Antioxidant Exploration in Cardamom Rhizome Potential as a Functional Food Ingredient

H Winarsi1*, A Yuniaty2, Warsinah3

1Department of Nutrition, Faculty of Health Sciences, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia
2Biology Faculty, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia
3Department of Pharmacy, Faculty of Health Sciences, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia

* Email correspondence: winarsi12@gmail.com

Abstract. Oxidative stress triggers the emergence of degenerative diseases. Antioxidant-rich products have been shown to reduce the degree of oxidative stress. Cardamom rhizome is reported to be rich in antioxidants, but the components and levels are unknown. This study aims to explore the flavonoids, vitamin C and essential oils content in cardamom rhizome, and its potential as functional food ingredients. The research method used is quantitative exploration. Cardamom rhizome is washed, thin sliced, oven dried at 50-60°C, then ground into flour. Cardamom rhizome flour was then analyzed for flavonoids, vitamin C and essential oils content. The results show that level of flavonoid is 324.51 mg/g extract, vitamin C 0.73 mg/g extract; and essential oil 0.22 ml/g extract. Cardamom rhizome flavonoid level is almost 3 times higher than that of leaf. Meanwhile cardamom leaf has been proven able to be formulated into functional drinks. On the other hand, flavonoids, vitamin C, and essential oils are known to many experts as potential antioxidant compounds, and are beneficial for health. Therefore, it is believed that cardamom rhizome can be used as a component of functional food. In conclusion, cardamom rhizome is rich in flavonoid antioxidant and has the potential as a functional food ingredient.

Keyword: cardamom rhizome, flavonoid, vitamin C, essential oil, functional food.

1. Introduction

Today the incidence of degenerative diseases is getting higher [1]. Oxidative stress is a major factor in the development of degenerative and chronic diseases, such as cancer, arthritis, aging, autoimmune, cardiovascular and neurodegenerative diseases [2]. Oxidative stress according to Prior [3] and Winarsi et al. [4] is an imbalance condition between antioxidant status and the amount of oxidants in the body, thus suppressing the antioxidant status at the cellular level. A further impact is the disruption of the body’s defense system, susceptibility to infection and various diseases, even premature aging. Physiologically the body is able to neutralize oxidative stress by stimulating the work of endogenous antioxidant enzymes, and the antioxidant compound can be naturally produced or supplied through dietary intake.

Endogenous and exogenous antioxidants work as free radical scavengers, by protecting cells from attacks by Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), or repairing them. These antioxidants can also increase immunological defenses, and reduce some of the risks of degenerative diseases [5]. In general there are 2 types of antioxidants, i.e. enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX) and glutathione reductase (GRx) enzymes, which directly involved in neutralizing ROS and RNS [6; 7; 8]. SOD is the first defense enzyme against free radical attack. It catalyzes the dismutation of superoxide (O2 •-) anion radicals to hydrogen peroxide (H2O2) through a reduction reaction. The resulted H2O2 oxidant is converted into water and oxygen by CAT or GSH-PX. GSH-PX selenoprotein enzyme removes H2O2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase is a flavoprotein enzyme responsible for regenerating GSH from GSSG, with NADPH as a reductor source. Besides hydrogen peroxide, GSH-PX also reduces lipids or non-lipid hydroperoxides [9].
Non enzymatic antioxidant consists of metabolic and nutrient antioxidants. Metabolic antioxidants are endogenous antioxidants produced through the body's metabolism. For example, lipoic acid, glutathione, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal chelating proteins, transferrin and others [6]. Nutrient antioxidants are exogenous antioxidants, compounds that cannot be produced by the body, but must be provided through food or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 fatty acids and omega-6, and others. Antioxidants from diets play an important role in helping endogenous antioxidants to neutralize oxidative stress. Therefore, nutritional antioxidant deficiency is one of the pathological causes of a number of chronic and degenerative diseases. It is reasonably relevant to repair or improve the antioxidant status. What needs to be understood is that each nutritional antioxidant is unique in its structure and function [6; 10]. It has been reported that natural antioxidant-rich food products can improve the antioxidant status [4; 11; 12]. It is important to explore natural ingredients rich in antioxidants, which allow them to be used as functional food ingredients. According to the POM Agency (2005) functional food is natural or processed food containing one or more compounds which based on scientific studies considered to have certain physiological functions beneficial to health. Functional food products are consumed like food or beverages, have sensory characteristics such as appearance, color, texture and taste acceptable to consumers, and do not provide contraindications and side effects on the metabolism of other nutrients when used in recommended amount [13]. In various countries, functional foods are often referred to by various other terms such as nutraceutical, vitafood, phytofood, pharmafood, designer food and food for specified health use. The criteria for functional food must be in the form of food products (not capsules, tablets or powders) derived from naturally occurring ingredients, suitable for consumption as part of daily food, and have certain functions when ingested, as well as providing certain roles in metabolic processes inside the body. The third criterion distinguishes functional foods from conventional foods. Functional properties in functional foods are caused by the presence of bioactive components found in plant materials, such as antioxidants, dietary fiber, inulin, and fructooligosaccharides, or animal ingredients such as eicosapentaenoic acids (EPA), docosahexaenoic acids (DHA), and conjugated linoleic acid (CLA). Bioactive components are found in many types of plants. Indonesia as a country rich in flora, has the potential as an originator and functional food developer.

Cardamom is often used by people in certain areas as cough and cold medicines. Processing cardamom include washing the whole plant, boiling, filtering and then the water is ready to drink. Cardamom, reported Nair et al. [14], contains phenolic antioxidant and flavonoids spread throughout the plant, i.e. root (rhizome), stem, leaf, fruit and seed. Winarsi et al. [15] reported that flavonoids in cardamom leaf was 129 mg/g, greater than those in cardamom stem. In vitro study conducted by Winarsi et al.[15] showed that the lowest IC50 was in cardamom fruit, followed by rhizome, stem, and leaf. IC50 is a value that indicates a concentration that can inhibit 50% oxidation (free radicals). The smaller the IC50 value, the higher the antioxidant activity is. A compound is said to be a very strong antioxidant if IC50 values <50, strong (50-100), moderate (100-150), and weak (151-200) μg/ml [16]. Therefore based on the IC50 values, it can be inferred that the highest antioxidant potential in cardamom is found in fruit, followed by rhizome, stem and leaf [15]. Based on these findings, it is clear that cardamom rhizome, stem and leaf can also be used for health. However, there is no data reveals how much the antioxidant compound content in cardamom rhizome is. Therefore this study aims to determine the levels of flavonoid, vitamin C and volatile oil in cardamom rhizome, as an effort to get functional food components.

2. Experimental Method
This quantitative exploration study used cardamom rhizome obtained from cardamom farmers in the village of Ciberem, Sumbang District, Banyumas Regency. After being cleaned from the roots and other impurities, cardamom rhizome was washed using running water, then sliced thinly, and dried using an oven at 50-60°C until dry. Dried rhizome was ground into flour, called cardamom rhizome flour (CRF). Furthermore, the CRF was tested for flavonoid content using a routine standard; vitamin C using the Yodometry method; and essential oils using HPLC. Data Analysis: the data were analyzed by using the F test. If there was a significant effect, the analysis was continued with Duncan’s Multiple Range Test (DMRT) at a 5% significance level.
3. Results and Discussion

3.1. Flavonoid content of cardamom rhizome flour

The flavonoid content in 1 g of cardamom rhizome extract was 324.51 mg, almost three times the level in cardamom leaf extract (130 mg) (Table 1). This finding was in accordance with the IC50 values in which the IC50 of cardamom rhizome extract was 3.86, lower than that of cardamom leaf extract (4.05) [15].

Table 1. Flavonoid, vitamin C and essential oil content in flour and cardamom rhizome extract

| Component     | Flour      | Cardamom Rhizome |
|---------------|------------|------------------|
| Flavonoid     | 7.46 mg/g  | 324.51 mg/g      |
| Vitamin C     | 1.67 µg/g  | 0.73 mg/g        |
| Essential Oil | 0.05% v/b  | 0.22% v/b        |

Flavonoid is phenolic or polyphenolic compounds which has one of the following characteristics, i.e. antioxidant [4; 11], antidiabetic [17; 18], hypcholesterolemic [19; 20], capable weight control [20; 21], and anti-inflammatory [22]. Nair et al. [14] stated that Cardamom contains phenolic antioxidant compounds and flavonoids about 50-100 mg in the form of quercetin, kaempferol, luteolin and pelargonidin. Cardamom leaf flavonoid has been proven as antidiabetic, controlling weight loss, hypcholesterolemic [21], decreases atherogenic index, blood sugar level [18], and decreased oxidative stress of alloxan induced diabetic mice [4]. Cardamom leaf has also been formulated and patented by Winarsi et al. [23] (as a functional drink rich in flavonoid and as an anti-atherogenic agents for women with type-2 diabetes mellitus). Cardamom leaf which contains flavonoid only one-third of cardamom rhizome can be made as functional drink and proven beneficial for patient with diabetes mellitus; surely, the cardamom rhizome can also be formulated as functional food ingredient beneficial for health. Based on their research, Winarsi et al. [24] have proven that cardamom rhizome extract flavonoid has a hypcholesterolemic effect and suppresses the LDL-ox level, as well as improves antioxidant and immune status of atherosclerotic mice [25].

3.2. Vitamin C content of cardamom rhizome

Vitamin C in cardamom rhizome flour is 1.67 µg / g, or only 0.15% of that in cardamom leaf flour (1.11 mg). When converted as cardamom rhizome extract, vitamin C content is 0.73 mg, which is also lower than cardamom leaf extract (20 mg) [15]. Vitamin C content of cardamom rhizome extract was only 3.65% of that in cardamom leaf extract. Although the level is low, vitamin C is a non-enzymatic antioxidant that can protect cell damage due to free radicals. Structurally, vitamin C is similar to glucose, which can replace glucose in various chemical reactions, making it effective for the prevention of glycosylation of non-enzymatic proteins [26]. Vitamin C, also called ascorbic acid, is a water soluble vitamin. Although the level in cardamom rhizome is low, but this vitamin is needed by the body for the synthesis of collagen, carnitine and neurotransmitters [27]. Health benefits of vitamin C include its role as antioxidant, anti-atherogenic, anti-carcinogenic, and immunomodulators. Vitamin C suppresses the occurrence of diseases such as stomach, lung and colon cancer. As an antioxidant vitamin C works synergistically with vitamin E to reduce free radicals and regenerate reduced vitamin E. However, high dose of vitamin C intake (2000 mg / day) or more is still a debate because it is feared to be pro-oxidant which is ultimately carcinogenic [27; 28]. Given the low vitamin C level, the potential of flavonoids is more dominant as a functional ingredient.

3.3. Cardamom rhizome essential oil

Essential oil content in cardamom rhizome flour is about 0.05% (v/w), lower than that in cardamom seed which is 1.2% (v/w) [29]. This is even much smaller than Haris's findings (1987) which has a range of 2-8%. Although the essential oil content of cardamom rhizome flour is low, it is higher than that of cardamom flour or leaf extract where the essential oil content is not detected [15]. According to Fachriyah and Sumardi [29] in the essential oils of cardamom seed there are 5 important compounds namely α-pinene, β-pinene, p-simena, 1,8-cineol and α-terpineol. Marongiu et al. [30] found that the essential oils of Elettaria cardamomum (L.) fruit (seeds) contain few waxes, alpha-terpiny acetate
42.3%, 1.8-cineole 21.4%, linalyl acetate 8.2%, limonene 5, 6%, and linalool 5.4%; limonene, 36.4%, 1.8-cineole 23.5%, terpinolene 8.6%, and myrcene 6.6%. It is also reported that in addition to essential oils, cardamom seeds also contain various minerals, i.e. Mg, Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Cu and Zn in various concentrations [16; 31]. In this study, the compounds contained in cardamom rhizome essential oil have not been analyzed, but it is assumed that the cardamom rhizome essential oil contain the same compounds although in different levels. There are various products which contain essential oils of cardamom seeds, such as cakes, flowers, perfumes, and medicines [32]. The addition of essential oils into these products is intended as flavoring, aroma enhancing, or hiding the bitter taste. Essential oils are also used as raw materials for making oil of cardamom which is sold as flavoring bottles and canned foods. It is clear that cardamom rhizome essential oil can also be used as a component of functional food. According to Winarti and Nurdjanah [13] functional food has several important benefits, i.e. to prevent several diseases, increase immunity, slow the aging process, and improve physical appearance. For industry, functional food does not limit the opportunity to innovate in formulating products that have added value to society. Furthermore for the government, the presence of functional food can reduce costs for public health maintenance.

4. Conclusion
Cardamom rhizome contains higher flavonoid antioxidant compounds and essential oils compared to cardamom leaf, but its vitamin C level is low. Flavonoids and vitamin C are potential for nutrition antioxidants, therefore it is believed that cardamom rhizome can be used as functional food ingredient. Essential oil in cardamom rhizome is also very beneficial for health. Thus the three components will complement each other as new functional food products. Cardamom rhizome has so far only been used as cardamom plant seed, but the success of this study allows cardamom rhizome to be formulated as functional food. This finding provides new hope for cardamom farmers and society in general. More than that, this finding also clarifies the reason people make use of the whole cardamom plants, i.e. for health purpose.

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