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COMPARISON OF IC50 AND AAI VALUE OF ETHANOL EXTRACT KOPASANDA LEAVES (CHORMOLAENA ODORATA) AS AN ANTIOXIDANT USING THE DPPH METHOD FOR PERCOLATION AND SOCKLETATION EXTRACTION

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ABSTRACT

The Kopasanda plant is a plantplant species that are easily found in various places that have themefficacy as an antioxidant.Objective: to determine the antioxidant value of the ethanol extract of kopasanda leaves using the DPPH method of percolation and soxhletation extraction which is expressed using IC50 and AAI in a quantitative descriptive manner. Kopasanda leaves were extracted using the percolation and soxhletation method with 70% ethanol solvent and phytochemical screening and antioxidant activity of the extract were carried out using the DPPH method. Data analysis uses linear regression testing. The phytochemical screening test showed that the ethanol extract of kopasanda leaves contained positive flavonoids, saponins, tannins and alkaloids. The average percent reduction of DPPH by the perchloation extraction method at concentrations of 40 ppm and 120 ppm: 40.87% and 63.94%, while the soxhletation extraction method: 39.82% and 67.42%. The average IC50 value of the ethanol extract of kopasanda leaves for the percolation extraction method: 74.1081 ppm and the soxhletation extraction method:68.1975 ppm. The results of the antioxidant activity test of kopasanda leaves using the DPPH method for percolation and soxhletation extraction are included in the strong antioxidant category with a medium antioxidant index.

Keywords: antioxidants; kapasanda leaves; percolation; soxhletation

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INTRODUCTION

The kopasanda plant (Chromolaena odorata L) is a type of plant that is easily found in various places. Kopasanda plants also act as inhibitor plants because they are competitors in absorbing water and nutrients which are detrimental to surrounding plants, but on the other hand this plant has many benefits for human life, for example in the agricultural sector it is useful as organic fertilizer, biopesticide and herbicide and in the health sector. Traditionally used as a medicine for wounds, diabetes, coughs and is useful in stopping bleeding. Kopasanda leaves are rich in polyphenols, tannins, saponins, flavonoids and alkaloids (Hermiati, et al., 2013). The kopasanda plant has the regional names Kirinyuh (Sunda), Takelan (Java), Lenga-lenga (North Sumatra), Kopasanda (Makassar), Census (Flores) and is a plant that is useful for treating sore throats, coughs, malaria medicine, antimicrobial, antidiarrheal, wound medicine that is often used by the public. Kopasanda leaf extract has an antioxidant effect because it has high levels of flavonoids which can inhibit the oxidation process(Putri et al., 2020).

Antioxidants are chemical compounds that can break free radical chain reactions and can inhibit biological processes in the body that can be dangerous and detrimental. Antioxidants have a very important influence, namely as protective factors because these compounds can delay or prevent lipid oxidation by inhibiting the oxidation chain reaction.(Setiabudi et al., 2020).The human body will be protected by natural antioxidants from damage caused by reactive oxygen compounds, able to prevent the occurrence of degenerative diseases. Synthetic antioxidants can cause side effects that are dangerous to health in the human body because they are carcinogenic. Therefore, antioxidant compounds are needed to reduce the negative effects of free radicals(Devitria, 2020).

According to researchPrincess of 2020against the antioxidant activity test of 70% ethanol extract of kopasanda leaves using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method with maceration extraction, very strong antioxidant activity was obtained with an Inhibitory Concentration or IC50 (Inhibition Concentration) value of less than 50 ppm, so it was concluded that with A high flavonoid content will result in a higher antioxidant inhibitory power in the oxidation process.Based on the benefits of kopasanda leaves, researchers need to test the antioxidant activity of the ethanol extract of kopasanda leaves using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method using two types of extraction, namely percolation and soxhletation with the aim of comparing the IC50 and AAI (Antioxidant Activity Index) values. Objective: to determine the antioxidant value of ethanol extract of kopasanda leaves using the DPPH method of percolation and soxhletation extraction expressed by IC50 and AAI.

METHOD

This type of research is experimental. The population in this study were kopasanda plants originating from Liliba Village, Kupang City. The sample in this study was the ethanol extract of kopasanda leaves. The dependent variable is antioxidant activity. The DPPH method is a method used to test the antioxidant activity of the ethanol extract of kopasanda leaves using a UV-Vis spectrophotometer to measure the antioxidant value. AAI was used to determine the antioxidant activity index of the ethanol extract of kopasanda leaves.The equipment used is a glass beaker (Pyrex), a crane analytical balance (type EW 220-3 NM), UV-Vis spectrophotometry (Shimadsu type UV-1700), a rotary evaporator (Eyela type N-1000), a measuring flask (Pyrex), percolation vessel, dropper pipette (Pyrex), test tube (Pyrex), blender (Philips), stir bar (Pyrex), vial (Glory), porcelain cup, parchment paper, filter paper, spatula, flannel cloth, oven, tissue, aluminum foil, percolator, soxhletation tool, mattress thread and scissors.The materials used were 1.1--diphenyl-2-picrylhydrazyl, kopasanda leaves, 70% ethanol (Onemed), 95% ethanol (Onemed), Mayer's reagent (Merck), Dragendrof's reagent, 1% FeCl3. Research procedures were carried out through several stages, including 1). Material collection. 2). Making simplicia powder (Octariani et al., 2021). 3). Extraction: Percolation and soxhletation. 4). Phytochemical screening: a). Identification of alkaloids: (Saraswati et al., 2019). b). Identification of flavonoids (Saraswati et al., 2019). c) Tannin identification: (Saraswati et al., 2019). d). Identification of saponins: (Saraswati et al., 2019). 5). Testing antioxidant activity: a) Preparation of DPPH solution (Sari et al., 2021). b). Determination of Maximum Wavelength (Sari et al., 2021). c). Preparation of test solution: (Sari et al., 2021). d). Absorbance Measurement (Sari et al., 2021). This research was conducted at the Laboratory of Chemistry, Pharmacognosy, Instrument Analysis, Pharmacy Study Program, Poltekkes Kemenkes Kupang in May - June 2023.

RESULTS

Table 1.
Phytochemical Screening Results of Ethanol Extract of Kopasanda Leavesin Liliba Village, Kupang City in 2023

Identification	Results	Literature	Conclusion
Flavonoids	Red	Extract + 5-6 drops of concentrated HCI + Mg powder forms a red color(Saraswati, 2019)	Positive
Saponin	There is foam	Extract + water until all parts are submerged, shake vigorously until foamy. If the foam remains constant for 15 minutes then the extract is positive for containing saponin compounds(Saraswati, 2019)	Positive
Tannin	Blackish green	Extract + 3 ml warm water + 1-2 drops of 1% FeCl3, a dark blue or blackish green color forms, indicating the presence of tannin group compounds(Saraswati, 2019)	Positive
Alkaloids	With Dragendrof's reagent: red precipitate, Mayer's reagent: yellow	Extract + HCl + 2-3 drops of Dragendorff's reagent, if there is a red precipitate then it is positive for alkaloids, but if added with 2-3 drops of Mayer's reagent it produces a yellow precipitate then it is positive for alkaloid compounds(Saraswati, 2019)	Positive

Table 1 above shows that the ethanol extract of kopasanda leaves contains active compounds, namely flavonoids, saponins, tannins and alkaloids.

Table 2.
Test Results of DPPH Reducing Activity of Ethanol Extract of Kopasanda Leaves Using Percolation and Soxhletation Extraction Methodsin Liliba Village, Kupang City in 2023

Extraction	Concentration (ppm)	Percent attenuation (%)			Mean percent attenuation (%) ± SD	Linear regression equation
		Replication I	Replication II	Replication III		
Percolation	40	35.98	30.61	56.04	40.87	y=0.2951x + 29.528 r= 0.9921
	60	46.91	31.60	62.39	46.96	
	80	49.14	44.22	68.84	54.06	
	100	57.92	46.82	74.75	59.83	
	120	62.39	51.56	77.88	63.94	
Soxhletation	40	60.79	26.23	32.35	39.82	y=0.3486x + 25.1 r= 0.9968
	60	62.02	36.67	37.24	45.31	
	80	67.23	43.68	46.78	52.56	
	100	75.71	57.94	52.89	59.83	
	120	79.78	66.74	55.74	67.42	

Table 2 showsthat the percent dampening of the ethanol extract of Kopasanda leaves by the percolation extraction method has different average values of the percent dampening of DPPH, where at a concentration of 40 ppm the average percent dampening is 40.87% and at a concentration of 120 ppm the average percent dampening is obtained. amounting to 63.94% with the linear regression equationy = 0.2951x+29.528 and correlation coefficient (r) = 0.9921, while the average percent attenuation of the soxhletation extraction method for a concentration of 40 ppm is 39.82% and a concentration of 120 ppm is 67.42% with the linear regression equationy = 0.3486x +25.1and correlation coefficient (r) = 0.9968.

Table 3.
IC50 Value of Ethanol Extract of Kopasanda Leaves Percolation Extractionand Soxhletation in Liliba Village, Kupang City Year 2023

Extraction	IC50 value			
	Replication I	Replication II	Replication III	Average
Percolation	74.0457 ppm	115.9044 ppm	32.3742 ppm	74.1081
Soxhletation	27.6694 ppm	83.4257 ppm	93.4975 ppm	68,1975

Table 3 showsThe IC50 value of the ethanol extract of Kopasanda leaves using the percolation method was 74.1081 and soxhletation was 68.1975. This value states that the ethanol extract of kopasanda leaves has strong antioxidant activity because the IC50 is <100 ppm.

Table 4.
AAI Value of Kopasanda Leaf Ethanol Extract Percolation and Soxhletation Extractionin Liliba Village, Kupang City in 2023.

Extraction	AAI value			Average
	Replication I	Replication II	Replication III	
Percolation	0.6752	0.4313	1.5444	0.8836
Soxhletation	1.8070	0.5993	0.5347	0.9803

Table 4 above shows that the average AAI value for the percolation method is 0.8836 and soxhletation is 0.9803.

DISCUSSION

Phytochemical Screening Results of Ethanol Extract of Kopasanda Leaves

The aim of the phytochemical screening test is todetermine the content of active compounds in the extract qualitatively, namelysecondary metabolite compounds such as fLavonoids, saponins, tannins and alkaloids are qualitatively found in kopasanda leaf extract with each active compound providing positive results when reacted with certain reagents. Flavonoids are polar compounds that can dissolve in polar solvents such as ethanol, where ethanol is able to free flavonoids from their salt form. The addition of concentrated hydrochloric acid functions to protonate flavonoids until flavonoid salts are formed. The formation of a red color occurs due to the addition of magnesium powder which binds to the carbonyl group of flavonoids, causing the flavonoid compounds to be reduced.

Saponin compounds containing glycosides when shaken easily form foam or froth that can last for 30 seconds. The tannin compound test using ferric chloride reagent showed positive because a dark blue or blackish green color was formed. Alkaloid compounds are also found in kopasanda leaf extract because alkaloids have alkaline properties which when reacted with Dragendorff or Mayer reagents will produce a yellow precipitate or turbidity. The solvent used is ethanol because it is polar so it is able to extract flavonoids, saponins, tannins and alkaloids which are compounds with functional groups, have nitrogen and oxygen atoms and polar double bonds (Fitriah et al., 2017). The results of this study are in line with research (Agustina and Resmayani, 2022) that the ethanol extract of kopasanda stems had flavonoid levels of 25.03 ± 4.448 mgQE/g. Supporting research according to (Nurjanah *et al*, 2020) is that the ethanol extract of kopasanda leaves contains qualitative flavonoids, saponins, tannins and alkaloids.

Test Results of DPPH Reducing Activity of Ethanol Extract of Kopasanda Leaves Using Percolation and Soxhletation Extraction Methods

Percolation is a process where the simplicia is refined and then extracted with the appropriate ingredients by passing it slowly through a column or in other words extraction with a new solvent and is generally carried out at room temperature. Meanwhile, soxcletation is an extraction method with a new solvent using a specific tool so that the extraction is stable using a reverse cooling tool. Based on the linearity values of percolation and soxhletation extraction, it can be concluded that there is a linear relationship between the concentration and absorbance of the grade series solutions prepared. If the linearity value approaches 1, the results obtained are more linear. DPPH's function is as a free radical, where the free radical will be reduced by samples containing antioxidants or flavonoids. The principles of the DPPH method are: Antioxidant compounds will donate hydrogen atoms to DPPH radicals, causing DPPH to become a non-radical reduction form. DPPH in nonradical form will lose its purple color. The antioxidant ability of the ethanol extract of kopasanda leaves can be observed from the reduction in the intensity of the purple color of the DPPH solution when it is reacted (Hujjatusnaini *et al*, 2021). This research is in line with the results of research (Princess, 2020) on the antioxidant activity test of 70% ethanol extract of kopasanda leaves using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method with maceration extraction, very strong antioxidant activity was obtained with an Inhibitory Concentration or IC_{50} (Inhibition Concentration) value of less than 50 ppm, so it was concluded that with a high flavonoid content will result in a higher antioxidant inhibitory power in the oxidation process.

IC50 Value of Extract and AAI Ethanol of Kopasanda Leaves Percolation Extractionand Soxhletation

The higher the concentration of the sample extract, the greater the percent attenuation. If the percent damping value is high, the lower the IC_{50} value. The IC_{50} value is the concentration at which the sample buffer inhibits free radicals by 50% which is obtained using the linear regression equation y = bx + a. The most active antioxidant activity value in this study was soxhletation extraction because it had a higher IC_50 value, namely 68.1975 ppm compared to percolation extraction with an IC_{50} value of 74.1081 ppm. The results of this study are also different from research (Agustina and Resmayani., 2022) that the activity of antioxidant compounds obtained an IC_{50} value of 156.22 ppm in the weak antioxidant category. The research results are in line according to (Fatmawati, 2019), that the IC_{50} calculation results from each percolation extract method were 33.49 μg/ml. The smaller the IC_{50} value, the stronger the antioxidant activity.

The same research according to (Latif., 2015) where the percent antioxidant activity in the 80% ethanol extract and the chloroform fraction at a concentration of 100 ppm was 64.93% and 54.9% respectively with IC_{50} values of 13.07 mg/mL and 81.9 mg/mL. The 80% ethanol extract and chloroform fraction have great potential when used as a source of natural antioxidants. Phytochemical tests show that the 80% ethanol extract contains flavonoids, alkaloids and tannins. Meanwhile, the chloroform fraction extract contains flavonoid compounds. This research is in line with the research results (Nurhasanawati, 2017) shows that there is an influence of the extraction method on the antioxidant activity of 70% ethanol extract of guava leaves. Even though both are classified as having very strong antioxidant activity, the best antioxidant activity was in the soxhletation method with an IC_{50} value of 37.67 ppm while the IC_{50} value

AAI Value of Kopasanda Leaf Ethanol Extract Percolation and Soxhletation Extraction

Antioxidants are useful for humans to reduce the negative effects of free radicals. How antioxidant b works is by giving hydrogen atoms from the antioxidant to free radicals, thereby reducing the reactivity of these radicals. AAI value of Kopasanda leaf ethanol extract shows that the average AAI value for the percolation method is 0.8836 and soxhletation is 0.9803. This value shows that the ethanol extract of kopasanda leaves has moderate antioxidant activity with an AAI value < 1. Results of this researchdifferent from the research results (Mamay et al., 2022) with resultquantitative antioxidant activity test of the ethanol extract of kopasanda leaves using the maceration method has very strong antioxidant activity with an AAI value>2.

CONCLUSION

Based on the results of the research above, it can be concluded that the ethanol extract of kopasanda leaves (Chormolaena odorata L) as an antioxidant using the DPPH method for percolation and soxhletation extraction, contains secondary metabolite compounds such as flavonoids, saponins, tannins and alkaloids. Sample extraction by percolation and soxhletation has DPPH reduction. Sample extracts by percolation and soxhletation have strong IC50 and AAI values.

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