Extract of fermented plants to increased content of vitamin c, total phenol and antioxidant of mustard greens

Rachmiwati Yusuf¹, Indra Fuadi², Ida Nur Istina¹, Sri Swastika¹ and Anis Fahri¹
¹Riau Assessment Institute For Agricultural Technology, Indonesia
²Agency for Agricultural Research and Development, Indonesia.
Email: rachmi_2608@yahoo.co.id

Abstract. Research on the application of fermented plant extracts to increase the antioxidants of mustard greens has taken in the experimental garden of Horticulture Seed Center in Riau Province, from June to October 2018. This study used a completely randomized design with six treatments and three replications. (A) = neem leaves; (B) = sour-sop leaves; (C) = the crown of the god; (D) = lemongrass; (E) = mixture of plant extracts (neem leaves, sour-sop leaves, god's crown fruit and lemongrass); and (F) = control. The parameters observed in this study were vitamin C content (mg / 100 g), total phenol content (mg/100 g) and total antioxidant activity (% inhibition). The purpose of this study was to determine the effectiveness of several kinds of plant extracts (neem leaves, soursop leaves, god's crown fruit and lemongrass and a mixture of several plant extracts) fermented with EM4 on levels of vitamin C, total phenol content and antioxidants of mustard greens. The results showed that in general, the fermented neem leaf treatment provided the highest effectiveness for all parameters compared to other plant extracts, namely vitamin C levels (72,846 mg/100 g), total phenol content (0.074 mg/100 g) and antioxidant (75.230%).

1. Introduction

Pests and diseases in plants are one of the most disturbing disturbances in agricultural business, especially vegetable crops. The attack can be sudden and explosive so that in a relatively short time, it can kill the entire plant and cause crop failure. In pest control is carried out by vegetable and fruit farmers, mostly chemically using synthetic pesticides, these chemicals can pollute the environment and food products and can reduce the content of antioxidant compounds. The use of organic pesticides has been shown to increase of antioxidant compounds, such as vitamin C and polyphenols; so that the use of it can be able to increasing the antioxidant content both of vegetables and fruits[1].

Antioxidants are chemicals that harm or inhibit oxidation damage. Antioxidants are primary compounds in maintaining a healthy body because they function as a fortress that can prevent various diseases. In a specific sense, antioxidants are substances that can obviate or prevent the occurrence of free radical autoxidation reactions. Examples free radicals diseases are degenerative diseases (deterioration of body functions),
cardiovascular disease, cancer, neurodegenerative diseases, diabetes, cataracts, heart attacks and premature ageing. It is caused by chronic free radicals so that it takes years to treat the disease [2,3,4]. Antioxidants are secondary metabolite compounds produced by plants. The secondary metabolite synthesized compounds obtained by genetics and the environment, such as conventional or organic environments [5]. In this case, free prevention prevents tissue damage caused by radicals by eliminating the formation of radicals, reducing or increasing their breakdown [6,7].

Antioxidants can achieve from internal enzymes such as superoxide dismutase (SOD), (GPX), catalase (CAT), (GSH), tocopherols, and β-carotene as well as from food intake or supplements such as vitamin C, vitamin A which are known as a synthetic antioxidant [8]. The use of antioxidants from synthetic materials is known to increase the occurrence of carcinogenesis [9], so it is more advisable to obtain them from natural ingredients. Natural antioxidants come from every part of the plant such as bark, stems, leaves, flowers, fruits and roots [10]. Supporting in production increased healthy vegetable crops and antioxidant content, the research needed on the effectiveness of plants that produce organic pesticides in increasing vitamin C, total phenols and plant antioxidants.

According to [11,12] a type of plant can have antioxidant activity if it contains compounds capable of warding off free radicals such as phenols, flavonoids, vitamins C and E, catechins, carotene and resveratrol. Several chemical compounds in plants that can act as antioxidants, including polyphenols, alkaloids, flavonoids, vitamin C, vitamin E and β-carotene. The used of synthetic pesticides chosen as the last alternative due to the impact caused, so it must be thought about as early as possible and retained to a minimum. Therefore, the policy of using environmentally friendly vegetable materials is the right choice to build future agriculture.

The results of literature search [13,14, 15, 16] obtained that various types of fermented plants can act as a source of natural antioxidants. The use of fermented plant extracts induces the formation of secondary metabolic compounds, such as vitamin C and total phenol, which further increases antioxidant activity. The chemical compounds contained in plants are broken down by microorganisms that contain in EM4 through a fermentation process, resulting in active phytohormones and their derivatives such as auxins, gibberellins, cytokinins and kinetins [17]. In this study, the plants used as organic pesticides were by selected from plants that have medicinal and antioxidant properties such as neem, the crown of gods, soursop and lemongrass. The purpose of this study was to determine the effectiveness of several kinds of plant extracts fermented with EM4 on levels of vitamin C, total phenol content and antioxidants of mustard greens.

2. Material and Methods

2.1 Place and Time

The research was divided into two activities. The mustard planting was done at the site garden of the Riau Province Food and Horticultural Seeds Institute. The second activity is the examination of vitamin content has been done at the FMIPA laboratory, Riau University. This research was conducted from June to October 2018.

2.2 Materials and Utilities

The materials used in this research were mustard seeds and plants, filter paper, fermented plant extracts (neem leaves, soursop leaves, god's crown fruit and lemongrass), Effective Microorganism (EM)4, brown sugar solution, Folin Cioucalteau reagent, Citric acid iodine and other ingredients. other chemical required. The tools used in this research include Spektronik 20 D, centrifugal, hand tractor, hoe, watering can, a set of stationery and other glass tools by work procedures.
2.3 Research design

This research consists of three stages of work. The first stage was making the Fermented Plant Extract (FPE), the second stage was sample preparation for field plants. The third stage was testing/observing predetermined parameters. Preparation for mustard planting starts from land clearing, tillage and construction of a 1x1 meter bed. This study used a completely randomized design (CRD) with six treatments and three replications. The fermented plant extract used were: (A) = neem leaves; (B) = soursop leaves; (C) = the crown of the god; (D) = lemongrass; (E) = mixture of plant extracts (neem leaves, soursop leaves, god's crown fruit and lemongrass); and (F) = control. The parameters observed in this study were vitamin C levels (mg / 100 g), total phenol content (mg / 100 g) and total antioxidant activity (% inhibition).

The fermented plant extract was made by weighing 250 g of plant material, then cut it into small pieces and put it in a 1500 ml aqua bottle. Next, add 10 ml of EM4, 10 ml of brown sugar solution (1: 1) and 1000 ml of water. Shake well and leave for 15 days in the shade at room temperature. Every day the bottle cap is opened to release gas. The characteristic of ready-to-use vegetable pesticides is when they smell a specific plant fragrance. For non-fermented vegetable pesticides, it is made by blending 250 g + a teaspoon of soap, then left for 24 hours before use. The application of vegetable pesticides is carried out by mixing 10 ml of extract in 1 litre of water and then pouring it all over the plant using watered can. Vegetable pesticides are applied to plants 7 days after planting.

2.4 Determination of Vitamin C Content

Determination of vitamin C is evaluated by the iodometric method. The mustard plant was weighed 25 grams and blended to form a homogenate. The homogenate was put into a 100 ml measuring flask and added with distilled water to the limit mark, then centrifuged for 20 minutes at 4000 rpm. 25 ml of the filtrate was taken and put into 125 ml Erlenmeyer and added with 2 ml of 1% starch solution. The sample titrated with a standard solution of 0.01 N iodine [18]. The volume of the iodine solution used recorded, the final result of vitamin C with iodine solution produces a blue solution.

1 ml iodine ~ 0.88 mg ascorbic acid

2.4 Determination of Total Phenolic Content

Total phenol can be measured using the modified Folin-Ciocalteau method [19]. The determination of total phenol uses gallic acid as standard. Absorption was measured at 755 nm using Spektronik 20 D. 100 g of Mustard greens homogenized in 300 ml distilled water for 20 minutes and centrifuged for 10 minutes at a rotation of 5000 rpm. To each tube was added supernatant (0.5 ml) and standard gallic acid (0, 40, 80, 120, 160 and 200 ppm), then added 0.5 ml of Folin-Ciocalteau reagent and 7.5 ml of distilled water. The mixture was stirred at room temperature for 10 minutes before adding 1.5 ml of the 20% sodium carbonate solution. The mixture was heated using a water bath at 40°C for 20 minutes, then cooled. The total phenol content determined based on the linear regression equation of the standard solution of gallic acid, where the standard mg of gallic acid is equivalent to 100 g of fresh sample weight of kale.

\[ Y = a x \]

Note:

\[ Y = \text{samples absorbant} \]
\[ x = \text{Total concentration of fenol (mg/100 g wet samples)} \]
2.5 Antioksidan activity determinant of Mustard Plant

Method of Antioksidan activity determinant used by [20] modified methods. Five grams of mustard greens blended in 10 ml of distilled water. 50 μl Linoleic acid added in a test tube containing 5 ml of ethanol, and 50 μl plant extract to the solution, then vortexed. It is allowed to stand in a dark place at 25°C for 24 hours. 100 μl of FeCl₂ (0.014 M) and 100 μl of 30% KSCN were adding to each tube. After that, the absorbance measured at a wavelength of 500 nm. The blank of it is measure at time 0 and after 24 hours. Plant extract blank is the plant extract itself added with FeCl₂ and KSCN.

\[
\% \text{ resistance} = \left(1 - \frac{(Abs_{Sample_{24}} - Abs_{Blanko Extract_{24}}) - Abs_{Sample_{0}}}{Abs_{Blanko_{24}} - Abs_{Blanko_{0}}} \right) \times 100\%
\]

2.6 Data analysis

Data were analyzed statistically with ANOVA to determine whether the treatment of several kinds of fermented plant extracts and control gave differences in the content of vitamin C, total phenol and antioxidants of mustard greens, if there were differences, it extended by the 5% Duncan Multiple Range Test (DMRT).

3. Result and Discussion

3.1. Content of Vitamin C (mg / 100 g)

The results of observations on vitamin C content given six treatments presented in Table 1.

| Treatment                                      | Content of Vitamin C (mg/100g) |
|------------------------------------------------|--------------------------------|
| Fermented neem leaf extract                    | 72.846a                        |
| Fermented soursop leaf extract                 | 71.720a                        |
| Fermented lemongrass extract                   | 66.932b                        |
| Fermented extract of the crown of the gods     | 66.635b                        |
| Extract fermented plant mixtures               | 65.930b                        |
| Control                                        | 62.360b                        |

The numbers on each column followed by the same small letter is not significant according DNMRT test at 5% level.

Table 1 shows that the highest vitamin C content founded in the neem leaf FPE sprinkling treatment followed by the FPE treatment of soursop leaves, lemongrass FPE and the lowest was the control, all treatments gave very different results compared with control. Neem FPE treatment with soursop leaf FPE, lemongrass FPE with the crown of Dewa fruit FPE and with a mixture of 4 fermented plants gave no significant difference, while other treatments gave significantly different results.

3.2 Total phenol content (mg / 100 g)

The results showed that total phenol from each fermented plant extract treatment in Table 2.
Table 2. Average total phenol content of mustard greens grown with various FPE treatments

| Treatment                               | Total Concentration of fenolik (mg/100 g) |
|-----------------------------------------|-------------------------------------------|
| Fermented neem leaf extract            | 0.074<sup>a</sup>                         |
| Fermented soursop leaf extract         | 0.058<sup>b</sup>                         |
| Fermented lemongrass extract           | 0.055<sup>b</sup>                         |
| Fermented extract of the crown of the gods | 0.054<sup>b</sup>                        |
| Extract fermented plant mixtures       | 0.052<sup>b</sup>                         |
| Control                                | 0.036<sup>c</sup>                         |

The numbers on each column followed by the same small letter is not significant according DNMRT test at 5% level.

From Table 2 showed that the treatment that gave the highest total phenol content was neem leaf FPE, followed by soursop leaf FPE, lemongrass FPE, crown of god fruit FPE, mixed FPE 4 plants and the lowest was control. Neem leaf FPE treatment and control gave significantly different results with all treatments while un-significantly different from the other.

3.3 Total antioxidant activity (%)

Determination of total antioxidant activity aims to determine the extent to which antioxidants in mustard greens can prevent oxidation reactions caused by free radicals. The total antioxidant activity influenced by the content of vitamin C, the total concentration of phenolic and other antioxidant compounds found in the mustard greens itself. The total antioxidant activity for each treatment was present in Table 3.

Table 3. The average antioxidant content of mustard greens grown with various FPE treatments

| Treatment                               | (% Resistance) |
|-----------------------------------------|----------------|
| Fermented neem leaf extract             | 75.230<sup>a</sup> |
| Fermented soursop leaf extract          | 74.962<sup>a</sup> |
| Fermented lemongrass extract            | 74.126<sup>a</sup> |
| Fermented extract of the crown of the gods | 70.830<sup>b</sup> |
| Extract fermented plant mixtures        | 70.236<sup>b</sup> |
| Control                                 | 65.214<sup>c</sup> |

The numbers on each column followed by the same small letter was not significant according DNMRT test at 5% level.

Table 3 show that the total antioxidant activity (% inhibition) founded at sprinkling neem leaf FPE followed by FPE treatment of soursop leaves lemongrass FPE and the lowest resistance found in control. The result of the further test showed that all treatments gave significantly different results from the control, but the treatments of FPE of neem leaves with FPE of soursop leaves and FPE of lemongrass, FPE of the crown of gods with mixed FPE of four plant extracts were un-significant each other.

3.4 Vitamin C content (mg / 100 g)

Vitamin C functions as an antioxidant, antioxidant, metal binding, reducing agent, and capturing oxygen. Watering plants with fermented plant extracts can increase the content of vitamin C, polyphenols, antioxidant activity and plant resistance to pests and diseases [21]. These bioactive compounds can induce genes in plants to form secondary metabolites such as antioxidants and polyphenols [22, 23]. The use of plant extracts mixed with EM4 is assumed to have a dual effect, namely that in addition to affecting plant pests, EM4 can also fertilize plants, increase plant production and provide nutrients
needed by plants. The high content of vitamin C in mustard greens with plant extract fermented in EM4 compare to control. It is due to the containing bioactive organic acids. The small phytochemical or metabolic molecules that are easily absorbed and needed for plant growth and protection. Against pests and diseases. Bioactive compounds can increase the synthesis of polyphenols, vitamin C and other antioxidant compounds [1].

[24] added that watering with FPE will nourish plants because of the presence of microorganisms that can produce hormones, vitamins and substances needed for plants. It will affect increasing nutrition for plants and increasing antioxidants. Plots watered by neem leaf FPE gave better results compared to other treatment plots. It is due to the bioactive compounds azadirachtin, meliantriol and galanin contained in neem leaves are suitable for stimulating the growth of mustard, one of them is plant height increase. These bioactive compounds will stimulate an increase in synthetic nitrogen which is beneficial for plants.

3.5 Total phenol content (mg / 100 g)
In the observation of total phenol, the sprinkling treatment with FPE also gave higher results compared to the control. It is because the microbes contained in Effective Microorganism (EM) can degrade organic compounds from plant extracts and sugars into simpler compounds that can be used by plants. In addition to increasing total phenol, watered plant growth with plant extracts also showed better growth, such as in plant height and the resulting leaf width. Leaves are the site of photosynthetic events for plants, the better plant growth (high production and low leaf damage) gives an idea that photosynthesis in these plants has been going well. It is in line with the statement by [23] which states that the content of phenolic compounds can increase due to the influence of environmental factors such as the high intensity of sunlight irradiation, soil fertility conditions and fertilization.

According to [22], the high intensity of sunlight received by plants will increase plant phenolic content by photosynthetic bacteria which contained in EM. [21], stated that the phenolic content in plants could increase due to the high intensity of sunlight irradiation. Mustard greens grown organically using FPE and bokashi fertilizer fermented with EM4 can also increase the phenolic content of these plants because the photosynthetic bacteria contained in EM4 will increase the photosynthetic capacity of the plant so that it can increase the phenolic content of the plant. The chemical content of neem leaf extract fermented with EM thought to have a high effect on the increase in bioactive compounds that can stimulate the synthesis of antioxidant content. This condition causes the antioxidant content to be higher and significantly different in giving fermented neem leaf extract compared to other treatments.

3.6 Total antioxidant activity (%)
The highest total antioxidant activity was in the FPE treatment. It is because the sprinkler solution is a fermented solution of beneficial plant groups. According to [25], the microbes contained in EM4 degrade organic compounds from plant extracts and sugars into simpler compounds that can be used by plants, besides that the metabolic produced such as hormones, vitamins and amino acids will encourage the formation of antioxidant compounds.

The content of vitamin C, total phenol concentration and total antioxidant activity of control were lower than that of the mustard greens planted with FPE watered. The low total antioxidant activity was due to water treatment, which resulted in the plants growing smaller and the vitamin C content in the plants being low which in turn affected the total antioxidant content as a whole. All plots treated with plant extract sprinkling gave significantly different results on the antioxidant content of mustard greens compared to the control. It shows that giving plant extracts can increase bioactive compounds that can stimulate the synthesis of antioxidant compounds.
Plots treated with single extracts gave best results. It is maybe due to an antagonistic chemical reaction by mixing the plant extracts so that it will reduce the effect on the chemical content of each plant. Plants that grown optimally and produced organically will produce healthy products, contain antioxidants and are safe for health because they do not contain pesticide residues. It indicated from the three components of antioxidants that observed, namely the content of vitamin C, total phenolic concentration and antioxidant activity.

Plants watered with fermented neem leaf extract showed the highest vitamin C content compared to other treatments. According to [1] plants that produced organically contain high vitamin C. It is presumably due to the non-oxidation of some of the vitamin C by chemical fertilizers and pesticides. Fertilizers, pesticides and other agricultural chemicals are free radical compounds that will continue to oxidize to stabilize themselves. The use of these chemicals will result in plant reactions to defend themselves, namely by providing the antioxidants they have, so that the free radical compounds get a partner and become stable.

4. Conclusion
The results of the study concluded that in neem plant extract fermented with EM4 gave the highest effectiveness for vitamin C levels, total phenol content and antioxidants of mustard greens. Planting the vegetables and fruits were recommended to watered using neem leaf extract fermented with EM4. It was enhanced in levels of vitamin C increasing, total phenol and antioxidant activity. Further research, on the increase in antioxidants and the effectiveness of neem plants as an organic pesticide, needs to be continued and developed on a broader scale, such as in food crops and other horticultural crops.

References

[1] Wood, M T, Miles R and Tahoro P 1999 Proceeding of fifth Internasional Conference on Kyusei Nature Farming and Effective Microorganisms for Agriculture and Environmental Sustainability 207-215
[2] Matteo V D and E Esposito 2003 Current Drug Targets-CNS & Neurological Disorders 2 95-107
[3] Touyz R M and E L Schiffrin 2004 Histochem Cell Biol.122 339-352
[4] Ardiansyah 2007 Antioksidan dan Peranannya Bagi Kesehatan Available from : http://www. Berita Iptek-2007-01-23. Antioksidan dan Peranannya Bagi Kesehatan.Shtme.
[5] Winter C K and Davis S F 2006 J.Food Sci 71 1-19.
[6] Young J S and J V Woodside 2001 J. of Clinical Pathology 54 176-186.
[7] Goldberg G 2003 Plants: Diet and Health Lowa State Press, Blackwell Publishing Iowa State Press, State Avenue: Blackwell Publishing Company
[8] Mates J M, C Perez-Gomez and I NDeCastro 1999 Clinical Biochemistry 32 595-603.
[9] Rohman A and S Riyanto 2005 Majalah Farmasi Indonesia 16 136-140
[10] Pratt D E 1992 Natural Antioxidants From Plant Material. Phenolic Compounds in Food and Their Effects on Health (Washington DC: American Society)
[11] Hernani and Rahardjo M 2005 Tanaman Berkhasiat Obat (Jakarta: Penebar Swadaya)
[12] Walujo E B 2008 Biodiversitas 9 59-63.
[13] Hakim E H, Y M Syah, L D Juliawati and D Mujahidin 2008 Jurnal Matematika dan Sains13 33-42.
[14] Rohman A, S Riyanto and D Utari 2005 Majalah Farmasi Indonesia 17 136-142.
[15] Amrun M and Umiyah 2005 Jurnal Ilmu Dasar 6 110-114.
[16] Praptiwi, M Harapini and I Astuti 2006 *Biodiversitas* 7 242-244
[17] Kato S, H L Xu, M Fujita, K Yamada, K Karase and Umemura 1996. *Effect Of Organic Fertilization on Growth Pattern* (Bangkok: Kyuse Nature Farming) pp 23-26
[18] Soedarmadji S, Haryono B and Suhardi 1997 *Prosedur Analisis untuk Bahan Makanan dan Pertanian* (Yogyakarta: Liberty)
[19] Chaovanalikit A and Wrolstad R E 2004 *J. Food Science*. 69: 67
[20] Lindsey I, Motsel I, M and Jager K A 2002 *J. Food Science*. 67: 2139-2130
[21] Jose C, Abdullah C A M, Armaini, Nurkholidah, Annisava A R 2006 *Natural Farming System: Its Influence on the Growth and Antioxidant of Vegetables. Prosiding Seminar UKM-UNRI Ke-4*, Fakulti Sains dan Teknologi, Malaysia: Universiti Kebangsaan Malaysia) pp 283
[22] Kalt W 2005 *J Food Science* 70 R11.
[23] Hounsome N, Hounsome B, Tomos D, and Edwards-Jones G 2008 *J. Food Science* 73 R48-R65.
[24] Asia Pacific Nature Agriculture Network 1997 *Pedoman Penggunaan EM bagi Negara-negara Asia Pacific Nature Agriculture Network (APNAN)*. Dalam Seminar Nasional Pertanian Organik, Jakarta, 03 April 1997.
[25] Higa T 1993 *Effective Mikroorganisms : Their Role in Kyuse Nature Farming and Sustainable Agriculture. Prosiding of Third International Conference*. Held in Santa Balbara, California USA 5-7 Oktober 1993.