervescent candy. Future studies should also investigate microplastic contamination throughout the pineapple production chain and explore the utilization of pineapple peel as a value-added livestock feed ingredient to support a circular bioeconomy while evaluating its potential role in limiting microplastic circulation within integrated crop–livestock systems. Collectively, these research directions may contribute to the achievement of Sustainable Development Goal 3 (Good Health and Well-being) and Sustainable Development Goal 12 (Responsible Consumption and Production).

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Introduction

The excessive generation of free radicals in the human body can lead to oxidative stress (Martemucci *et al*., 2022), a condition associated with various degenerative diseases such as neurological, renal, hepatic, and cardiovascular disorders (Chaudhary *et al*., 2023; Kıran *et al*., 2023; Tumilaar *et al*., 2024). When the body produces more reactive oxygen species (ROS) than its natural antioxidant defense system can handle, oxidative stress occurs (Nakai and Tsuruta, 2021).

The Nrf2/KEAP1 signalling pathway (nuclear factor erythroid 2-related factor 2/Kelch-like ECH-associated protein 1) is one of the fundamental pathways involved in cellular antioxidant defense. Under basal conditions, KEAP1 binds to Nrf2 and promotes its degradation, whereas under oxidative stress conditions, Nrf2 is released and activates antioxidant response pathways (Alzain *et al*., 2023; Li *et al*., 2019; Tonolo *et al*., 2020). Furthermore, Sirtuin 1 (SIRT1) increases cellular antioxidant capacity and promotes mitochondrial biogenesis by deacetylating transcription factors, thereby reducing oxidative stress (Iside *et al*., 2020; Sellitto *et al*., 2022).

Pineapple (*Ananas comosus* (L.) Merr.) is a tropical fruit widely cultivated in Indonesia and has various reported health benefits (Hossain, 2016; Mohsin *et al*., 2020). Previous studies have reported DPPH IC_{50} values for pineapple extracts from different plant parts, including the pulp and peel, which are known to differ in phytochemical composition. Pineapple pulp contains various secondary metabolites, including steroids, phytosterols, flavonoids, tannins, polyphenols, terpenoids, saponins, and alkaloids (Rahman *et al*., 2020; Sharma *et al*., 2024). Methanolic extracts of *A. comosus* pulp have been reported to show IC_{50} values of 7.56 mg mL^{-1}, 8.19 mg mL^{-1}, and 2.90 mg mL^{-1}, respectively (Jovanović *et al*., 2018). Another study reported that 50 % methanolic extracts of *A. comosus*, from three different varieties cultivated in Malaysia (Josephine, Morris, and Sarawak), exhibited DPPH IC_{50} values of 29.0 mg mL^{-1}, 12.4 mg mL^{-1}, and 18.4 mg mL^{-1}, respectively (Yuris and Siow, 2014).

Hermayanti *et al*. (2025) reported that *A. comosus* extract can reduce adverse health effects in laboratory rats exposed to microplastics (MPs), specifically PET. This finding is supported by previous *in vivo* studies on MPs-contaminated potatoes conducted by Garfansa *et al*. (2024) and Hermayanti *et al*. (2024). In addition, our research team has reported that food ingredients in Indonesia are contaminated with MPs, including potatoes (Setyobudi *et al*., 2024a, 2024c, 2025), 33 brands of edible oil (Damat *et al*., 2025; Setyobudi *et al*., 2024b), five brands of commercial red palm oil (Saati *et al*., 2025), goat milk and water drinking, as well as livestock production facilities (Iswahyudi *et al*., 2025; Wahyudi *et al*., 2025), and paddy (Iswahyudi *et al*., 2024). These findings highlight the broader context of food-derived exposures and reinforce the importance of investigating natural antioxidant sources.

Therefore, this study aims to characterize the profile of bioactive compounds in *A. comosus* ethanolic fruit extract using liquid chromatography–high-resolution mass spectrometry (LC–HRMS), identify functional groups using Fourier Transform Infrared Spectroscopy (FTIR), evaluate antioxidant activity using the DPPH assay, and predict potential interactions between selected metabolites and the proteins KEAP1 and SIRT1 through molecular docking.

Materials and Methods

Extraction of pineapple fruits

An amount of 1 kg of *A. comosus* fruit was cleaned, the pulp was separated, cut into small pieces, homogenized in a blender (GETRA Heavy Duty Blender KS-778, Indonesia), and then mixed with 3.0 L of 95 % ethanol. The mixture was stirred (Eberbach e7200 universal lab stir, Germany) until homogeneous. The mixture was incubated at 4 °C for 24 h, then filtered to separate the filtrate from the residue. The filtrate was evaporated at 40 °C with a rotation speed of 10 rpm to 20 rpm (1rpm = 1/60 Hz) using a rotary evaporator (Rotavapor® R-180, USA) under reduced pressure, until no further ethanol condensation was observed in the receiving flask. The resulting concentrated extract was collected and used for further analyses.

Antioxidant activity assay using the DPPH method

The antioxidant activity of *A. comosus* fruit extract and vitamin C (as positive control) was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. A 0.1 mM DPPH solution was prepared by dissolving an accurately weighed amount of DPPH powder in ethanol p.a., adjusting to the desired volume, and incubating for 30 min in the dark to ensure radical stabilization. The maximum absorbance wavelength was determined by scanning the DPPH solution from 500 nm to 540 nm, and the wavelength corresponding to the maximum absorbance was used for subsequent measurements (Damat *et al.*, 2021).

A stock solution of *A. comosus* extract was prepared at a concentration of 1 000 mg L^{-1} by accurately weighing the sample and dissolving it in ethanol p.a. The stock was diluted in series to obtain five concentration levels for analysis. Each concentration was prepared in triplicate to ensure reproducibility. For each concentration, 1 mL of the sample solution was mixed with 1 mL of a 0.1 mM DPPH solution in a closed container and incubated at room temperature in the dark for 30 min. The concentration range used for the analysis was adjusted to adequately capture the full dose–response relationship required for IC_{50} determination.

The absorbance of each solution was measured at the maximum wavelength using a UV–Vis spectrophotometer (New UV-1900 UV-Vis Spectrophotometer, Japan). The percentage of DPPH radical scavenging activity was calculated according to Equation (1):

$$\% \text{ Inhibition} = (A_0 - A_s) / A_0 \times 100 \text{ ..... (1)}$$

Where:
A0 = Absorbance of the control (DPPH solution without sample)
As = Absorbance of the sample or vitamin C solution

The relationship between sample concentration and DPPH radical scavenging activity (%) was analyzed using a four-parameter logistic (4PL) model to determine the IC_{50} value (the concentration required to achieve 50 % inhibition) (Baj *et al.*, 2023; Song *et al.*, 2012). The 4PL model was selected due to its suitability in describing sigmoidal dose–response relationships commonly observed in antioxidant assays. All absorbance values were corrected using appropriate solvent blanks prior to analysis. The model fitting was performed using the SciPy library in Python (curve fit function) (Virtanen *et al.*, 2020), based on the following Equation (2):

$$Y = D + (A-D)/(1+(x/C)^B) \text{ ..... (2)}$$

Where:
y = response (% inhibition),
x = sample concentration (mg L^{-1}),
A = minimum asymptote (lower response limit),
D = maximum asymptote (upper response limit),
C = inflection point corresponding to the IC_{50} value, and
B = slope or Hill coefficient describing the curve's steepness.
The parameters A, B, C, and D were estimated using the nonlinear least squares regression method. The coefficient of determination (R2) was calculated to assess the goodness-of-fit between the experimental data and the predicted curve. Data were visualized using Matplotlib with error bars representing the standard deviation (SD) of the measurements (Hunter, 2007). A horizontal and vertical reference line was added to indicate the predicted IC_{50} value on the fitted curve.

FTIR analysis of pineapple extract

Functional group analysis of the pineapple fruit extract was performed using Fourier Transform Infrared Spectroscopy (FTIR, Shimadzu ATR; Shimadzu IRSpirit). The sample was placed on the ATR crystal,

ensuring optimal contact, and then pressed using a rotary press. The instrument recorded the infrared spectrum to identify the functional groups present in the extract.

Bioactive compound analysis using LC– HRMS

The bioactive compounds in pineapple extract were analyzed using a Vanquish™ UHPLC system coupled with a Q Exactive™ Hybrid Quadrupole-Orbitrap™ HRMS detector (Thermo Scientific™, Germany). Chromatographic separation was performed on a Thermo Scientific™ Accucore™ Phenyl-Hexyl column (100 mm × 2.1 mm, 2.6 µm) with MS-grade solvents. The mobile phases consisted of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B), at a flow rate of 0.3 mL min^{-1}. The phase B gradient was programmed to increase from 5 % to 90 % over 16 min, held for 4 min, and then returned to 5 % by minute 25. HRMS analysis was carried out in positive ion mode (m/z 66.7–1000) with a resolution of 70 000 using XCalibur 4.4. Data were processed using Compound Discoverer 3.2 and identified through the mzCloud and ChemSpider databases. Only compounds with confirmed names and structures in the databases were included in the results. As MS/MS fragmentation analysis was not performed, the identified compounds should be considered as tentatively assigned.

Molecular docking

The target protein structures were obtained from the Protein Data Bank (http://rcsb.org) with PDB IDs 4L7B (Keap1) (Jnoff *et al.*, 2014) and 5BTR (Sirt1) (Cao *et al.*, 2015). Protein preparation was performed using Biovia Discovery Studio Visualizer 2021 to obtain a single protein and its native ligand for docking. The validation of the docking method was performed by redocking the native ligand into its respective protein (Table 1). The validation results are summarized in Table 5. The method was considered valid if the RMSD value was < 2 Å (Anggraeni *et al.*, 2023). The 3D structures of ligands were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) and prepared using the GFN-xTB (Bannwarth *et al.*, 2019). The docking result selected was the conformation with the lowest binding energy within the most populated cluster (Muchtaridi *et al.*, 2018). The interactions between ligands and target proteins were analyzed using LigPlot+ 2.3.1 (Laskowski and Swindells, 2011). Then, the hydrogen bond and hydrophobic interaction were analyzed with Matplotlib (Hunter, 2007) and Seaborn (Waskom, 2021) in Python. The docking results were interpreted as predictive models to estimate potential ligand–protein interactions and do not represent direct evidence of biological activity.

Table 1: *Docking parameter for each protein*

Docking parameter	KEAP1	SIRT1
Grid box coordinates		
x	-3.687	-18.588
y	2.183	57.871
z	-28.014	6.360
Grid box dimension	50	45
Number of GA runs	100	100
Maximal number of energy evaluations	2500000	2500000

Results and Discussion

Antioxidant activity assay

The antioxidant activity of *A. comosus* fruit extract and vitamin C was analyzed using the DPPH radical scavenging method (Table 2), and the concentration–response relationship was modelled using the four-parameter logistic (4PL) regression (Table 3 and Figure 1). The fitted equations and correlation coefficients (R^2) demonstrated excellent agreement between the experimental and predicted values for both samples (R^2 > 0.999), confirming the suitability of the 4PL model for describing the dose–response behaviour. The relatively moderate IC_{50} value suggests that the concentration of active phenolic and flavonoid constituents in the fruit portion may be lower compared to other pineapple parts, such as the peel, which have been reported to contain higher levels of antioxidant compounds (García *et al.*, 2021). However, the extract still has a detectable capacity to scavenge radicals. This potential could be further explored through purification or combination with other pineapple fractions rich in antioxidants.

The curves represent the four-parameter logistic (4PL) regression fit to the experimental data, showing the relationship between concentration and the percentage of DPPH inhibition. The horizontal and vertical dashed lines indicate the IC_{50} values predicted from the fitted model. Error bars represent the standard deviation (n = 3).

FTIR analysis of pineapple extract

The FTIR spectrum of the pineapple extract shows several characteristic absorption bands (Figure 2). A broad band at 3 282.95 cm^{-1} indicates O–H stretching vibrations, corresponding to hydroxyl

groups commonly found in organic acids and sugars. The absorption peak at 2 926.79 cm^{-1} corresponds to aliphatic C–H stretching, while the strong band at 1 708.97 cm^{-1} represents C=O stretching, characteristic of carboxylic acids or aldehydes. The band observed at 1 634.29 cm^{-1} is attributed to C=C stretching or O–H bending vibrations. Additionally, absorption bands in the range of 1 249 cm^{-1} to 1 002 cm^{-1} correspond to C–O or C–O–C stretching, indicating the presence of ether or alcohol functional groups within the compound structures.

Table 2: *DPPH assay result of A. comosus and vitamin C as a control*

Sample	Concentration (mg L^{-1})	Replication			Average	SD
		1	2	3		
A. comosus	273.7319	53.6455	54.3094	54.5009	54.15193	0.448914
	129.7489	42.0154	41.9516	41.9133	41.9601	0.051578
	61.501	37.2791	37.2281	37.2025	37.23657	0.038996
	29.1515	34.7003	34.7514	34.7769	34.74287	0.039006
	13.8178	34.2408	34.8152	34.9046	34.65353	0.360222
Vitamin C	2.7655	70.4763	70.4016	70.439	70.43897	0.03735
	1.3108	57.1776	57.1776	57.2647	57.20663	0.050287
	0.6213	49.3951	49.3453	49.3453	49.3619	0.028752
	0.2945	44.8252	44.8127	44.8626	44.8335	0.025965
	0.1396	42.2974	42.285	42.3223	42.30157	0.018996

Table 3: *IC50 predicted result from 4PL regression*

	A	B	C	D	R^2	IC_{50} predicted
Vitamin C	39.1254	0.8003	40.0997	336.6362	0.9999	0.6726 mg L^{-1}
A. comosus	34.0674	1.3868	908.3413	160.1159	0.9991	225.3471 mg L^{-1}

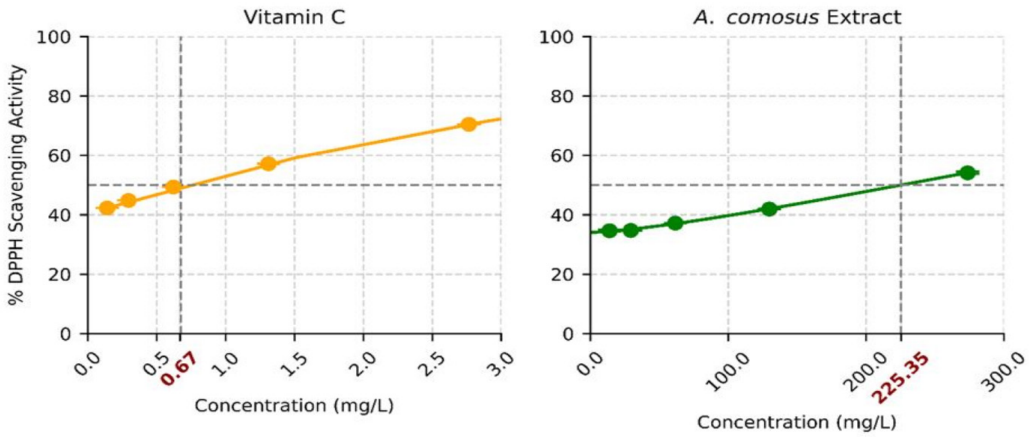


Figure 1: *DPPH radical scavenging activity of A. comosus fruit extract and vitamin C.*

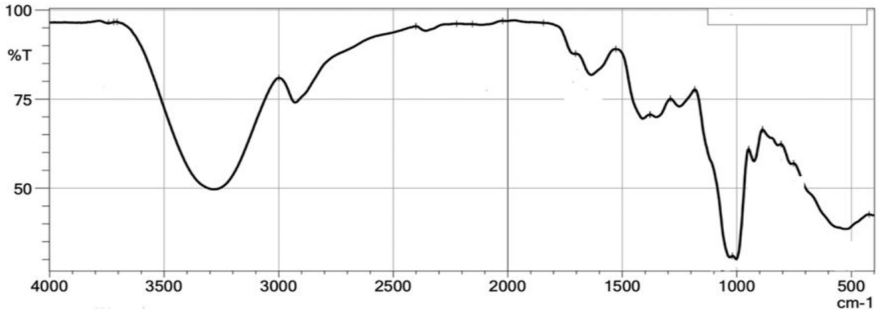


Figure 2: *Infrared spectra of A. comosus ethanolic extract*

Table 4: *LC–HRMS-based identification of bioactive compounds in A. comosus fruit extract*

No	Retention time (min)	% Area	Measured m/z	Proposed metabolite	Pubchem ID
1	0.767	10.21	103.09982	Choline	305
2	0.783	16.37	342.11596	A,α-Trehalose	7427
3	0.809	0.43	115.0634	D-(+)-Proline	8998
4	0.844	4.41	137.04758	Methyl isonicotinate	227085
5	0.893	30.58	192.02635	Citric acid	311
6	1.046	0.34	122.04803	Nicotinamide	936
7	1.071	0.12	131.09455	L-Isoleucine	6306
8	1.252	4.14	206.04208	3-Hydroxy-3-(methoxycarbonyl)pentanedioic acid	12566215
9	1.298	2.07	228.02432	2-(2-thienyl)-4H-chromen-4-one	240093
10	1.358	0.28	165.07891	L-Phenylalanine	6140
11	1.645	2.31	126.03167	5-Hydroxymethyl-2-furaldehyde	237332
12	2.441	0.67	242.03998	10-methoxy-4H-benzo[4,5]cyclohepta[b]thiophen-4-one	2825162
13	3.405	0.68	216.08939	2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid	98285
14	4.487	0.82	224.06815	Sinapinic acid	1549091
15	5.238	2.37	164.04726	4-Coumaric acid	322
16	8.451	0.09	201.20894	N,N-Dimethyldecylamine N-oxide	62452
17	8.906	0.92	157.14655	2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	549976
18	11.143	0.54	266.15529	Dodecyl sulfate	8778
19	11.417	0.38	414.20397	Bis(4-ethylbenzylidene)sorbitol	-
20	11.75	0.70	211.04521	Acridine-9(10H)-thione	2818474
21	11.989	2.57	356.2919	Monoolein	-
22	12.689	0.24	234.16178	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	73219
23	14.069	0.74	354.27658	1-Linoleoyl glycerol	5283469
24	14.275	1.33	255.25594	N,N-Diethyldodecanamide	18783
25	14.349	2.61	278.22433	A-Linolenic acid	5280934
26	14.673	2.57	356.2919	Monoolein	5283468
27	15.316	4.63	358.30751	1-Stearoylglycerol	22035687
28	15.604	3.06	283.28725	Stearamide	31292
29	16.093	0.74	292.23982	9(Z),11(E),13(E)-Octadecatrienoic Acid methyl ester	21718552
30	16.554	0.16	306.25539	Linolenic acid ethyl ester	5367460
31	16.876	0.27	270.25566	Methyl palmitate	8181
32	17.182	0.24	296.27114	Cis-12-Octadecenoic acid methyl ester	12963317
33	17.282	2.41	390.27645	Bis(2-ethylhexyl) phthalate	8343

The LC–HRMS analysis of *A. comosus* extract identified 33 major compounds with retention times ranging from 0.7 min to 17.3 min (Table 4).

The most abundant metabolites were citric acid (30.58 %), α,α-trehalose (16.37 %), and choline (10 %). The dominant presence of citric acid is consistent with previous research, which supports its antioxidant potential (Krueger *et al.*, 1992). α,α-Trehalose and choline, as abundant sugars and quaternary ammonium compounds, respectively, play important physiological roles in osmoprotection and membrane stabilization, which may also contribute to antioxidant defense. However, as compound identification was based on accurate mass analysis without MS/MS confirmation, these compounds should be considered as tentatively identified.

Overall, the LC–HRMS results indicate that the antioxidant activity observed in the DPPH assay may be associated with the synergistic effects of multiple metabolites, particularly organic acids, phenolics,

and unsaturated fatty acids. The coexistence of both polar and non-polar compounds in the ethanolic extract supports the broad spectrum of antioxidant mechanisms in pineapple, ranging from radical scavenging to modulation of redox-related signalling proteins.

Table 5: *Results of docking method validation for KEAP1 and SIRT1 proteins.*

RMSD range (Å)	KEAP1	SIRT1
0.0 to 1.0	5	33
1.0 to 2.0	91	67
2.0 <	4	0

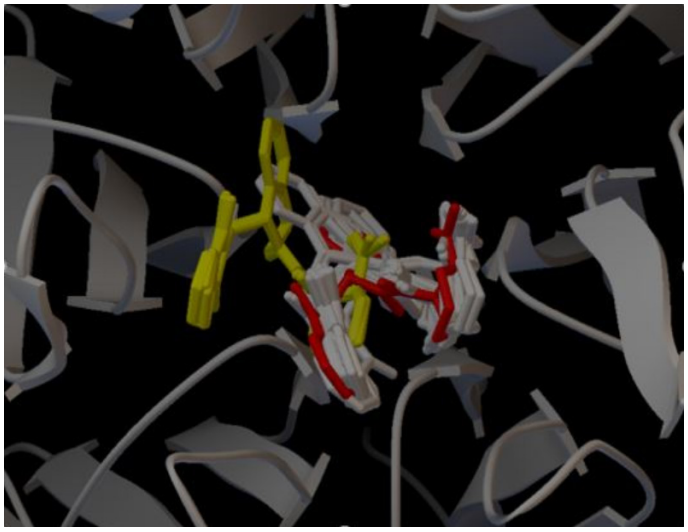


Figure 3: *The result of the re-docking of the native ligand into the KEAP1 protein.*

The red color represents the original crystallographic position of the native ligand, the white color indicates the 96 poses that met the RMSD criteria, and the yellow color shows the 4 poses that did not meet the RMSD criteria.

The red color represents the original crystallographic position of the native ligand, while the white color indicates the 100 poses that met the RMSD criteria.

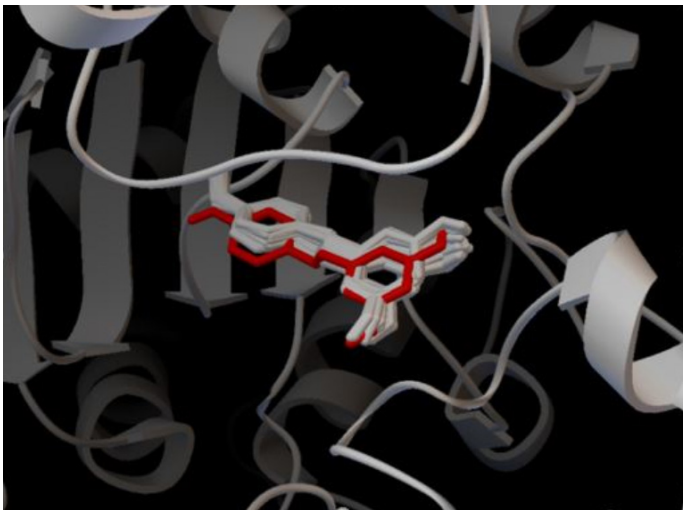


Figure 4: *The result of the re-docking of the native ligand into the SIRT1 protein.*

Six representative compounds were selected based on their relative abundance, structural diversity, and previously reported relevance to antioxidant-related activity. Molecular docking results showed that the native ligands exhibited the highest binding affinities toward both target proteins (Table 6). Among the six tested metabolites, α-linolenic acid demonstrated the strongest affinity toward both targets, with ΔG = −5.94 kcal·mol^{-1} (Ki ≈ 44.24 μM) for KEAP1 and ΔG = −5.66 kcal·mol^{-1} (Ki ≈ 70.98 μM) for SIRT1. 4-Coumaric acid ranked second against KEAP1 with ΔG = −5.06 kcal·mol^{-1} (Ki ≈ 195.35 μM). The other compounds (citric acid, α,α-trehalose, choline, and 5-hydroxymethyl-2-furaldehyde) exhibited weaker binding energies, with Ki values ranging from hundreds of micromolar to the millimolar range.

Table 6: *Result of molecular docking analysis from six sselected A. comosus secondary metabolites to KEAP1 and SIRT1 proteins*

Molecules	KEAP1		SIRT1	
	Binding Energy (kcal mol^{-1})	Ki	Binding Energy (kcal mol^{-1})	Ki
Citric acid	-4.48	522.77 μM	-3.68	1.99 mM
α,α-Trehalose	-2.24	22.96 mM	-3.65	2.10 mM
Choline	-2.33	19.67 mM	-3.12	5.14 mM
α-Linolenic acid	-5.94	44.24 μM	-5.66	70.98 μM
4-Coumaric acid	-5.06	195.35 μM	-4.43	562.01 μM
5-Hydroxymethyl-2- furaldehyde	-3.87	1.46 mM	-4.32	683.91 μM
Native ligand*	-8.56	531.09 nM	-7.14	5.80 μM

Native ligand: KEAP1 (1S,2R)-2-{[(1S)-1-[(1,3-dioxo- 1,3-dihydro-2H-isoindol-2-yl)methyl]-3,4-dihydroisoquinolin-2(1H)-yl] carbonyl}cyclohexanecarboxylic acid, Sirt1 Resveratrol*

These binding affinities are within the micromolar range, indicating moderate interactions and should therefore be interpreted with caution.

All tested ligands bound in the same binding pocket as the native ligand, forming both hydrophobic and hydrogen-bond interactions, as shown in the molecular docking interaction map for KEAP1 (Figure 5). Citric acid and 4-coumaric acid had the most similar interaction profiles to the native ligand, as indicated by the heatmap and hierarchical clustering. Meanwhile, α-linolenic acid exhibited distinct interaction features dominated by hydrophobic contacts, suggesting a different binding orientation within the same pocket. These results suggest that several pineapple metabolites, particularly citric acid, 4-coumaric acid, and α-linolenic acid, may potentially be associated with antioxidant-related pathways through partial or allosteric interaction with the KEAP1 target despite their moderate binding affinities.

From the SIRT1 clustering result (Figure 6), α,α-Trehalose exhibited an almost identical interaction profile to the native ligand. α-linolenic acid and

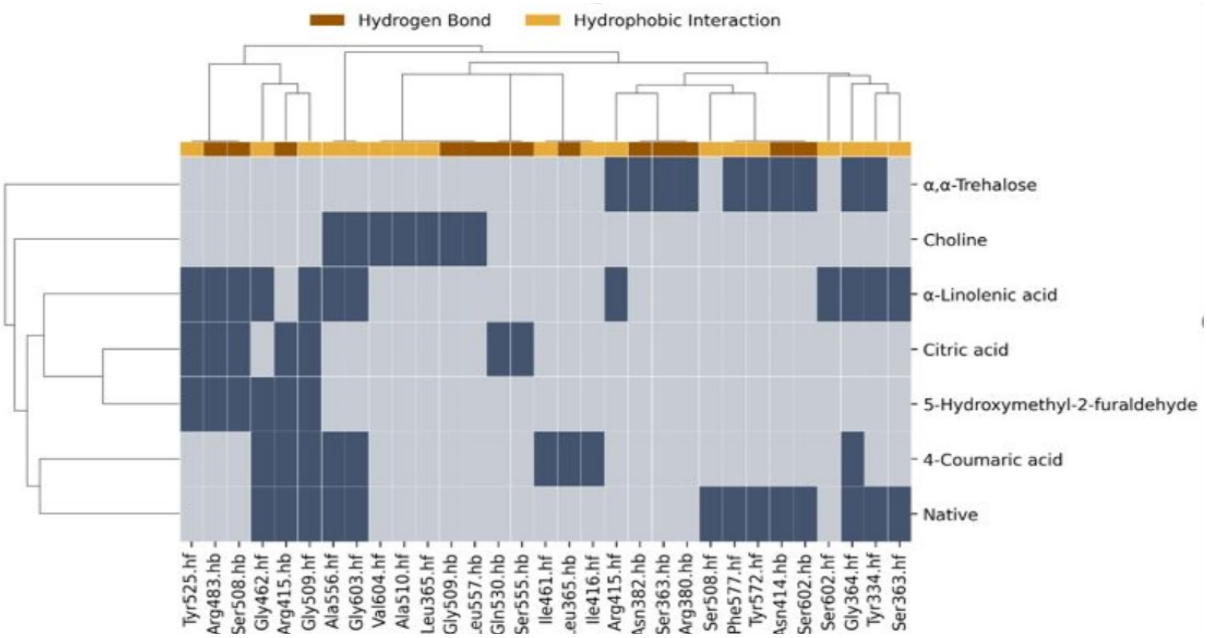


Figure 5: *Hydrogen binding and hydrophobic interaction result from molecular docking analysis and the clustering against the KEAP1 protein. Dark grey indicated interaction, and light grey indicated no interaction.*

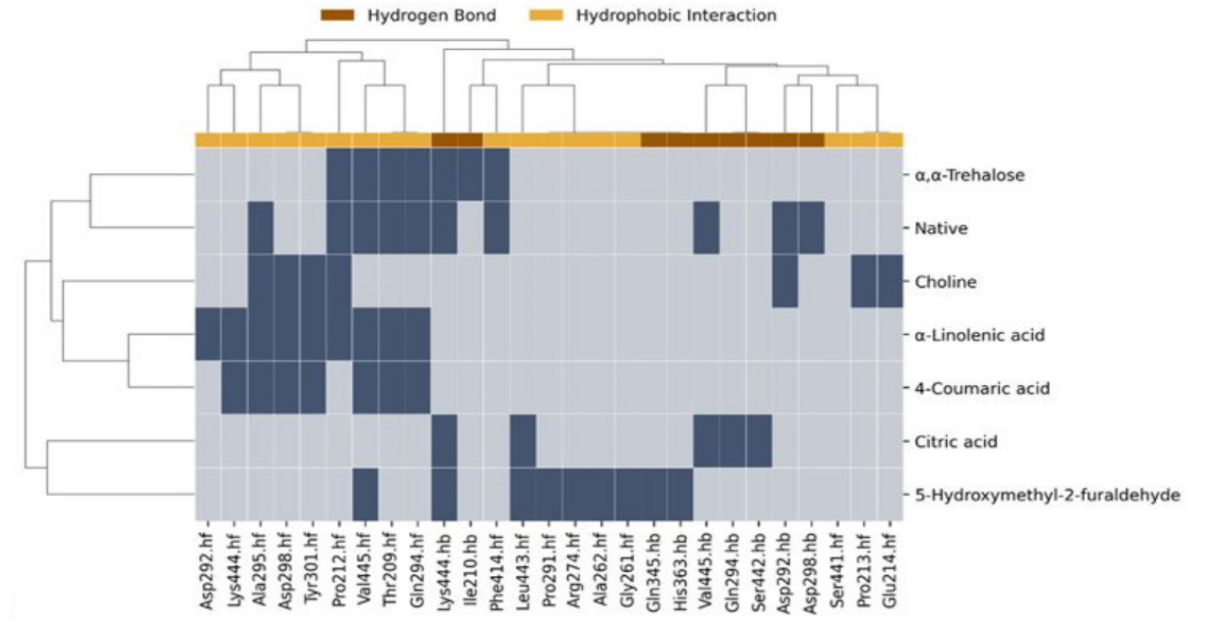


Figure 6: *Hydrogen binding and hydrophobic interaction result from molecular docking analysis and the clustering against the SIRT1 protein. Dark grey indicated interaction, and light grey indicated no interaction.*

4-coumaric acid showed broader interactions with key residues, including Asp292, Lys444, Ala295, Asp298, and Tyr301. Among these compounds, α-linolenic acid demonstrated the most similar interaction topology to the native ligand, suggesting it potential interaction with SIRT1, although this remains predictive and requires further validation.

From this molecular docking study, α-linolenic acid and 4-coumaric acid emerged as the most promising candidates for interaction with KEAP1, while α-linolenic acid also showed moderate affinity toward SIRT1. This result is in accordance with previous studies reporting that α-linolenic acid can inhibit KEAP1 in Leydig cells of male chicken testes (Zhao *et al*., 2023) and activate SIRT1 in mice (Bossio *et al*., 2023). However, these findings are based on animal models and should be interpreted with caution, as their relevance to human systems remains to be established.

Conclusions

This study demonstrated that *Ananas comosus* fruit extract possesses measurable antioxidant activity, as confirmed by the DPPH assay and supported by LC–HRMS phytochemical profiling and FTIR characterization. The application of a four-parameter logistic (4PL) model provided a robust and reliable approach for IC_{50} determination, enabling accurate characterization of the concentration–response relationship. Phytochemical profiling revealed diverse metabolites, including organic acids, phenolics, sugars, and unsaturated fatty acids, which may collectively contribute to the observed antioxidant activity. Molecular docking analysis further suggested that α-linolenic acid and 4-coumaric acid are promising candidates for interaction with KEAP1 and SIRT1, although these computational predictions require further biological validation. Overall, this study provides an inte-grated experimental and computational framework supporting the potential of *A. comosus* fruit as a natural antioxidant source for oxidative stress-related applications.

Future Perspectives

Future studies should extend these findings through dose optimization, pharmacokinetic evaluation, safety assessment, and well-designed clinical studies to facilitate the translation of pineapple-derived antioxidants into functional food and health-related products. Further investigation is also warranted to isolate and characterize the principal bioactive compounds and to develop innovative formulations with improved consumer acceptability and stability, including functional confectionery and effervescent candy, which may provide practical and convenient delivery systems for pineapple-derived antioxidants (Hermayanti *et al*., 2025).

Considering the increasing concern regarding environmental contamination of agricultural products, future research should also evaluate the occurrence and characteristics of microplastic contamination throughout the pineapple production chain, including agricultural soils, cattle manure used as organic fertilizer, irrigation water, and harvested fruits (Setyobudi *et al*. 2026). Such investigations are essential to ensure that pineapple-derived functional foods do not inadvertently become carriers of microplastics to humans.

In addition, pineapple peel, which represents a major agro-industrial by-product, deserves further investigation as a sustainable source of bioactive compounds and as a potential livestock feed ingredient. Valorization of pineapple peel could reduce environmental pollution while supporting a circular bioeconomy. Future studies should also evaluate whether its utilization in livestock feeding systems can contribute to minimizing microplastic transfer throughout the food chain, including into cattle-derived products such as milk, meat, and manure (Setyobudi, 2026). Such integrated research will not only accelerate the development of pineapple-derived antioxidant products but also promote a circular bioeconomy through sustainable utilization of pineapple biomass, strengthen food safety, and contribute to the achievement of Sustainable Development Goals 3 (Good Health and Well-being) and Sustainable Development Goals 12 (Responsible Consumption and Production).

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Novelty Statement

This study presents an integrated approach combining LC–HRMS-based phytochemical profiling, FTIR analysis, quantitative antioxidant modelling using a four-parameter logistic (4PL) model, and molecular docking targeting KEAP1 and SIRT1 in *Ananas comosus*. While previous studies have primarily focused on antioxidant activity, this work provides a combined experimental and computational perspective to explore potential molecular interactions associated with oxidative stress-related pathways.

Author's Contribution

Diah Hermayanti: Conceptualized and designed the study, elaborated the intellectual content, performed literature search, manuscript preparation, and manuscript revision.
Roy Hendroko Seyobudi: Performed literature search, materials research, manuscript preparation, review, and manuscript revision.
Meddy Setiawan and **Maizirwan Mel**: Research supervision and manuscript review
Desy Andari, Muhammad Artabah Muchlisin, and Engrid Juni Astuti: Analytical laboratory and manuscript review
All authors have read and approved the final manuscript.

Generative AI and AI-assisted technology statement
The authors declare that no generative AI was used in the generation, analysis, or interpretation of research data. AI-assisted tools were used solely for language editing and improving clarity of the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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