Antioxidant assay and component identification using lc-hrms of ecoenzyme products from *sandoricum koetjape* and *pometia pinnata* fruit peel waste

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Abstract. Kecapi (Sandoricum koetjape) and matoa (Pometia pinnata) fruit peel, which are often considered as waste, have the potential as a source of natural antioxidants. This study examines the antioxidant potential of kecapi fruit peel and matoa fruit peel in ecoenzyme production using the DPPH method and reducing power using the FRAP method. The chemicals content in the ecoenzyme samples were also identified using LC-HRMS. The EcoE-2 sample had the highest total phenolic content of 1613.962 ppm eq. gallic acid and also the highest total flavonoid content of 66.653 ppm eq. quercetin. The antioxidant power using the DPPH method for the EcoE-2 product (crude solution) was also greater than EcoE-1, the IC_{50} value is 2633.0 ppm, the IC_{50} for the EcoE-2 paste (without water/ethanol) was 119.3 ppm. For the reduction power with the FRAP method, data obtained that EcoE-2 also has a better reduction power than EcoE-1. It can be concluded that the higher content of matoa fruit peel will increase the antioxidant power of this ecoenzyme product. There are 118 putative chemical substances identified from the EcoE-2 sample, including several flavonoids and phenolic derivatives such as catechin, coumarin derivatives, and benzofuran compounds, supporting the observed antioxidant activity.

1 Introduction

Fruit is a vital source of food and nutrition for humans, and is an essential necessity utilized daily by people, both for direct consumption and processed into various products. This utilization process often leaves waste in the form of peels and unused fruit scraps, which become garbage. Disposing of waste carelessly or without adequate processing can negatively impact environmental cleanliness and potentially endanger health. Furthermore, this waste can also pollute the environment. In general, organic waste management still faces

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significant challenges because it is often directly disposed of in trash bins around residential areas, awaiting transport to final disposal sites (TPA). In some areas, piles of organic waste are often found on roadsides, riverbanks, and gutters. This can cause various problems, such as greenhouse gas emissions and methane gas produced from the decomposition process of organic substances. Furthermore, leachate produced from waste has the potential to pollute the environment because environmental conditions become increasingly dirty and produce unpleasant odors [1]. Blocked waterways become breeding grounds for insects such as mosquitoes and flies, which can impact residents' health. The government has implemented various programs to clean up, but it seems that the public is not very concerned because they feel that the remaining fruit and vegetable peels are useless, even though this paradigm is wrong. To support government programs in preserving healthy environment and maintaining public health, efforts are being made to reduce the problem of organic waste which has become a common problem encountered around us, both in rural and urban areas. Therefore, fruit waste and peels need to be processed into more useful products with economic value, one of which is the production of ecoenzymes [2] in this study. Hopefully, this research will also contribute to building public understanding of organic waste and strengthening public interest in processing and obtaining economic and health benefits from ecoenzyme (EcoE) products.

The Kecapi tree (Sandoricum koetjape) is a large, lush tree, reaching 15–30 meters tall, with branches and smooth, straight young leaves. The kecapi tree's trunk can reach 90 cm in diameter. It can be found in primitive and secondary forests at altitudes of 1,200 m or more and is also found in lowland forests [3]. The Kecapi tree has many benefits, including treating roundworms, lowering fever, treating bloating and diarrhea, strengthening the mother's body after childbirth [4], and possessing antioxidant properties [5]. The Matoa plant (Pometia pinnata) is a woody plant from the Sapidantheaceae family found in Southeast Asia and the Pacific. The Matua tree is also recognized as one of Indonesia's endemic plants, particularly in the Papua region [6]. Parts of this plant, including the leaves, fruit, bark, fruit skin, and roots, can be used medicinally. Phytochemical analysis showed that ethanol extract of matoa bark contains secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids. Phenolic compounds such as flavonoids, tannins, and saponins exhibit high antioxidant activity [7].

Ecoenzymes (EcoE) are complex organic substances consisting of polypeptide, enzymes, organic acids, and mineral salts, which have function in structural components of cell, or as catalyst. EcoE is a fermentation product obtained from fruit and vegetable waste and brown sugar or molasses. During the fermentation process, carbohydrates derived from molasses are converted into volatile acids, while organic acids and enzymes are extracted from fruit peels to form an enzyme solution [8]. The process of making EcoE is quite simple and can be done at home using organic waste from households. EcoE does not require complex or extensive media. You only need to use used plastic bottles or regular plastic or glass containers. The biggest challenge in making EcoE is the time required, which is approximately 3 months from the manufacturing process until it is ready for use [9]. EcoE produced from fruit peels has a wide range of applications in everyday life. Generally, EcoE can be used in the food industry as a natural and environmentally friendly alternative to chemical catalysts. Overall, the utilization of EcoE from fruit peels is a sustainable and economical solution to address various real problems, such as reducing waste and environmental pollution, as well as increasing health or economic benefits.

Antioxidants are substances needed by the body and generally function to inhibit fat oxidation. Free radicals are formed as a byproduct of the energy production process in the body. Antioxidants are included in a group of chemicals that protect biological systems from the potential harmful effects caused by oxidation reactions, through various mechanisms to prevent damage caused by these radicals [10]. Antioxidants play a very important role in

maintaining human health, especially in preventing damage due to oxidation. The oxidative process involves chemical reactions in the body that involve the transfer of electrons, which in turn produce free radicals. Antioxidants are easily oxidized, so free radicals will oxidize these antioxidants, which function to protect other molecules in the cell from damage caused by oxidation by free radicals [11].

Liquid chromatography-high-resolution mass spectrometry, commonly known as LC-HRMS, is a sophisticated instrumental analytical technique frequently applied in various scientific fields, including analytical chemistry, biology, pharmacy, and medicine. One of the main advantages of LC-HRMS is its high sensitivity and ability to analyze very complex samples. Thanks to these capabilities, LC-HRMS has become a crucial tool in modern science, helping researchers and practitioners answer various scientific and medical questions more precisely and accurately. Liquid chromatography-mass spectrometry is an analytical method that integrates two main techniques to separate and analyze the components contained in a sample.

The purpose of this study was to examine the potential of fermented fruit peel waste from plants that thrive and are endemic to Indonesia, namely kecapi and matoa, to become antioxidant agent. This study was conducted to determine the total phenolic and flavonoid values, antioxidant activity, and chemical content in EcoE products using a combination of organic waste raw materials that have never been used before as far as our current literature study.

2 Methods

2.1 Tools and Materials

The materials used in this study were kecapi and matoa fruit peel, distilled water, palm sugar, DPPH (1,1-diphenyl-2-picrylhydrazyl) (Merck), ethanol p.a (Merck), Folin-Ciocalteu solution (Merck), gallic acid (Merck), Na_2CO_3 (Merck), aluminum chloride (Merck), quercetin, $FeCl_3$ (Merck), phosphate buffer solution pH 6.6 (Merck). Fe (III) $6H_2O$, 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ), HCl 37%, distilled water, sodium acetate, glacial acetic acid, gallic acid, methanol. The tools used are laboratory glassware such as test tubes, Erlenmeyer flasks, petri dishes, beakers in several brands and non-glass equipment such as spatulas, magnetic rods, micropipettes, tips, 2 ml microtubes, 15 mL centrifuge tubes, ovens, hotplates, microplate readers, UV-Vis spectrophotometers, and LC-HRMS (Liquid chromatography - Orbitrap high-resolution mass spectrometry - Thermo Scientific™ Q Exactive™ Hybrid Quadrupole Orbitrap™ High Resolution Mass Spectrometer). LC-HRMS testing is carried out according to the standard procedures of PT. INVILAB biotechnological analysis.

In the production of ecoenzymes, variations were made in the weight ratio of kecapi fruit peel to matoa fruit peel. EcoE-1 is a variation in the amount of matoa fruit peel 400 g and kecapi fruit peel 200 g. EcoE-2 is a variation in the amount of matoa fruit peel 200 g and kecapi fruit peel 400 g. The dependent variable in this study is the ability of ecoenzymes from kecapi fruit peel and matoa in inhibiting the formation of free radicals with the DPPH method and the ability to reduce Fe^{3+}. Concentration variations were made to determine the IC_{50} value.

2.2 Procedures

2.2.1 Ecoenzyme Production

The matoa and kecapi fruit peels are cut into small pieces and placed in a fermenter container. 3 liters of distilled water and 0.5 kg of refined palm sugar are added. The fermenter container is made from a plastic gallon jug and equipped with a hose and bottle to channel the gas formed during the fermentation process to prevent it from exploding or breaking. The fermenter container is stored in a safe, dark place, out of direct sunlight. The ecoenzyme is stirred weekly while monitoring any changes. After 10 weeks of fermentation, the ecoenzyme is harvested by filtering the liquid from the fruit peel pulp. This process prepares the ecoenzyme for further processing. The EcoE pulp is spread in the students' gardens as fertilizer.

2.2.2 Total Phenolic Acid Test

Total phenolic content was calculated using a spectroscopic method with Folin-Ciocalteu reagent. The total phenolic content was measured as gallic acid equivalents (mgGAE) per gram of dry sample. Gallic acid was prepared in a series of concentrations of 20, 40, 60, 80, and 100 ppm. A 0.3 ml gallic acid solution was mixed with 1.5 mL of 10% Folin-Ciocalteu reagent and 1.2 mL of 7.5% Na_2CO_3. The solution was then vortexed and stored in the dark at room temperature for 30 minutes. A blank solution was also prepared, and its absorbance was recorded using a spectrometer at 765 nm [12]. For the triplet experiment, 1 mL of EcoE solution was diluted 20-fold with ethanol before reaction, and its absorbance was measured to match the standard curve used, and calculations were adjusted accordingly.

2.2.3 Total Flavonoid Test

The total flavonoid content test was conducted using the aluminum chloride technique. This test quantifies the total flavonoid content of the eco-enzyme. A 2 mL sample of quercetin was taken and mixed with 2 mL of 2% aluminum chloride before vortexing. A calibration curve was created using serial concentrations of quercetin. The absorbance of the mixture was measured using a spectrophotometer at 415 nm. The number of flavonoids was expressed as mg QE/g sample [12]. Each analysis was performed three times. A 1 mL Eco-E solution was diluted 10-fold with ethanol before reaction, and its absorbance was measured to match the standard curve used, and calculations were adjusted accordingly.

2.2.4 LC-HRMS Analysis

The test sample was prepared by dissolving 0.1 mL of the sample into a 10 mL volumetric flask, then adding ethanol solvent and homogenizing after ultrasonication for 30 minutes. Next, a 0.22 μm GHP/PTFE filter membrane was inserted into the instrument. The LC setup used a ThermoScientific™ Accucore™ Phenyl-Hexyl Column 100 mm × 2.1 mm ID × 2.6 μm. Eluent A – water containing 0.1% formic acid and Eluent B – methanol containing 0.1% formic acid. Flow rate – 0.3 mL/min. MS Settings: Operation Mode: ESI (-) / ESI (+), acquisition range: 66.7–1000 m/z.

2.2.5 Ecoenzyme Antioxidant Assay

The free radical scavenging activity of EcoE and the standard quercetin was determined using the DPPH radical scavenging method [13]. The IC50 value of EcoE was determined using varying concentrations. For antioxidants, the IC50 value of quercetin was determined using varying concentrations. Pipette 80 μL of sample and standard solutions into 96-well plates, starting from the highest concentration to the lowest. Each concentration was added to three wells (triplicate). Empty three wells for blank media (the blank media contained 80 μL of solvent and added 80 μL of 0.1 mM DPPH solution). Transfer the 0.1 mM DPPH solution to a plastic container (sufficient volume). Add 80 μL of 0.1 mM DPPH solution using a multichannel pipette to the wells of the plate containing the standard and sample. Cover the microplate with aluminium foil and incubate for 30 minutes at 25°C (dark). After

30 minutes, the solution mixture was read on a microplate reader at a wavelength of 492 nm for 30 seconds with a vibration shake medium [14].

$$\% \text{ inhibition} = \frac{\text{Abs Blank - Abs sample}}{\text{Abs Blank}} \text{ x } 100\% \qquad (1)$$

2.2.6 Ecoenzyme Reducing Power Assay

The Ferric Reducing Antioxidant Power Assay (FRAP) was investigated using a modified method by Tang et al. [13]. Pipette 20 µL of sample and standard gallic acid solutions into 96-well plates, starting from the highest concentration to the lowest. Each concentration was added to three wells (triplicate). Transfer the FRAP DYE working solution to the reservoir (sufficient volume). Add 280 µL of the solution using a multichannel pipette to the wells containing the standard and sample. Note: The solution was added in the dark. Cover the well plate with aluminum foil and incubate for 10 minutes at room temperature (dark conditions). After 10 minutes, the solution mixture was read on a microplate reader at a wavelength of 492 nm for 30 seconds with a vibration shake medium. The FRAP DYE solution consisted of sodium acetate solution, TPTZ solution, and Fe(III) solution in a 10:1:1 ratio.

2.2.7 Research Data Analysis

The data analysis results were processed using MS Excel to obtain the average value and standard deviation for each measurement. Chemical content data was analyzed using LC-HRMS using the Compound Discoverer 3.2 dan Sirius 6.2.2 application.

3 Results and Discussion

3.1 Analysis of Total Flavonoids and Total Phenolics EcoE

The total flavonoid content test (Figure 1) is an analytical method to calculate the total amount of flavonoids in ecoenzymes using the aluminium chloride technique.

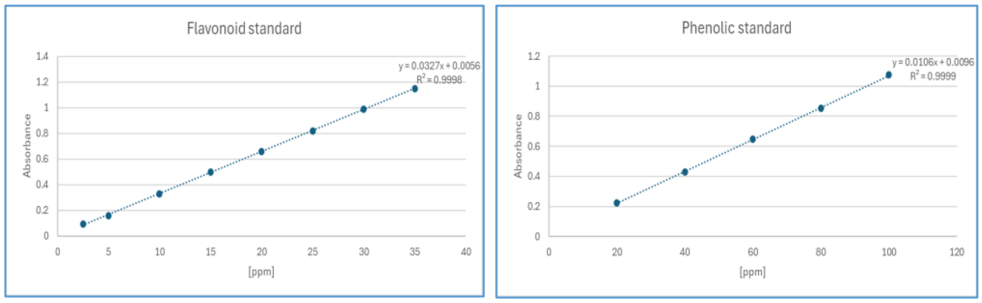

Fig.1. Standard curve of total flavonoid (quercetin standard) and total phenolic (gallic acid standard) tests.

The test results for total flavonoids and total phenolics of EcoE-1 and 2 are shown in Tables 1 and 2.

Table 1. Results of total flavonoid tests of EcoE-1 and 2 with quercetin as standards

Sample	Absorbance			Avg	Conc. (ppm eq. Quercetin)	Average Conc. (ppm)	SD
	Abs 1	Abs 2	Abs 3				
EcoE-1	0.138	0.240	0.102	0.160	47.217		

Sample	Absorbance			Avg	Conc. (ppm eq. Quercetin)	Average Conc. (ppm)	SD
	Abs 1	Abs 2	Abs 3				
EcoE-1	0.132	0.234	0.102	0.156	45.994	46.673	0.622
EcoE-1	0.136	0.238	0.102	0.159	46.809		
EcoE-2	0.255	0.333	0.078	0.222	66.177		
EcoE-2	0.260	0.338	0.078	0.225	67.197	66.653	0.513
EcoE-2	0.257	0.335	0.078	0.223	66.585		

Note: diluted 10 times (1 mL EcoE + 9 mL ethanol) before testing. Avg = Average

Table 2. Results of total phenolic tests of EcoE-1 and 2 with gallic acid as standards

Sample	Absorbance			Avg	Conc. (ppm eq. galic acid)	Average Conc. ppm	SD
	Abs 1	Abs 2	Abs 3				
EcoE-1	0.762	0.768	0.777	0.769	1432.830		
EcoE-1	0.759	0.768	0.771	0.766	1427.170	1428.428	3.928
EcoE-1	0.758	0.767	0.77	0.765	1425.283		
EcoE-2	0.872	0.86	0.852	0.861	1606.415		
EcoE-2	0.861	0.875	0.869	0.868	1619.623	1613.962	6.803
EcoE-2	0.866	0.856	0.875	0.866	1615.849		

Note: diluted 20 times (0.5 mL EcoE + 9.5 mL ethanol) before testing. Avg = Average

Based on the results of the total phenolic content, it was seen that both of ecoenzymes had high total phenolic content, i.e. EcoE-1 = 1428.428 ppm equivalent of gallic acid and EcoE-2 = 1613.962 ppm equivalent of gallic acid, but the total flavonoid content was small which means flovonoid content is low. But it is important to aware that Folin–Ciocalteu assay is non-specific because it responds to any compound capable of reducing the reagent, not only phenolic compounds. In fermentation-derived samples, this limitation becomes critical because fermentation generates and concentrates many non-phenolic reducing substances—such as reducing sugars, organic acids, amino acids, peptides, vitamins, and other microbial or plants metabolites—that can strongly contribute to the Folin–Ciocalteu signal.

3.2 Analysis of EcoE Antioxidant Test DPPH Method

The results of the EcoE-1 and 2 antioxidant tests, as well as quercetin as a standard, are shown in Tables 3 and 4 below. The EcoE solution used for this antioxidant test was the result of fermentation (called crude solution) that had been filtered with a 0.22 µm microfilter at various volumes and diluted with ethanol. The concentration was measured based on the specific density of the fermented EcoE solution. The specific density was determined using the pycnometer method (triplicate).

Table 3. Results of the DPPH method antioxidant activity test for EcoE-1 (crude solution).

Vol. EcoE (mL)	Conc. EcoE (ppm)	Abs1	Abs2	Abs3	Avg	SD	% Inhibition	IC_{50} (ppm)
0	0.00	0.881	0.854	0.891	0.875	0.019	0	
0.2	1998.04	0.535	0.629	0.557	0.574	0.049	34.46	
0.4	3996.08	0.39	0.38	0.335	0.368	0.029	57.92	3902.1

0.6	5994.12	0.196	0.216	0.217	0.210	0.012	76.05	
0.8	7992.16	0.126	0.135	0.117	0.126	0.009	85.60	

Note: EcoE used for antioxidant testing was diluted 1:10 with ethanol p.a. Abs = Absorbance. Avg = Average

Table 4. Results of the DPPH method antioxidant activity test for EcoE-2 (crude solution).

Vol. EcoE (mL)	**Conc. EcoE** (ppm)	**Abs1**	**Abs2**	**Abs3**	**Avg**	**SD**	**% Inhibition**	**IC_{50}** (ppm)
0	0.00	1.105	1.013	1.067	1.062	0.046	0	
0.2	1006.18	0.899	0.859	0.844	0.867	0.028	18.3	
0.4	2012.35	0.583	0.621	0.598	0.601	0.019	43.4	2633.0
0.6	3018.53	0.466	0.436	0.423	0.442	0.022	58.4	
0.8	4024.70	0.294	0.296	0.293	0.294	0.002	72.3	

Note: EcoE used for antioxidant testing was diluted 1:20 with ethanol p.a. Abs = Absorbance, Avg = Average

For the quercetin antioxidant test, standard quercetin solids were used, dissolved in ethanol according to the concentration variations used. The results of the antioxidant test are shown in Table 5.

Table 5. Results of the DPPH method antioxidant activity test for quercetin solution

Quercetin Conc. (ppm)	**Abs1**	**Abs2**	**Abs3**	**Avg**	**SD**	**% Inhibition**	**IC_{50}** (ppm)
0.00	1.105	1.013	1.067	1.062	0.046	0	
16.67	0.722	0.758	0.727	0.736	0.020	30.7	
33.33	0.464	0.482	0.467	0.471	0.010	55.6	32.7
50.00	0.252	0.234	0.267	0.251	0.017	76.4	
66.67	0.097	0.069	0.088	0.085	0.014	92.0	

Note: Abs = Absorbance, , Avg = Average

Antioxidant activity of EcoE-1 and 2 crude solution is low as we see from the IC_{50} in ppm, but if we see the volume of EcoE crude solution that is needed to inhibit DPPH radical is quite low compared to the total volume of ecoenzyme we have produced. For EcoE only about 0.4-0.5 mL EcoE capable to inhibit 50% of DPPH radical activity, while we can produce easily 2000 mL of ecoenzyme, it means that volume capacity of ecoenzyme as antioxidant is bigger, because ecoenzyme is cheaper, easy to produce, and having better economics benefit if to applied in large scale antioxidant applications.

We can then evaluate the antioxidant of ecoenzyme after all the solvent i.e., water have been evaporated leave only paste of ecoenzyme. The EcoE product was freeze-dried to evaporate all water and small number of ethanol solvents. The freeze-drying method was chosen to ensure that the active chemical components contained in EcoE still remain in the EcoE paste. The EcoE paste was collected and then measured for antioxidant activity using the DPPH method and for reducing activity using the FRAP method. The results are shown in Tables 6 and 7. This antioxidant activity test was conducted to determine the antioxidant and reducing activity of EcoE-1 and 2 products and can be compared to the antioxidant activity test of the EcoE solution still contained in solvents from fermentation. Calculation of antioxidant and FRAP reducing power tests using this paste will provide more accurate view about the activity of the active compound and it can be seen in the IC_{50} value. The IC_{50} value for DPPH inhibition of EcoE-1 is 168.63 ppm and EcoE-2 is 119.26 ppm.

Table 6. DPPH antioxidant activity test of EcoE-1 paste (water/EtOH solvent free)

Conc. (ppm)	% Inhibition			Avg	SD	IC_{50} (ppm)
	1	2	3			
200	53.071	55.038	58.637	55.58	2.82	
50	17.850	14.587	14.875	15.77	1.81	
25	7.246	7.582	8.109	7.65	0.44	168.6
12.5	3.83	3.791	3.263	3.63	0.32	
6.25	0.672	5.182	3.503	3.12	2.28	

Note: SD = Standard Deviation, Avg = Average

Table 7. DPPH antioxidant activity test of EcoE-2 paste (water/EtOH solvent free)

Conc. (ppm)	% Inhibition			Avg	SD	IC_{50} (ppm)
	1	2	3			
200	70.777	71.737	72.265	71.59	0.75	
100	42.658	42.083	43.762	42.83	0.85	
25	7.870	7.054	9.261	8.06	1.12	119.3
12.5	4.367	1.919	5.374	3.89	1.78	
6.25	1.775	0.960	2.447	1.09	1.80	

Note: SD = Standard Deviation, Avg = Average

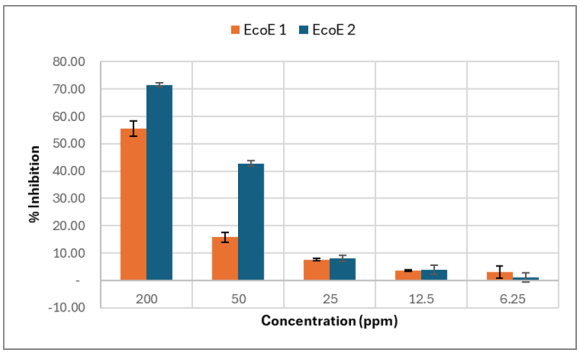

Fig. 2. The percentage of inhibition of radical DPPH by EcoE-1 and EcoE-2.

EcoE-2 seems to have better antioxidant activity compared to EcoE-1, for both form of solution or paste (solvent free) (Figure 2). Even though based on the pair T-test 2 tail calculation on Excel program, we observed that the p-value is > 0.05, which means that the antioxidant activities of both EcoE paste are not significantly different. The EcoE paste antioxidant power is approximately 4-5 times weaker than standard pure quercetin (IC_{50} = 32.65 ppm).

A FRAP test was also performed on Fe(III) to determine the ability of EcoE to release electrons and reduce iron ions. This ability was compared against a gallic acid standard. The reducing power data for EcoE-1 and 2 are shown in Figure 3. Table 8 shows the reducing power for gallic acid.

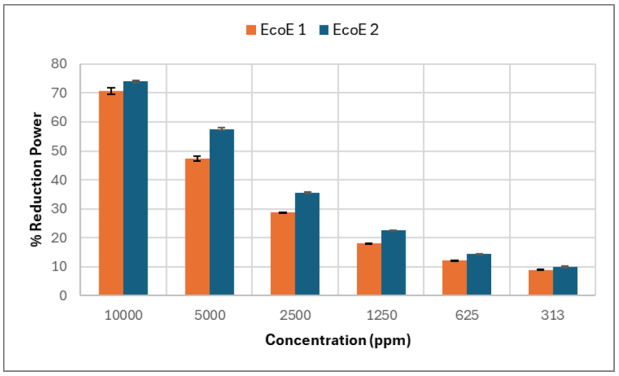

Fig. 3. The percentage of reduction power by EcoE-1 and EcoE-2.

Based on the pair T-test 2 tail calculation on Excel program, we observed that the p-value is < 0.05 i.e. 0.02 which means that the reduction power of both EcoE is significantly different. The EcoE-1 (RP_{50} = 5473.2 ppm) has weaker reduction power than the EcoE-2 (RP_{50} = 3969.9 ppm). Both of EcoE paste still have weaker reduction power in comparison to gallic acid (RP_{50} = 92.4 ppm). The finding support assumption that EcoE-2 which has more flavonoid content and phenolic content will show better antioxidant and reduction power compared to EcoE-1.

Table 8. Results of the FRAP reduction power test of gallic acid

Conc. (ppm)	Absorbance			Reduction Power (%)			Avg	SD	RP_{50} (ppm)
	1	2	3	1	2	3			
1000	2.500	2.530	2.557	92.233	93.343	94.354	93.31	1.06	
250	1.964	1.979	1.972	72.465	73.022	72.753	72.75	0.28	
125	1.594	1.615	1.617	58.830	59.598	59.672	59.37	0.47	
62.5	1.059	1.112	1.106	39.078	41.033	40.793	40.30	1.07	92.4
31.25	0.647	0.663	0.666	23.889	24.450	24.583	24.31	0.37	
15.63	0.412	0.430	0.436	15.214	15.875	16.089	15.73	0.46	
7.81	0.268	0.299	0.308	9.886	11.022	11.358	10.76	0.77	

Note: SD = Standard Deviation, Avg = Average

The results of this study indicate that EcoE produced from kecapi fruit and matoa fruit peels has weak to moderate antioxidant activity, as indicated by the IC_{50} values from the DPPH test, reduction power, and the total phenolics and flavonoid observed.

3.3 Component Identification in EcoE using LC-HRMS

To determine the chemical components contained in EcoE, an LC-HRMS test was conducted. The Total Ion Chromatogram (TIC) results of EcoE-2 are shown in Figure 4. Both of EcoE used the same fermentation component/ingredients so EcoE-2 was chosen with better antioxidant and reduction power for the LC-HRMS analysis. There were 19 putative chemical components that were identified based on the molecular weight of the compounds (Δ mass < ±3 ppm) as compared to the database using the Compound Discoverer 3.2. But based on criteria for annotation of putative compounds on the mzCloud best match value is > 60% as recommended by ThermoFisher Product Communication, only 10 compounds that satisfied this requirement as shown in Table 9. Unfortunately, 10 putative identified compounds are too few to describe characteristics of the ecoenzyme. Because of this we

decided to used Sirius 6.2.2 application program to identify more putative compounds based on the raw data from LC-HRMS instrument. The result was shown in Table 10.

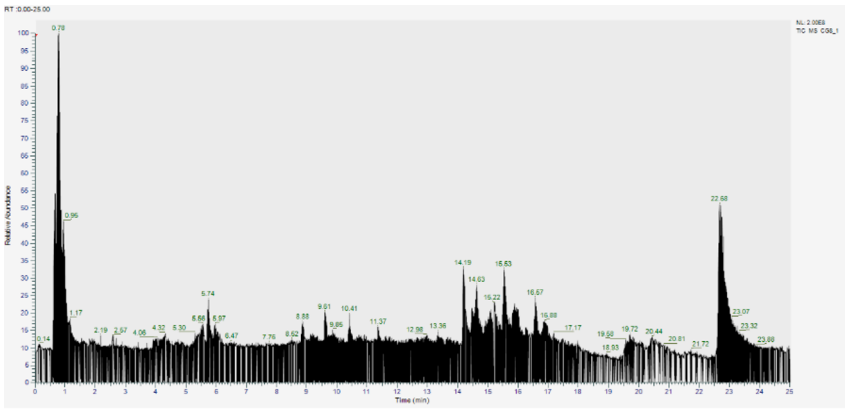

Fig. 4. Total Ion Chromatogram (TIC) of LC-HRMS EcoE-2.

Some setting in Sirius 6.2.2 compute system are as follow: instrument is orbitrap, MS2 mass accuracy is 5 ppm, fallback adduct is $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, search databases are all listed including PubChem, except for PubMed. Other settings are left as default. From the Sirius results, only decent and good overall Feature Quality, Sirius Score Normalized value > 0.75 are accepted. The duplication was filtered, and we got 108 putative identified compounds.

Table 9. List of putative compounds identified using LC-HRMS and analysed in Compound Discoverer 3.2.

No	Name	Formula	Annot. Δ Mass [ppm]	Calc. MW	RT [min]	Area (Max.)	mzCloud Best Match
1	2-Amino-1,3,4-octadecanetriol	$C_{18}H_{39}NO_3$	-0.99	317.2927	9.743	6683271.01	75.6
2	Tiaprofenic acid	$C_{14}H_{12}O_3S$	0.79	260.0509	0.979	10840735	60.6
3	N,N-Diethyldodecanamide	$C_{16}H_{33}NO$	-0.97	255.256	15.854	27818322.1	88.9
4	N,N-Diisopropylethylamine (DIPEA)	$C_8H_{19}N$	0.36	129.1518	1.944	33043655.8	78.2
5	Bis(4-ethylbenzylidene)sorbitol	$C_{24}H_{30}O_6$	-1.65	414.2036	11.372	40861047.1	99.8
6	Dibenzylamine	$C_{14}H_{15}N$	-0.9	197.1203	5.808	52339669.3	99.9
7	Methyl isonicotinate	$C_7H_7NO_2$	-0.39	137.0476	0.799	57649756.1	98.1
8	2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	$C_9H_{19}NO$	-0.55	157.1466	9.208	70344617.2	92.7
9	1-Stearoylglycerol	$C_{21}H_{42}O_4$	-1.85	358.3077	15.533	90032358.1	96.3
10	Stearamide	$C_{18}H_{37}NO$	-2.02	283.2869	15.865	182718975	97.9

Table 10. List of putative compounds identified using LC-HRMS and analysed in Sirius 6.2.2 in EcoE2

No	Molecular Formula	adduct	Name	Ion Mass	RT (Min)
1	$C_{24}H_{30}O_6$	$[M + H]^+$	(1R)-1-[(2R,4S,4aR,6R,8aR)-2,6-bis(3,4-dimethylphenyl)-4,4a,8,8a-tetrahydro-	415.211	11.629

			[1,3]dioxino[5,4-d][1,3]dioxin-4-yl]ethane-1,2-diol		
2	$C_9H_{18}O_8$	$[M + K]^+$	(2S,3S,4S,5R,6R)-3-(2,3-dihydroxypropoxy)-6-(hydroxymethyl)oxane-2,4,5-triol	293.063	0.751
3	$C_{19}H_{26}$	$[M + H]^+$	(3E,5E,7E,9E,12Z)-3,5,7,9-tetramethyl-11-methylidenetetradeca-1,3,5,7,9,12-hexaene	255.211	13.484
4	$C_{29}H_{48}$	$[M + H]^+$	(3S,3aR,5aR,5bR,7aS,11aS,11bR,13bS)-5a,5b,8,8,11a-pentamethyl-3-prop-1-en-2-yl-2,3,3a,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydro-1H-cyclopenta[a]chrysene	397.382	18.518
5	$C_{23}H_{39}NO_2$	$[M + H]^+$	(5Z,8Z,12Z,15Z)-N-(2-hydroxyethyl)henicosa-5,8,12,15-tetraenamide	362.305	15.526
6	$C_8H_{14}O_8$	$[M + Na]^+$	[(3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] ethaneperoxoate	261.058	0.984
7	$C_{10}H_8O_3$	$[M + H]^+$	[2-(3-Oxoprop-1-enyl)phenyl] formate	177.055	5.468
8	$C_5H_9N_4O_4P$	$[M + H]^+$	[3-hydroxyimino-3-(1H-1,2,4-triazol-5-yl)propyl]phosphonic acid	221.042	0.742
9	$C_9H_{12}N_4O_2$	$[M + Na]^+$	[4-[[1-(Hydroxymethyl)pyrazol-4-yl]methyl]pyrazol-1-yl]methanol	231.084	0.975
10	$C_{21}H_{33}N_3O_3$	$[M + H]^+$	1,3,5-Tris(2,2-dimethylpropionylamino)benzene	376.259	10.893
11	$C_{15}H_{14}O_{10}$	$[M + H]^+$	1-{[3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}propane-1,2,3-tricarboxylic acid	355.066	4.405
12	$C_{20}H_{38}O_3$	$[M + H]^+$	10-(6-Hydroxydec-8-enoxy)dec-2-en-5-ol	327.289	15.535
13	$C_{13}H_{16}$	$[M + H]^+$	1-But-1-ynyl-4-propan-2-ylbenzene	173.132	13.484
14	$C_8H_{19}N$	$[M + H]^+$	1-Butanamine, N-(2-methylpropyl)-	130.159	1.963
15	$C_9H_6O_3$	$[M + H]^+$	1-Hydroxy-3H-2-benzopyran-3-one	163.039	7.658
16	$C_{20}H_{39}NO$	$[M + H]^+$	1-Isocyanatononadecane	310.31	15.973
17	$C_4H_8O_2$	$[M + H]^+$	1-Methoxyethyl(methylidene)oxidanium	89.06	5.747
18	$C_{10}H_{14}N_2$	$[M + H]^+$	1-methyl-2-(pyridin-3-yl)pyrrolidin-1-ium	163.123	0.136
19	$C_{26}H_{31}NO$	$[M + H]^+$	1-Methyl-3,5-bis[(4-propan-2-ylphenyl)methylidene]piperidin-4-one	374.247	16.169
20	$C_{15}H_{20}O_4$	$[M + H]^+$	2-(2,3-Dimethylbut-2-enyl)-5,6-dimethoxy-3-methylcyclohexa-2,5-diene-1,4-dione	265.143	4.722
21	$C_9H_{17}NO_4$	$[M + H]^+$	2-(2-Aminoethyl)malonic acid diethyl ester	204.123	0.788
22	$C_7H_{13}NO_4$	$[M + H]^+$	2-(2-Aminoethyl)pentanedioic acid	176.092	0.872
23	$C_6H_4O_5$	$[M + H]^+$	2-(3-Hydroxy-5-oxofuran-2-ylidene)acetic acid	157.013	0.947
24	$C_{14}H_{31}NO$	$[M + H]^+$	2-(Dodecylamino)ethanol	230.248	9.662
25	$C_7H_9F_2NO$	$[M + H]^+$	2,2-Difluoro-2-(3-methylfuran-2-yl)ethan-1-amine	162.073	13.009
26	$C_{29}H_{37}NO$	$[M + H]^+$	2,4-Di-tert-butyl-6-(dibenzylaminomethyl)phenol	416.294	17.614
27	$C_{15}H_{22}O_2$	$[M + H]^+$	2,5-Di-tert-butyl-3-acetyl-2,4-cyclopentadiene-1-one	235.169	12.655
28	$C_{23}H_{41}NO$	$[M + H]^+$	2,6-DI-Tert-butyl-4-[(dibutylamino)methyl]phenol	348.326	12.962
29	$C_{16}H_{35}NO_3$	$[M + H]^+$	2-[(2,2-Diethoxyethyl)(octyl)amino]ethan-1-ol	290.269	9.784
30	$C_{19}H_{30}$	$[M + H]^+$	2-[(3E,5E)-3,7-dimethylocta-3,5,7-trienyl]-1,3,3-trimethylcyclohexene	259.242	14.761
31	$C_8H_{13}NO_6$	$[M + H]^+$	2-[(3-Hydroxy-2,2-dimethylpropanoyl)amino]propanedioate	220.082	0.872

32	$C_7H_8N_2O_4S_2$	$[M + K]^+$	2-[(5-Methyl-1,3,4-thiadiazol-2-yl)sulfanyl]butanedioic acid	286.957	0.742
33	$C_{12}H_{21}NO_6$	$[M + H]^+$	2-[(R)-1-(Nitromethyl)-2-methylpropyl]malonic acid diethyl ester	276.144	0.993
34	$C_{11}H_{18}N_2O_5$	$[M + H]^+$	2-[[(2S)-piperidine-2-carbonyl]amino]pentanedioic acid	259.129	0.937
35	$C_{10}H_{18}O_8$	$[M + Na]^+$	2-[2-Hydroxy-3-(hydroxymethyl)cyclopropyl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	289.09	1.003
36	C_8H_8O	$[M + H]^+$	2-ethenylphenol	121.065	15.544
37	$C_{19}H_{32}O_3$	$[M + H]^+$	2-Methyl-3-prop-2-enoylpentadec-2-enoic acid	309.242	14.64
38	$C_8H_9O_3P$	$[M + Na]^+$	2-Phenylethenyl dihydrogen phosphite	207.017	22.666
39	$C_{20}H_{35}NO$	$[M + H]^+$	2-tert-Butyl-6-(dibutylaminomethyl)-4-methylphenol	306.279	11.266
40	$C_8H_{13}NO_6$	$[M + H]^+$	3-(2-Carboxyethylamino)pentanedioic acid	220.082	0.76
41	$C_7H_{13}NO_4$	$[M + H]^+$	3-(Aminomethyl)-3-methylpentanedioic acid	176.092	0.77
42	$C_{24}H_{22}O_{14}$	$[M + H]^+$	3,4-bis({[3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy})-2,5-dihydroxyhexanedioic acid	535.108	4.414
43	$C_{15}H_{22}O$	$[M + H]^+$	3,5-di-tert-butylbenzaldehyde	219.174	12.99
44	$C_9H_{18}N_2O$	$[M + H]^+$	3-Cyclohexyl-1,1-dimethylurea	171.149	22.983
45	$C_{12}H_{23}NO_7$	$[M + H]^+$	3-methyl-2-({[2,3,4-trihydroxy-5-(hydroxymethyl)oxolan-2-yl]methyl}amino)pentanoic acid	294.155	0.984
46	$C_8H_{17}NO_2$	$[M + H]^+$	4,4-Diethoxybutan-1-imine	160.133	0.788
47	$C_6H_8O_3$	$[M + H]^+$	4-hydroxy-3,5-dimethyl-3H-furan-2-one	129.055	1.17
48	$C_7H_6N_2O_3S_2$	$[M + H]^+$	5-Acetyl-4-methylsulfonyl-1,3-thiazole-2-carbonitrile	230.99	0.956
49	$C_{24}H_{43}NO_2$	$[M + H]^+$	6,6-bis[(Z)-non-2-enoxy]hexanenitrile	378.335	15.898
50	$C_9H_6O_3$	$[M + H]^+$	6-Hydroxycoumarin	163.039	5.3
51	$C_{10}H_8O_2$	$[M + H]^+$	6-methyl-2H-chromen-2-one	161.06	14.752
52	$C_7H_7NO_2$	$[M + H]^+$	6-Methylnicotinic acid	138.055	0.788
53	$C_9H1_7NO_4$	$[M + H]^+$	Acetylcarnitine	204.123	0.937
54	$C_9H_6O_3$	$[M + H]^+$	Benzofuran-4-carboxylic acid	163.039	1.702
55	$C_{20}H_{43}NO_2$	$[M + H]^+$	Bis(2-hydroxyethyl)hexadecylammonium	330.336	11.722
56	C10H8O4	$[M + H]^+$	Buxuletin	193.05	5.607
57	C15H14O6	$[M + H]^+$	Catechin	291.086	3.342
58	$C24H_{20}O_9$	$[M + H]^+$	Catechin-[5,6-E]-4Beta-(3,4-Dihydroxyphenyl)Dihydro-2(3H)-Pyranone	453.118	6.437
59	$C_{27}H_{44}$	$[M + H]^+$	Cholestyne	369.351	17.772
60	$C_{24}H_{38}O_4$	$[M + H]^+$	Dehp	391.284	17.502
61	$C_{16}H_{22}O_4$	$[M + H]^+$	Dibutyl Phthalate	279.159	13.363
62	$C_8H_{19}N$	$[M + H]^+$	dibutylamine	130.159	22.526
63	$C_{22}H_{43}NO$	$[M + H]^+$	docos-13-enamide	338.341	16.98
64	$C_{30}H5_8O_4S$	$[M + H]^+$	dodecyl 3-{[3-(dodecyloxy)-3-oxopropyl]sulfanyl}propanoate	515.412	20.028
65	$C_{21}H_{41}NO$	$[M + H]^+$	Eicosylisocyanate	324.326	16.532

66	$C_{17}H_{35}NO$	$[M + H]^+$	heptadecanamide	270.279	14.64
67	C16H33NO	$[M + H]^+$	Hexadecyl(oxo)azanium	256.263	15.553
68	$C_{18}H_{34}O$	$[M + H]^+$	Hexadecylketene	267.268	15.218
69	$C_{11}H_{19}NO_6$	$[M + H]^+$	Isoleucinopine	262.129	0.937
70	$C_6H_5NO_2$	$[M + H]^+$	Isonicotinic Acid	124.039	0.965
71	$C_{18}H_{32}O_{16}$	$[M + Na]^+$	Lactosucrose	527.158	0.751
72	$C_6H_{10}O_8$	$[M + Na]^+$	Mucic Acid	233.027	0.751
73	$C_{10}H_{23}NO$	$[M + H]^+$	N-(2-butoxyethyl)butan-1-amine	174.185	2.569
74	$C_{20}H_{37}NO$	$[M + H]^+$	N-(2-methylpropyl)hexadeca-2,4-dienamide	308.294	15.414
75	$C_{24}H_{41}NO$	$[M + H]^+$	N-(2-phenylethyl)hexadecanamide	360.325	16.197
76	$C_9H_{12}N_4O_2$	$[M + Na]^+$	N'-(4-methoxypyrimidin-2-yl)cyclopropanecarbohydrazide	231.084	0.798
77	$C_{11}H_{21}NO$	$[M + H]^+$	N-(cyclopropylmethyl)-N-pentylacetamide	184.17	9.131
78	$C_{21}H_{37}NO_2$	$[M + H]^+$	N,N-bis(prop-1-ynoxy)pentadecan-1-amine	336.289	15.414
79	$C_{20}H_{35}NO_2$	$[M + H]^+$	N,N-bis(prop-1-ynoxymethyl)dodecan-1-amine	322.273	14.248
80	$C_{16}H_{17}NO$	$[M + H]^+$	N,N-Dibenzylacetamide	240.138	10.501
81	$C_{16}H_{19}NO$	$[M + H]^+$	N,N-Dibenzylethanolamine	242.154	5.738
82	$C_{30}H_{45}NO$	$[M + H]^+$	N,N-dibenzylhexadecanamide	436.357	17.949
83	$C_{24}H_{49}NO$	$[M + H]^+$	N,N-dibutylhexadecanamide	368.388	17.902
84	$C_{18}H_{37}NO$	$[M + H]^+$	N,N-Dimethylhexadecanamide	284.294	15.535
85	$C_{13}H_{17}NO$	$[M + H]^+$	N-[(1Z,4Z,6Z)-1-[(1E)-buta-1,3-dienoxy]octa-1,4,6-trien-2-yl]methanimine	204.138	12.403
86	$C_{19}H_{33}NO_2$	$[M + H]^+$	N-[bis(prop-1-ynoxy)methyl]dodecan-1-amine	308.258	13.838
87	$C_{15}H_{15}NO$	$[M + H]^+$	N-Benzyl-2-phenylacetamide	226.122	10.408
88	$C_{23}H_{39}NO$	$[M + H]^+$	N-Benzylhexadecanamide	346.31	16.299
89	$C_{25}H_{43}NO$	$[M + H]^+$	N-benzyloctadecanamide	374.341	17.147
90	$C_{10}H_{21}NO$	$[M + H]^+$	N-butyl-N-(3-methylbutyl)formamide	172.17	9.131
91	$C_{12}H_{25}NO$	$[M + H]^+$	N-butyl-N-heptylformamide	200.201	11.704
92	$C_9H_{19}NO$	$[M + Na]^+$	N-cyclopentyloxy-2-methylpropan-1-amine	180.136	8.87
93	$C_{19}H_{39}NO$	$[M + H]^+$	Nonadecyl(oxo)azanium	298.31	15.712
94	$C_{21}H_{43}NO$	$[M + H]^+$	N-pentylhexadecanamide	326.341	16.57
95	$C_{23}H_{47}NO$	$[M + H]^+$	N-pentyloctadecanamide	354.372	17.138
96	$C_9H_{15}N_3O_2$	$[M + H]^+$	N-Propyl-L-histidine	198.124	0.826
97	$C_3H_4N_4O_4$	$[M + H]^+$	O-(1-nitroimidazol-4-yl)oxyhydroxylamine	161.03	0.732
98	$C_{18}H_{32}O$	$[M + H]^+$	Octadec-17-yn-2-one	265.252	15.712
99	$C_{18}H_{34}O$	$[M + H]^+$	Octadec-5-yn-1-ol	267.268	16.001
100	$C_{18}H_{37}NO$	$[M + H]^+$	Octadecyl(oxo)azanium	284.294	16.532
101	$C_{18}H_{35}NO$	$[M + H]^+$	Oleamide	282.279	14.612
102	$C_9H_{17}NO_5$	$[M + H]^+$	Pantothenate	220.118	1.431
103	$C_{18}H_{39}NO_3$	$[M + H]^+$	PEG-3 lauramine	318.3	9.97

104	$C_5H_8O_2$	$[M + Na]^+$	Pent-2-yne-1,5-diol	123.04	22.712
105	$C_8H_4O_3$	$[M + H]^+$	Phthalic Anhydride	149.023	23.132
106	$C_{12}H_{22}O_{11}$	$[M + Na]^+$	Sucrose	365.105	0.751
107	C2H6N2O4	$[M + H]^+$	Trihydroxymethylurea	123.04	0.648
108	$C_{36}H_{75}N$	$[M + H]^+$	Tris-decyl(hexyl)azanium	522.597	19.161

From Table 10, we can observe 7 flavonoids and phenolics compounds were putatively identified i.e. 1-Hydroxy-3H-2-benzopyran-3-one, Catechin, Catechin-[5,6-E]-4Beta-(3,4-Dihydroxyphenyl)Dihydro-2(3H)-Pyranone, Buxuletin, 6-Hydroxycoumarin, and 6-methyl-2H-chromen-2-one, and Benzofuran-4-carboxylic acid. The chemical structure of the 6 phenolics and flvonoid are shown in Figure 5. The flavonoid and phenolics are known to have antioxidant activity.

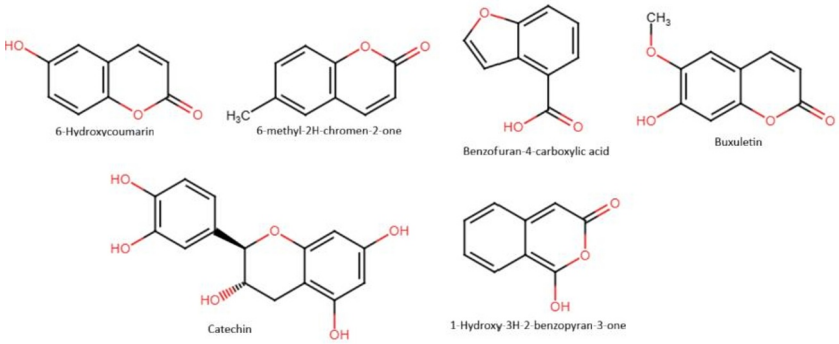

Fig. 5. The six putative identified flavonoid and phenolics compounds from Ecoenzyme (EcoE2) registered in PubChem.

We can observe the Pharmacokinetics, Druglikeness and Medicinal Chemistry of the six flavonoid and phenolics compounds as listed in Table 11 based on SwissADME databased analysis (http://www.swissadme.ch/index.php).

Table 11. The SwissADME results for 6 flavonoid and phenolics identified in EcoE2.

Molecule	#H-bond acceptors	#H-bond donors	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	log Kp (cm/s)	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	Bioavailability Score	PAINS #alerts	Brenk #alerts	Leadlikeness #violations
1-Hydroxy-3H-2-benzopyran-3-on	3	1	High	Yes	No	Yes	-6.56	0	1	0	0	1	0.55	0	0	1
6-Hydroxycoumarin	3	1	High	Yes	No	Yes	-6.56	0	1	0	0	1	0.55	0	1	1
6-methyl-2H-chromen-2-one	2	0	High	Yes	No	Yes	-6.03	0	0	0	0	1	0.55	0	1	1
Benzofuran-4-carboxylic acid	3	1	High	Yes	No	Yes	-5.98	0	1	0	0	1	0.85	0	0	1
Buxuletin	4	1	High	Yes	No	Yes	-6.39	0	0	0	0	1	0.55	0	1	1
Catechin	6	5	High	No	Yes	No	-7.82	0	0	0	0	0	0.55	1	1	0

We can predict the biological activities of the compound by using PASS online Way2Drug Prediction (https://way2drug.com/PassOnline/predict.php) and the result was shown in Table 12.

Table 12. The biological activity of 6 flavonoid and phenolics identified in EcoE2.

Compound Name	Biological activities Prediction	Pa Value
1-Hydroxy-3H-2-benzopyran-3-one	Neuropeptide Y4 antagonist	0,969
	Aspulvinone dimethylallyltransferase inhibitor	0,934
	CYP2C12 substrate	0,900
6-Hydroxycoumarin	CYP2C12 substrate	0,972
	CYP2A11 substrate	0,937
	4-Nitrophenol 2-monooxygenase inhibitor	0,929
	Chlordecone reductase inhibitor	0,927
	Membrane integrity agonist	0,926
	Aspulvinone dimethylallyltransferase inhibitor	0,925
	CYP2A4 substrate	0,921
	Aryl-alcohol dehydrogenase (NADP+) inhibitor	0,909
	CYP2B5 substrate	0,902
6-methyl-2H-chromen-2-one	CYP2C12 substrate	0,952
	CYP2A11 substrate	0,937
	CYP2B5 substrate	0,918
	4-Nitrophenol 2-monooxygenase inhibitor	0,914
Benzofuran-4-carboxylic acid	Methylenetetrahydrofolate reductase (NADPH) inhibitor	0,902
Buxuletin	CYP2C12 substrate	0,958
	Chlordecone reductase inhibitor	0,938
	Aspulvinone dimethylallyltransferase inhibitor	0,931
	CYP2A11 substrate	0,926
	4-Nitrophenol 2-monooxygenase inhibitor	0,921
	UGT1A6 substrate	0,903
Catechin	Membrane integrity agonist	0,983
	Mucomembranous protector	0,962
	TP53 expression enhancer	0,959
	HMOX1 expression enhancer	0,939
	Sulfotransferase substrate	0,927
	CYP1A1 substrate	0,927
	CYP2C12 substrate	0,909

From 108 putative identified compounds, there are 93 compounds that have SMILES in PubChem database. These 93 compounds were analyzed by ADMETLab 2.0 to observe the acceptance of the compounds for 4 drug candicate rules i.e. Lipinski's, Pfizer, GSK, GoldenTriangle rules. There are 17 compounds that accepted in all 4 rules as shown in Table 13.

Table 13. The compounds that accepted based on 4 rules for drugs candidate

No	CompoundName	Lipinski	Pfizer	GSK	GoldenTriangle
1	(5Z,8Z,12Z,15Z)-N-(2-hydroxyethyl)ericosa-5,8,12,15-tetraenamide	Accepted	Accepted	Accepted	Accepted
2	(3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)ethanperooxide	Accepted	Accepted	Accepted	Accepted

3	(3-hydroxyimino-3-((1H-1,2,4-triazol-5-yl)propyl)phosphoric acid	Accepted	Accepted	Accepted	Accepted
4	(4-((1-(hydroxymethyl)pyrazol-4-yl)methyl)pyrazol-1-yl)methanol	Accepted	Accepted	Accepted	Accepted
5	2-((3-Hydroxy-2,2-dimethylpropanoyl)amino)propanedioate	Accepted	Accepted	Accepted	Accepted
6	2-((5-Methyl-1,3,4-thiadiazol-2-yl)sulfany)butanedioic acid	Accepted	Accepted	Accepted	Accepted
7	2-(((2S)-piperidine-2-carbonyl)amino)pentanedioic acid	Accepted	Accepted	Accepted	Accepted
8	5-Acetyl-4-methylsulfonyl-1,3-thiazole-2-carbonitrile	Accepted	Accepted	Accepted	Accepted
9	Corvalen	Accepted	Accepted	Accepted	Accepted
10	Isoleucine	Accepted	Accepted	Accepted	Accepted
11	Music Acid	Accepted	Accepted	Accepted	Accepted
12	N'-(4-methoxypyrimidin-2-yl)cyclopropanecarbohydrazide	Accepted	Accepted	Accepted	Accepted
13	N,N-Diterglycetalamide	Accepted	Accepted	Accepted	Accepted
14	N,N-Ditethylanola-mine	Accepted	Accepted	Accepted	Accepted
15	N-((1Z,4Z,6Z)-1-(1-(5-butyl-1,3-dienoxy)octa-1,4,6-trien-2-yl)methanamine	Accepted	Accepted	Accepted	Accepted
16	N-Benzyl-2-phenylacetamide	Accepted	Accepted	Accepted	Accepted
17	Pantothenate	Accepted	Accepted	Accepted	Accepted

We can predict the biological activities of these 17 compounds by using PASS online Way2Drug Prediction. The Table 14 shows the predicted bioactivity of the 17 compounds predicted as the highest Pa value.

Table 14. The biological activities of the 17 compounds

No	**CompoundName**	**Biological activity Prediction Way2drug**
1	(5Z,8Z,12Z,15Z)-N-(2-hydroxyethyl)ericosa-5,8,12,15-tetraenamide	Fucosterol-epoxide lyase inhibitor Antieczenatic
2	((3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl)ethanperooxide	Exoribonuclease II inhibitor Levanase inhibitor
3	(3-hydroxyimino-3-((1H-1,2,4-triazol-5-yl)propyl)phosphoric acid	Glutamate-5-semialdehyde dehydrogenase inhibitor
4	(4-((1-(hydroxymethyl)pyrazol-4-yl)methyl)pyrazol-1-yl)methanol	5-O-(4-coumaroyl)-D-quinate 5'-monooxygenase inhibitor Carboxypeptidase Taq inhibitor

5	2-((3-Hydroxy-2,2-dimethylpropanoyl)amino)propanedioate	Fucosterol-epoxide lyase inhibitor Protein-disulfide reductase (glutathione) inhibitor
6	2-((5-Methyl-1,3,4-thiadiazol-2-yl)sulfany)butanedioic acid	Insulin promoter
7	2-(((2S)-piperidine-2-carbonyl)amino)pentanedioic acid	Proline racemase inhibitor
8	5-Acetyl-4-methylsulfonyl-1,3-thiazole-2-carbonitrile	Mucomembranous protector
9	Corvalen	Membrane integrity agonist Mucomembranous protector
10	Isoleucinphrine	Chymosin inhibitor Acryloylindropepsin inhibitor
11	Music Acid	Aspartate 4-decarboxylase inhibitor Levanase inhibitor
12	N'-(4-methoxypyrimidin-2-yl)cyclopropanecarbohydrazide	HMGCS2 expression enhancer
13	N,N-Dibenzylacetamide	CYP2H substrate Membrane integrity agonist
14	N,N-Dibenzylethanolamine	Glycosylphosphatidylinositol phospholipase D inhibitor Photic disorders treatment
15	N-((1Z,4Z,6Z)-1-(1-(5-butyl-1,3-dienoxy)octa-1,4,6-trien-2-yl)methanamine	Fatty-acyl-CoA synthase inhibitor GGTP substrate
16	N-Benzyl-2-phenylacetamide	Photic disorders treatment Mucomembranous protector
17	Pantothenate	Benzoate-CoA ligase inhibitor Leukopoiesis stimulant

4 Conclusion

The results of this study indicate that EcoE from kecapi and matoa fruit peels has low to moderate antioxidant activity. EcoE-2 has better antioxidant and reducing power than EcoE 1, so it can be seen that the high portion matoa fruit peel content increases the antioxidant and reduction power of ecoenzymes. More than 100 putative organic compounds have been identified by LC-HRMS including sereval flavonoids and phenolics. The total experimental phenolic content of this ecoenzyme is quite high. Although high Folin–Ciocalteu values were observed, these results should be interpreted as overall reducing capacity rather than absolute phenolic content due to the non-specific response of the assay in fermentation-derived matrices. The findings highlight the potential of ecoenzyme fermentation as a sustainable approach to valorize fruit peel waste while underscoring the importance of complementary analytical techniques, such as LC-HRMS, for accurate bioactive compound characterization. Future studies should focus on targeted quantification of key antioxidant compounds and biological validation to better define the functional applications of ecoenzymes.

Acknowledgement

The authors express their heartfelt appreciation to the 2024 Student Creativity Program (PKM), supported by the Directorate General of Higher Education, Research, and Technology through the Directorate of Learning and Student Affairs, for funding this research. Gratitude is also extended to SDAE division of Universitas Pelita Harapan for their additional financial support. We thank We thank Lembaga Penelitian dan Pengabdian Masyarakat (LPPM) Universitas Pelita Harapan for supporting this research by some funding allocated from research proposal grand number of P-062-FIP/XII/2024. Also, LPPM UPH has supported for conference in 2025 and publication fee. Thank you for Happy Chelsy Betaria Napitupulu and Misael Ivan Pangela for helping their junior friends.

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