

Antioxidant and Antielastase Activity of *Kaempferia Rotunda* and *Curcuma Zedoaria*

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Abstract

Curcuma zedoaria and *Kaempferia rotunda* are known in Indonesia with the common name: *temu putih* and *kunyit putih*. Both plants are often used in *Jamu*, known as traditional Indonesia medicine, to treat various diseases. The aim of this research was to determine the antioxidant activity of rhizomes and leaves of *K. Rotunda* and *C. Zedoaria* and its potential as an inhibitor elastase. Both activities can be used as a preliminary test for antiaging, so the utilization of these plants can be developed as herbal cosmetics. Both dry leaves and rhizomes were refluxed with ethanol 96%. The extracts were evaporated and the antioxidant activity was determined using DPPH methods. The best antioxidant results continued with inhibitor elastase assay based on the formation of *p*-nitroaniline and can be measured at 405 nm.

The IC₅₀ values of DPPH scavenging activity of Vitamin C, rhizome and leaves of *K. rotunda* as well as rhizome and leaves of *C. zedoaria* were 2.24; 193.71; 126.99; 72.61 and 45.75 ppm respectively. The ability of inhibition elastase of *K. rotunda* and *C. zedoaria* leaves in 150 ppm was 40,82 % and 49,24 % with EGCG used as control positive at 23 ppm gave activity of 85.72%. This investigation gave the promising effect to emphasize the importance of *C. zedoaria* leaves as antiaging cosmetic ingredient because it gave the best results.

Keywords: *Kaempferia Rotunda* L., *Curcuma zedoaria* (Christm.) Roscoe, Antioxidant, Inhibitor elastase.

Introduction

Zingiberaceae is the largest tribe in Zingiberales, it contains about 300 genera and more than 1000 known species. Zingiberaceae is one of the major sources of food producers, seasoning, herbs, dyes, perfumes and cosmetics. In general, Zingiberaceae produces essential oils and oleoresins that have high economic value as well as flavonoid, polifenol and terpenoid having extensive activities such as antimicrobial, anti-arthritis, antioxidant, anticancer, anti-inflammatory, antidiabetic, anti HIV, neuroprotector, larvacidal, antimelanogenic etc.^{1,4,9,12,13}

In Indonesia, the Zingiberaceae family is a major component in herbal formulation named *Jamu*. *Jamu* is the

Indonesian traditional herbal medicine that has been practiced for many centuries in the Indonesian community to maintain good health and to treat diseases. *Jamu* has acquired a potential benefit both economically and clinically⁵. Besides beneficial in medicine, some Zingiberaceae plants are also often used as traditional cosmetics. Tundis et al¹⁰ reviewed that extracts and pure compounds from Fabaceae, Asperaceae and Zingiberaceae families have shown particular interest and appear most promising in the development of anti-aging products.

Tu et al⁹ reported studies about essential oils from the leaves of *Alpinia zerumbet* (Zingiberaceae) had strong anti-aging activity by inhibiting collagenase, tyrosinase, hyaluronidase and elastase as well as showed strong antioxidant activities against DPPH and nitric oxide, hydroxyl radical scavenging activity and xanthine oxidase inhibition.

From the PROSEA (Plant Resources of South-East Asia Foundation) base, *Curcuma zedoaria* (Christm.) Roscoe and *Kaempferia rotunda* L (Zingiberaceae) are known in Indonesian with the common name : *temu putih*, *kunir putih* and *kunyit putih*. Both rhizomes are widely used as stimulant, anti-emetic, stomachic, carminative, anti-diarrhoeal, diuretic, anti-pyretic and depurative, after childbirth, to clean and cure ulcers, wounds and other kinds of skin disorders. The rhizomes are also chewed against halitosis and a decoction is drunk against stomach-ache, indigestion and colds. The major use of rhizomes is for starch. In Indonesia, the heart of young shoots is used as a vegetable, young rhizome parts are eaten raw and inflorescences cooked. The leaves are used for flavouring foods¹¹.

In this study, we examined the antioxidant and antielastase activity of rhizome and leaves of *Curcuma zedoaria* (Christm.) Roscoe and *Kaempferia rotunda* L. Both activities were employed as preliminary test for anti skin aging. The enzymes involved in the destruction of the skin extracellular matrix components such as collagen, elastin and hyaluronic acid are collagenases, elastases and hyaluronidases. When this enzyme is excessive, it will cause the skin aging process³. When UV rays are absorbed by the skin, they cause radical oxygen species (ROS) increasing and oxidative stress inducing. This causing oxidative damage and mitochondrial DNA damage changes in shape of the lipid peroxide and modification of genes that ultimately leads to changes in protein structure and function. The high amount of ROS also increases the activity of collagenase, hyaluronidase and elastase⁷.

The development of in vitro methods to test the antioxidant activity and inhibition of elastase enzyme can be used in anti-aging activity screening test. In vitro testing is a screening test which is a rapid and rational method of reducing the number of in vivo trials and the risks associated with human subjects; then this could reduce the cost of research and development⁸. This study was to examine antioxidant capacity of both rhizomes and leaves extract using DPPH scavenging method². Antielastase was assayed using spectrophotometric method⁵ with slight modifications.

Material and Methods

Reagent and Material: *Kaempferia rotunda* L. and *Curcuma zedoaria* (Christm.) Roscoe leaves and rhizomes were collected from the Research Institute for Spice and Medicinal Crops (BALITTRO) in Bogor, Indonesia and determined in Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia. The plants were harvested in October 2016. Buffer Trizma base (Sigma, T1503), Porcine pancreatic elastase (Sigma, E7885), Substrat N-Succinyl-Ala-Ala-Ala-p-nitroanilide (SANA) (Sigma, S4760), Epigallocatechin gallat (EGCG) (Sigma, E4143), DPPH (1,1-difenil-2-pikrilhidrazil) (Sigma), vitamin C, methanol p.a, aquadest, etanol 96% were purchased and used as such.

Preparation of plant extracts: Dried rhizomes and leaves were extracted with ethanol 96% by reflux for one hour (7-8 times). The extracts were filtered through Whatmann paper and concentrated using a vacuum rotary evaporator.

Phytochemical screening: Phytochemical screening represents a set of colorimetric methods that can lead to detect the presence or lack of secondary metabolites and should be realized on extracts. Qualitative test is to detect alkaloids, tannins, flavonoids, saponin, quinone, coumarin, steroids and triterpenoids and volatile oil.

Antioxidant Activity Test: The DPPH radical-scavenging activity was determined using the method proposed by Blois et al² with slight modification. DPPH was dissolved in methanol p.a (0.4 mM). The DPPH solution (1 ml) was added to serial concentration of extracts in methanol and added to methanol 5.0 mL. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min.

The decrease in absorbance of the resulting solution was monitored at 516.5 nm at spectrophotometry UV-VIS (UV 1800-Shimadzu). All determinations were performed in triplicate. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = $\frac{[(\text{Abs neg control} - \text{Abs sample}) / (\text{Abs neg control})] \times 100}$ where Abs neg control is the absorbance of DPPH radical and methanol; Abs

sample is the absorbance of DPPH radical + extract/control with Vitamin C as reference.

Antielastase assay: Antielastase assay using spectrophotometric method⁶ used with slight modification was using porcine pancreatic elastase (PPE) with the substrate N-Succ-(Ala)3-p- nitroanilide (SANA). The product reaction, p-nitroaniline for 20 min at 25°C was monitored by measuring the absorbance at 405 nm with microplate reader (Elx 800). Reaction mixture were contained 0.2 M Tris-HCl buffer (pH 8.0), 1µg/mL elastase, 0.8 mM SANA as shown in table 1.

It was pre-incubated for 15 min at 25°C and the reaction was started by adding substrate. Blanks contained all the components except the enzyme. The EGCG (epigallocatechin gallat) was used as a positive control. Each treatment was triplicated. The percentage of inhibition was calculated as:

$$\text{Inhibition (\%)} = (1 - B/A) \times 100$$

where A is the enzyme activity without inhibitor and B is the activity in the presence of inhibitor.

Results and Discussion

In this study, Zingiberaceae species was tested in the composition of Jamu as antielastase and radical scavenger activity for the development of anti-wrinkle skin material in cosmetic formulation. The yield of reflux ethanol extraction (table 2) showed that ethanol soluble matter of *C. zedoaria* was more than *K. rotunda*.

Results of phytochemical screening of all sample (Table 3) reveal abundant amount of flavonoids, steroid/triterpenoid and volatile oil. These plants appear to have high potential bioactivity. Free radical scavenging was measured using the DPPH color test which showed high antioxidant activity in the sample. Antioxidant test results are presented in figure 1. These results are compared with Vitamin C as a standard. The test results obtained from the IC50 value showed that *C. zedoaria* is more potent than *K. rotunda* and leaves of *C. zedoaria* gave the best antioxidant activity.

Elastase is one of the proteolytic enzymes in the dermis responsible for the degradation of elastin in the extracellular matrix. Elastin loss is one of the main causes of aging signs visible (sagging and wrinkles) in the skin. Both leaves ethanol extracts tested had the moderate potential of antielastase (fig. 2) and showed that the *C. zedoaria* leaves have substantial potent compounds as antioxidants and antielastases.

The potential use of Jamu as a base for skin care products will be very interesting as well as to find out if cosmetic ingredients used traditionally in traditional medicine have bioactivity that may be useful in modern formulations.

Table 1
Antielastase assay composition

Reagent	Well microliter plate		
	Control (µL)	Sample (µL)	Blank sample (µL)
Buffer Tris HCl 0,1 M pH 8,0	120	90	110
Sample solution	-	30	30
Elastase enzyme	20	20	-
N-SucAla ₃ -pNA	10	10	10

Table 2
Yields of extract

<i>K. rotunda</i> leaves	14.84%
<i>K. rotunda</i> rhizome	8.36%
<i>C. zedoaria</i> leaves	14.93%
<i>C. zedoaria</i> rhizomes	18.57%

Table 3
Results of phytochemical screening

Phytochemical test	Leaves <i>K. rotunda</i>		Rhizome <i>K. rotunda</i>		Leaves <i>C. zedoaria</i>		Rhizome <i>C. zedoaria</i>	
	Dry powder	extract	Dry powder	extract	Dry powder	extract	Dry powder	extract
Alkaloid	-	-	-	-	-	-	-	-
Flavonoid	+	+	+	+	+	+	+	+
Saponin	-	-	+	+	+	+	+	+
Tannin	+	+	-	-	-	-	-	-
Steroid/Triterpenoid	+	+	+	+	+	+	+	+
quinone	-	-	-	-	+	+	+	+
coumarin	-	-	-	-	-	-	-	-
Volatile oil	+	+	+	+	+	+	+	+

+: presence; -: absence

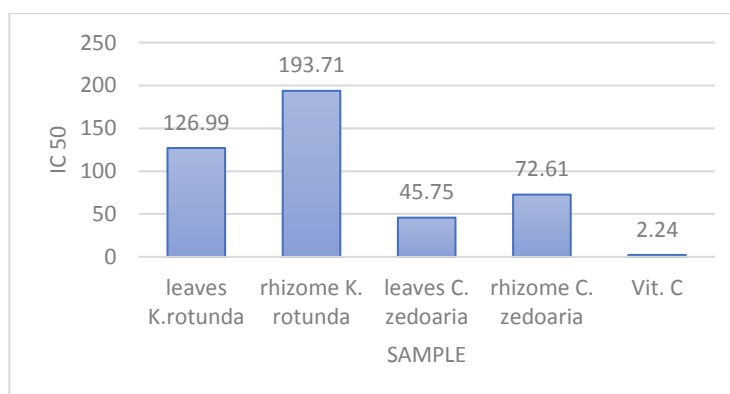


Fig. 1: Result of antioxidant activity using the DPPH test.

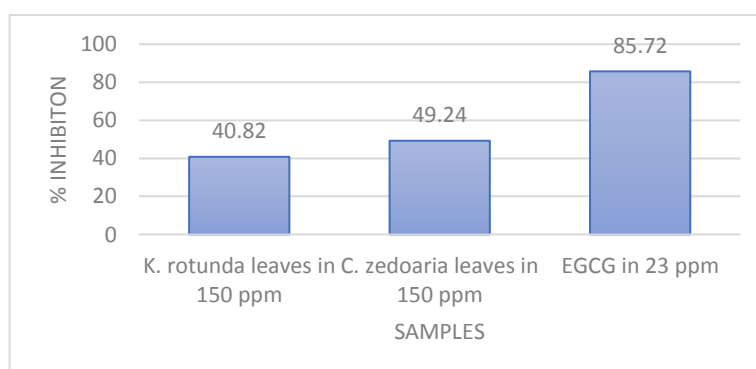


Fig. 2: Result of antielastase assay

Conclusion

Results of phytochemical screening showed the presence of flavonoids, steroids, triterpenoids and essential oil and *C. zedoaria* leaves have potential activity as antioxidant and antielastase.

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