# Phytochemical Characteristic and Antimicrobial Activity of Coconut Coir Extract on Various Solvents

Karseno[1(✉)], Pepita Haryanti[1], and Reza Kezia Poetri[2]

[1] Food Technology Study Program, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto, Indonesia
karseno@unsoed.ac.id

[2] Alumni of the Food Technology Study Program, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto, Indonesia

**Abstract.** Coconut coir is a by-product of the coconut that known contains phytochemical compounds and antimicrobial activities. This study aims to determine the phytochemical characteristics and the antimicrobial activity of coconut coir extracts. Completely Randomized Design was used in this study and the effect of maturity level of coconut coir and the solvent type were investigated. The maturity level of coconut coir consists of young and old coconut coir, and the solvent type were aquadest, ethanol, ethyl acetate and n-hexane. The phytochemicals characteristic, pH and antimicrobial activity of coconut coir extract were determined. The results of this study indicated that young coconut coir extract containing tannins, flavonoids and steroids. Meanwhile, the old coconut coir extract containing tannins, flavonoids, steroids and terpenoids. Both of young and old coconut coir extracts showed antimicrobial activity against *S. cerevisiae*, *A. aceti* and the microbes isolated from fermented sap. Old coconut coir extract showed antimicrobial activity stronger than young coconut coir extract. The old coconut coir extracted with aquadest show the highest antimicrobial activity against all microbial tested. This extract contains a total phenolic of 76.04 mg GAE/g, total flavonoid of 1.57 mg QE/g and total tannin of 522.95 mg TAE/g, respectively. In addition, based on the absorbance spectrum, it was confirmed that this extract contains tannins, flavonoids and steroids.

**Keywords:** antimicrobial activity · coconut coir · phytochemical compounds

## 1 Introduction

Coconut (*Cocos nucifera* L.) is a plant that well known growth well in tropical areas such as Indonesia. Coconut also known as three of life since all part of coconut can be used. Coconut coir is a by-product of coconut with 35% of the total weight of coconuts [1]. In Indonesia, coconut coir waste reaches to 1,8 million tons of coconut fiber and 3.3 million tons of coconut powder [2]. Coconut coir contains bioactive such as tannin compounds that can inhibit the antimicrobial activity in the palm roomie by binding to


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enzymes and proteins produced from microbes so that the microbes become inactive. Coconut coir was thought to contain triterpenoid and flavonoid compounds that act as antibacterial substances. Literature studies shows that there is potential in the coconut coir as an antimicrobial source because of the content of secondary metabolites that can be used as natural preservatives.

According to [3], the maturity level of coconut is an important factor that affects the composition and amount of phytochemical compounds. [4] stated that old and young coconut coir extracts were positive containing tannins, phenols and flavonoids. These bioactive compounds are able to act as antimicrobials with different inhibitory mechanisms.

Utilization of the bioactive components contained in coconut coir will be easier when the extraction process was carried out. Extraction is the process of separating a substance from its mixture using a solvent. The result of extraction process is influenced by several factors such as the level of polarity of the solvent, the ratio of the material and solvent, and the length of the extraction time. The selection of the appropriate solvent will make the extraction process efficient [5]. According to the principle of "like dissolves like", a solvent will tend to dissolve compounds that have the same level of polarity. Polar solvents will dissolve polar compounds and non-polar solvents will dissolve non-polar compounds [6]. According to [7], n-hexane is non-polar, ethyl acetate is semi-polar, ethanol and aquadest are polar. So far, the effect of solvent polarity on the content of phytochemical compounds in young and old coconut coir extracts is unknown and related to this research is still limited. Therefore, it is necessary to conduct research to determine the appropriate type of solvent to obtain the highest phytochemical compounds in coconut coir extract. This study aims to determine the characteristics of phytochemical compounds and antimicrobial activity in young and old coconut coir extract in different solvent types.

## 2 Materials and Methods

### 2.1 Materials

Young and old coconut coir were obtained from traditional market in Banyumas regency. Young coconut coir is obtained from coconuts with a harvest age of 7 to 9 months that commonly consumed as young coconut drink. Meanwhile, the old coconut coir is obtained from coconuts with a harvest age of 11 to 12 months, and usually used for the production of grated coconut or coconut milk. *S. cerevisiae* and *A. aceti* culture were purchased from Gadjah Mada University culture collection. All chemical reagents including medium were purchased from Sigma and Merck except as mentioned in the text. The equipment used includes cabinet dryer, waterbath shaker, UV-Vis spectrophotometer, incubator andglassware.

### 2.2 Preparation of Coconut Coir Extract

The young and old coconut coirs were cut into a size of 1 × 1 cm and washed thoroughly with water. Furthermore, the coconut coir is dried in a cabinet dryer (24 h for young

coconut coir, 16 h for old coconut coir). The dried coconut coir was ground and sieved with a 14 mesh to obtain young and old coconut coir powder. 10 g of young and old coconut coir powder then added 100 mL of solvent (aquadest, ethanol, ethyl acetate, n-hexane) as 10% concentration in an Erlenmeyer. The extraction process was carried out using a water bath shaker at 28 °C for 30 min. Extraction results were filtered using filter paper, then all extracts were tested for phytochemical characteristic and antimicrobial activity.

### 2.3 pH Extract Determination

The pH of the extract of was carried out using pH paper. The pH paper is dipped in the extract for a few seconds, and then the pH paper is removed. The color on the dipped pH paper was determined according to pH indicator value.

### 2.4 Qualitative Phytochemical Determination [8]

#### 2.4.1 Tannin Identification

2 mL of sample extract was put into a test tube, then 2 mL of aquadest and 2-3 drops of 5% $FeCl_3$ were added. If a green precipitate is detected, the sample is positive for tannin compounds.

#### 2.4.2 Flavonoids Identification

1 mL of sample extract was put into a test tube and 1 mL of 10% lead (IV) acetate was added. If a yellow or orange color is detected, the sample is positive for flavonoid compounds.

#### 2.4.3 Terpenoids Identification

A total of 2 mL of sample extract was put into a test tube and then 2 mL $(CH_3CO)_2$ and 2–3 drops of concentrated $H_2SO_4$ were added. If a dark red color is detected, the sample was positive contains terpenoid compounds.

#### 2.4.4 Saponin Identification

5 mL of sample extract was put into a test tube and 5 mL of aquadest was added and then heated. If the foam detected on the surface, the sample is positive for saponin compounds.

#### 2.4.5 Steroid Identification

2 mL of sample extract was put into a test tube, then 2 mL of chloroform ($CHCl_3$) and 2 mL of concentrated sulfuric acid ($H_2SO_4$) were added. If a red-brown ring is detected between the layers of the solution, the sample is positive for steroid compounds.

**Table 1.** Qualitative phytochemical characteristics of coconut coir extract in various solvents type

| No. | Treatment | Compounds | | | | | |
|---|---|---|---|---|---|---|---|
| | | Tanin | Flavonoid | Terpenoid | Saponin | Steroid | Alkaloid |
| 1. | B1P1 | ++ | ++ | – | – | ++ | – |
| 2. | B1P2 | ++ | + | – | – | ++ | – |
| 3. | B1P3 | + | + | – | – | + | – |
| 4. | B1P4 | – | – | – | – | – | – |
| 5. | B2P1 | +++ | +++ | – | – | ++ | – |
| 6. | B2P2 | +++ | ++ | + | – | ++ | – |
| 7. | B2P3 | – | – | – | – | + | – |
| 8. | B2P4 | – | – | – | – | – | – |

Description: - = not detected; + = weak; ++ = strong; +++ = very strong; B1 = young coconut coir; B2 = old coconut coir; P1 = aquadest; P2 = ethanol; P3 = ethyl acetate; P4 = n-hexane.

#### 2.4.6 Alkaloid Identification

2 mL of sample extract was put into a test tube and then a few drops of Hager's reagent were added. If a yellow precipitate is detected, the sample is positive for alkaloid compounds.

### 2.5 Quantitative Phytochemical Determination

#### 2.5.1 Total Phenolic Content [9]

A total of 600 μL of the sample was put into a test tube and 3 mL of 10% Folin-Ciocalteu reagent was added. After 10 min of incubation, 2.4 mL of 7.5% $Na_2CO_3$ was added. The samples were then incubated for 1 h at room temperature in the dark. The absorbance of the sample was measured at 765 nm. Gallic acid was used as standard. The results are expressed in milligrams of gallic acid equivalent per gram of sample (mg GAE/g) (Table 1).

#### 2.5.2 Total Flavonoids [10]

2 mL of the sample was put into a test tube and the same volume of $AlCl_3$ was added. Then the sample was incubated for 10 min and the absorbance was measured at a wavelength of 365.7 nm. Samples were replaced with standard series quercetin concentrations of 0, 4, 8, 12, 16, and 20 ppm for blank measurements. Then the absorbance was measured at 365.7 nm. The measurement results are expressed in milligrams of quercetin equivalent per gram of sample (mg QE/g).

#### 2.5.3 Total Tannins

0.1 mL of the sample was put into a 10 mL volumetric flask. The sample was added with 7.5 mL of aquadest, 0.5 mL of Folin-Ciocalteu reagent and 1 mL of 35% $Na_2CO_3$

solution. Then, distilled water is added to exactly 10 mL. The solution was shaken until homogeneous and incubated at room temperature for 30 min. The absorbance of the sample was measured at 700 nm. For measurement of blanks, samples were replaced with standard tannic acid series concentrations of 0, 50, 100, 150, 200 and 250 ppm. Then the absorbance was measured at 700 nm. The measurement results are expressed in milligrams of tannic acid equivalent per gram of sample (mg TAE/g sample).

#### 2.5.4 Antimicrobial Activity Determination

Antimicrobial activity determination was carried out using the agar diffusion method (clear zone) referring to [11]. *S. cerevisiae, A. aceti*, and microbial cultures from fermented sap were used. 1 ose of microbial culture inoculated into PGY liquid media in a test tube and then vortexed until homogeneous. The cultures were incubated (20 h, 37 °C). Then, 100 μl cultures were inoculated on PGYA media using the pour plate technique. 50 μl of coconut coir extract was dropped onto the paper disc and left for a while until the extract diffused into the paper disc, then placed on the media containing the culture, and incubated for 20 h at 37 °C. Antimicrobial activity was determined by measured the diameter of the clear zone in the media.

## 3 Results and Discussion

### 3.1 Qualitative Phytochemical Characteristics.

The results of the qualitative phytochemical test showed that tannin and flavonoid compounds were detected in aquadest, ethanol, ethyl acetate extract for young coconut coir, and also detected in aquadest and ethanol extracts for old coconut coir. Steroid compounds were detected in the extracts of aquadest, ethanol and ethyl acetate in both young and old coconut coir. Terpenoid compounds were only detected in the ethanol extract for old coconut coir. Meanwhile, saponins and alkaloids were not detected in all solvent type. In the old coconut coir extract, tannin and flavonoid compounds were detected and the color intensity stronger than the young coconut coir extract. This is because the level of maturity is an important factor that affects the composition and amount of phytochemical compounds in plants [3]. Aquadest and ethanol solvents confirmed to be more effective in extracting phytochemical compounds than ethyl acetate and n-hexane solvents. This is indicated that the phytochemical compounds in coconut coirs are dominated by polar compounds, so the effective solvents for extracting phytochemical compounds are polar solvents such as aquadest and ethanol.

### 3.2 pH Extract

Based on Fig. 1, the aquadest extract has the lowest pH value compared to other extracts, namely 4.5. The lowest pH value in the coconut coir extracted with aquadest was indicated that the phytochemical compounds in the coconut coir were able to be extracted optimally by the aquadest solvent.

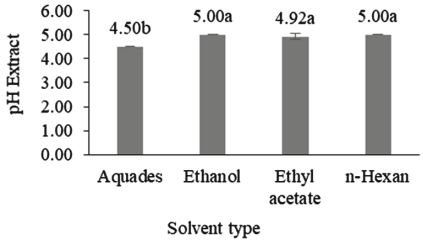


**Fig. 1.** The pH value of coconut coir extract in various solvents type. The values that followed by the same letter indicate no significant difference ($p > 0.05$).

**Table 2.** The antimicrobial activity of coconut coir extract against *S. cerevisiae*, *A. acetii*, and microbes isolated from the fermented sap

| No | Treatment | Diameter of inhibition zone against *S. cerevisiae* | Diameter of inhibition zone against *A. aceti* | Diameter of inhibition zone against microbes isolated from fermented sap |
|---|---|---|---|---|
| 1. | B1P1 | 2.34 b | 3.06 b | 3.69 b |
| 2. | B1P2 | 0.00 c | 2.23 c | 5.42 a |
| 3. | B1P3 | 0.00 c | 0.00 d | 0.00 d |
| 4. | B1P4 | 0.00 c | 0.00 d | 1.85 c |
| 5. | B2P1 | 2.91 a | 3.74 a | 5.21 a |
| 6. | B2P2 | 2.78 a | 3.43 ab | 3.82 b |
| 7. | B2P3 | 0.00 c | 0.00 d | 0.00 d |
| 8. | B2P4 | 0.00 c | 0.00 d | 2.48 bc |

Description: = B1 = young coconut coir; B2 = old coconut coir; P1 = aquadest; P2 = ethanol; P3 = ethyl acetate; P4 = n-hexane. The values that followed by the same letter indicate no significant difference ($p > 0.05$).

### 3.3 Antimicrobial Activity

According to [12], tannin compounds have acidic properties and have strong activity at weak acid pH. [13] stated that flavonoids are slightly acidic. Based on these literature, it is suspected that most of the phytochemical compounds have acidic properties and this is the reason for the lowest in pH that occurred in the coconut coir aquadest extract.

Table 2 shows that antimicrobial activity of coconut coir extract against *S. cerevisiae* was lower than to other microbes test. It is suspected that this is because *S. cerevisiae* is a group of microbes that is quite strong, has strong fermentative properties, biochemical stability, and ability to reproduce well in a propagation medium, more tolerant of acidic environments and has several important enzymes in the decomposition of organic compounds [14].

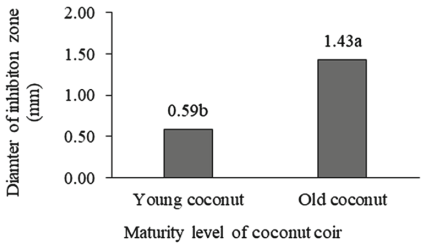


**Fig. 2.** The average diameter of the inhibition zone of coconut coir extract against *S. cerevisiae* at various levels of coconut coir aging. The values that followed by the same letter indicate no significant difference ($p > 0.05$).

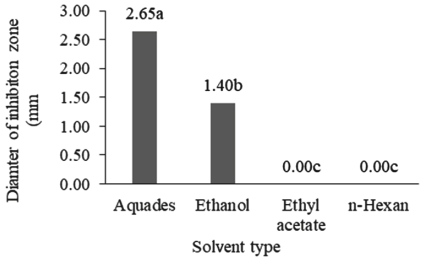


**Fig. 3.** The average diameter of the inhibition zone of coconut coir extract against *S. cerevisiae* in various types of solvents. The values that followed by the same letter indicate no significant difference ($p > 0.05$).

### 3.3.1 Antimicrobial Activity Against Microbes Isolated from Fermented Sap

The old coconut coir aquadest extract and old coconut coir ethanol extract shows higher antimicrobial activity against *S. cerevisiae* than other treatments, with inhibitory zone diameters of 2.91 mm and 2.78 mm, respectively. Both of these inhibition zones are included in the weak category (Fig. 2).

The diameter of the inhibition zone produced by old coconut coir extract was higher than young coconut coir. The larger diameter of the inhibition zone of old coconut coir showed a positive correlation with the results of qualitative phytochemical tests, where the old coconut coir extract was positive for tannins, flavonoids, steroids and terpenoids with stronger intensity than young coconut coir extract. However, this result is different from [4] which stated that the antimicrobial activity of young coconut coir ethanol extract was higher than old coconut coir aquadest extract against *S. aureus* and *E. coli* tested microbes. It is suspected that this difference in results is due to differences in the test microorganisms used. Because each microorganism has its own characteristics and different sensitivity to antimicrobial substances (Fig. 3).

The diameter of the inhibition zone produced by aquadest extract was the highest when compared to other solvent extracts. This is due to the effect of the pH of the extract and the phytochemical content of the coconut coir extract. The low pH value of the aquadest extract contributed to inhibiting the growth of *S. cerevisiae*. The content of phytochemical compounds in coconut coir is dominated by polar compounds, so these

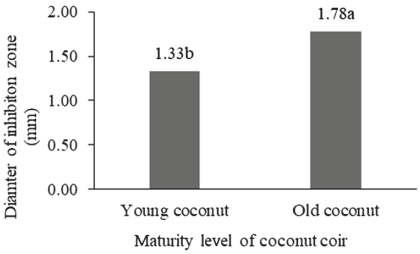


**Fig. 4.** The average diameter of the inhibition zone of coconut coir extract against *A. aceti* at various levels of coconut coir aging. The values that followed by the same letter indicate no significant difference ($p > 0.05$).

compounds are more extracted using polar solvents such as aquadest. [15] tried to extract gedang limes using ethanol, water, ethyl acetate and n-hexane as solvents. The results obtained showed that the phytochemical compounds of alkaloids, flavonoids, reducing sugars, phenols, proteins, amino acids, saponins, tannins, terpenoids and glycosides were more extracted using ethanol and water as solvents than ethyl acetate and n-hexane as solvents. The results of this study strengthen this research, where aquadest and ethanol solvents have a greater ability to extract phytochemical compounds.

### 3.3.2 Antimicrobial Activity Against *A. aceti*

The diameter inhibition zone of coconut coir extract against *A. aceti* was greater than the inhibition zone of coconut coir extract against *S. cerevisiae*, but smaller than inhibition zone of coconut coir extract against microbes isolated from fermented sap. It is suspected due to *A. aceti* which is included in the group of gram-negative bacteria whose cell walls contain 10–20% peptidoglycan. Outside the cell wall is a capsule. The function of capsules is to defend the cell from antibiotic produced by other microbes [16]. The presence of a peptidoglycan layer and capsule on *A. aceti* caused this bacterium to be more resistant to antimicrobial agent from coconut coir extract. Antimicrobial activity of old coconut coir with aquadest extract was highest antimicrobial activity against *A. aceti* with an inhibition zone diameter of 3.74 mm. This inhibition zone is included in the weak category (Fig. 4).

The diameter of the inhibition zone of aquadest extract was the highest among other extracts, followed by ethanol extract. This is due to the effect of the pH of the extract and the phytochemical content of the coconut coir in aquadest extract (Fig. 5).

### 3.3.3 Antimicrobial Activity Against Microbes Isolated from Fermented Sap

The diameter of the inhibition zone of coconut coir extract against microbes isolated from fermented sap was the largest compared to other tested microbes. This is presumably because the microbial resistance of the fermented sap to coconut coir extract is quite low. According to [17], the most dominant microorganism found in sap is yeast from the genus *Saccharomyces* sp., while the bacteria found in sap come from the genera *Acetobacter, Sarcina, Leuconostoc, Brevibacterium, Serratia* and *Pediococcus*. The yeast from the genus *Saccharomyces* are known as microorganisms that can ferment glucose

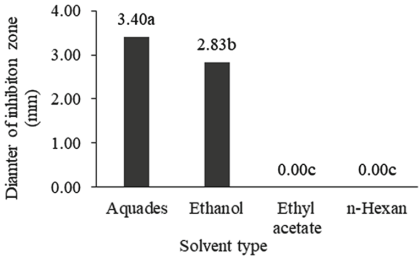


**Fig. 5.** The average diameter of the inhibition zone of coconut coir extract against *A. aceti* in various types of solvents.

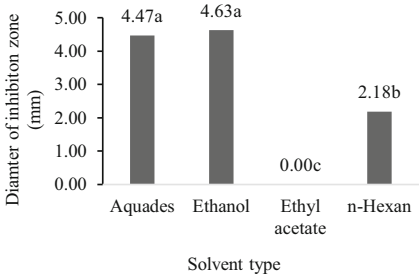


**Fig. 6.** The average diameter of the inhibition zone of coconut coir extract against microbes from damaged sap in various types of solvents. The values that followed by the same letter indicate no significant difference ($p > 0.05$).

into ethanol. Bacteria of the genus *Acetobacter* can oxidize ethanol to acetic acid. From the sap fermentation process, the accumulation of acid formed will lower the pH, besides that the alcohol formed can inhibit the growth of microorganisms in the fermented sap. Therefore, the antimicrobial activity of coconut coir extract against microbes isolated from fermented sap was much stronger than the other test microbes used in this study.

The young coconut coir ethanol extract and old coconut coir aquadest extract show higher antimicrobial activity against microbes isolated from fermented sap with inhibitory zone diameters of 5.42 mm and 5.41 mm, respectively. Both of these inhibition zones are included in the weak category (Fig. 6).

The ethanol and aquadest extracts showed a higher diameter of the inhibition zone than the ethyl acetate and n-hexane extracts. This is because the phytochemical compounds in coconut coir can be extracted optimally with ethanol and aquadest as solvents. The treatment of young coconut coir ethanol extract and old coconut coir aquadest extract was a treatment with higher antimicrobial activity against microbes isolated from fermented sap than other. This shows that both treatments have the same effectiveness as antimicrobials.

**Table 3.** Qualitative phytochemical characteristics of coconut coir extract in various solvents type.

| No | Treatment | Total phenolic (mg GAE/g) | Total flavonoids (mg QE/g) | Total tannins (mg TAE/g) |
|---|---|---|---|---|
| 1. | B1P1 | 28.46 | 1.15 | 165.08 |
| 2. | B1P2 | 9.54 | 0.51 | 69.95 |
| 3. | B2P1 | 76.04 | 1.57 | 522.95 |
| 4. | B2P2 | 85.33 | 0.99 | 522.47 |

Description: = B1 = young coconut coir; B2 = old coconut coir; P1 = aquadest; P2 = ethanol. The values that followed by the same letter indicate no significant difference ($p > 0.05$).

## 3.4 Quantitative Phytochemical

### 3.4.1 Total Phenolic Content

Based on Table 3, old cocout coir extract produced higher total phenolics than young coconut coir extract. The total phenolic content in plants is affected by many factors including the age of the plant, soil conditions, application of fertilizers and environmental conditions both biologically, physically and chemically (Khadijah *et al.,* 2017). In general, the total phenolic content of coconut coir extract was higher in ethanol and aquadest extracts. According to (18), phenolic compounds are classified as polar to semi-polar compounds so that more phenolic compounds will be extracted in polar to semi-polar solvents such as ethanol, methanol and aquadest.

### 3.4.2 Total Flavonoids Content

Based on Table 3, it is known that in the same solvent, old coconut coir extract contains higher total flavonoids than young coconut coir. According to [19], the morphology and age of plants will affect the content of secondary metabolites and bioactive compounds produced, including total flavonoids. In the same material, the aquadest extract contained higher total flavonoids than the ethanol solvent. It is suspected that this is affected by the polarity of the flavonoid compounds and the polarity of the solvent used during extraction. According to [20], flavonoids are polar compounds because they have a number of unsubstituted hydroxyl groups, so a polar solvent is needed to extract them.

### 3.4.3 Total Tannin Content

Based on Table 3, in the same solvent, old coconut coir extract contained higher total tannins than young coconut coir. These results are in line with the results of the qualitative phytochemical tests that have been carried out. The results of the qualitative tannin test showed that in the same solvent the tannin color intensity of the old coconut coir extract was higher than that of the young coconut coir. In the same material, the aquadest extract contained higher total tannins than the ethanol solvent. The higher total tannin yield in the distilled water extract was due to the fact that tannins are polar compounds that can be easily extracted using polar solvents. In this total tannin test, aquadest solvent has

**Table 4.** The range of wavelengths of phytochemical compounds according to the literature

| Maximum wavelength (nm) | Phytochemical identification | References |
|---|---|---|
| 203.9–276 | Steroid | [21] |
| 310–560 | Flavonoid | [22] |
| 600–850 | Tanin | [23] |

**Table 5.** Peak point and identification of phytochemical compounds.

| Treatment | Peak points (nm) | Absorbance | Prediction of phytochemical compound |
|---|---|---|---|
| B1P1 | 735.0 | 0.092 | Tannin |
| | 701.5 | 0.096 | Tannin |
| | 321.5 | 4.000 | Flavonoid |
| | 256.0 | 4.000 | Steroid |
| B1P2 | 741.0 | 0.064 | Tannin |
| | 701.0 | 0.064 | Tannin |
| | 430.0 | 0.542 | Flavonoid |
| | 317.0 | 4.000 | Flavonoid |
| B2P1 | 701.5 | 0.179 | Tannin |
| | 383.5 | 4.000 | Flavonoid |
| | 365.5 | 4.000 | Flavonoid |
| | 353.0 | 4.000 | Flavonoid |
| | 341.5 | 4.000 | Flavonoid |
| | 334.5 | 4.000 | Flavonoid |
| | 321.5 | 4.000 | Flavonoid |
| | 312.5 | 4.000 | Flavonoid |
| | 254.0 | 4.000 | Steroid |
| | 223.5 | 4.000 | Steroid |
| B2P2 | 701.5 | 0.257 | Tannin |
| | 445.0 | 3.934 | Flavonoid |
| | 349.0 | 4.000 | Flavonoid |
| | 329.5 | 4.000 | Flavonoid |

Description: B1 = young coconut coir; B2 = old coconut coir; P1 = aquadest; P2 = ethanol.

a higher polarity than ethanol solvent, so more tannin compounds are extracted using aquadest as a solvent. In other words, the solvent plays an important role in the extraction process.

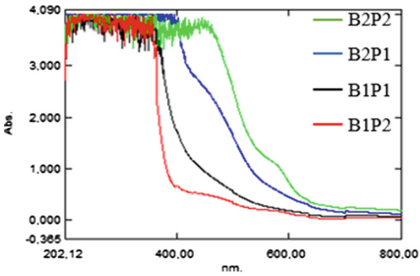


**Fig. 7.** UV-Vis spectrum of selected treatment coconut coir extract.

### 3.4.4 Extract Absorbance Spectrum

UV-Vis spectrum analysis of coconut coir extract was carried out to determine the maximum wavelength of each selected treatment and to relate it to the library of maximum wavelengths of phytochemical compounds. Each compound has a different maximum wavelength. Table 4 shows the wavelength range of several phytochemical compounds quoted from several libraries.

Figure 7 shows that the coconut coir extract have similar curve shape but with different peak points. The results of the peak point and identification of phytochemical compounds in the selected coconut coir extract can be seen in Table 5.

## 4 Conclusion

The results of this study indicated that young coconut coir extract containing tannins, flavonoids and steroids. Meanwhile, the old coconut coir extract containing tannins, flavonoids, steroids and terpenoids. Both of young and old coconut *coir* extracts showed antimicrobial activity against *S. cerevisiae*, *A. aceti* and the microbes isolated from fermented sap. Old coconut coir extract showed antimicrobial activity stronger than young coconut coir extract. The old coconut coir extracted with aquadest show the highest antimicrobial activity against all microbial tested. This extract contains a total phenolic of 76.04 mg GAE/g, total flavonoid of 1.57 mg QE/g and total tannin of 522.95 mg TAE/g, respectively. In addition, based on the absorbance spectrum, it was confirmed that this extract contains tannins, flavonoids and steroids.

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