PROFILE OF CHEMICAL COMPOUNDS AND ANTIOXIDANT ACTIVITY OF PAPAYA LEAF EXTRACT (*CARICA PAPAYA L.)* LIVING IN PEATLANDS

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Abstract

Papaya leaves (*Carica Papaya L)* are empirically widely used by the community to treat dengue fever, reduce fever, improve digestion, this is because of the content of carpine substances in papaya leaves. Peatlands are soils made from piles of dead and decayed plant remains, so that the carbon content in peat soil is very large. Very low pH acidic peat between 2,7 – 5,0. Carbon content 5 – 80 mg and nitrogen content ranging from 0,10 – 1,97%. Mineral content in the soil will affect the content of secondary metabolites and their pharmalogical activities. One of them is antioxidant activity. The purpose to determine the content of chemical compounds and to determine the antioxidant activity of papaya leaf extract (*Carica Papaya L*) living on peatlands. This research uses the maceration method using 96% ethanol as a solvent. Soaking for 3x24 hours. The extract obtained was then put in a rotary evaporator to form a thick extract. The extract was then identified for its chemical composition using Thin Layer Chromatography and antioxidant activity was measured using the DPPH method *(2.2 diphenyl-1 pikrylhydrazil*). The results of the content of chemical compounds in papaya leaves that grow on peat soil include flavonoids, alkaloids, and steroids. Antioxidant testing obtained IC50 results of 20.564 µg/mL. In Conclusion Papaya leaves (*Carica Papaya L*) living on peatlands have a very strong category of antioxidant activity.

Keywords: Antioxidant, papaya leaf (*Carica Papaya L),* DPPH, peatlands

Introduction

Indonesia has a lot of biodiversity and consists of various islands, one of which is the island of Borneo. According to research data by Barchia (2012), the area of peatlands in Kalimantan is around 4,699,004.61 with a percentage of 25% more than other provinces in Indonesia. Formation Peat is formed from piles of dead plant remains, both weathered and not. Plants that die and rot gradually form a layer which then becomes a transition layer between the peat layer and the mineral soil underneath. This difference in soil conditions will affect the content of chemical compounds with medicinal properties it contains and will also differ in the pharmacological effects produced.

One type of traditional plant that is often used as a nutritious plant is papaya leaf (*Carica Papaya L*). Empirically papaya plant (*Carica Papaya L*) is one type of medicinal plant and can be used for both

fruit, roots and leaves. Papaya leaves (*Carica Papaya L*) contain flavonoid secondary metabolites (Nugroho, 2017) alkaloids (Julianti, 2014) and steroid compounds (Ayoola, 2008). According to research by Seigher et al (2012) papaya leaves (*Carica Papaya L*) contain bioactive compounds that can increase the amount of antioxidants in the blood and can reduce the level of lipid peroxidation. These compounds include papain, simopapain, cystatatin, ascorbic acid, flavonoids, cyanogens, glycosides and glucosinolates.

Antioxidants are substances that are needed by the body to ward off free radicals against the body in normal cells, proteins and fats such as degenerative diseases, namely cancer. Cancer can be caused due to pollution, radiation exposure, unhealthy lifestyle. One way to prevent cancer is to consume nutritious plants, namely papaya leaves (Yahya, 2012). The antioxidant content of the California papaya was accumulated at 71.15% and the flavonoid content was 46.02 g. This type of California papaya has a high amount of antioxidants and flavonoid levels.

Papaya plants (*Carica Papaya L*) that grow on peatlands may have different active compounds from papayas that usually grow on soil in general. This is what underlies the research on chemical compound testing and compound separation using thin layer chromatography (TLC), secondary metabolite tests and antioxidant activity on papaya plants growing on peatlands.

Materials and Methods

Materials

The materials used in this study were papaya leaf extract, ethanol 96%, distilled water, chloroform, anhydrous acetic acid, Alcl3, Hcl, acetone, boric acid, oxalic acid, ether p, Dragendroff's reagent, Mayer's reagent, Liebermann Burchard's reagent, 2,2 *diphenyl-1 picrylhydrazil* (DPPH), quercetin, Wagner's reagent, Mg powder, Hot Plate, hydrochloric acid, acetic acid, filter paper, Klt plate silica gel F_{254}.

Methods

The type of research used is descriptive observational.

Peatlands Inspection Test

The test is carried out by checking the peat soil, moisture, and acidity of the peat soil by taking a sample of the peat soil to be tested. Soil sample checks were carried out in Balai Riset dan Standarisasi Industri Banjarbaru (Baristand).

Simplicity Making

The collection of raw materials, the process of sorting the results when the plants are still fresh. Sorting is done on soil and gravel, grass. The process of washing simplicia raw materials is carried out to clean the dirt that is attached to the soil or contaminated with pesticides. The cutting of the simplicia material is done in order to facilitate the process of drying, packing and grinding. Newly harvested plants are not allowed to be chopped directly but must first be dried in one piece for 1 day (Agoes, 2009). Then drying is done to reduce the water content so that the material is not easily overgrown with mold and bacteria. Dry sorting is the selection of materials that have undergone a drying process. Simplicia need to be placed in a separate container so as not to mix with each other simplicia with one another.

Extraction

Papaya leaves (*Carica Papaya L*) are selected which are still fresh, washed, cut into pieces and dried by aerating. A total of 250 grams of papaya leaf simplicia powder (*Carica papaya L*) was weighed, 5 liters of 96% ethanol at room temperature for 3 x 24 hours and then filtered and obtained. The liquid extract filtrate obtained was then thickened using a vacuum rotary evaporator at a temperature of less than 60◦ , speed of 70 rpm, and pressure of 0.7 bar to obtain a thick extract. After obtaining a thick extract, then weighed with an analytical balance.

Identification of Secondary Metabolic Compounds

Identification of secondary metabolites with eluent from thin layer chromatography (TLC) method. Sample preparation on papaya leaves was selected using maceration extraction method and 96% ethanol solvent. The selection of the stationary phase for identification or separation of a good compound is by TLC plate, while the selection of the mobile phase or eluent is based on the chemical nature of a secondary metabolite compound to be studied, namely the level of polarity of the substance.

Table 1. Eluent used

Compound	Eluent	Comparison
Flavonoids	N-butanol : acetic acid : water (Niwarna, dkk 2015)	4 : 1 : 5
Alkaloids	Chloroform : Ethyl acetate	1 : 3
Terpenoids	Toluene: ethyl acetate: chloroform (Arundina, 2015)	5 : 1 : 4

Alkaloids Identification

Two (2) ml of the test solution was evaporated over the cup. The resulting residue was then dissolved with 5 ml of 2N HCl. The solution obtained was divided into 5 test tubes. The first tube was added with 3 drops of Dragendorff's reagent, the second tube was added with Mayer's reagent, the third tube was added with 3 drops of Hager's reagent, and the fourth tube was added with 3 drops of Wagner's reagent. The formation of an orange precipitate in the first tube, a white precipitate in the second tube, a yellow precipitate in the third tube, and a brownish red precipitate in the fourth tube indicated the presence of Alkaloids. The test solution contains alkaloids if at least a precipitate is formed using the two groups of experimental solutions used (Depkes RI, 1995).

Flavonoids Identification

The test was carried out by taking 2 ml each. Papaya leaf samples that have been extracted with ethanol solvent. Then heated for about 5 minutes. After being heated, 0.1 grams of Mg powder and 5 drops of concentrated HCl were added. An orange yellow color is formed, then it is positive for flavonoids (Mustikasari dan Ariyani 2010).

Steroid Identification

Steroid examination was carried out with Liebermann-Burchard reagent. Test solution as much as 2 ml. evaporated in a vaporizer. The residue was dissolved with 0.5 ml of chloroform, then 0.5 ml of anhydrous acetic acid was added. Then 2 ml of concentrated sulfuric acid was added through the tube wall. The formation of a brownish or violet ring on the boundary of the solution indicates the presence of terpenoids, whereas a greenish blue ring indicates the presence of sterols (Ciulei, 2014).

Antioxidant Activity Testing

The DPPH sample was prepared by making a mother liquor then weighed the 15.7 mg DPPH powder (2.2 Diphenyl 1-Pikrihidrazil) dissolved with 100 ml of 96% ethanol into the flask. (Brand,1995).

Determination of the Maximum Wavelength of DPPH

Determination of the maximum wavelength of DPPH then from the mother liquor is taken using a pipette volume of 2 ml then dissolved using 96% ethanol as much as 2 ml Then vortexed for 1 minute the solution is then run using a UV-Vis spectrophotometer at a wavelength of 450-550 nm (Molyneux, 2018).

Determination of Operating Time

Determination of the DPPH solution dissolved in 15.7 Mg with the addition of 2 ml of the mother liquor that has been made then up to the mark on the volumetric flask using a volume pipette with 2 ml of quercetin standard solution. 2 minutes until a stable absorbance is obtained (Alifni, 2017).

Determination of IC_{50} Value of Quercetin Comparison Solution

Ten (10) mg of quercetin powder was weighed on an analytical balance after that it was dissolved in 100 ml of 96% ethanol solvent, then the grade series was made with a concentration of 20 ppm 2 ml, 30 ppm 3 ml, 40 ppm 4 ml and 50 ppm 5 ml. A total of 2 ml of the mother liquor of 15.7 mg DPPH antioxidant was added to each concentration of quercetin solution and then left in the dark for 30 minutes. After 30 minutes, the absorbance of each solution was read using a UV-Vis spectrophotometer instrument at the maximum wavelength of DPPH that had been obtained (Mardiah, 2017).

Determination of IC_{50} Value of Papaya Leaf Extract

The mother liquor was made as much as 10 mg of the extract sample dissolved in 96% ethanol to 100 ml in a flask. Then made each series of extract concentrations of 20 ppm 2 ml, 30 ppm 3 ml, 40 ppm 4 ml, and 50 ppm 5 ml. The DPPH solution from the mother liquor was added with each concentration of 2 ml of the sample solution. The solution was left in the dark for the operating time at 40 minutes to be stable and the absorbance was read at a predetermined wavelength.

Results and Discussion

Results

Peatlands Test

Soil sampling was carried out directly around the papaya plant (Carica Papaya L) that lives on peat soil at a depth of 40-50 cm. Soil was taken and weighed as much as 4 kg of soil for examination of the soil in Balai Riset Dan Standarisasi Industri (Baristand). The results showed that the pH of acidic peat soil was 4.82 with the potentiometric test method, the test results obtained carbon (C) 8.56% by the spectrophotometric method and Nitrogen (N) 0.96% by the Kjedahl method.

Yield of Papaya Leaf Extract (Carica Papaya L) on Peatlands

$$\text{Extract Yield Calculation} = \frac{\text{Total weight of extract (gr) x 100 \%}}{\text{Total weight of simplicia powder (g)}}$$

$$= \frac{65 \text{ gr x } 100 \%}{250 \text{ gr}}$$

$$= 0.26\%$$

Phytochemical Screening Test of Ethanol Extract of Papaya Leaves (Carica Papaya L) on Peatlands

The method used is the test tube method and the results can be seen in the following table:

Table 2. Phytochemical Screening Results

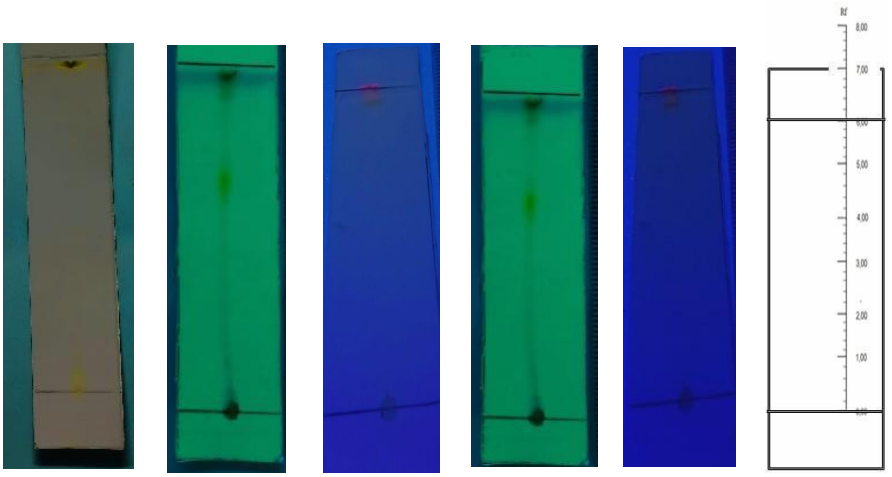
Secondary metabolites	Result	Information	Reactor
Flavonoids	+	Formation of yellow-orange color	Mg and HCL
Alkaloids	+	Orange precipitate	Dragendorff
	+	White precipitate	Mayer
	+	Yellow precipitate	Hager
	++	Brown/violet ring at the solution boundary	Wagner
Steroids	++	Brownish/violet ring at the boundary of the test solution	Lieberman Burchard

Information : (+) looks clearly
: (++) looks very clear

Chemical Compound Screening Test with Thin Layer Chromatography method

Identification of Flavonoid Test

The flavonoid test was carried out by thin layer chromatography with the mobile phase of N-Butanol : Acetic Acid : Water (4 : 1: 5) and Silica Gel 60 F_{254} stationary phase.



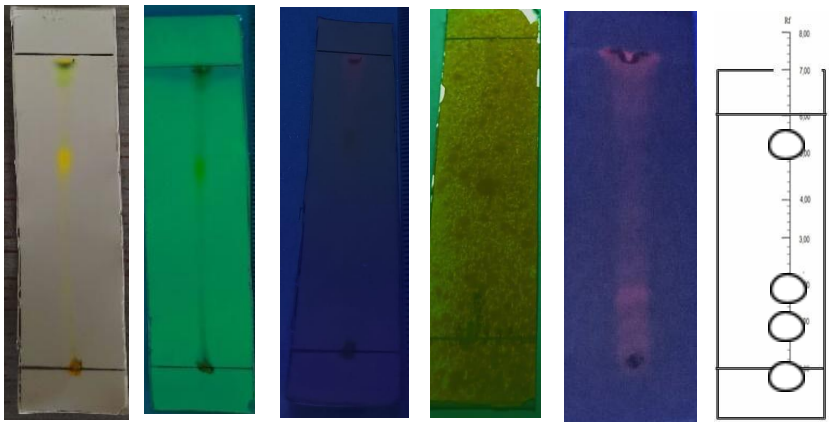
(a) (b) (c) (d) (e) (f)

Figure 1. Results of TLC Identification of Flavonoid Group Compounds

Information:
(a) Observations in visible light
(b) Observation on 254 UV light
(c) Observation at 366 UV light
(d) Observation at 254 UV light after being sprayed using Alcl3 reagent
(e) Observation at 366 UV light after being sprayed using Alcl3 reagent
(f) Observations on the spots seen on the TLC plate

Identification of Alkaloid Test

In the identification test of alkaloids, the mobile phase used was Chloroform 1: 3 Ethyl Acetate and silica gel 60 F_{254} was used as the stationary phase. The results of the identification test of alkaloid compounds using thin layer chromatography can be seen in the following figure:



(a) (b) (c) (d) (e) (f)

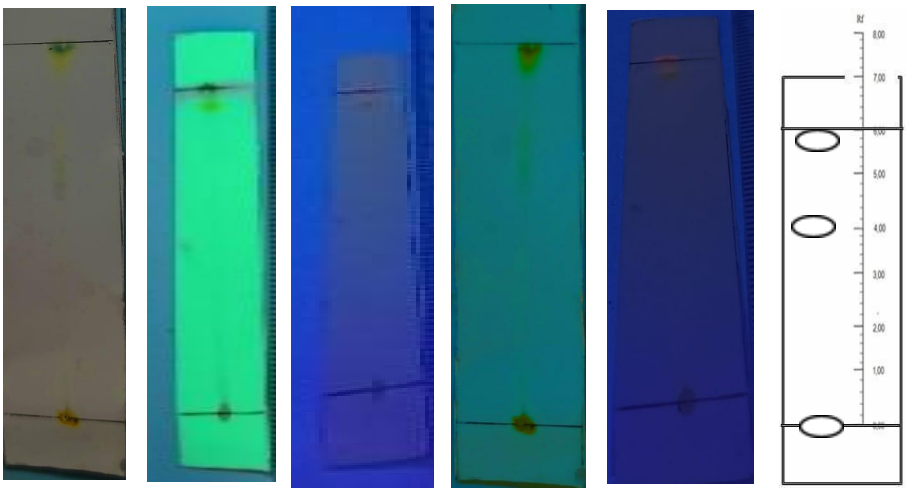
Figure 2. Results of TLC Identification of Alkaloid Group Compounds

Information:
(a) Observations in visible light

(b) Observation on 254 UV light
(c) Observation on 366 UV light
(d) Observation on 254 UV light after spraying using Dragen dolf reagent
(e) Observation on 366 UV light after spraying using Dragen dolf reagent
(f) Observation of the spots seen on the plate after being sprayed using Dragen Droff reagent.

Identification of Steroid Test

In the identification test, the mobile phase steroid used was Toluene: Ethyl Acetate: Chloroform 5: 1: 4 and the stationary phase used Silica gel 60 F_{254} using Liebermann Burchard spray.



(a) (b) (c) (d) (e) (f)

Figure 3. Results of Identification of Steroid Group Compounds

Information:
(a) Observations in visible light
(b) Observation on 254 UV light
(c) Observation on 366 UV light
(d) Observation on 254 UV light after spraying using Liebermann Burchard reagent
(e) Observation on 366 UV light after spraying using Liebermann Burchard reagent
(f) Observation of the visible spots on the plate after spraying using Liebermann Burchard's reagent

Preliminary Test Results of Antioxidant Activity by TLC

Qualitative testing of antioxidants by TLC method with mobile phase Butanol: Acetic acid: Water (BAA) in a ratio (6:2:2) with Silica Gel 60 F_{254} stationary phase (visible light) shows a yellow-purple background.

Antioxidant Activity Testing

Determination of Wavelength 2.2 Diphenyl 1-1 Picryhydrazil (DPPH)

The maximum wavelength of the DPPH measurement shows a wavelength of 514 nm. According to Molyneux (2018) the maximum wavelength of dpph measurement is 450-550.

Absorbance Measurement Results 2.2 Diphenyl 1-1 Picryhydrazil (DPPH)

The measurement test for the blank solution was carried out three times with replications showing the results of 0.869, 0.868, 0.869 with an average of 0.869.

Operating Time

The time used to react DPPH with an antioxidant compound requires a stable time, the time constant in this study was at 40 minutes as shown in the graph below:

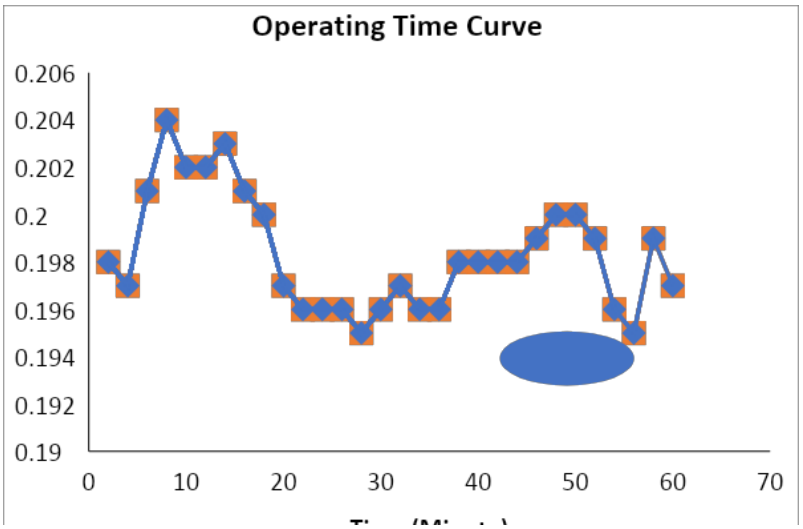


Figure 4. Operating Time Curve

Determination of IC_{50} Value of Quercetin Comparison Solution

Table 3. Results of IC_{50} Determination of Quercetin Comparative Solution

Sample	Concentration (ppm)	% Inhibition	IC_{50}
Quercetin	20	51.208	16.831 ppm
	30	63.176	
	40	75.25	
	50	78.71	

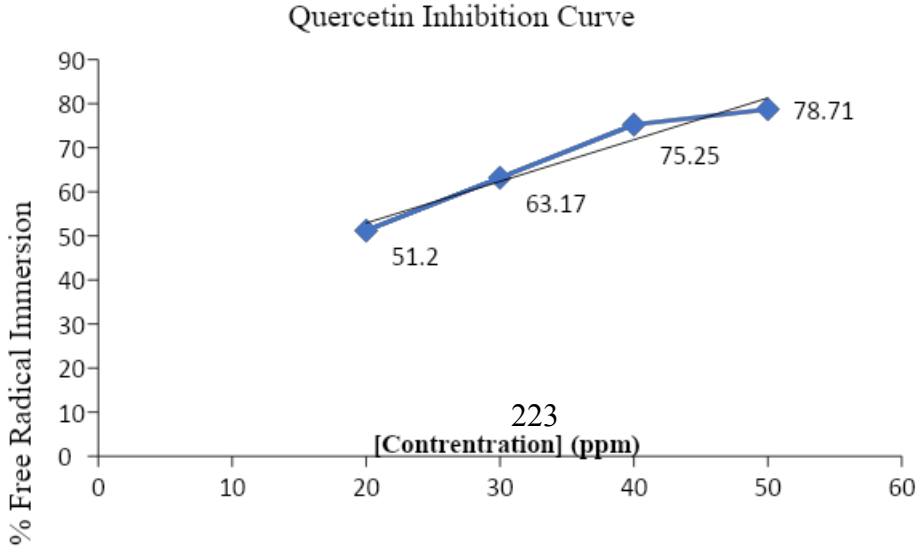


Figure 4. Determination of the IC_{50} value of the quercetininhibition inhibitory solution

Determination of IC_{50} Value of Papaya Leaf Ethanol Extract

Table 4. Results of IC_{50} Determination of Papaya Leaf Ethanol Extract

Sample	Konsentrasi (ppm)	% Inhibition	IC_{50}
Papaya Leaf Ethanol Extract (*Carica Papaya L.)*	20	41.08 %	20.564 ppm
	30	44.76 %	
	40	48.21 %	
	50	56.27 %	

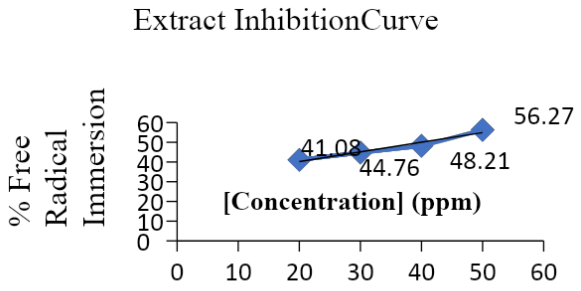


Figure 5. Determination of inhibition of IC_{50} value of papaya leaf ethanol extract

Discussion

This research was conducted by extracting papaya leaf powder that has become simplicia using the maceration method, sample extraction was carried out by maceration immersion, weighed 250 grams of papaya leaf powder *(Carica Papaya L*) then soaked in 500 ml of 96% ethanol for 3 x 24 hours and then filtered with filter paper to obtain the filtrate.

In the flavonoid test, magnesium was added to the extract and then concentrated HCl was added, the results obtained were the formation of a reddish-orange color in the extract. The mechanism that occurs in this test is the addition of Mg and HCl metals to reduce the benzopyron core which is a compound contained in the flavonoid structure, therefore the formation of red or orange flavilium salts.

Testing for alkaloids, namely the extract added with Dragendorff's reagent produces an orange precipitate, this indicates that the extract is positive for alkaloids. The mechanism that occurs in the detection process with Dragendorff is as follows. From this analysis, a precipitation reaction occurs due to ligand replacement. The free electrons in the alkaloids will replace the iodine ions with Dragendorff's reagent containing bismuth nitrate and potassium iodide in a solution of glacial acetic acid. Researchers also tested alkaloids with extracts added with Mayer reagent, the results obtained were positive for the formation of a white precipitate. The extract alkaloid test was added to Hager's reagent, a yellow precipitate was formed under the bottom of the tube, the results obtained were positive, and finally the researchers also tested the alkaloids with the extract added with Wagner's reagent. The results were positive with the formation of a brownish/violet ring at the boundary of the solution in the tube.
Testing of steroids on the extract by adding Liebermann-Burchad reagent plus concentrated HCl and concentrated sulfuric acid based on the analysis of positive results so that the sample results obtained formed a bluish green ring at the boundary of the test solution. The results of this study were positive extracts containing steroids.

Qualitative test of antioxidant activity of papaya leaves (*Carica Papaya L*) using a TLC plate that has been eluted sprayed with DPPH 2,2 diphenyyl 1-picrihydrazil reagent in this study, the formation of color after spraying with DPPH solution as a detection of compounds having antioxidant activity. In this study, it was marked by the change of the sample spots to a greenish yellow color with a clearly visible purple background. This color change occurs due to the reaction of antioxidant compounds in reducing DPPH radical compounds in TLC plates, so it can be interpreted that papaya leaves have effectiveness as an antioxidant determination.

This research was then continued with quantitative testing of antioxidants. The maximum wavelength is determined from the measurement of the absorbance of the solution. The DPPH solution in this study was read at a wavelength of 514 nm, this is in accordance with Molyneux's theory (2018), that the maximum wavelength range of DPPH is 450-550 nm.

The measurement of operating time in this study was carried out in a time interval of 2 minutes in order to obtain a stable absorbance of operating time. The test material was extract with a positive control of quercetin which was reacted with DPPH solution and observed its absorbance at a wavelength of 514 nm in the 0 minute to the 60th minute with an interval of 2 minutes. The results showed that at the 40th minute the relative absorbance was constant, so that the antioxidant activity test at the next stage was carried out at the 40th minute.

Determination of IC_{50} value of quercetin comparison solution was used as a control or positive comparison of quercetin antioxidant activity compounds including flavonoid compounds. Based on the results of linear regression analysis between the relationship between quercetin concentration and the percentage of inhibition obtained, the equation Y = 0.9461x + 33.969 with a coefficient of relation (r) 0.9533. The IC_{50} value of quercetin obtained was 16.831 g/ml. The results showed that the comparison sample of quercetin had a very strong activity, namely 16.831 g/ml. According to Lung (2015) the level of strength of the intensity of antioxidant activity is very strong in the IC_{50} value range of less than 50 g/ml, which means that the comparison test for quercetin in this study is in accordance with the theory.

Determination of the IC_{50} value of papaya leaf extract (Carica Papaya L) on peatlands in South Kalimantan obtained a regression equation between the relationship between papaya leaf extract concentration and % inhibition, namely Y = 0.4902x + 30.432 with a coefficient of relation (r) 0.9527. The IC_{50} value of papaya leaf extract was 20.564 µg/ml antioxidant ethanol extract of papaya leaves on peatlands in South Kalimantan showed IC50 of 20.564 µg/ml so it could be categorized as having very strong antioxidant activity <50 µg/ml.

Conclusion

Based on the results of the study, it can be concluded that the results of the screening test of chemical compound profiles in papaya leaves (*Carica Papaya L*) growing on peatlands are positive for flavonoids, alkaloids and steroids. Quantitative testing of antioxidant activity levels showed clear results that papaya leaf extract growing on peatlands had strong antioxidant activity with an IC50 value 20.564 µg/ml.

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