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COMPARISON OF CHEMICAL AND PHARMACOLOGICAL RESEARCH DATA BETWEEN *VITEX TRIFOLIA* L. AND *VITEX ROTUNDIFOLIA* L.

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ABSTRACT

Both *Vitex trifolia* L. and *Vitex rotundifolia* L. known to have a number of flavonoids and flavonoid containing both casticin. The research looked at the comparison of chemical constituents of the extracts ethanol plant based phytochemical screening, tlc, and total flavonoid content, whereas pharmacological effect seen on the anti-inflammatory effect. Both extracts also tested on their antioxidant activities. The total flavonoid was analysed by using ultraviolet-visible spectrophotometer with aluminum chloride as the shifting-reagent and quercetin as the standard. Antiinflammatory activity assay was performed by using the carrageenan induced-method in five groups of rats. The tested groups were negative control (PGA 2%), positive control (acetocal 10 mg/kg of body weight), ethanol extracts of both viteces dose 500 mg/kg of body weight, 1000 mg/kg of body weight, and 2000 mg/kg of body weight, respectively. Results of the analysis of total flavonoid each *Vitex trifolia* and *Vitex rotundifolia* was 0.045% and 0.048% of the dry weight. TLC results and phytochemical screening showed an equivalent spot. Antioxidant properties of extracts of *Vitex trifolia* slightly better than *Vitex rotundifolia*. Anti-inflammatory activity of the ethanol extract of fruit of *Vitex trifolia* and *Vitex rotundifolia* respectively indicated by inhibition of inflammation by 21.44%, 30.78%, and 41.46% for the dose of 500 mg extract/KgBW, 1000 mg/kg, and 2000 mg/KgBW. and 21.40%, 30.88% and 41.47% for the dose of 500 mg extract/KgBW, 1000 mg/kg, and 2000 mg/KgBW. These results indicate that the use of extracts of *Vitex trifolia* L interchangeable with *Vitex rotundifolia* extract as an anti-inflammatory agent.

Keywords: *Vitex rotundifolia*, *Vitex trifolia*, flavonoids, quercetin, anti-inflammatory.

INTRODUCTION

Indonesian society has long recognized the use of plants for the treatment of disease. This is interesting to the researchers to scientifically test the efficacy of plants in order to determine the chemical content and biological activity. One of the plants is interesting to study *Vitex trifolia* L. are quite widely available in Indonesia. Legundi plants (Indonesian) or *Vitex trifolia* L. empirically used as a cure for stomach cramps, coughs, wounds, tonsillitis, puerperal fever, and typhus (Astuti and Ruspandi, 1998). Hosozawa *et al.* (1974) stated that *Vitex trifolia* efficacious as antimicrobial, anti-cytotoxic and anti-feeding activity. In other countries such as Thailand and China plants are widely used traditionally to have efficacy that is equivalent to *Vitex trifolia* is *Vitex rotundifolia. Vitex rotundifolia (*alaban tanah, Indonesian) said to be efficacious antibacterial, leukemia, anticarcinogenic and anti-mutagenic (Kawazoe *et al*., 2001; Ko *et al*., 2000).

Several studies have reported the chemical content of the fruit and leaves *Vitex trifolia* the flavonoid compounds (casticin; 3,6,7-trimethyl quersetagetin; vitexin; artemetin; 5-methyl artemetin; 7-desmetil artemetin; luteolin; luteolin-7-O-β-D-glucuronide; luteolin-3-O-β-D-glucuronide and isoorientin) (Zeng *et al*., 1996). Yang *et al* (2011) reported that Casticin-induced apoptosis involves death receptor 5 upregulation in hepatocellular carcinoma cells. Ye *et al*., 2010 mentioned that casticin also contained in *Vitex rotundifolia*. It said flavonoids are secondary metabolites that have potential as antiatherosklerosis agents, anti-inflammatory, antioxidant, anti-thrombogenic, antitumor, antiosteoporosis, and antiviral (Nijveld *et al*., 2001). Our previous study (Mustarichie *et al.*, 2012) reported on the in-silico prediction of the Interaction of Quercetin and Casticin With H4R, Anti-Inflammatory Receptor, As Supporting Data for Anti-Inflammatory Herbal Medicine.

MATERIAL AND METHODS

Plant Material: The material used in this study, *Vitex trifolia* L. was obtained from coastal areas in Karawang Barat, Indonesia while *Vitex rotundifolia* L. obtained from the shore of Merak, Indonesia.

Chemicals: Chemicals used in these experiments was 95% Ethanol, Acetosal, Vitamin C, distilled water, ammonia, Mayer reagent, Dragendorff, Bourchardat, Lieberman-Bouchard, magnesium metal powder, 2N HCl, amyl alcohol, 1% gelatin solution, $FeCl_3$, ether, a solution of 10% vanillin-sulfuric acid, 5% KOH,, $AlCl_3$, quersetin, PGA, Carrageenan. All chemicals used in these experiments unless otherwise was p.a grade.

METHODS

Material Preparation: Fruit *Vitex rotundifolia* and *Vitex trifolia* dried so that it becomes a form simplicia, then chopped or mashed.

Chemical methods

Extraction: Powdered fruit each viteces weighed and then extracted with soxhletation method using 95% ethanol. Liquid extract then thickened with a rotary evaporator at a temperature of 50-60^{o}C, followed by evaporation of residual solvent in the freeze dryer to obtain a constant weight viscous extract. Final extract obtained weighed and then calculated its yield.

Phytochemical screening: The method used was according to Farnsworth (1966), Dirjen POM (1979) and Mustarichie *et al*. (2011). Alkaloid Compounds tested with reagent Bouchardat, seen from the formation of red-brown color. Flavonoid compounds tested with amyl alcohol reagent, evidenced by the formation of a red color. Tannins and polyphenolic compounds tested with a solution of 1% $FeCl_3$ gives blue and black. Tannin tested with gelatin solution gives a white precipitate. Saponin compounds tested with the shuffle and is characterized by the formation of a stable foam in the filtrate. Triterpenoids and steroid compounds tested with reagent Liebermann-Bouchardat marked with purple and green to blue triterpenoids for steroids. Quinone compounds tested with KOH solution, evidenced by the formation of a yellow color.

TLC test

For Thin Layer Chromatography (TLC), silica gel plates prepared. Extract spotted on the plate that has given the start line and the finish line a few times. At the TLC eluent used 2 types, namely a mixture of n-hexane: EtOAc (4:6) and chloroform: methanol (95:5). Observations were made with visible light, UV 254, UV 366, stain $AlCl_3$ with heating and H_2SO_4 + warming.

Analysis of Total Flavonoids

Flavonoids content in the Ethanolic extract was Analyzed using modification of Chang *et al*., 2002) method. Quantification of total flavonoids was done using standard addition method with the help of shifting-$AlCl_3$ reagent. Total flavonoids calculated as quercetin equivalent (QE) w/w sample.

PHARMACOLOGICAL METHODS

Antioxidant activity assay: Antioxidant activity test was carried out by DPPH method as follows.

Preparation of DPPH solution: DPPH as much as 6 mg dissolved in 200 mL of ethanol to obtain stock solution of DPPH with the concentration of 30 ppm. DPPH solution was made fresh and kept at low temperature protected from light.

Wavelength measurements: As many as 3 mL DPPH solution dissolved in 2 mL of ethanol, homogenized, and measured at a wavelength of 400-750 nm.

Preparation of DPPH Standard Curve: DPPH solution was diluted to 12, 15, 18, 21, 24, 27 and 30 ppm. DPPH solution was allowed to stand 40 minutes each concentration and measured absorbance at a wavelength of maximum standard

Vitamin C as a comparison solution was diluted to 4, 5, 6, 7, 8, 9, and 10 ppm. Vitamin C solution of 2 mL of DPPH solution plus as much as 3ml, homogenized, and allowed to stand for 40 minutes at room temperature in a dark room. Absorbance was measured at a wavelength maximum of DPPH. The experiments were carried triplets. Used as a blank solution of 3 mL of ethanol and 2 mL of vitamin C.

Calculation of IC50 values

Percent inhibition of DPPH by antioxidants in the sample was calculated by the formula:

curve is then created.

$$\% \text{ Inhibition} = [\, 1 - (A_{test} / A_{control})] \times 100\ \%$$

Where:

A_{test} = Absorbance of DPPH solution in the sample

$A_{control}$ = Absorbance of DPPH solution ethanol (3 mL DPPH and 2 mL ethanol)

(Utami *et al*., 2005)

Preparation of test solution and Vitamin C

Condensed extract ethanol from viteces and the comparison of vitamin C weighed as much as 20 mg and dissolved in 50 mL of ethanol in order to get the mother liquor 400 ppm. Mother liquor was then diluted to a concentration.

Determination of DPPH Operating Time

DPPH solution 40 mg / ml mixed into the test sample with a ratio of DPPH: sample (3:2) and placed in a reservoir. Absorbance of this solution were observed at λ 450-650 nm at during a certain time span. The resulting absorbance data was then observed and presented in graphical form.

Extract Concentration Orientation

This procedure was performed to determine the minimum concentration of extract that could inhibit the activity of DPPH radical. The extract was diluted to a concentration of 2 ppm, 8 ppm and 16 ppm. Each extract as much as 2 mL plus 3 mL DPPH 30 ppm, shaken, and allowed to stand 40 minutes at room temperature in a dark room. Absorbance was measured at a wavelength of DPPH obtained. The experiments were carried triplets. If it was not be able to measure , the concentration was increased to 20 ppm, 40 ppm and 60 ppm.

Absorbance measurements of samples

Test solutions were diluted from stock solution to 80, 100, 120, 140, and 160 ppm. Test solution to 2 mL of DPPH solution plus as much as 3ml, homogenized, and allowed to stand for 40 minutes at room temperature in a dark room. Absorbance was measured at a wavelength maximum of DPPH. The experiments were carried triplets.

Acquired percent inhibition datas was plotted against the concentration of the extract. These data were evaluated using linear regression. IC_{50} value describes the concentration of extract required to inhibit 50% DPPH radical activity. IC_{50} values was obtained from the graph of extract concentration against the percent inhibition of DPPH.

Anti-inflammatory Activity Assays

Antiinflammatory activity assays performed using the method of induction of edema in the feet of mice with the aid of pletismometer. Experimental animals were divided into five groups: negative control, positive control acetosal 10 mg/KgBW, I test dose of 500 mg extract/KgBW, extract II test dose of 1000 mg/KgBW, and III trials extract dose 2000 mg/KgBW. Each test animals given the test substance was suspended in 2% PGA orally. An hour later 1% carrageenan induced increments of 0.05 ml subcutaneously in the foot test animals. Foot volume was measured before inducing rat carrageenan (Vo) and every hour after inducing over a span of five hours (Vt). Mean increase in paw edema volume compared

with the experimental initial leg volume, Percent inhibition of inflammation obtained by comparing the average volume of edema test against a negative control group.

RESULT AND DISCUSSION

Materials Processing

Fruit of *Vitex rotundifolia* L. and *Vitex trifolia* L. washed first to remove the possibility of impurities that could affect the results of the analysis of this study, such as dust, soil, or other debris that sticks to the surface of the fruit. Once clean, drying at room temperature. The purpose of drying was to remove water from the previous wash to prevent mushrooming, as well as to stop the enzymatic reaction and hydrolysis in the cells and tissues of plants. Water in plant tissues will evaporate and form pores that can be traversed by extracted solvent. Fruit that had been dried and then crushed into powder form simplicia. This was done so that when the extraction takes place, the surface area of simplicia that could be submerged by the more extensive solvent so that secondary metabolites that were interested could be optimized.

Extraction

Extraction is to extract active substances of particular secondary metabolites in plant parts. Extraction on research carried out by using soxhlet apparatus. This was chosen because the soxhletation was an effective way to attract secondary metabolites present in simplicia as extraction done by repeated. In addition, the expected secondary metabolites may be taken from a simplicia are heat resistant, so that by means of Soxhlet extraction method was one effective way.

Solvents used in the extraction process was 95% ethanol. Ethanol was chosen as solvent since ethanol is a solvent that can attract almost all secondary metabolites contained in simplicia and ethanol is relatively more secure because not toxic like methanol. Soxlehtation was done till colourless distillate.

Liquid extract obtained from this soxhletation process thickened and then evaporated using rotary evaporator on the water bath until thick extract obtained constant weight. Extract heavy viscous constant, indicating that there was no solvent in the condensed extract. Having obtained extract thick with a weight of constant, it was then calculated to yield fruit simplicia *Vitex rotundifolia* L. and *Vitex trifolia* L. using the formula:

Yield = (weight in condensed extract) / (Weight simplicia) x 100%

It was found viscous extract gained 2.29%. and 3.18% for *v. rotundifolia* and *v. trifolia*,

respectively.

Phytochemical screening

Phytochemical screening carried out to determine the class of secondary metabolites what was contained in the fruit of *Vitex rotundifolia* L. and *Vitex trifolia* L. Phytochemical screening on the research done on simplicia and extracts. Results of phytochemical screening can be seen in Table 1.

Table 1 The results of phytochemical screening Fruit *Vitex trifolia* L. and *Vitex rotundifolia* L.

Phytochemicals	*Vitex rotundifolia*		*Vitex trifolia*	
	Plant	Extract	Plant	Extract
Alkaloid	+	+	+	+
Polyphenol				
Tannin				
Flavonoid	+	+	+	+
Monoterpenoids				
Sesquiterpenoids				
Steroid				
Triterpenoid				
Quinon				
Saponin	+	+	+	+

Phytochemical screening results of fruit *Vitex rotundifolia* L. and *Vitex trifolia* L. extracts shows the similarity of their secondary metabolite content.

TLC Analytical results using chromatographic techniques using thin plate with solvent mixture of both plant samples shown in Figure 1.

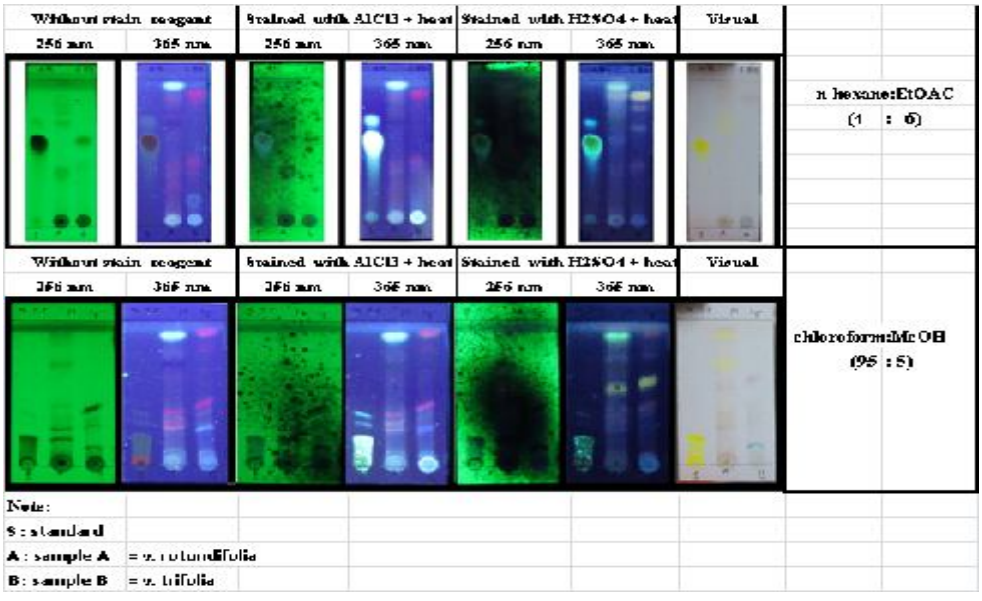


Figure 1. TLC of *Vitex rotundifolia* L. and *Vitex trifolia* L.

At the TLC 2 types eluent used, namely a mixture of n-hexane : EtOAc (4:6) and chloroform:methanol (95:5). Perhaps because of the concentration of flavonoids are low when compared to standard casticin, not too clear sighting stain. It provides guidance that the analysis of total flavonoid levels need to be done with the standard addition method. Nevertheless, the appearance of stains on *v.rotundifolia* extract (sample A) and *v.trifolia* (sampelB) showed the stains to match, either with or without stains reagents of $AlCl_3$ or H2SO4.

This result is equivalent to that of phytochemical screening of the extracts contain equal metabolites.

Analysis of Total Flavonoids Content

Analysis of total flavonoid content in fruit *Vitex rotundifolia* L. and the fruit of *Vitex trifolia* L performed using ultraviolet-visible spectrophotometer instrument. Flavonoids itself is a compound having the structure of a typical basis, ie two aromatic rings form a chromophore called ring A and ring B. Here is a picture of the structure of flavonoids:

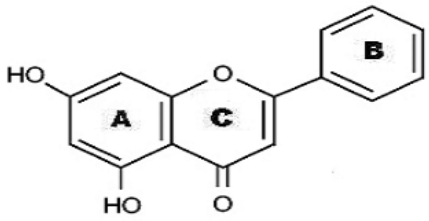


Figure 2. Flavonoid structure

When the the ultraviolet or visible wave passed flavonoid compounds, there will be a transition of an electron from the π to π * in ring A and ring B. This is why flavonoids in general may issue a peak at a specific wavelength. Generally be detected two maxima for the flavonoid, which is in the range of 240-285 nm are called ribbon II and in the 300-400 nm region known as the ribbon I (Malesev and Kuntic, 2007). Wavelength range will be different for each type of flavonoid depends auxochrome attached to the chromophore group in the flavonoid structure. Auxochrome attached to the chromophore will give bathochromic shift causing different wavelengths.

For the quantitative analysis of flavonoids, the colorimetric method is more commonly used (Wahyuningrum, 2006). Indonesian Ministry of Health (DepKes RI, 2000) guides the determination of total flavonoids based on standard parameters common medicinal plant

extracts. Quantitative analysis of total flavonoid done with standard addition method with the help of sliding $AlCl_3$ reagent. Because of the samples to be analyzed in the form of extracts, the matter content in the sample not only flavonoids alone. Therefore the standard addition method was chosen to equalize the conditions of the sample solution and matrix standard solution, so the calculation is obtained more accurate levels. The use of sliding $AlCl_3$ reagent aim to confirm the flavonoids present in a sample. Flavonoids form acid-resistant complex between the hydroxyl group and the neighboring ketone and form the non-resistant acid complexes with othodihydroxyl (Mabry *et al*, 1970). This causes auxochrome changes in the structure of the flavonoids, which in turn affects the resulting wavelength shift.

Estimation of total flavonoids content in this study was calculated as quersetin. Determination of total flavonoid using quercetin as standard due to quercetin belong to flavonol groups and as flavonoids identifier. It has been commonly used to analyze total flavonoids content (Chang *et al*., 2002; Umar, 2008).

Analyte solution made by extracting 0.1 grams of powder sample in 50 mL of ethanol 95% for 3x24 hours. Ethanol was used as a solvent because it was inert and did not interfere with the reading of the spectrum measured by the instrument. Analyte solution that had made as many as 50 mL and then added to the slide $AlCl_3$ reagent with the analyte solution ratio of 10 mL : 1 mL. Parent stock solutions were made in the concentration of 200 ug / mL (ppm). For measurement, a concentration of 50 ppm standard was made by diluting 5 mL of mother liquor with 95% ethanol by volume to 20 mL.

For sample preparation, made 5 different treatment of the analyte. Analyte solution was reacted with $AlCl_3$ reagent slide put into 20 mL volumetric flask as each 5 mL. Then into each flask was added a solution of 50 ppm standard number 0 mL, 1 mL, 1.5 mL, 2 mL, and 2.5 mL and diluted with ethanol until the volume reached 20 mL volumetric flask. It was measured using ultraviolet-visible spectrophotometer. Spectral measurements carried out at the wavelength range 240-480 nm due to the peak wavelength of flavonoids especially quersetin be seen clearly.

Absorbance values obtained by addition of standard to the sample can be seen in Table 2.

Table 2. Absorbance value obtained based on standard added to the sample

Amount of Samples (mL)	Amounts of standard (mL)	Concentration of standard (ppm)	Absorbance at 375 nm
5	0	0	0,0100
5	1	2,5	0,2129
5	1,5	3,75	0,3362
5	2	5	0,4205
5	2,5	6,25	0,5200

The standard addition curve was made to see the effect of concentration on the absorbance of the resulting samples. Standard addition curve obtained can be seen in Figure 3.

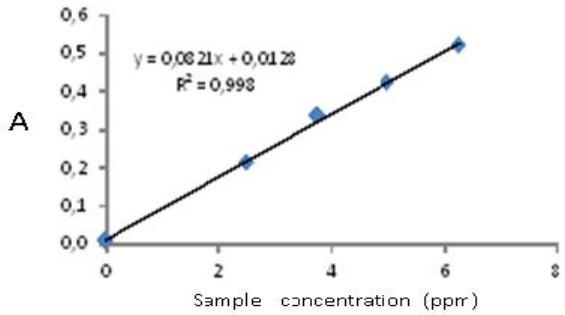


Figure 3. Standard addition curve

Standard addition curve obtained had a line equation y = 0.0821x - 0.0128 with R2 = 0.998, which indicated that the concentration was able to explain the diversity of the absorbance at 99.8%, and only about 0.2% was due to other factors. Based on this standard curve, the total flavonoid content could be determined and calculated as quercetin. At crosses the x-axis with a value of y = 0 could be considered as the maximum concentration of flavonoids present in the sample solution. When taking measurements with the calibration curve method, when there is no absorbance value at the maximum wavelength, the standard concentration values are zero. Meanwhile, if using a calibration curve method, when there is no valuce of the absorbance at the maximum wavelength at which point there is a difference between the value of the analyte concentration and the standard. Total flavonoid concentrations were calculated as quercetin be known when there was no absorbance value (y = 0) on the curve. Value of y = 0 can be put into the equation y = 0.0821x - 0.0128 to get the total flavonoid

content in the analyte. After getting the total flavonoid content, the percentage content of flavonoids in the sample can be calculated using the equation:

% total flavonoid content = (flavonoid concentration)/(sampel weight) x 100 %

Concentration of flavonoids were obtained from the 0.1 grams of sample analyzed were 31.2 mg. So that it can be obtained percentage of total flavonoid content in sample were 0.048% w/w and 0,045% w/w for *Vitex rotundifolia* and *Vitex trifolia,* respectively. This indicate that both samples has similar total flavonoids content.

Antioxidant test

To find an operating time which is an effective search time of measurement with DPPH shown in Table 3.

Table 3. Operation time of DPPH

Minute	A_1	A_2	A_3	A_{mean}	
0	0,7177	0,7170	0,7171	0,7173	
5	0,6991	0,6967	0,6988	0,6982	0,0191
10	0,6866	0,6873	0,6875	0,6871	0,0111
15	0,6807	0,6803	0,6797	0,6802	0,0069
20	0,6740	0,6750	0,6739	0,6743	0,0059
25	0,6700	0,6702	0,6702	0,6701	0,0042
30	0,6671	0,6659	0,6652	0,6661	0,0041
35	0,6625	0,6634	0,6634	0,6631	0,0030
40	0,6607	0,6609	0,6592	0,6603	0,0028
45	0,6574	0,6568	0,6572	0,6571	0,0031
50	0,6545	0,6560	0,6545	0,6550	0,0021
55	0,6538	0,6538	0,6533	0,6536	0,0014
60	0,6528	0,6526	0,6522	0,6525	0,0011
65	0,6512	0,6510	0,6499	0,6507	0,0018
70	0,6500	0,6494	0,6504	0,6499	0,0008
75	0,6482	0,6495	0,6496	0,6491	0,0008
80	0,6480	0,6490	0,6480	0,6483	0,0008
85	0,6485	0,6473	0,6476	0,6478	0,0005
90	0,6477	0,6475	0,6472	0,6475	0,0003
95	0,6468	0,6468	0,6471	0,6469	0,0006
100	0,6464	0,6471	0,6463	0,6466	0,0003
105	0,6454	0,6482	0,6454	0,6463	0,0003
110	0,6454	0,6451	0,6442	0,6449	0,0014
115	0,6442	0,6456	0,6455	0,6451	-0,0002
120	0,6446	0,6444	0,6441	0,6444	0,0007

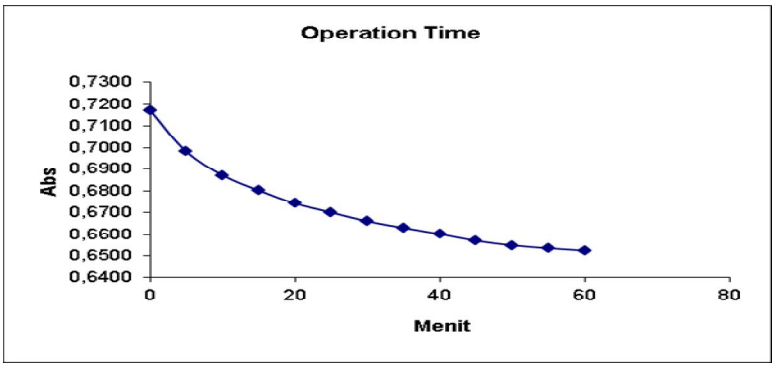
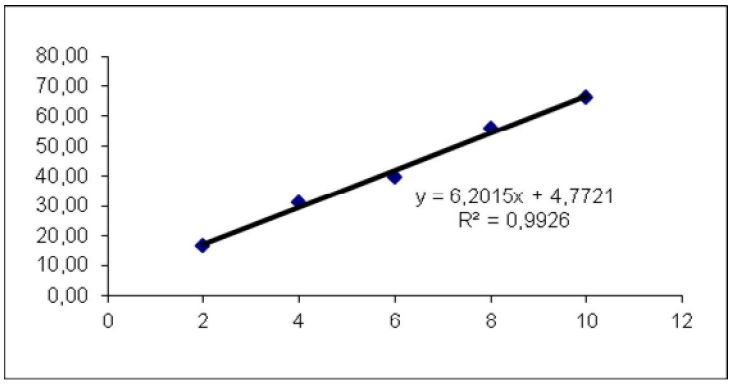


Figure 3. DPPH operation time

Based on the operation time curve, then the time chosen for the reaction time was 30 minutes.

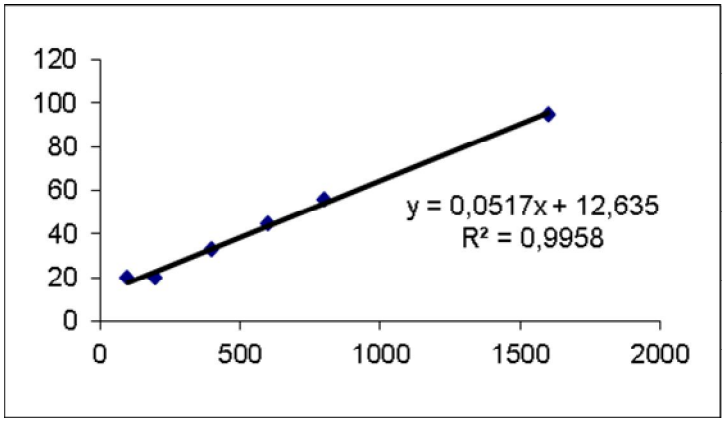
For Vitamin C, it was found the following data:

Vit C (ppm)	A1	A2	Amean	% Inhibition
DPPH	0.7703	0.7653	0.7678	
2	0.6398	0.6386	0.6392	16.75
4	0.5278	0.5288	0.5283	31.19
6	0.4635	0.4642	0.4639	39.59
8	0.3376	0.3376	0.3376	56.03
10	0.2593	0.2575	0.2584	66.35
IC_{50}	**50**	**7.293.058**		



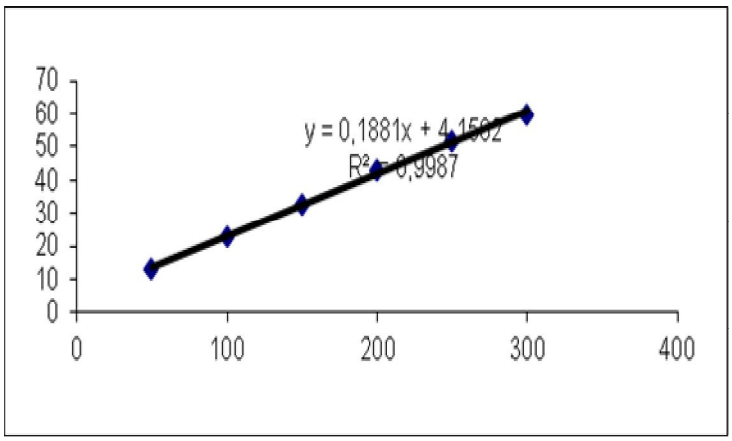
For *Vitex rotundifolia* , it was obtained the following data:

A ppm	A1	A2	A rata2	% inhibisi
DPPH	0,6875	0,6876	0,68755	
100	0,5538	0,5539	0,55385	19,44586
200	0,5507	0,551	0,55085	19,88219
400	0,4605	0,4602	0,46035	33,04487
600	0,3825	0,3825	0,3825	44,36768
800	0,304	0,3039	0,30395	55,79231
1600	0,0387	0,037	0,03785	94,49495
IC 50	**50**	**722,7273**		



For *Vitex trifolia* was:

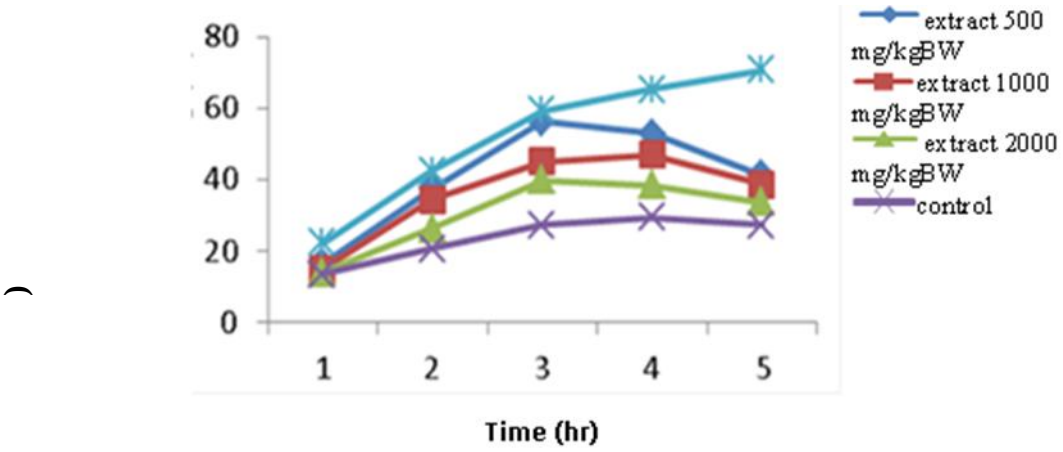
B ppm	A1	A2	A rata2	% inhibisi
DPPH	0,5731	0,5727	0,5729	
50	0,4981	0,4972	0,49765	13,13493
100	0,4421	0,4423	0,4422	22,81375
150	0,3864	0,387	0,3867	32,50131
200	0,3284	0,3284	0,3284	42,67761
250	0,2773	0,2777	0,2775	51,56223
300	0,231	0,2311	0,23105	59,6701
IC 50	**50**	**243,7523**		



The results of this test shows that both extracts *v.trifolia* and *v. rotundifolia* have weak antioxidant properties when compared with vitamin C. From IC_{50} obtained, the antioxidant effect of *v.trifolia* slightly better than *v. rotundifolia.*

Antiinflammatory Assay

Anti-inflammatory activity of the test results with the method using the carrageenan induced edema in the legs mice showed anti-inflammatory activity of the extract given by *Vitex trifolia* and *Vitex rotundifolia* as shown below.



Figue 4. Curve of % Inflammation against Time

Anti-inflammatory effects of the test material is comparable to the more extensive small percentage curve inflammation. From Figure 4 it can be seen that the outer curve of the positive control has the least curve which means the positive control group is the most excellent. From these data it can be calculated percentage inhibition of inflammation of each test sample. Comparison a test dose to the positive control can be seen in Figure 5.

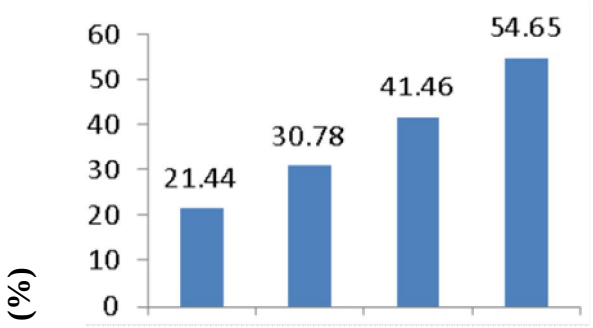


Figure 5 Comparison of Percentage Inhibition of Inflammation Each Test Group

Both extracts were treated in the same way and the results was that anti-inflammatory activity of the ethanol extract of fruit of *Vitex trifolia* and *Vitex rotundifolia* respectively indicated by inhibition of inflammation by 21.44%, 30.78%, and 41.46% for the dose of 500 mg

extract/KgBW, 1000 mg/kg, and 2000 mg/KgBW and 21.40%, 30.88% and 41.47% for the dose of 500 mg extract/KgBW, 1000 mg/kg, and 2000 mg/KgBW

CONCLUSIONS

Results of the analysis of total flavonoid calculated as quercetin of *Vitex trifolia* and *Vitex rotundifolia* was 0.045% and 0.048% of the dry weight, respectively. TLC results and phytochemical screening showed an equivalent spot. Antioxidant properties of extracts of Vitex trifolia slightly better than *Vitex rotundifolia.* Anti-inflammatory activity of the ethanol extract of fruit of *Vitex trifolia* and *Vitex rotundifolia* respectively indicated by inhibition of inflammation by 21.44%, 30.78%, and 41.46% for the dose of 500 mg extract/KgBW, 1000 mg/kg, and 2000 mg/KgBW. and 21.40%, 30.88% and 41.47% for the dose of 500 mg extract/KgBW, 1000 mg/kg, and 2000 mg/KgBW. These results indicate that the use of extracts of *Vitex trifolia* interchangeable with *Vitex rotundifolia* extract as an anti-inflammatory agent.

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