Phytochemical Screening and Antioxidant Activity in Ecoenzymes with Variations in Carbon Sources

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Abstract. Ecoenzyme is a fermented solution from a mixture of fruit skin waste, vegetables, water and sugar. The fermentation process of various types of natural ingredients is known to be able to increase the content of various phytochemical compounds and antioxidants in these products. Phytochemicals are secondary metabolites that act as antioxidants. Antioxidants are compounds that can overcome oxidative damage due to reactive oxygen compounds that play a role in capturing free radicals. The purpose of this study was to determine the phytochemical and antioxidant content of each ecoenzyme. This type of research is descriptive. Ecoenzyme extraction was carried out by maceration method using three different solvents namely distilled water, methanol and ethanol. Phytochemical screening was carried out by qualitative methods. The antioxidant activity test was measured using the DPPH (1,1-Diphenyl-2-Prikrylhidrazil) method. The results showed that the ecoenzymes identified alkaloids, flavonoids and saponins. The antioxidant activity of the ecoenzyme with methanol extract (IC50 value) was 100.052 ppm (moderate activity category), while the antioxidant activity of the other ecoenzyme sample extracts had weak antioxidant properties. The conclusion from this study is that ecoenzyme contains phytochemicals and antioxidant activity that can be applied to some field research.

1 Introduction

Waste has always been a problem that is difficult to overcome amidst society's consumerist culture. The rest of daily consumption becomes organic waste which continues to accumulate. Currently, Indonesia produces up to 30 million tons of waste per year and 40% of it is organic waste such as fruit and vegetable peels (SIPSN Ministry of Environment and Forestry, 2021). One method of processing organic waste from fruit and vegetable peels that can be implemented easily is by fermenting the waste into ecoenzymes [1].

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Ecoenzyme is a fermented solution from a mixture of fruit peels, vegetables, water and sugar. Ecoenzyme was first discovered by a researcher from Thailand named Dr. Rasukon Poompanvong [2]. The ecoenzyme solution has a brown color, with a fresh sour fermented aroma. Ecoenzyme has many benefits, for the agricultural sector, ecoenzyme can be used as organic fertilizer and natural pesticide. Luzik et al. reported all ecoenzyme variants to have an acidic environment and high protein content, favorable for microbial activity and IAA production [3]. IAA concentrations ranged from 26.73 µg/mL to 30.26 µg/mL, with the sour orange peel variant exhibiting the highest production [3]. In the environment, ecoenzymes can be used as natural cleaners, including as plant growth factors, detergent mixtures, pesticide residue cleaners, scale cleaners and producers of antimicrobial compounds [4].

Carbon sources are sources of energy that microorganisms need for their growth. One of the carbon sources needed during fermentation is sugar. The types of sugar that can be used to make ecoenzymes are molasses, brown sugar, coconut sugar and palm sugar [5].

The type of carbon source used can also cause differences in the compound content of the fermentation results. According to Gusdiansyah, ecoenzyme with a carbon source from cane sugar produce ecoenzyme fermentation products with better antimicrobial activity than other types of sugar [6]. Other fermentations in making breadfruit leaf tea kombucha using brown sugar affect the alcohol and titratable acid content [7].

Therefore, it is likely that ecoenzyme products from the same organic material but different carbon sources will contain different phytochemical compounds and antioxidant activity. Until now there has been no research on the content of phytochemical compounds and the antioxidant activity of ecoenzymes made from various carbon sources.

2 Materials and method

2.1 Tools and materials

The tools used in the research were a rotary evaporator (Heidolph), water bath (Memmert wtb11), UV-vis spectrophotometer, analytical balance, and incubator. The materials used in the research were ecoenzyme (fruit and vegetable peels, molasses, brown sugar, coconut sugar), ethanol, 2N sulfuric acid methanol, distilled water, anhydrous acetic acid, concentrated hydrochloric acid, concentrated sulfuric acid, 0.05N ammonia-chloroform, magnesium powder, potassium iodide, iodide, mercuric chloride, wagner, glacial acetic acid, 2,2-diphenyl-1-picryl hydrazyl (DPPH).

2.2 Research procedure

2.2.1 Ecoenzyme collection

Ecoenzymes were collected from several practical work results in biochemistry courses in the biology department, at Padang State University. The number of ecoenzymes produced in this practicum was 25 types of samples with three types of carbon sources (molasses, coconut sugar and brown sugar), with organic ingredients such as dragon, orange, kale, cucumber and lettuce.

Table 1. Ecoenzyme combinations

No.	Ecoenzyme Source	Sample Code
1.	Source 1	SA1
2.	Source 2	SA2
3.	Source 3	SA3

2.2.2 Extraction

The samples to be used were previously macerated. Ecoenzyme maceration was carried out using ethanol, methanol and distilled water as solvents. 100 ml of each ecoenzyme was macerated using 500 ml of solvent. The maceration process was carried out in a dark glass container for 3 days. The maceration results are obtained next filter using filter paper until a filtrate is obtained. The filtrate obtained later evaporated using a rotary evaporator at a temperature of 60°C at a speed of 50 rpm until a thick and concentrated extract is obtained.

2.2.3 Phytochemical screening

2.2.3.1 Alkaloid test

The concentrated ecoenzyme extract is put into a test tube. Then 1 mL of chloroform and 0.5 mL of ammonia were added. The sample was shaken and then several drops of 2N sulfuric acid were added. Then the sample is shaken again until two layers are formed, namely the top layer is the acid layer and the bottom layer is the chloroform layer. The top layer is taken and then dripped onto the drip plate. After that, the drop plate was dripped with Mayer's reagent and Dragendorf's reagent, so that an orange or brown precipitate was formed and the formation of a white precipitate showed a positive test result for alkaloids [8].

2.2.3.2 *Flavonoid test*

Take the concentrated ecoenzyme extract then place it on the drip plate. On the drop plate containing ecoenzyme, add 0.1 g of magnesium powder and 2 drops of concentrated hydrochloric acid. The formation of an orange to red color indicates a positive test result for flavonoids [8].

2.2.3.3 Steroids and triterpenoids

50g of *ecoenzyme* concentrated extract was taken and placed on a drop plate. Then, add 5 drops of anhydrous acetic acid and 3 drops of concentrated sulfuric acid to form a green or blue color. The green or blue color indicates a positive test result for steroids, while the formation of a red or violet color indicates a positive test result for triterpenoids [8].

2.2.3.4 Saponin

The concentrated *ecoenzyme* extract is put into a test tube. Then, add 2 ml of aquades, boil in a water bath for 2-3 minutes. After that, cool the sample and shake until foam forms. The formation of foam and the persistence of the foam for 5 minutes indicate a positive test result for saponin [8].

2.2.4 Total antioxidant activity test

2.2.4.1 Preparation of DPPH solution

A total of 4 mg DPPH was dissolved in 100 ml methanol pa then, the solution was stored in a dark place.

2.2.4.2 Making test solutions

Each sample of thick extract was weighed at 10 mg and then dissolved in 10 ml of methanol. From the mother liquor, a concentration series of 50 ml, 100 ml, 150 ml, 200 ml and 250 ml was made.

2.2.4.3 Testing

A total of 1.5 ml of the test solution was added with 2.5 ml of DPPH. Then, the solution was incubated in a dark room for 30 minutes. Then the absorbance was measured at a wavelength of 517 nm. For control, 2.5 ml of DPPH solution was used, then 1.5 ml of methanol solution was added based on the absorbance obtained, and %inhibition was calculated using the formula:

$$\% \text{ inhibition} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\% \quad (1)$$

Table 2. Antioxidant properties based on IC $_{50\ \text{Value}}$ [9]

IC value $_{50}$	Antioxidant Properties
50 ppm<	Very strong
50 ppm-100 ppm	Strong
100 ppm-150 ppm	Currently
150 ppm-200 ppm	Weak

3 Results and discussion

Based on the results of research that has been carried out regarding phytochemical screening and antioxidant activity in ecoenzymes with various carbon sources.

Table 3. Phytochemical screening

Sample	Solvent	Alkaloids	Flavonoids	Saponin
SA1	Aquades	positive	negative	positive
	Methanol	positive	negative	negative
	Ethanol	positive	negative	negative
SA2	Aquades	positive	negative	positive
	Methanol	positive	positive	negative
	Ethanol	positive	positive	negative
SA3	Aquades	positive	negative	negative
	Methanol	positive	positive	negative
	Ethanol	positive	positive	negative

The content of secondary metabolite compounds or phytochemicals can be influenced by the content of the carbon source. In the fermentation process, nutritional requirements are very important for the growth of microorganisms, so the treatment of carbon sources and nitrogen sources greatly influences this process. Carbon sources are a source of energy needed by microorganisms for their growth, while nitrogen sources are needed for protein synthesis. In general, the carbon sources used in the fermentation process are monosaccharides such as glucose and disaccharides such as sucrose and lactose. Sucrose found in carbon sources is one of the compounds that can increase secondary metabolite compounds [10]

Phytochemical screening in this study was carried out using the sample maceration method. Maceration is an extraction method by soaking the material in a solvent. Based on the overall results of phytochemical screening on ecoenzymes from several carbon sources, it shows the presence of alkaloids, flavonoids and saponins.

Table 4. Antioxidant activity on ecoenzyme extracts from several carbon sources [9]

Sample	Solvent	IC $_{50}$ (ppm)	Information
SA1	Aquades	217.327	Weak
	Methanol	100.052	Currently
	Ethanol	208.575	Weak
SA2	Aquades	230.479	Weak
	Methanol	178.39	Weak
	Ethanol	262.665	Weak
SA3	Aquades	173.395	Weak
	Methanol	193.444	Weak
	Ethanol	205.812	Weak

In research analyzing the antioxidant activity of *ecoenzyme* extracts from several carbon sources. It can be seen that the higher the antioxidant activity, the color of the reacting solution becomes pale yellow, but in *ecoenzymes* the color changes from dark purple to pale purple. No visible change in color to yellow. One method that can be used to measure antioxidant activity is measuring the ability of extracts to reduce DPPH free radicals. DPPH is a free radical found in stable organic nitrogen with a dark purple color which, when reduced by antioxidants, turns colorless. The DPPH method used in the antioxidant activity test was chosen because this method is simple, fast, easy and sensitive.

Based on research results (Table 4), the highest antioxidant activity was produced by ecoenzyme SA1 methanol with IC50 100.052 ppm, while the lowest antioxidant activity was produced by ecoenzyme SA2 distilled water with IC50 230.479 ppm. Based on the results of the IC 50 value, methanol ecoenzyme SA1 has moderate antioxidant properties, while the other ecoenzymes have weak antioxidant properties. The best antioxidant properties are produced by the molasses sugar ecoenzyme with methanol solvent [11]. This research also reported that the antioxidant activity of a variety of orange peels is relatively weak. Solvent concentration can affect antioxidant activity. According to Fathurrachman, the solvent concentration used has different polarities and is related to the content of secondary metabolites filtered during the extraction process. The presence of secondary metabolites can influence antioxidant activity [12]. However, Fevria et al. also reported the addition ecoenzyme liquid into spinach (*Amaranthus* sp.), which cultivate by hydroponically, showed vitamin C levels increased significantly [13].

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and nitrogen sources greatly influences this process. Carbon sources are a source of energy needed by microorganisms for their growth, while nitrogen sources are needed for protein synthesis. In general, the carbon sources used in the fermentation process are monosaccharides such as glucose and disaccharides such as sucrose and lactose. Sucrose found in carbon sources is one of the compounds that can increase secondary metabolite compounds [9]. Natasya et al. report the eco-enzyme that had better quality was the eco-enzyme derived from pineapple flesh (dominated by parenchyma tissue). So, it can be said that the eco-enzyme produced from tissue dominated by parenchyma tissue has better quality than that which is dominated by epidermal tissue [14].

Phytochemical screening in this study was carried out using the sample maceration method. Maceration is an extraction method by soaking the material in a solvent. Based on the overall results of phytochemical screening on ecoenzymes from several carbon sources, it shows the presence of alkaloids, flavonoids and saponins in all the samples. This is the reason why *ecoenzyme* can speed up biochemical reactions in nature and produce enzymes that are useful for various daily needs, ranging from cleaning, biofertilizer and health [15].

4 Conclusion

Based on the results of research that has been carried out regarding phytochemical screening and antioxidant activity in ecoenzymes with various carbon sources phytochemical compounds contained in ecoenzyme extracts from several carbon sources are alkaloids, flavonoids and saponins. Ecoenzymes from several carbon sources have average activity characteristics weak antioxidant, and moderate antioxidant properties. The highest antioxidants are found in ecoenzymes with carbon sources molasses, while sugar has low antioxidant activity in coconut.

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