IDENTIFICATION OF CHEMICAL COMPOUNDS, TOTAL PHENOLIC COMPOUND CONTENT AND ANTIOXIDANT POTENTIAL OF *Acorus calamus* L. LEAF AND RHIZOME EXTRACT

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Acorus calamus L. is one type of swamp plant that grows wild. Antioxidant potential and initial examination of chemical compound and total phenolic of *Acorus calamus* L. are important to study to estimate potential of these plants for various application. This study tested the potential of leaves and rhizome of *Acorus calamus* L. which were processed into extracts by maceration immersion method with ethanol 70% solvent. Identification of groups of chemical reactions and qualitative testing processes for several groups of chemical compounds. Determination of total phenolic content was determined by Folin-Ciocalteu method. Antioxidant activity test was carried out using DPPH method. The results showed that extraction of leaves and rhizomes of *Acorus calamus* L. yielded yields of 10.42 %(w/w) and 11.82 %(w/w), respectively. The chemical compounds identified in fresh *Acorus calamus* L. leaves were alkaloids, flavonoids, steroids and phenolics, while in fresh *Acorus calamus* L. rhizome the presence of alkaloids, flavonoids, terpenoids and phenolics was identified. The total phenolic content of *Acorus calamus* L. leaves was measured 5.72% and that of *Acorus calamus* L. rhizome was 2.42%. Antioxidant activity of extract of *Acorus calamus* L. leaves was 137.39 mg/L and 96 mg/L for *Acorus calamus* L. rhizome. The conclusion of this study showed that leaves and rhizome of *Acorus calamus* L. have potential to be used as antioxidants with a higher value was in *Acorus calamus* L. rhizome which have strong potential of antioxidant activity.

Key words: *Acorus calamus* L., antioxidant activity, *Acorus calamus* L. leaves, *Acorus calamus* L. rhizome, phenolic content

INTRODUCTION

Antioxidant is chemical compound that can stop or control the process of chemical substance's oxidation. There are many chemical compounds in plants that have a function as antioxidants. Chemical compounds that are classified as secondary metabolite compounds can act as antioxidants. These compounds include: phenolic compound. Phenolic compounds are found in many plants. Phenolic compounds and polyphenolic compounds have antioxidant activity due to the hydroxyl groups present in the compounds (Zeb, 2020). Antioxidant have a very important function and role in human body health. Sources of antioxidants come from inside and outside human body. Inside human body, source of antioxidant are limited and decreased in number as

humans age, therefore human need antixodant from other source for consumption. Antioxidant are found in various plants like fruit, vegetables and many potentially medicinal plants which are found in abundance in nature, especially in Indonesia as a country which is rich in various type of plants.

Acorus calamus L. is one type of swamp plant that grows wild that have many of pharmacological effect like antioxidant. The pharmacological effect of *Acorus calamus* L. are antibacterial activity, antifungal activity, antioxidant activity, antidiabetic activity, anti-inflammatory, anticancer, alagesic, antimutagenic, anti-asthmatic, antispasmodic, anti-ulcer, and antidiarrhoeal acitivity (Sharma et al. 2014). Phytochemical components of *Acorus calamus* extracts from rhizome in several differents solvent are: alkaloids, steroids, saponins, terpenoids in ethanol; alkaloids, phenolic, saponins, terpenoids and glycosides in ethyl acetate; flavonoids, phenolic, terpenoids in chloroform; flavonoids, alkaloids, phenolic, tannins, steroids, saponins, terpenoids, glycosides in methanol; alkaloids, saponins, glycosides in acetone. Phenolic is one of chemical compound in *Acorus calamus* L. rhizome extracts (Elshikh et al. 2022). This research was conducted to determined the chemical compounds in leaves and rhizome of *Acorus calamus* L., antioxidant activity and phenolic content as the main antioxidant compound in natural raw material.

METHODS

Sample

Sample of this research, leaves and rhizomes of *Acorus calamus* L. obtained from mangrove forest area of Muara Sungsang, Banyuasin, Sout Sumatra-Indonesia. *Acorus calamus* L. leaves and rhizomes that have been collected were washed with water, chopped and dried by air. After that, the rhizome were continued to dried process with an oven at a temperature of 50 ^{o}c. Dried leaves and rhizome of *Acorus calamus* L. were crushed into powder.

Identification of Chemical Compound

Identification of chemical compounds were determined by standard procedure for fresh sample of leaves and rhizomes of *Acorus calamus* L includes: groups of alkaloid, flavonoid, phenolic, saponin, steroid and terpenoid. Identification of flavonoid and phenolic compound were carried out on extract of leaves and rhizomes of *Acorus calamus* L.

Extraction

Each of leaves and rhizome powdered of *Acorus calamus* L. were weighed at 150 g and ready to be used as a sample for extraction process. Extraction was carried out using solvent extraction method by maceration. Each sample was put into a maceration bottle, ethanol 70% as a solvent was added until all the samples were submerged. Extraction was carried out in a place protected from sunlight and left for 3 x 48 hours, and was stirred occasionally. After maceration process, the liquid extract was processed by evaporated the solvent and extract ready to be used for antioxidant test.

Analysis of Total Phenolic Compounds

Determination of total phenolic content of *Acorus calamus* L. leaves and rhizome extract were carried out using Folin-Ciocalteu method with gallic acid as the standard.

Antioxidant Activity Test

Antioxidant activity test were carried out using the DPPH (2,2-dyphenyl-1-picrylhydrazil) free radical scavenging method as reported by Brand-Williams et al. (1995) and Sari et al. (2023) with modification. Test solution for antioxidant activity was made by extract sample with varying concentration: 50, 100, 150, 200 and 250 mg/L. The comparator as antioxidant used was ascorbic acid (5, 10, 15, 20 and 25 mg/L). DPPH 0.05 mM solution (3.8 mL) was reacted with the solution of extract and ascorbic acid (0.2 mL) in spectrophotometer UV-Vis at maximum wavelength of DPPH blank.

Absorbance values of DPPH before (A_0) and after (A_1) the reaction were used to calculate percentage of DPPH inhibition with the following equation (Hasanah et al. 2016):

$$\%\ inhibition = \frac{A_0 - A_1}{A_0} x100\% \quad \ldots\ldots \text{ equation 1.}$$

The strength of antioxidant activity was known from IC_{50} value (the concentration of sample that can inhibit 50% of DPPH as free radical). IC_{50} was obtained from a linear curve between percent inhibition for various sample concentration (test solution).

RESULT AND DISCUSSION

Leaves and rhizome of *Acorus calamus* L. were identified first before extraction (the picture of leaves and rhizome *Acorus calamus* L. show at **Figure 1**). The chemical compounds contained in *Acorus calamus* L. fresh leave and rhizome were identified in this research and the results showed in **Table 1**.

Table 1. The result of chemical compound identification in fresh sample of *Acorus calamus* L.

Chemical compound	Leaves	Rhizome
Phenolic	+	+
Saponin	-	-
Terpenoid	-	+
Steroid	+	-
Alkaloid	+	+
Flavonoid	+	+

Several things that can influence extraction of plant samples are the choice of solvent, time and temperature of extraction process (Ingle et al. 2017). The extraction used in this research was processed used solvent extraction by maceration method with ethanol 70% as the solvent. Choosing the right solvent is the important thing for solvent extraction process. Ethanol is usually used in the initial extraction process because ethanol is universal solvent for phytochemical investigation (Zhang et al. 2018). Extraction processed of fresh leave and rhizome *Acorus calamus* L. was obtained leave extract with yield value of 10.42 %(w/w) and rhizome extract with yield value of 11.81 %(w/w).

Figure 1. Fresh sample of leave (A) and rhizome (with the roots) (B) of *Acorus calamus* L.

Ethanol extract of leaves and rhizome were identified contained flavonoid and phenolic from the test in this research. Identification process of extract was continued by determining the total phenolic content in it. Total phenolic content in leaves was obtained 5.72% and 2.42% in rhizome compared with gallic acid in determining process from the linear curve of the absorbance data spectrophotometer UV-Vis of gallic acid and sample that showed in **Table 2**.

Table 2. Absorbance of gallic acid and samples on spectrophotometer UV-Vis

Number	Solution test	Concentration (mg/L)	Absorbance		
1	Gallic acid standard	3.125	0.055	0.046	0.068
		6.25	0.082	0.084	0.092
		12.5	0.155	0.164	0.158
		25	0.31	0.308	0.323
		50	0.593	0.621	0.597
		100	1.104	1.118	1.124
2	Extract leaves	1000	0.308	0.284	0.29
3	Extract rhizome	1000	0.657	0.657	0.656
4	Blank	0	0.015	0.014	0.014

Antioxidant test of extract leaves and rhizome were processed by DPPH assay with spectrophotometer UV-Vis. DPPH assay method is a very simple method to set up (Niederlander et al. 2008). DPPH absorbance was observed at the maximum wavelength of DPPH blank that previously checked, at 516.6 nm with the absorbance of DPPH blank was 0.6669. The changes in DPPH absorbance indicate the changes in DPPH concentration in the test solution. The antioxidant activity of chemical compound in the extract has reduced the DPPH concentration as seen from the decrease in absorbance value of solution test. Visually, this change in concentration is observed from the colour change of DPPH solution before and after the reaction. The color of the purple DPPH solution changes to pale yellow after being added or reacted with extract solution, as show in **Figure 2.**

Figure 2. The change of DPPH solution before and after reacted with extract solution

The substance with antioxidant in it will donate a hydrogen atom to free radical DPPH, the DPPH will loss its violet colour become pale yellow as colour from pycryl group still present in it. DPPH reduced (decolorised by molecule of the reductant from antioxidant (Molyneux, 2004).

Table 3. Absorbance of DPPH before and after reaction with extract leaves and rhizome

Concentration (mg/L)	Absorbance, A_1 (average value)	
	Extract leaves (A_0=0.6667)	Extract rhizome (A_0=0.6711)
250	0.0570	0.0466
200	0.2015	0.1265
150	0.3173	0.2194
100	0.4326	0.3246
50	0.5136	0.4322

DPPH absorbance values before and after reaction with samples extract at various concentrations (**Table 3)** were used to calculate the inhibition percentage for extract leaves and rhizome (equation 1) that the results were explained in **Table 4**, then a graph was made by that data as shown in **Figure 3**. The result at **Table 4** showed that the higher extract concentration value, the higher inhibition value will be, because the antioxidant compound in the extract is greater so it more effective in eliminating free radicals.

Table 4. Inhibition percentage of extract leaves and rhizome

Concentration (mg/L)	% Inhibition	
	Extract leaves	Extract rhizome
250	91.45	93.06
200	69.78	81.15
150	52.42	67.31
100	35.13	51.62
50	22.98	35.29

Both of the linear curve of extract leave and rhizome respectively were satisfied the equation y=0.3432x + 2.875 (R^2=0.9908) and y=0.2901x + 22.172 (R^2=0.9961). Percentage inhibition of ascorbic acid as comparator antioxidant at various concentration (5, 10, 15, 20 and 25 mg/L), the values respectively were 55.97; 46.77; 37.19; 27.81; 15.08 %.

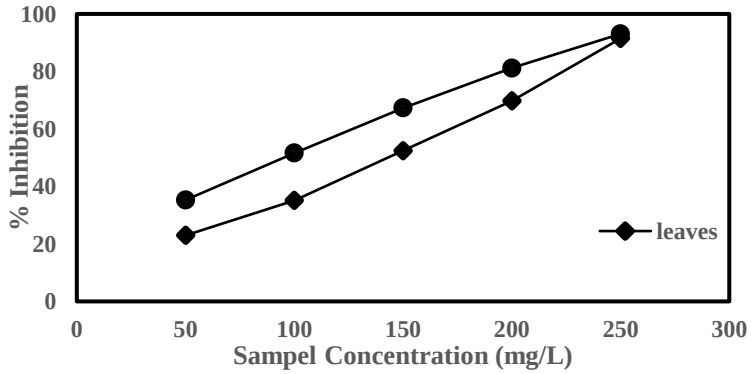

Figure 3. Inhibition percentage at various concentration of extract sample curve

The linear regression of ascorbic acid curve satisfied the equation y = 2.0148x + 6.342 (R^2=0.9956). From all the curve equation of extract and ascorbic acid, the IC_{50} as antioxidant activity strength was calculated. The IC_{50} value of leave extract was obtained 137.39 mg/L (moderate antioxidant activity), rhizome extract was obtained 96 mg/L (strong antioxidant

activity) and ascorbic acid was obtained 21.677 mg/L (very strong antioxidant activity).

The strength antioxidant activity of rhizome *Acorus calamus* L. (96 mg/L, strong category) is not much different compared to other rhizome antioxidant, such as *Rynchanthus beesianus* ethanol 70% extract which have an IC_{50} 69.48 mg/L (strong antixodant) (Jia et al. 2023). Compared with another extract, rhizome of *Acorus calamus* L. has higher antioxidant activity like *Podophyllum hexandrum* Royle that has an IC_{50} values is 215.37 mg/L with methanol as the solvent extraction (Lone et al. 2023).

Antioxidant of material is influenced by the chemical compound contained in the material. The examples of chemical compound in natural material that influenced antioxidant activity is phenolic compound. phenolic content is correlated with the high antioxidant activity of an extract (Narita and Inouye., 2012). Phenolic are widely found in many type of plants and good for the development of effective specific drugs as antioxidants and anti-inflammatory. The simply classified of phenolic compound group are flavonoids and non-flavonoids (Saparbekova et al. 2023). Phenolic compounds have an ideal chemical structure for free radical scavenging activites. Flavonoids are the mayor phenolic components of naturally occuring phytochemical as antioxidants. Types of flavonoids are flavonol, flavone and isoflavone. Other phenolic that also act as antioxidants are phenolic acid, tannin and phenylpropanoids. The activity of phenolic compounds as antioxidant is by donating hidrogen atom (H) or electrons to scavenging free radical (Rice-Evans et al. 1997).

Chemical compound in *Acorus calamus* L. besides phenolic are monoterpenes, xanthone glycosides, lignans, steroids, flavones, triterpenoid, saponins and essential oil (Chandra and Prasad, 2017). Rhizome of *Acorus calamus* L. contain very strong aromatic compounds with a sweet odour and bitter taste (Subha et al. 2011). Essential oil of rhizome of *Acorus calamus* L. contain 78 compounds and the major components are 75.8% asarone, 25.8% trans-ß-Ocimene, 20.5% isocalamendiol, 20.1% methyleugenol, 22.6% 3-carene, 17.40% ß-asarone. The presence of polyphenolic compound such as flavonoids and flavonols in the stucture of *Acorus calamus* L. can be considered to show antioxidant activity (Atalar and Turkan, 2018). One of monoterpen compounds in *Acorus calamus* L. that has antixodant activity is para-cymene. That compound, *p*-cymene that also known as *p*-cymol or *p*-isopropyltulene is an alkyl-substituted aromatic compound usually obtained from essential-oil. *P*-cymene have pharmacological properties include: antioxidant activities (Balahbib et al. 2021). Compounds in phenolic acid group that repeatedly as antioxidant are caffeic acid and chlorogenic acid. Other phenolic that have antixodant activity are rosmaridiphenol, linoleic acid, polyhydroxylated chalcones and ubiquinol (Larson, 1988).

CONCLUSION

This research proves that both of leave and rhizome of *Acorus calamus* L. have antioxidant acitivity. The antioxidant potential of rhizome of *Acorus calamus* L. is higher than leave. The strength of antioxidant activity of rhizome was on strong category and total phenolic content is 2.42% compared with gallic acid. Rhizome of *Acorus calamus* L. can be used for raw material of various health products and also as bioreductor for oxidation-reduction process in reactions needed to produce many health products.

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