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Antioxidant Capacity Profile of *Dewandaru* Leaf (Extract *Eugenia uniflora L.*): Part of Usadha Bali

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Abstract

Bali has around 50,000 usadha palm oil which is a source of untreated herbal medicine. One of the plants that have the potential as an antioxidant and is traditionally used by the community is Dewandaru (Eugenia uniflora L.) plant. Dewandaru containing tannins, flavonoids, and anthocyanins. Dewandaru plants have antibacterial activity, antioxidants, and antidotes to free radicals. Making extract with six different solvents, among them methanol, ethyl acetate, n-butanol, chloroform, and n-hexane. The measurements were taken of the reduction of free radical activity by observing the absorbance at the maximum wavelength with a UV-Vis spectrophotometer alternately on the six samples. Secondary metabolites contained in Dewandaru leaves, namely flavonoids, tannins, and quinones. The value obtained is IC50 methanol extract 5,857 ppm; n-butanol extract 8,893 ppm; ethyl acetate extract 15,203 ppm; n-hexane extract 162.7315 ppm; and chloroform extract 75,873 ppm. Methanol extract, n-butanol, and ethyl acetate have very strong antioxidant activity because <50 ppm; n-hexane extract has weak antioxidant activity (ranging from 150-200 ppm), and chloroform extract has strong antioxidant activity (ranging from 50-100 ppm). Major conclusions: Dewandaru leaves has the potential as a natural antioxidant in the treatment section of Usadha Bali.

Contents

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1 Introduction

Various sources of free radicals can be found in our daily life, such as vehicle and factory smoke, radiation, food, and the result of the body's oxidation process. Free radicals are atoms or groups which have one or more unpaired electrons. The antioxidant is a compound which can obstruct the oxidation reaction by binding free radicals and molecule which are very reactive, therefore cell damage can be obstructed. This compound has lightweight of molecule but it can inactivate the development of oxidation reaction by preventing radical forming.

Usadha Balinese traditional healing science has not been scientifically developed either for the purpose of healing or for local drink. Moreover, it has not been developed globally yet. Bali has about 50.000 *lontar usadha*, the literature of herbal healing, which is not well developed. If it was developed properly, it could improve people's health and economic issues because its cost is relatively low and it does not have chemical impacts (Sutomo & Iryadi, 2019).

A health problem can be reviewed by health economic science. One of the medical plants which are used as medicine by Balinese traditional healer is *Dewandaru*. The use of traditional medical plants has not been developed scientifically. Therefore, nobody knows how its contents can heal people (Akrishnan, 2018). Its use is just based on people's beliefs, experience, and testimonies from the healed patients (Rasna *et al.,* 2017).

Nowadays many people seek alternative medication by using traditional medicine such as herbal medicine because its process is more natural, original, and relatively safe without any side effects like the synthesized medicines (Ekor, 2014). Traditional medicine is a medicine made of plants, animals, minerals of the mix of them (Yuan *et al.,* 2016). A plant can have a healing effect because it has phytochemical contents or secondary metabolites (Mulyani *et al.,* 2016).

One of the plants which potentially functions as antioxidant and has been traditionally used by the society is *Dewandaru* (*Eugenia uniflora* L.). It contains tannin, vitamin C, an essential compound like cineol, citronella, sesquiterpene, flavonoid, and anthocyanin. *Dewandaru* has an antibacterial, antioxidant, free radical antidote, hydrolysis and enzyme oxidation resistor, and anti-inflammation (Santoso, 2018).

The use of synthetic antioxidants nowadays gets serious attention because it is harmful and carcinogenic (Mbah *et al.,* 2019). Therefore, currently, a research is conducted regarding antioxidant development which made of natural resources that can be easily found, cheap and safe to be consumed by people (Atta *et al.,* 2017). However, scientific research toward phytochemical content and antioxidant activity of *Dewandaru* leaves extract has never been done. Although there are a lot of *Dewandaru* leaves available in Bali which is one of medical plants as mentioned in *Usadha* Bali.

From the explanation above, the researcher is interested in researching *Dewandaru* leaf extract which grows in Bali province. First of all, the researcher will do a phytochemical screening of *Dewandaru* leaves extract to find out secondary metabolites contained in Dewandaru leaves extract. Then conducting antioxidant activity tests as scientific proof to discover the property of that plant (Oliveira *et al.,* 2006; Consolini *et al.,* 1999; Victoria *et al.,* 2012; Mielke & Schaffer, 2010).

2 Materials and Methods

Research plan
This research is a laboratory descriptive research using the DPPH method to examine the antioxidant activity of *Dewandaru* leaves extract by using solvents such as methanol, n-butanol, ethyl acetate, n-hexane, and chloroform.

Materials
A sample that used in this research is *Dewandaru* leaves which taken from all over Bali which has been determined at LIPI. Chemical materials which used in this research such as ethyl acetate (Merck), n-butanol (Merck), methanol (Merck), standard DPPH (*2,2-difenil-1-pikrilhidrazil*) (Merck), ethanol 96% (Merck), HCl 2N (Merck), dragendorff reagent (Merck), mayer reagent (Merck), Pb acetate 10% (Merck), aquadest (Merck), FeCl 5% or FeCl 10% (Merck), chloroform (Merck), anhydrous acetic acid (Merck), dense sulfuric acid (Merck), and NaOH 1N (Merck).

Method
Dewandaru leaves extraction

Dewandaru leaves are picked from Bali areas (Abiansemal, Jl. Cargo Tanan No. 20 Denpasar, and Renon), they are then dried in the oven with temperature 40^{o}C. After that, they are blended into a powder. 200-gram simplistic of *Dewandaru* leaves powder is weighed. Then it is macerated by using solvent (n-buthanol, methanol, ethyl acetate, chloroform, and n-hexane) as much as 600 ml in a glass jar and stirred constantly for one hour. And then it is covered by aluminum foil and plastic wrap and hushed for three days (it must be stirred every day for 30 minutes). After three days, the extract is filtered manually and dried in the oven with temperature 40^{o}C.

Phytochemical Screening
Phytochemical screening of *Dewandaru* leaves extract includes an examination of compound groups such as Alkaloid, Flavonoid, Saponin, Tannin, Steroid/Triterpenoid, and Quinone.

a) Alkaloid
   2 ml test solution is veporized on the porcelain cup until obtaining the residue. The residue is then dissolved with 5 ml HCl 2N. After it is cold, then it is filtered. The obtained solution then divided into 3 test tubes. The first test-tube functions as blank. The second test tube is added with 3 drops of dragendorff reagent and the third test tube is added with 3 drops of Mayer reagent (through the tube wall). The formation of orange sediment in the second tube and yellow sediment in the third tube show the existence of Alkaloid (Susanti *et al.,* 2014).
b) Flavonoid
   1 ml test solution is added with 1 ml Pb acetate 10% (lead acetate), it positively contains flavonoid if there was yellow sediment (Natalia, 2016).
c) Saponin
   50 ml test solution is added with 5 ml aqua dest, then it is shaken for 10 seconds. If the foam is formed (1-10 cm height) in less than 10 minutes and it does not vanish when 1 drop of HCl 2N is added, it shows the existence of Saponin (Susanti *et al.,* 2014).
d) Tannin
   2 ml test solution is added with several drops of $FeCl_3$ 5% or $FeCl_3$ 10%. The formation of dark green or blue solution shows the existence of tannin (Natalia, 2016).
e) Steroid/Triterpenoid
   2 ml test solution is veporized in a vaporizer cup. The residue is dissolved with 3 ml chloroform, then moved into the test tube. After that, it is added by 3 ml anhydrate acetate acid and 2 ml dense sulfuric acid through the tube wall. The formation of a brown or violet ring on the solution border shows the existence of a triterpenoid, while if greenness blue rings appeared it shows the existence of steroids (Susanti *et al.,* 2014).

f) Quinone
5 ml test solution is added with several drops of NaOH 1 N, the formation of the red solution shows the existence of the quinone compound (Natalia, 2016).

Antioxidant activity test
Antioxidant activity test toward *Dewandaru* leaves extract is conducted by using ethyl acetate and n-buthanol solvent as seen in the following steps:

a) The Making of Master Raw Solution
Dewandaru leaves extract with ethyl acetate solvent is macerated with n-butanol to create a master raw solution in concentration 100 ppm as much as 50 ml. 5 mg dry extract is dissolved by methanol inside the volumetric flask 50 ml until meeting the border mark. Then it is shaken until homogeneous.
b) The Making of Standard Working Solution
Dewandaru leaves extract with ethyl acetate and n-buthanol 100 ppm diluted into concentration 20 ppm. 4 ml of master raw solution 100 ppm is dissolved by methanol inside volumetric flask 10 ml until meeting the border mark. Then it is shaken until homogeneous.
c) The Making of Test Sample Solution
From standard working solution 20 ppm ethyl acetate and n-buthanol extract of *Dewandaru* leaves are made into concentration 2; 4; 6; 8; 10 and 12 ppm by taking 1; 2; 3; 4; 5 and 6 ml. Each of them is put inside a volumetric flask 10 ml. Then it is added by methanol until meeting the border mark. After that, it is shaken until homogeneous.
Solution 20 ppm is taken 1, 2, 3, 4, 5, and 6 ml. Then each of them is put inside volumetric flask 20 ml and added by methanol until meeting the border mark. That solution is shaken until it creates a test solution with concentrations 1, 2, 3, 4, 5, and 6 ppm.
25 ml of 100 ppm solution is dissolved with methanol inside volumetric flask 50 ml until meeting the border mark. Then it is shaken until homogenous and becomes 50 ppm solution. 50 ppm solution of n-hexane and chloroform extract is then taken 1, 2, 3, 4, 5, 6, and 7 ml. After that, each of them is put into a volumetric flask 10 ml and added by methanol until meeting the border mark. That solution is shaken, so it becomes a test solution with concentration 5, 10, 15, 20, 25, 30, and 35 ppm.
d) The Making of Master Raw Solution DPPH with Concentration 100 ppm
Scaling 5 mg of DPPH powder then put it into volumetric flask 50 ml. It is added by methanol ad 50 ml and shaken until homogenous.
e) The Making of Standard Working Solution DPPH with Concentration 40 ppm
20 ml standard solution DPPH concentration 100 ppm is taken and put into volumetric flask 50 ml and added by methanol until meeting the border mark. It is shaken until homogenous.
f) The Determination of Maximum Wavelength of DPPH 40 ppm Solution
4 ml standard solution DPPH 40 ppm is taken and put into the cuvette, then its absorption spectrum is observed in wavelength 400-800 nm with spectrophotometer UV-Vis. 4 ml methanol is used for the blank solution. The maximum wavelength can be determined from the absorption curve.
g) The Measurement of DPPH Absorbance
4ml standard solution DPPH 40 ppm is taken 4 ml and put into the cuvette, then its absorption spectrum is observed in wavelength 400-800 nm with spectrophotometer UV-Vis. 4 mL methanol is used for the blank solution. The maximum wavelength can be determined from the absorption curve.
h) The Measurement of DPPH Free Radicals Damping Activity with Spectrophotometer UV-Vis
The measurement of free radical activity is done by taking 2 ml liquid DPPH 40 ppm. It is put into the test tube and added by 2 ml test solution from each concentration, shaken until homogenous and then hushed for 30 minutes. After that, observing the absorption in the maximum wavelength by using spectrophotometry UV-Vis in turn toward six concentrations for each test sample. The absorption of each extract is noted.
i) Determination of IC50 Value and the Making of Calibration Curve
From the absorbance result of each tested concentration, the damping percentage value is obtained by using the following formula:

$$\%\text{damping}=\frac{\text{absorbance DPPH} - \text{absorbance of test sample}}{\text{absorbance DPPH}} \text{x } 100\%$$

From the damping percentage on each concentration, then the regression curve is made, so the equation is y = bx + a and the result will be IC_{50} by the calculation in a linear regression where the extract concentration (ppm) as a basis (the x-axis) and subtuance percentage as ordinate (the y-axis) *(Astawa et al., 2017; Arora et al.,* 1998; Jayaprakasha *et al.,* 2003; Matalon *et al.,* 1990; Al-Mamary *et al.,* 2002). The value of IC_{50} is found from the calculation of a damping percentage of 50%.

From the subtuance percentage of each concentration, then regression curve is made, so the equation is found y = bx + a and the result will be IC_{50} by the calculation in a linear regression where the extract concentration (ppm) as a basis (the x-axis) and subtuance percentage as ordinate (the y-axis). The value of IC_{50} is found from 50% subtuance.

3 Results and Discussions

3.1 The result of simplistic and maceration production

Dried *Dewandaru* leaves are refined by using a blender. Simplisia powder of *Dewandaru* leaves is then macerated by using ethyl acetate and n-butanol solvent. After the maserat is obtained, then it is concentrated which produces dried *Dewandaru* leaves extract with ethyl acetate solvent 2.5742 gram and dried *Dewandaru* leaves extract with n-butanol solvent 1.3558 gram.

3.2 Phytochemicals screening result

Phytochemicals screening is done to find out the secondary metabolite content of *Dewandaru* leaves extract. Screening results of a secondary metabolite which positively contained in *Dewandaru* leaves in methanol, n-butanol, dan ethyl acetate extract are flavonoids, tannin, and quinone. Whereas its n-hexane and chloroform extract only positively contain flavonoid (Chhabra *et al.,* 1984; Tona *et al.,* 1998; Naz & Bano, 2013).

Based on phytochemicals screening results of the extract mentioned in table 1, it shows that methanol, n-butanol, and ethyl acetate of *Dewandaru* leaves extract (*Eugenia uniflora* L.) positively contain flavonoids, tannin, and quinone compound. While in alkaloid testing, saponin, steroid, and terpenoid show a negative result. The screening result of n-hexane and chloroform only positively contain flavonoid. It is due to no color change or sediment when it is added by reagents in the testing process. Flavonoids, phenolic, and tannin are phenol compounds that have a bunch –OH which tied in aromatic ring carbon. Flavonoid ability is very potential for antioxidant because of its molecule structure and hydroxyl bunch position (Agati *et al.,* 2012).

The result of the research shows that *Dewandaru* leaves positively contain flavonoids since there is yellow sediment on the sample which is reacted with Pb acetate condensation 10%. It is because the flavonoids have a benzene ring which has a hydroxyl bunch (Natalia, 2016).

Tannin testing is done by adding $FeCl_3$ which reacts with one of the hydroxyl rings that exist in tannin (Natalia, 2016). From this research, *Dewandaru* leaves positively contain tannin which can be seen from the formation of dark green solutions. Quinone testing is done by adding several drops of NaOH 1 N into the test solution. The result shows it positively contains the quinone compound by the formation of a red solution. This research shows that *Dewandaru* leaves extract to contain a quinone compound because of the formation of a brownish-red solution.

3.3 The result of the antioxidant activity test

Antioxidant activity test in *Dewandaru* leaves extract with methanol solvent is done in concentration 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm, dan 6 ppm. Ethyl acetate extract and n-butanol are done in concentration 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm, dan 12 ppm. N-hexane dan chloroform extract is done in concentration 5, 10,

15, 20, 25, 30, and 35 ppm which added by DPPH standard solution 40 ppm. The absorption is then measured by spectrophotometer UV-Vis. The measurement result of test sample absorption is presented in the following table:

Table 1
Screening Result of Dewandaru Leaves Extract

Test	Reactor	Observation Result				
		Methanol	n-butanol	ethyl acetate	n-hexane	Chloroform
Alkaloids	Dragendorff	(-)	(-)	(-)	(-)	(-)
	Mayer	(-)	(-)	(-)	(-)	(-)
Flavonoids	Pb acetate 10%	(+)	(+)	(+)	(+)	(+)
Saponin	Aquadest + HCl 2N	(-)	(-)	(-)	(-)	(-)
Tannin	$FeCl_3$ 5% or $FeCl_3$ 10%	(+)	(+)	(+)	(-)	(-)
Steroid/Triterpenoid	Anhydrant Acetate Acid + Concentrated Sulfuric Acid	(-)	(-)	(-)	(-)	(-)
Quinone	Solution NaOH 1 N	(+)	(+)	(+)	(-)	(-)

Table 2
The Absorbance of Dewandaru Leaves Extract Concentration with Some Solvent

No	Solution	Absorbance				
		Methanol Solven	n-Butanol Solven	Ethyl Acetate Solvent	n-Hexane Solvent	Chloroform Solvent
1	Control	0.387	0.426	0.375	0.378	0.274
2	1 ppm	0.344	-	-	-	-
3	2 ppm	0.322	0.391	0.357	-	-
4	3 ppm	0.289	-	-	-	-
5	4 ppm	0.254	0.372	-	-	-
6	5 ppm	0.220	-	-	-	0.268
7	6 ppm	-	0.354	0.337	-	-
8	8 ppm	-	0.331	0.326	-	-
9	10 ppm	-	0.311	0.313	-	0.259
10	12 ppm	-	0.274	0.298	-	-
11	15 ppm	-	-	-	0.38	0.26
12	20 ppm	-	-	-	0.37	0.23
13	25 ppm	-	-	-	0.36	-
14	35 ppm	-	-	-	0.34	-

Based on the table above it can be discovered that the absorbance of each compound is decreased. The bigger the concentration of the test sample solution, the smaller the absorbance of DPPH 40 ppm. It means DPPH as the free radicals have been able to be reduced by the antioxidant which exists in test sample solution which marked by the decrease of the absorbance.

3.4 The calculation of damping percentage

Based on the absorbance which obtained from the six concentration sample, then damping percentage is calculated by using the following formula:

$$\%\text{damping} = \frac{\text{DPPH absorbance} - \text{test sample absorbance}}{\text{DPPH absorbance}} \text{x } 100\%$$

Table 3
Damping percentage of Dewandaru leaves extract with some solvent

No	Solution	Damping Percentage (%)				
		Methanol Solven	n-Butanol Solven	Ethyl Acetate Solvent	n-Hexane Solvent	Chloroform Solvent
1	1 ppm	11.11	-	-	-	-
2	2 ppm	16.79	8.22	4.8	-	
3	3 ppm	25.32	-	-	-	-
5	5 ppm	43.15	-	-	-	2.18
6	6 ppm	-	16.90	10.13	-	-
7	8 ppm	-	22.30	13.06	-	-
8	10 ppm	-	26.99	16.53	-	5.47
9	12 ppm	-	35.68	20.53	-	-
10	15 ppm	-	-	-	0.26	6.56
11	20 ppm	-	-	-	2.38	14.59
12	25 ppm	-	-	-	5.29	-
13	35 ppm	-	-	-	11.37	-

Based on the table above, it can be seen that the damping percentage of each concentration increases. The bigger the concentration of the test sample solution, the higher the damping percentage. It means that the more the antioxidant in the test sample solution, the more the free radicals damping. It is marked by the increase of free radicals damping percentage.

3.5 The calculation of IC_{50} value

The calculation of IC_{50} value is done by making a relationship curve between the concentration of the test sample and damping percentage so that the linear regression equation is y = bx + a, where x is ppm concentration and y is IC_{50} percentage (Okoh *et al.,* 2014). The result can be seen in Figures 3 and 4.

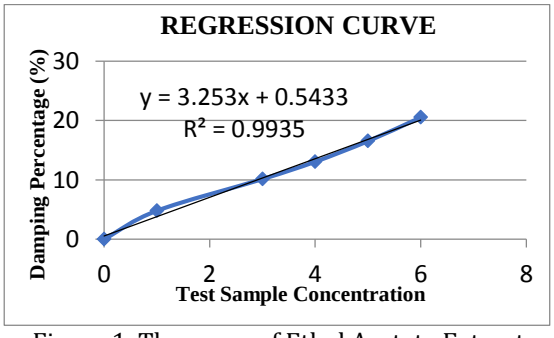

Figure 1. The curve of Ethyl Acetate Extract

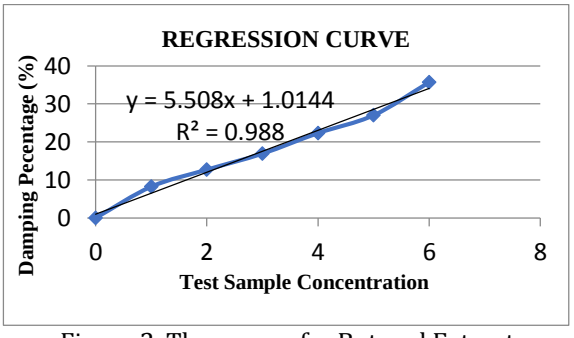

Figure 2. The curve of n-Butanol Extract

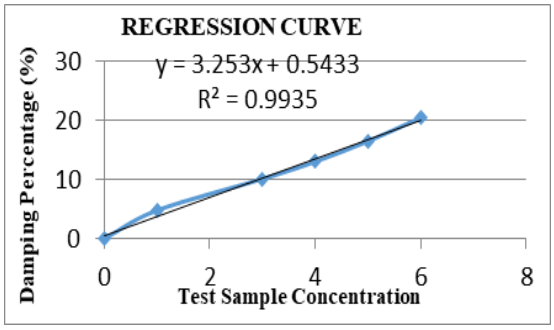

Figure 3. The curve of Methanol Extract

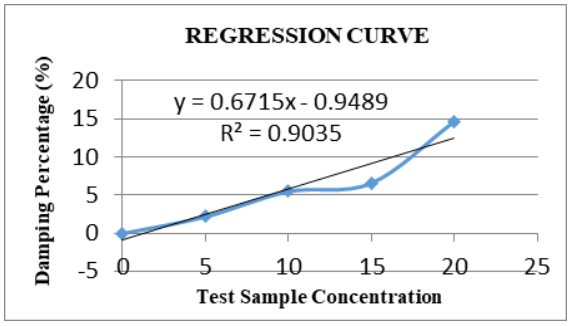

Figure 4. The curve of Chloroform Extract

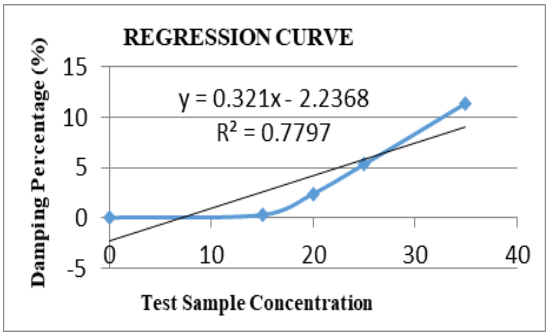

Figure 5. The curve of n-Hexane Extract

Based on Figure 1, relationship curve between the concentration of test solution and damping percentage, the regression equation is y = 3,253x + 0,5433, R^2 = 0,9935. From that equation, then the calculation of IC_{50} value is done by replacing the value of y = 50.

Based on Figure 2, relationship curve between the concentration of test solution and damping percentage, the regression equation is y = 5,508x + 1,0144, R^2 = 0,988. Based on Figure 3, relationship curve between the concentration of test solution and damping percentage, the regression equation is y = 3.253x + 0.5433, R^2 = 0.9935. Based on Figure 4, relationship curve between the concentration of test solution and damping percentage, the regression equation is y = 0.6725x – 0.9489, R^2 = 0.9035. Based on Figure 5, relationship curve between the concentration of test solution and damping percentage, the regression equation is y = 0.321x – 2.2368, R^2 = 0.7797.

This research shows that *Dewandaru* leaves extract has an antioxidant activity which marked by the existence of flavonoid secondary metabolite content which functions to muffle free radicals. The value of IC_{50} *Dewandaru* leaves methanol extract = 5.857 ppm, the value of IC_{50} *Dewandaru* leaves n-butanol extract = 8.893 ppm, the value of IC_{50} *Dewandaru* leaves ethyl acetate extract = 15.203 ppm, the value of IC_{50} *Dewandaru* leaves n-hexane extract = 162.7315 ppm, and the value of IC_{50} *Dewandaru* leaves chloroform extract = 75.873 ppm. Therefore, *Dewandaru* leaves extract is potentially used as part of *Usadha* Bali treatment as a natural antioxidant that can be used by society.

Table 4
Antioxidant properties based on IC_{50} value

IC_{50}Value	Antioxidant properties
50 ppm <	Very Strong
50 ppm – 100 ppm	Strong
100 ppm – 150 ppm	Medium
150 ppm – 200 ppm	Weak

The result of the research shows that methanol extract, n-butanol and ethyl acetate of *Dewandaru* leaves are classified into a very strong category of the antioxidant compounds because of the value of IC_{50} is less than 50 ppm. Meanwhile, chloroform extract is classified into a strong category of the antioxidant compound and the n-hexane extract is classified into a medium category of the antioxidant compound.

4 Conclusion

Based on the research result, it can be concluded that:

a) Based on the result of the phytochemical screening test of *Dewandaru* leaves with methanol solvent, ethyl acetate, and n-butanol, it is found that *Dewandaru* leaves positively contain flavonoids, tannin and quinone compound. While n-hexane and chloroform extract positively contain flavonoids only.
b) Dewandaru leaves have antioxidant activity.

Based on antioxidant activity test of *Dewandaru* leaves, it shows that the use of n-butanol solvent produces IC_{50} value = 8.893 ppm, ethyl acetate solvent produces IC_{50} value = 15.203 ppm, *Dewandaru* leaves methanol extract produces IC_{50} value = 5.857 ppm, *Dewandaru* leaves chloroform extract produces IC_{50} value = 75.873 ppm, IC_{50} value of Dewandaru leaves' ethyl acetate extract is 15.203 ppm. Based on the table of *Dewandaru* leaves' antioxidant properties, methanol, n-butanol, and ethyl acetate extract are classified into a very strong category of the antioxidant compound because its IC_{50} value is less than 50 ppm, while the chloroform extract classified into a strong category of the antioxidant compound and n-hexane extract classified into a medium category of the antioxidant compound.

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