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Identification of compound content and antioxidant activity test of ethanol extract of *Stachytarpheta jamaicensis* (l.) vahl. leaf abts method

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Abstract
Stachytarpheta jamaicensis (L.) Vahl leaves contains secondary metabolites in the form of flavonoids, which can be used as a source of antioxidants. All flavonoids have been reported to undergo some form of degradation with exposure to physico-chemical environmental factors. This study aims to identify the compound content and determine the antioxidant activity of horse whip leaf extract. Identification of compound content by TLC method and antioxidant activity in reducing ABTS radicals using a UV-VIS spectrophotometer which is then expressed in IC50 value. Extraction by maceration method placed at room temperature, namely 30ºC. The results of the identification of the compounds contained in the horse whip leaf extract were positive for flavonoids, saponins, tannins and terpenoids. ABTS method antioxidant activity with IC50 value 39.41 ppm is included in the very strong antioxidant category because it is < 50 ppm compared to Vitamin C which IC50 value is 4.07 ppm. It can be concluded that the very strong activity of the ethanol extract of horse whip leaves is thought to be due to its compound content, one of which is the antioxidant compound content, namely flavonoids.

Keywords: *Stachytarpheta jamaicensis* (L.) Vahl, antioxidants, ABTS

Introduction
The horse whip plant is one of the abundance of flora in Indonesia. The horse whip plant is a weed that can grow anywhere and has not been widely used by the community. This plant is able to live in areas with tropical climates throughout the year, both in lowland and highland areas (Setiawan, 2019; Kumala *et al*, 2016) [13, 21]. Setiawan (2019) [21] reports that *Stachytarpheta jamaicensis*contains various secondary metabolite compounds that can be used as a source of antioxidants. Content biochemicals found in this plant include flavonoids, phenols, tannins, alkaloids, saponins and antioxidants (Liew and Yoke 2016) [14].
The biochemical content of flavonoids and phenolics found in *Stachytarpheta Jamaicensis* can be used as a source of antioxidants (Setiawan, 2019) [21]. Antioxidants are compounds that are able to overcome the negative effects of Oxidation in living things such as damage to the vital elements of cells in a way donating one electron to compounds that are oxidative so activities can be hampered. Antioxidants play a role in the radical defense system free caused by external factors such as temperature, soil pH, UV radiation, air pollution in the environment, and other pollution (Neldawati, 2013) [16].
Several studies have been conducted on horse whip plants, including measurement of specific and non-specific parameters of simplicia and horse whip leaf extract to ensure the quality of the samples to be further tested (Utami. Y.P, *et al*, 2022) [23]. Then Rante *et al*, (2020) [19] shows it is getting higher The concentration of the ethanol extract of horse whip leaves, the stronger the inhibition of activity the DPPH. Free radical scavenging activity ability of leaf ethanol extract This horse whip plant is proven by the IC50 value very strong at 16.66 µg/mL. According to Sharma *et al*, (2015) [22] antioxidant activity is also influenced by total amount of flavonoids and phenolics found in horsetail leaves synergize in inhibiting free radicals formed from environmental pollution.
Various methods can be used to test this antioxidant activity, including: metode ABTS (2,2'-*donkey* (3-*etilbenzoatiazolin*-6-*sulfonic acid*). Metode ABTS has the advantage that it can be used in water-based as well as solution systems organic matter that is polar, semi-polar, and non-polar, stable, and requires less reaction time (Amir *et al*. 2020) [1]. Therefore, the researcher is interested to identify the compound content and test the antioxidant activity of the extract horse whip leaves with the ABTS method.

Research methods
Tools and materials
The tool used is an autoclave maceration vessel *(When®)*, chamber, petri dish, Erlenmeyer *(Pyrex®)*, Incubator *(Memmert®)*, spirit lamp, UV lamp 254 nm and 365 nm *(Spectroline®)*, round ose, oven *(Falc®)*, tweezers, capillary tube, 1 ml syringe, 10 ml syringe, test tube *(Pyrex®)*, and analytical balance *(Mettler toledo®)*.
The materials used are ABTS, acetic acid, aquadest, raw vitamin C, *Stachytarpheta jamaicensis* (L.) Vahl. Leave, 70% ethanol, ethanol p.a, ethyl acetate, filter paper, chloroform, methanol, n-butanol, and potassium persulfate.

Sample collection and processing
The sample used is part of the horse *Stachytarpheta jamaicensis* (L.) Vahl obtained from Poasia District, Kendari City, Southeast Sulawesi Province.
As much as 3.25 kg of horse whip leaf samples were collected and sorted by wet sorting to separate dirt or other foreign material from the sample. Then washing is carried out to remove impurities attached to the sample. After tha, chopping is done to speed up the drying process. Drying is

done in aerated way. After that, dry sorting was carried out to separate the damaged simplicia from the previous process. The good simplicia is then stored in a container.

Extraction of *Stachytarpheta jamaicensis* (L) Valh Leaves
The simplicia of horse whip leaves was extracted by maceration method using 70% ethanol solvent. Weighed 250 g of simplicia into the maceration vessel and then added 70% ethanol until all of the simplicia was submerged with a solvent ratio of 1:10. Let stand for 3x24 hours while stirring occasionally. Then filtered, macerate in the form of liquid extract and then collected. Then the resulting residue is re-macerated for 2x24 hours in the same way using a new solvent. The liquid extract obtained was evaporated at 50ºC by using rotary evaporator until a thick extract is obtained. The viscous extract obtained was then weighed to determine the yield.

$$\%\ Yield\ = \frac{\text{Bobot extracte}}{\text{Bobots extracted from simplisia}}\ X\ 100\%$$

Extract Storage
The extract was stored at room temperature 30ºC and then the antioxidant capacity was measured using the ABTS method.

Phytochemical Screening Test with Thin Layer Chromatography Method

1. Alkaloid Test
The ethanol extract of horse whip leaves is dissolved in 70% ethanol, then spotted on a GF254 silica plate. The plate was then put into a chamber containing ethyl acetate, methanol and water with a ratio (100:13,5:10) v/v. A positive reaction is shown by some alkaloids giving blue or yellow fluorescence (Hanani, 2014) [6]. The chromatograms or plates were observed under UV 254 and 366 nm and then sprayed using a stain-seeking reagent between. Another reagent used to identify alkaloid group compounds, namely Dragendorf, if the sample contains positive alkaloids, an orange color appears on a yellow background (Harborne, 1984) [7].

2. Saponin Test
The ethanol extract of horse whip leaves is dissolved in 70% ethanol, then spotted on a GF254 silica plate. The plate was then put into a chamber containing chloroform: methanol (9:1) v/v eluent. Liebarmann burchard spray reagent was used for the appearance of the spots. Positive results if a blue to blue violet color is formed, sometimes in the form of red, yellow, dark blue, purple, green, or brown yellow spots in visible light (Wagner & Bland, 1996) [26].

3. Tanin Test
The ethanol extract of horse whip leaves is dissolved in 70% ethanol, then spotted on a GF254 silica plate. The plate was then put into a chamber containing the eluent methanol: water (6:4) v/v with FeCl stain remover.35%, the positive reaction that is formed is a black stain under UV 366 (Hayati *et al*, 2012).

4. Flavonoid Test
The ethanol extract of horse whip leaves is dissolved in 70% ethanol, then spotted on a GF254 silica plate. The plate was then put into a chamber containing the eluent n-butanol: acetic acid: water (4:1:5) v/v with a stain remover using ammonia vapor. Observation if positive on visible light shows yellow, green yellow or brown, light blue, red or orange. At UV 366 it shows yellow green fluorescence, or bright blue green fluorescence light blue according to the type of flavonoid (Hanani, 2014) [6].

5. Test triterpenoids and steroids
The ethanol extract of horse whip leaves is dissolved in 70% ethanol, then spotted on a GF254 silica plate. The plate was then put into a chamber containing the eluent chloroform: methanol (9:1), with the appearance of the Liebarmann Burchard reagent stain accompanied by heating at 105^{TWO}C for 5 minutes. A positive reaction is indicated by the presence of a blue-green stain seen under UV 366 (Kristanti *et al*, 2008) [12].

Antioxidant Activity Test with the ABTS Method

1. Preparation of Stock solution
Preparation of ABTS Stock Solution
Solution a: Weighed 7.1015 mg of ABTS, dissolved in 5 ml of distilled water, incubated for 12 hours. Solution b: Weigh 3,500 mg K2S2TWO8, dissolved in 5 ml of distilled water, incubated for 12 hours. Solutions a and b are mixed in a dark room and make up the volume with absolute ethanol to 25 ml (Sami and Rahimah, 2018) [20].

Preparation of Vitamin C Stock Solution
A 1000 ppm stock solution was prepared by weighing 10 mg of vitamin C and dissolving it in ethanol p.a, the final volume being sufficient for a 10 ml volumetric flask.

Preparation of Stock Solution of Horse Whip Leaf Extract
A stock solution of 1000 ppm was prepared by weighing 10 mg of thick horsetail leaf extract and dissolving it in ethanol while homogenizing, the final volume was added with ethanol to 10 ml in a volumetric flask.

2. Measurement of Antioxidant Activity
ABTS Blank Absorption Measurement
One ml of ABTS solution was pipetted and the volume was made up to 5 ml with ethanol p.a in a volumetric flask. This solution was then measured by UV-Vis spectrophotometry at a wavelength range of 600-800 nm. Measurement

Comparative Antioxidant Activity of Vitamin C
Tests were carried out by pipetting each of 5 µl, 10 µl, 15 µl, 20 µl and 25 µl of 1000 ppm pure vitamin C stock solution, the mixture was added to 1 ml of ABTS solution and then the volume was made up to 5 ml with absolute ethanol to obtain solutions with concentrations of 1 ppm, 2 ppm, 3 ppm, 4 ppm and 5 ppm. Then it was homogenized and then the absorption was measured by UV-Vis spectrophotometry at a wavelength of 667 nm.

Measurement of Antioxidant Activity of *Stachytarpheta jamaicensis* (L) Valh. Leaves
Pipette stock solution of 1000 ppm horsetail leaf extract sample, 100 µl, 150 µl, 200 µl, 250 µl and 300 µl, the mixture was added 1 ml of ABTS solution and then the volume was made up to 5 ml with absolute ethanol to obtain solutions with concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm. Then it was homogenized and then the absorption was measured by UV-VIS spectrophotometry at a wavelength of 667 nm.

Analysis of Data

The percentage of inhibition of ABTS produced by each of the concentrations of horse leaves and vitamin C is calculated by the formula:

$$\% \text{ inhibition} = \frac{\text{Blanco absorption} - \text{Blanco absorption of the sample}}{\text{Blanco absorption}} \times 100\%$$

The IC_{50} value for obtaining the strength of antioxidant activity of horseback leaves was calculated using a linear regression equation between % inhibition and concentration. The value of IC_{50} (Inhibitory Concentration) 50 is obtained from a line cut between 50% of the concentration axis barrier power using a linear equation (y = bx + a) where y = 50 and x indicates IC_{50} (Molyneux, 2004) [15].

Results and discussion

The antioxidant activity test of *Stachytarpheta jamaicensis (*L.) Vahl leaves from Poasia sub-district, Kendari city, Southeast Sulawesi. This study aims to determine the antioxidant activity of horsetail leaf extract stored at various temperatures and different storage times by looking at the profile and thin layer chromatography analysis, followed by testing the antioxidant activity using the ABTS method.

The dried simplicia of horse whip leaves was macerated with 70% ethanol, the maceration method was chosen because long immersion in the maceration process allows the breakdown of cell walls and membranes due to differences in pressure inside and outside the cell, so that the secondary metabolites present in the cytoplasm will dissolve in the solvent (Harborne, 1987) [7]. Flavonoids are a class of compounds that are thought to have a role in the antioxidant activity of horse whip leaves. Flavonoid compounds are polar so that polar solvents are needed (Gillespie and Paul, 2001). The effectiveness of the extraction of a compound by a solvent is highly dependent on the solubility of the compound in the solvent, according to the principle *like dissolve like* i.e. a compound will dissolve in a solvent with the same properties. Ethanol is a polar solvent so that the expected active substance can be maximally extracted according to its polarity. The maserate is then concentrated with *rotary evaporator*and obtained a thick extract of 38.499 grams, with a percent yield value of 15.4%.

Identification of Compound Content by TLC Method

According to Liew and Yoke (2016) [14] horse whip is rich in secondary metabolites. There are several major groups of secondary metabolites found in horse whip plants, including alkaloids, flavonoids, tannins, phenols, steroids, and terpenoids. To confirm the presence of these compounds, TLC analysis was carried out. TLC analysis is a separation of chemical components based on the principle of adsorption and partition which is determined by the stationary phase (adsorbent) and the mobile phase (eluent) (Alen *et al*, 2017) [2].

Compounds detected according to the mobile phase will appear as spots with the polarity of the mobile phase used (Harbone, 1987). Identification of chemical compounds by thin layer chromatography of ethanol extract of horsetail leaves includes groups of flavonoids, alkaloids, steroids, tannins and saponins which can be seen in table 1.

Table 1: Results of Phytochemical Screening by Thin Layer Chromatography Method

Compound	Observation Based on literature	UV 254	UV 366	Visual After Reagent Spraying (Visible Light)	Information
Alkaloid	Orange with background yellow back. Observed below UV 366 (Harborne,1984).	It's dark	Red	Dragendorf: Chocolate	Negative
Flavonoid	In visible light it shows yellow, green, yellow or brown, blue light, red or orange. On UV 366 shows green fluorescence yellow, or light blue fluorescent blue green light blue (Hanani, 2014)	It's dark 0,51	Blue time 0,73 Green Yellow 0,51	Ammonia: Yellow 0.73 Yellow green 0,51	Positive
Saponin	Positive result if formed blue to blue-violet sometimes in the form of red, yellow, dark blue, purple, green, or in the form of yellow brown in visible light (Wagner & Bland, 1996).	It's dark 0,73	Red 0,73 0,87	Dear man Burchard: Green 0,73	Positive
Tannin	Colored stains black under UV 366 light (Biological*et al*, 2012).	It's dark 0,90	Black 0,90	$FeCl_3$5%: Chocolate 0,90	Positive
Steroid	Green, blue seen under UV 366 (Kristanti*et al*., 2008).	It's dark 0,51	Blue 0.51	Dear man Burchard: Green 0,51	Positive

The TLC test on alkaloid compounds showed negative results after spraying with Dragendorf reagent. At UV 254, it showed dark fluorescence and at UV 366, it showed red fluorescence.

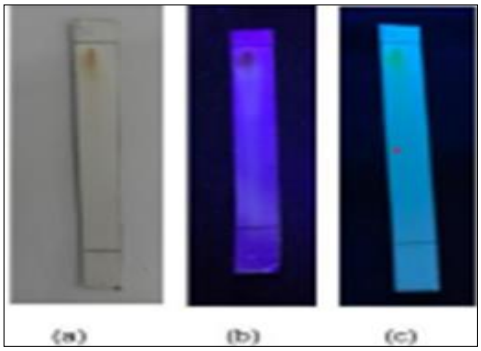

Fig 1: Alkaloid test results, captions: (a) visible light observations, (b) 366 nm UV light observations, (c) 254 nm UV light observations.

The TLC test for flavonoids was carried out with the mobile phase of n-butanol: acetic acid: water (4:1:5) with an ammonia stain and obtained positive results according to the literature. UV 366 shows bright light blue fluorescence at a spot with Rf 0.73, in Figure 2.

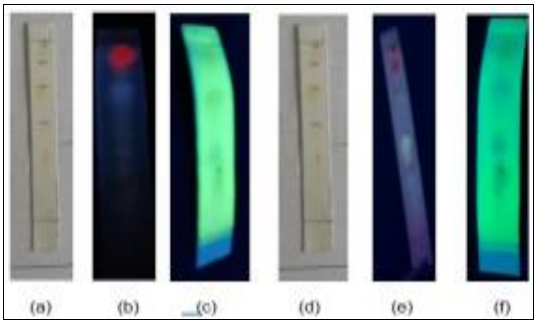

Fig 2: Flavonoid Test Results, Description: (a) visible light observations, (b) observations under UV light 366 nm, (c) observations under UV light 254 nm, (d) observations under visible light after being sprayed with ammonia, (e) observations under UV light 366 nm after being sprayed with ammonia, (f) observations under UV light 254 nm after being sprayed with ammonia.

There is an interpretation of the color of the spots that arise in terms of the structure of the flavonoids before and after being evaporated and observed in UV light there are light blue fluorescence stains and after being evaporated with ammonia the color changes little or no change (Yuda *et al*., 2017). As for the relationship between spotting with structure of the flavonoid where light blue fluorescence is seen in UV and after being sprayed with ammonia a yellow green or blue green fluorescence appears. The type of flavonoid suspected is the flavones and flavonones which do not have free 5-OH or flavonols which do not have free 5-OH, but there is a substitution in 3-OH. In UV light if it is not visible and after being evaporated with ammonia it forms light blue fluorescence then it probably contains isoflavone group flavonoids which do not have free 5-OH (Hanani, 2014) [6].

Identification of saponin compounds using Liebermann Burchard reagent resulted in spots on the chromatogram in the form of red, dark blue, purple,

green, or in the form of yellow-brown (Wagner and Bland, 1996) [26]. The results of the chromatogram for the identification of saponins showed that the positive horsetail leaf extract contained saponins in the presence of dark spots at UV 254 and red spots at UV 366 in Figure 3.

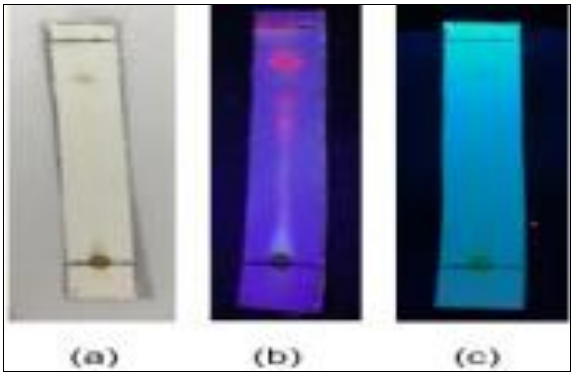

Fig 3: Saponin Test Results, Description: (a) observations in visible light, (b) observations in UV light 366 nm, (c) observations in UV light 254 nm.

A positive reaction for tannin compounds in the TLC test is the formation of black stains (Banu and Nagarajan, 2014) [3]. FeCl3as a spray reagent shows blackish purple spots this is caused by the formation of a complex between phenol and Fe compounds in the FeCl reagent$_3$.Testing for tannins by TLC showed positive results for containing tannin compounds, in Figure 4.

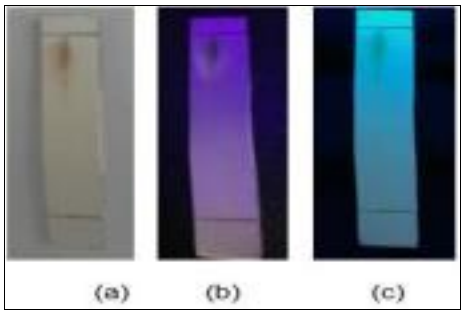

Fig 4: Tannin Test Results, Description: (a) observations in visible light, (b) observations in UV light 366 nm, (c) observations in UV light 254 nm.

In the identification of steroid compounds by TLC, the results of separation with Rf 0.51 were obtained, which were suspected to be steroid compounds due to the presence of blue spots after spraying with the Liberman-Burchard reagent seen in UV 366, in Figure 5. This is in accordance with the literature which states that positive reactions to steroid group compounds are marked by the presence of blue-green stains (Kristanti *et al*, 2008) [12].

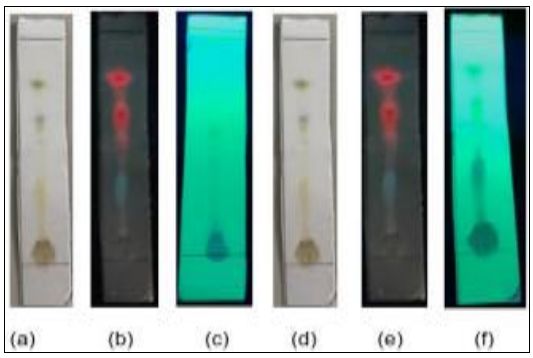

Fig 5: Steroid Test Results, Description: (a) visible light observation, (b) observation under UV light 366 nm, (c) observation under UV light 254 nm, (d) light observation after being sprayed with Liebermann-Burchard, (e) observation under UV light 366 after being sprayed with Liebermann-Burchard, (f) observation under UV light at 254 nm after being sprayed with Liebermann Burchard.

The results of the TLC analysis showed that the ethanol extract of horsetail leaves contains compounds belonging to the class of flavonoids, saponins, tannins and steroids. The results of this study are in line with the research of Utami., Y.P, *et al* 2022 [23], namely the results of the chemical content test showed that simplicia and extracts contained flavanoid, tannin, quinone, saponin, and steroid compounds. The biochemical content of flavonoids found in horse whip plants can be used as a source of antioxidants (Setiawan, 2019) [21]. According to Liew and Yoke (2016) [14] the ethanol extract of horsetail leaves has compounds with antioxidant properties mainly derived from phenolic acids, flavonoids and polyphenols. Derivatives of polyphenols as antioxidants can stabilize free radicals by complementing

the electron deficiency of free radicals and inhibiting the occurrence of chain reactions from the formation of free radicals (Hattenschwiller and Vitousek, 2000) [8]. As antioxidants, flavonoids can capture a number of oxidative ions, including superperoxide anions, hydroxyl radicals or peroxy radicals. Flavonoids can also quench singlet oxygen. One of the flavonoids with antioxidant activity is isoflavone compounds which are secondary metabolite compounds that are synthesized by plants (Irianti *et al*. 2018) [9].

Antioxidant Activity of *Stachytarpheta jamaicensis* (L) Valh. Leaves Extract

Measurement of antioxidant activity was carried out using the ABTS method. Condensed extract that has been stored at a storage temperature of 30^{TWO}C, the antioxidant activity was measured. The ABTS method is a method for determining antioxidant activity obtained from the oxidation of potassium persulfate with ABTS diammonium salt. The presence of antioxidant activity from the sample is indicated by the disappearance of the blue color in the ABTS reagent (Molyneux, 2004) [15]. The amount of antioxidant activity is indicated by the IC value50. IC value50shows the concentration value of the sample solution needed to reduce 50% of ABTS free radical activity.

The principle of testing antioxidant activity using the ABTS method is the removal of the color of the ABTS cation to measure the antioxidant capacity which directly reacts with the ABTS cation radicals. ABTS is a radical with a nitrogen center which has a characteristic blue-green color, which when reduced by antioxidants will change to a non-radical form from colored to colorless. The ABTS method is very sensitive to light, even the formation of ABTS requires an incubation time of 12-16 hours in the dark (Setiawan *et al*. 2018) [21].

Determination of the maximum wavelength was carried out by measuring the absorbance of the ABTS compound in the wavelength range of 600-800 nm and the wavelength that produced the maximum absorption of 667 nm was obtained. Measurements are made at the peak of the curve because at that peak the sensitivity is the highest. The results of measurements of ABTS free radical scavenging activity for vitamin C in table 2 show the IC value504.07 ppm which is included in the antioxidant category is very strong as a comparison, because vitamin C is the most powerful antioxidant substance in counteracting various free radicals (Amir *et al*. 2020) [1].

Table 2: Vitamin C Antioxidant Activity Test Table

Concentration (ppm)	absorbance	% Inhibition Concentracion	IC50 (ppm)
1	0,6290	12,68%	4,07
	0,6295		
	0,6294		
2	0,5892	18,25%	
	0,5891		
	0,5895		
3	0,5124	28,86%	
	0,5128		
	0,5129		
4	0,3819	47,00%	
	0,3820		
	0,3821		
5	0,2276	68,41%	
	0,2281		
	0,2275		
Blank	0,7202		

Sample measurements were carried out at various concentrations, the results of the measurements showed that the antioxidant activity of the ethanol extract of horsetail leaves in reducing ABTS radicals at a concentration of 20-60 ppm of ethanol extract gave an IC value.50of 39.41 ppm which is included in the very strong antioxidant category. This value indicates that horsetail leaf extract has very strong antioxidant activity close to its counterpart, which is less than 50 ppm in table 3.

Table 3: Antioxidant Activity Test of Horse Whip Leaf ABTS Method

Concentration (ppm)	absorbance	% Inhibition Concentracion	IC50 (ppm)
20	0,5353	25,71%	39,41
	0,5346		
	0,5359		
30	0,4423	38,78%	
	0,4409		
	0,4399		
40	0,3341	53,66%	
	0,3336		
	0,3348		
50	0,2437	66,25%	
	0,2432		
	0,2426		
60	0,2238	68,92%	
	0,2246		
	0,2235		
Blank	0,7204		

The increase in the percentage of inhibition in each ABTS test indicates that the greater the concentration of the extract, the greater the percent inhibition. This is supported by the research of Hernani and Roharjo (2005) which states that the percentage of inhibition (percent inhibition) of free radical activity will also increase with increasing concentration.

According to Liew and Yoke (2016) [14] the ethanol extract of horsetail leaves has compounds with antioxidant properties mainly derived from phenolic acids, flavonoids and polyphenols. The biochemical content of flavonoids and phenolics found in horsetail leaves can be used as a source of antioxidants (Setiawan, 2019) [21].

Conclusion

Identification of the compounds contained in horsetail leaf extract using the TLC method, which contained flavonoids, saponins, tannins and terpenoids with antioxidant activity using the ABTS method with an IC value5039.41 ppm is included in the very strong antioxidant category because it is < 50 ppm compared to Vitamin C which is 4.07 ppm. Activity very strong ethanol extract of horsetail leaf allegedly because it contains flavonoids which are antioxidants.

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