**THE POTENCY OF NUTGRASS RHIZOMES (*Cyperus rotundus* L.) EXTRACT AS ANTIOXIDANT AGENT**

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**Abstract**

**Purpose:** This study aims to identify the potency of antioxidant agent in nutgrass rhizomes (*Cyperus rotundus* L.) extract.

**Research Methodology: S**ample of nutgrass rhizomes were taken from Lampung Province and was extracted with ethanol and chloroform solvent. Later, those extracts were analyzed by Gas Chromatography Mass Spectrometry (GCMS) and the chemical compound identified was compared to literature to know the potency of the antioxidant agent.

**Results:** This study shows that in the nut grass root extract using ethanol solvent, there were 116 compounds with 6 main compounds, meanwhile in the nut grass rhizomes extract using chloroform solvent, of 142 compounds, there were 11 main compounds. Of the 6 main compounds of ethanol extract and 11 main compounds of chloroform extract of nut grass rhizomes, which is proven to have potential as an antioxidant agent is α-Copaene.

**Limitations:** This study is limited to the identification of potential antioxidant activity of the main compound.

**Contribution:** This study is potential to be used in the field of herbal medicine and other fields related to traditional medicinal plants.

***Keywords:*** *antioxidant, nutgrass rhizomes extract, GCMS*

**1. INTRODUCTION**

 Nut grass (Cyperus rotundus L.) is a weed that can absorb large amounts of nutrients compared to other plants. This plant is easy to grow anywhere, easy to maintain, and has high resistance to various external influences. This nature makes nut grasses often destroyed so as not to interfere with the growth of other plants (Erwin *et al*., 2017). As a plant, nut grass produces secondary metabolites that function to protect it from environmental threats, such as insect, bacteria, fungi and other types of pathogens. The content of secondary metabolite compounds found in nut grass includes alkaloids, flavonoids, tannins, glycosides and furochromones, as well as many new sesquiterpenoids (Dewoto, 2007; Lawal and Oyedeji, 2009; Sholekah, 2017).

The benefits of these secondary metabolites have been widely studied by scientists. There are several known effects of nut grass, including as an antimutagen and radiation, antimalarial, antidiabetic, and natural antioxidant (Meena *et al.*, 2010; Singh *et al.*, 2012; Sivapalan, 2013).

Antioxidants are compounds that prevent cell damage due to the oxidation process of other compounds. Oxidation reactions produce free radicals which are very reactive. These free radicals are the target of antioxidants. The body always maintains an antioxidant defense system to respond to the negative effects of oxidative stress due to the formation of reactive oxygen species (ROS). This oxidative stress is a key factor that plays an important role in the progression of various pathological diseases. Oxidative stress is caused by an imbalance between antioxidants and ROS, so that antioxidant supplements are needed to maintain health and prevent the occurrence of various pathological diseases (Pal *et al.*, 2014). Thus, this study aims to identify the potency of antioxidant agent in nutgrass rhizomes (*Cyperus rotundus* L.) extract.

**2. LITERATURE REVIEW**

Nutgrass (*Cyperus rotundus* L.) was one of widely distributed weeds and could be found in tropical, subtropical, and other areas with temperate climate at 1,000 mdpl heights. Based on literature, nutgrass was originally from Indian region and spread to the world, including South Africa, Korea, China, Japan, Malaysia, Indonesia, and other regions in South East Asia (Singh *et al.*, 2012). Nutgrass was belongs to Cyperaceae family and Cyperusgenus. Nutgrass could grow until 40 centimeters high. The stem is soft, triangular in shape, forms rhizomes, and is pale green in color. The leaves are single, tapered tip, flat edge, 50 centimeters long, 5 milimeters wide, and green. The flower is located at the end of the stem, in the shape of a grain, 1-3 centimeters long, 2 milimeters wide, and brown. The roots are fibrous and dirty white. While the rhizome is the size of a pinkie, round or oval in shape, wrinkled or notched, the outside is brown and the inside is white (Amalia *et al*., 2014).

There are two types of metabolites in plants, namely primary metabolites and secondary metabolites. Primary metabolites which are important factors for the growth and life of living things, and are formed in limited numbers. Secondary metabolites are not used by plants for growth, and they function more to protect plants from their environment, such as attacks by insects, bacteria, fungi and other types of pathogens (Dewoto, 2007; Lawal and Oyedeji, 2009; Sholekah, 2017). Based on the literature, secondary metabolite compounds contained in nut grass include alkaloids, flavonoids, tannins, glycosides and furochromones, as well as many new sesquiterpenoids. The benefits of these secondary metabolites have been widely studied by scientists. A study in Sudan found that apart from having an antimicrobial effect with different effects on different microorganisms, ethanol extract of the nut grass plant also had moderate cytotoxic effects on Vero cells, with IC50> 100 µg / mL (Lawal & Oyedeji, 2009; Kabbashi et al. , 2015). Scientists are still doing research on the compound content and effects of nut grass, many of which are thought to be unknown. Apart from being antimicrobial and anticancer, there are several more known effects of nut grass, for example as an antimutagen and radiation, antimalarial, anti-diarrheal, antidiabetic, and natural antioxidant (Meena et al., 2010; Singh et al., 2012; Sivapalan, 2013 ).

Antioxidants are compounds that prevent cell damage due to the oxidation process of other compounds. Oxidation reactions produce free radicals which are very reactive. Once formed, free radicals will start a reaction. These free radicals are the target of antioxidants. Antioxidants will work more effectively if there is more than one type of antioxidant that is synergized (Pal et al., 2014).

 The body always maintains an antioxidant defense system to respond to the negative effects of oxidative stress due to the formation of reactive oxygen species (ROS). This oxidative stress is a key factor that plays an important role in the progression of various pathological diseases. Oxidative stress is caused by an imbalance between antioxidants and ROS, so that antioxidant supplements are needed to maintain health and prevent the occurrence of various pathological diseases (Pal et al., 2014).

Currently, antioxidants are of great interest because of their relationship to free radicals and oxidative stress, cancer prophylaxis and therapy, and life expectancy. The use of antioxidants can protect the body from cardiovascular disease, neurological diseases, and carcinogenic diseases, and delay the onset of chronic diseases such as cataracts. Diseases caused by oxidative stress include cancer, coronary heart disease, obesity, type 2 diabetes mellitus, and hypertension (Yadav et al., 2016).

Based on the source, antioxidants are classified into 2 major groups, namely natural and artificial / synthetic antioxidants. Natural antioxidants are synthesized in the body through the process of metabolism or obtained from other natural sources. Natural antioxidants can be further divided into 2, enzymatic and non-enzymatic antioxidants. Examples of enzymatic antioxidants are superoxide dismutase, kalatase, glutathione peroxidase, and glutathione reductase (Pal et al., 2014).

Non-enzymatic antioxidants can be further divided into several classes, namely minerals, vitamins, carotenoids, polyphenols, and other antioxidants that are protein and non-protein. Minerals work as enzymatic antioxidant co-factors. Examples of mineral antioxidants are Mg, Cu, Fe, Zn, and Mn. Vitamins that function as antioxidants are Vitamins A, C, and E. Lycopene, β-carotene, lutein, and zeaxanthin are included in the carotenoid antioxidant class. Polyphenols are phytochemicals that have antioxidant activity, for example flavonoids, gingerols, and curcumin. Artificial antioxidants are synthesized using a variety of techniques. Which includes artificial antioxidants are EDTA, BHT, BHA, and ethoxyquin (Pal et al., 2014).

**3. RESEARCH METHODOLOGY**

This research is a descriptive study with a cross sectional design. The population is nut grass growing in Talang Padang, Tanggamus Regency, at the coordinate point -5.377263, 104.792544. The grass samples were then determined and extracted at the Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Lampung. The extract of nut grass was made using ethanol and chloroform as solvents. After that, the nut grass extract was sent to the Integrated Research and Testing Laboratory (LPPT) of Universitas Gadjah Mada (UGM) Yogyakarta for analysis of its compound content using GCMS. The GCMS results were then analyzed to identify compounds with potential as antioxidants, based on the literature.

**4. RESULTS AND DISCUSSIONS**

The location for sampling of nut grass rhizomes is in Talang Padang, Tanggamus Regency, at the coordinate point -5.377263, 104.792544. The main compounds identified from the ethanol extract and chloroform of nut grass rhizomes using the GCMS test can be seen in Tables 1 and 2.

In the extract of nut grass rhizomes using ethanol solvent, obtained 116 compounds with 6 main compounds, namely d-Selinene, Caryophyllene oxide, Longiverbenone, (3aR, 4R, 7R) -1,4,9,9-Tetramethyl-3,4,5 , 6,7,8-hexahydro-2H-3a (Cyperotundone), 2,5-Octadecadiynoic acid, methyl ester, and 2-Cyclohexene-1-carboxylic acid, 1,3-dimethyl-2- (3-methyl-7 -o (Methyl trisporate B).

**Table 1**. The main compounds detected in the ethanol extract of nut grass rhizomes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No. | Nomor Puncak | Senyawa | Waktu Retensi (menit) | Area Puncak (%) |
| 1. | 14 | d-Selinene | 20.07 | 7.62 |
| 2. | 38 | Caryophyllene oxide | 24.63 | 3.04 |
| 3. | 54 | Longiverbenone | 26.82 | 3.75 |
| 4. | 58 | (3aR,4R,7R)-1,4,9,9-Tetramethyl-3,4,5,6,7,8-hexahydro-2H-3a (Cyperotundone) | 27.22 | 11.21 |
| 5. | 91 | 2,5-Octadecadiynoic acid, methyl ester | 31.86 | 4.07 |
| 6. | 118 | 2-Cyclohexene-1-carboxylic acid, 1,3-dimethyl-2-(3-methyl-7-o (Methyl trisporate B) | 37.57 | 2.13 |

In the nut grass root extract using chloroform solvent, from 142 compounds, there are 11 main compounds. The compound is α-Copaene; 1H-Cyclopropa [a] naphthalene, 1a, 2,3,3a, 4,5,6,7b-octahydro-1 (β-Maaliene); 1,4,6-Trimethyl-1,2,3,3a, 4,7,8,8a-octahydro-4,7-ethanoazulene (Rotundene); Guaia-1 (10), 11-diene (α-Bulnesene); (1aR, 3aR, 4R, 7R, 8aS) -1a, 4,9,9-Tetramethyloctahydro-3a, 7-met; Caryophyllene oxide; 4,6,6-Trimethyl-2- (3-methylbuta-1,3-dienyl) -3-oxatricyclo [5.1 .; Longiverbenone; (3aR, 4R, 7R) -1,4,9,9-Tetramethyl-3,4,5,6,7,8-hexahydro-2H-3a (Cyperotundone); cis-Z-a-Bisabolene epoxide; 5,8-Dimethyl-1,4,6,7-tetrahydronaphthalene.

**Table 2**. The main compounds detected in the chloroform extract of nut grass rhizomes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No. | Nomor Puncak | Senyawa | Waktu Retensi (menit) | Area Puncak (%) |
| 1. | 24 | α-Copaene  | 19.45 | 2.11 |
| 2. | 27 | 1H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1 (β-Maaliene) | 20.11 | 33.02 |
| 3. | 34 | 1,4,6-Trimethyl-1,2,3,3a,4,7,8,8a-octahydro-4,7-ethanoazulene (Rotundene) | 21.6 | 2.28 |
| 4. | 39 | Guaia-1(10),11-diene (α-Bulnesene) | 22.44 | 1.3 |
| 5. | 47 | (1aR,3aR,4R,7R,8aS)-1a,4,9,9-Tetramethyloctahydro-3a,7-met  | 23.35 | 1.15 |
| 6. | 58 | Caryophyllene oxide | 24.65 | 2.78 |
| 7. | 64 | 4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)] octane | 25.52 | 1.66 |
| 8.  | 74 | Longiverbenone  | 26.85 | 2.91 |
| 9. | 78 | (3aR,4R,7R)-1,4,9,9-Tetramethyl-3,4,5,6,7,8-hexahydro-2H-3a (Cyperotundone) | 27.26 | 11.49 |
| 10. | 103 | cis-Z-a-Bisabolene epoxide | 30.17 | 1.08 |
| 11. | 139 | 5,8-Dimethyl-1,4,6,7-tetrahydronaphthalene | 37.59 | 2.35 |

 From Tables 1 and 2, it can be seen that the most dominant compound, both in the ethanol extract and chloroform extract of nut grass rhizomes, is Cyperotundone. Cyperotundone is an organic compound that belongs to the sesquiterpenoides group and is contained in nut grass (Cyperus rotundus). Apart from Cyperotundone, the compounds contained in both types of extracts are Caryophyllene oxide and Longiverbenone. Both of these compounds have cytotoxic and antimicrobial activity (Rahman and Anwar, 2008; Yagi *et al.*, 2016). There are no studies that discuss the antioxidant activity of these three compounds.

The other three main compounds in the ethanol extract of nut grass rhizomes, namely d-Selinen; 2,5-Octadecadiynoic acid, methyl ester; and Methyl trisporate B, there is also no discussion about its antioxidant activity. A study shows that Solanum spirale essential oil has antioxidant and cytotoxic activities, where the main compounds contained in these essential oils are other types of Selinene, namely α-Selinene and β-Selinene (Keawsa-ard et al., 2012). While there has been no research on the biological activity of 2,5-Octadecadiynoic acid, methyl ester, Methyl trisporate B, is known to have biological activity as a pheromone in the Zygomycetes fungi (Nakamura *et al*., 2017).

A tricyclic sesquiterpene compound detected in chloroform extract, α-Copaene, has cytotoxic and antioxidant activity. Research on the cytotoxic effect of α-Copaene on N2a neuroblastoma cells shows that α-Copaene has antioxidant activity, as evidenced by an increase in total antioxidant capacity (TAC) and at higher doses increases total oxidative status (TOS) in N2a-NB neuron cell cultures (Turkez *et al.*, 2014) . Other studies on the effects of copaene on human lymphocyte cells in vitro have shown that copaene increases the antioxidant capacity of human lymphocyte cultures(Turkez, Celik and Togar, 2014).

There have been no studies looking into the effects of isolated β-Maaliene compounds, but there are studies on chemical analysis and biological activity of plant essential oils of Valerianaceous species that show moderate antioxidant activity. Where the main compounds of these essential oils include α-Selinene and β-Maaliene (Wang *et al.*, 2010). Meanwhile, the cis-Z-a-Bisabolene epoxide compound has biological activity as a pheromone or hormone to increase sexual activity(Ganesh and Mohankumar, 2017).

Literature studies on phytochemical compounds and biological activity of Patchouli essential oil, of which the main compound is α-Bulnesene, shows that Patchouli essential oil has potential antioxidant activity. However, data on α-Bulnesene itself has not been found (Swamy and Sinniah, 2015). There has been no research on the biological activity of other compounds, namely Rotundene; (1aR, 3aR, 4R, 7R, 8aS) -1a, 4,9,9-Tetramethyloctahydro-3a, 7-met; 4,6,6-Trimethyl-2- (3-methylbuta-1,3-dienyl) -3-oxatricyclo [5.1.0.0 (2,4)] octane; and 5,8-Dimethyl-1,4,6,7-tetrahydronaphthalene.

**5. CONCLUSION**

This study concluded that of the 6 main compounds of ethanol extract and 11 main compounds of chloroform extract of nut grass rhizomes, which is proven to have potential as an antioxidant agent is α-Copaene.

**LIMITATION AND STUDY FORWARD**

This research is limited to the identification of potential antioxidant activity of the main compound. Hence, it is necessary to do further research to explore the potential of other compounds detected in the ethanol and chloroform extract of nuts grass.

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