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Editors • Peng Zhang and Tri Agus Siswoyo

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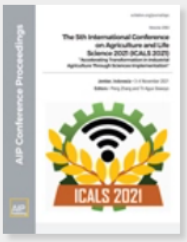
Table of Contents

PREV NEXT



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Date : October 1, 2021

To.
Dr.Ir. I Ketut Budaraga.MSi

Letter of Acceptance

Reff : 144/LoA/ICALS/X/2021

Dear Authors

We take the pleasure to inform you that your submitted abstract as specified below:

Tittle: Study of Antioxidants by-products of Gambir Leaves into Tea with the Addition of Red Ginger Powder (Zingiber officinale Var. Rubrum)

Has been officially accepted for oral presentation in The 5th International Conference on Agriculture and Life Sciences 2021 which will be held on November 3 - 4, 2021.

In order to help us in organizing a well-prepared and successful international conference, please submit your full paper manuscript that matches with the format and follow the guidelines posted at our website before October 12, 2021. Please note that the final decision for publication media will be based on the reviewer's selection board. Your kind cooperation is highly appreciated. The presented and selected paper will be published in **AIP Conference Proceedings** **with publication fee USD 120 / Rp 1.750.000.**

We are delighted to acknowledge your registration for the conference and are looking forward to your participation. More information is available on our conference website: **https://icals.unej.ac.id/** .

However, if you have any questions please feel free to contact us.

Sincerely yours,

Ir. Didik Pudji Restanto, MS., Ph.D
Chairman of the Organizing Committee The 5th ICALS

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Antioxidant study of the gambier leaves by-products into tea with red ginger powder addition (*Zingiber officinale var. Rubrum)*

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Antioxidant Study of the Gambier Leaves By-Products into Tea with Red Ginger Powder Addition (*Zingiber officinale Var. Rubrum)*

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Abstract. Indonesia has been the largest gambier producing country in the world and West Sumatra is the largest gambier producing center in Indonesia, it has become the province's leading commodity. Gambier sap production always produces by-products in the form of leftover leaves that can be developed into food products in the form of teabags. This study focuses in determining the antioxidant activity of by-products of gambier leaves with the addition of red ginger powder. This research was conducted at the Agricultural Product Technology Laboratory, Universitas Ekasakti, Padang from October to November 2020. The design used in this study was a simple completely randomized design with 5 treatments and 3 replications. The treatment in this study was the addition of dry ginger powder in gambier leaf tea, namely: A = 0%; B = 10%; C = 20%; D = 30%; E = 40%. The results of observations were analyzed by variance analysis (ANOVA) and Duncan's New Multiple Range Test (DNMRT) follow-up test at a 5% level. The results showed that the addition of red ginger powder to the tea by-products of gambier leaves had a significant effect between treatments on the antioxidant content. The entire parameters have met the quality requirements of dry tea set by SNI and red ginger gambier leaf tea is the most preferred by panelists in treatment E with 40% red ginger powder addition.

INTRODUCTION

Indonesia has a very important position as the largest gambier producer in the world by supplying 80% of the world's gambier needs. Based on Trade Map data in 2014, West Sumatra is the largest gambier center in Indonesia and can supply 80% to 90% of the total national gambier production with Lima Puluh Kota Regency as the largest producer of gambier (70.39%)[1]. Gambier is one of the leading commodities specifically for West Sumatra intending to export markets. Gambier as an export commodity contributes to the Gross Regional Domestic Product (GDP) of West Sumatra. Gambier is a people's commodity and is the main source of income for farmers in production centers, namely Limapuluh Kota and Pesisir Selatan Districts. The government's guidance is directed at the gambier production and processing subsystem to increase farmers' income. The aim is to increase production so that the export value increases. Gambier is often exported to India, Pakistan, Singapore, Bangladesh, Taiwan, Germany, and Japan, and others[2].

The Gambier part that is often used is the dry sap extract which is obtained from the leaves and stems through the compression and drying process so that it becomes a dry block. The use of gambier in various modern products is needed as a product diversification so that it is easier to consume and is of interest to the wider community as well as to increase the added value[3]. Based on the survey results of several gambier processing farmers in West Sumatra, in producing every 5 kg of gambier sap, they will get ± 2 kg of gambier by-product, so that the by-product of gambier produced annually is estimated at 5,582,000 kg.[4]

To increase the use-value of the by-products of gambier, it is necessary to use it widely in the industrial sector including the animal feed industry, fertilizer, and food industry. Utilization of gambier by-products requires

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conditioning before use because it is easily damaged and is characterized by the growth of white fungus[5] There need to be innovations to increase the added value of gambier farming, namely in the form of processing gambier by-products into gambier tea. Gambier also contains tannins, but less than tea leaves (*Camellia sinensis*). Therefore, it is necessary to make efforts to process gambier as a substitute for tea (*Camellia sinensis*) with a taste and aroma that is not inferior to tea (Camellia sinensis). Genetically, gambier plants contain more catechins than tannins, while tea plants contain more tannins than catechins.[6].

Catechins and tannins have different benefits, catechins are more useful in the fields of health, cosmetics, pharmaceuticals, and food, while tannins are used as skin tanning agents. 90% of the world's produced tannins are used for leather tanning[7]. In terms of health, it should be better to drink gambier leaf tea than Camellia sinensis tea, because gambier leaf tea contains higher catechins[8]. Gambier leaves also contain high levels of polyphenolic compounds similar to tea leaves (Camellia sinensis). One form of utilization and exploration of gambier leaves is making gambier leaf tea, in North Sumatra, especially in the Pak Pak Bharat Regency area, gambier leaf tea has been produced in the form of gambier tea bags through SMEs located in the area. Gambier leaf tea has the potential to be developed for health[9].

The marketing reach of this gambier leaf tea bag can be said to be low because consumer demand for this gambier leaf tea bag is low. This is because the aroma and taste of gambier tea bags are less favored by consumers with the distinctive aroma and taste of gambier and bitterness (astringency). For this reason, it is necessary to remove tannin compounds and non-polyphenolic compounds from gambier so that gambier polyphenol concentrates are obtained which have high polyphenols[10]. Spices are very aromatic because the content of essential oils is quite high as a component forming specific flavors, besides that spices also contain compounds that are good for health. Spices are often used by the community in the manufacture of traditional drinks such as herbs and other beverages. To eliminate the bitterness (astringency) and bitterness (bitterness) caused by tannin compounds and increase the ability of gambier as an antioxidant, it is necessary to innovate from gambier leaf tea with the addition of ginger.

Ginger is a rhizome-type spice that contains 0.25-3.3% essential oil. Ginger also contains gingerols and shogaols which cause a spicy taste, so that it can affect the aroma and taste of the gambier leaf tea. The ginger oleoresin contains about 33% gingerols, ginger rhizome contains about 6-8% fat, 9% protein, 50% more carbohydrates, vitamins, especially niacin and vitamin A, several types of minerals, and amino acids[11]. Ginger has quite a variety of uses, including as a spice, essential oil, flavoring agent, or as a medicine. The use of ginger as a beverage mixture is usually used in powder form so that it is easy to use and has a longer shelf life[12]. The research objectives are a. Knowing the effect of adding red ginger to the antioxidant content of gambier leaf tea, b. Knowing the right amount of red ginger in addition to producing quality gambier leaf tea and being liked by consumers.

MATERIALS AND METHODS

This research was carried out at the Agricultural Product Technology Laboratory, Padang Universitas Ekasakti, West Sumatra from October to November 2020. The main material used in this study was the by-product of gambier leaves (*Uncaria Gambier Robx*), taken directly from the results of the it is puzzling forging of farmers in Nagari Inderapura Pancung Problem District, Pesisir Selatan Regency, and red ginger were purchased directly at Pasar Raya Padang City. The materials used for the chemical analysis of the antioxidant test were distilled water, Follin ciocalteu reagent, Na2CO3 7%. The tools used in the research for tea processing are tempering, knife, blender, pot, scale, stirring spoon, and glass. The tools used for chemical analysis. Antioxidant tests are test tubes, vortex, UV-Vis spectrophotometer.

The design used in this study was a completely randomized design with 5 levels of treatment and 3 replications. The observed data were analyzed for variance (ANOVA) with the F test and Duncan's new multiple range test (DNMRT) at a 5% significance level. The treatment in this study was the addition of dry ginger powder in gambier leaf tea, namely: A = 0%; B = 10%; C = 20%; D = 30%; E = 40%. The formulation of gambier leaf tea was made by varying the concentration with ginger powder. Gambier leaf tea formulations can be seen in Table 1.

TABLE 1. Formulation of addition of ginger powder in gambier leaf tea

Ingredients	Unit	Treatment				
		A	B	C	D	E
Gambier tea powder	g	50	50	50	50	50
Ginger powder	g	0	5	10	15	20
Amount	g	50	55	60	65	70

(Modification, Widiastuti, 2017).

RESEARCH IMPLEMENTATION

The raw materials used in this study were by-products of gambier leaves which were taken directly from the presses of farmers in Nagari Inderapura, Pancung Problem District, Pesisir Selatan Regency, and red ginger from Pasar Raya, Padang City.

The Process of Making Gambier Leaf Tea Powder

As for how to make gambier leaf tea powder is as follows [3]:

1. Preparation of by-products of gambier leaves as much as 2 kg.
2. Gambier leaves are chopped and then washed with clean water for ± 20 minutes and drained.
3. Drying is done using the sun's heat with an average temperature of 27-30ºC for 4 hours.
4. Dried gambier leaves are ground using a blender until they become powder so that it is easy to serve as tea.
5. Then sieved using a sieve with a size of 60 mesh.

Process of Making Ginger Powder

As for how to make ginger powder is as follows [13]:

1. Preparation of red ginger ingredients as much as 1 kg.
2. Wash ginger until clean.
3. Sort the ginger and weigh it.
4. Size reduction using 5 mm thick slices
5. Drying is done using the sun's heat with an average temperature of 29-30ºC for 7 hours, reversed 3 times.
6. Do dry weighing.
7. Grind dry ginger using a blender.
8. The ginger powder was sieved using a 60 mesh sieve.

The Process of Mixing Gambier Leaf Tea with Red Ginger Powder

1. Mix gambier leaf powder and red ginger powder.
2. Enter the mixture of gambier leaf powder and red ginger powder according to the treatment.
3. Gambier red ginger leaf tea.

Antioxidant Analysis IC50

Analysis of gambier leaf tea with the addition of red ginger powder, namely antioxidant IC50 with the following analytical procedure [14] :

- **Preparation of 0.5 mM. DPPH solution**

The reagent solution is 0.5 mM DPPH solution in ethanol solvent. This solution was prepared by weighing 10 mg of DPPH powder, put into a 50 ml volumetric flask, then 96% ethanol was added, some of it was then shaken to dissolve the DPPH powder and then 96% ethanol was added to the mark.

- **Preparation of test solution**

The test solution was made with a concentration of 1000 ppm as the mother liquor. The preparation of the test solution was carried out by weighing the ethyl acetate fraction of the red ginger rhizome ethanol extract as much as

100 mg, put into a 100 ml volumetric flask and add 96% ethanol partially then shake until homogeneous, then add 96% ethanol to the limit mark. After that, it was made into a test solution of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm.

- **Preparation of vitamin C positive control**

Weighed 5 mg of vitamin C dissolved with 96% ethanol in a 50 ml volumetric flask then added with 96% ethanol up to the mark. Then the dilution was made into 4 concentration series, namely 4 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm.

- **Determination of operating time**

The test solution of gambier tea with the addition of red ginger was made in several concentrations. The concentrations of the test fractions made were 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. The test fraction was taken with the lowest concentration of 10 ppm, operating time was performed. Operating time is done by adding 4 ml of the test fraction plus 1 ml of 0.5 mM DPPH solution. The test solution was measured at 0.10,20,30,40,50, and 60 minutes at a maximum wavelength of 517.6 nm

- **DPPH radical scavenging absorbance measurement**

Blanks, test solutions, and positive controls that have been made in several concentrations, each taken as much as 4 ml, added 1 ml of 0.5 mM DPPH reagent, put in a vial, and shaken. The solution was allowed to stand for 30 minutes and then read the absorption at a maximum wavelength of 517.6 nm. The blank used was 96% ethanol and the positive control used was vitamin C. The absorbance measurement results using a UV-Vis spectrophotometer were used to calculate the percentage of free radical scavenging of DPPH. Percentage of DPPH free radical reduction is calculated using the formula:

$$\%\ Damping = \frac{Abs\ blanko - Ab\ \ sampel}{Abs\ blanko}\ x\ 100\ \%$$

Information: Abs blank : Absorbance DPPH + ethanol 96%
Abs sample : Absorbance sample + DPPH

The antioxidant activity of gambier tea with the addition of red ginger (Zingiber officinale var. Rubrum) and vitamin C and each IC50 value was calculated using linear regression analysis

$$Y = a + bx$$

The calculation results are entered into the regression equation with the extract concentration as the abscissa (X-axis) and the percentage of attenuation as the ordinate (Y-axis). The results of linear regression analysis in the form of the value of x were entered into the formula IC_{50} = Antilog x and determined by the level of antioxidant strength based on the IC_{50} value.

RESULTS AND DISCUSSION

The data on the measurement of DPPH inhibitory antioxidant activity can be seen in Table 2.

TABLE 2. Results of Antioxidant Analysis of IC_{50} Gambier Tea with the Addition of Red Ginger Powder

Treatment	Sample concentration (ppm)	% Antioxidant
A	10	9.54
	20	27.38
	30	48.31
	40	68.92
	50	86.15
B	10	15.69
	20	33.23
	30	52.31
	40	71.69
	50	87.08
C	10	21.23
	20	42.15
	30	59.69
	40	75.08
	50	88.92
D	10	35.08
	20	48.62
	30	65.54
	40	77.23
	50	92.92
E	10	40.00
	20	52.00
	30	66.46
	40	78.77
	50	94.46
Vitamin C	4	45.23
	6	52.00
	8	59.38
	10	66.77
	12	73.54

Based on Table 2, it can be seen that the antioxidant values are based on different concentration levels (ppm). Antioxidant testing was carried out at five concentrations and used vitamin C as a comparison. The results showed that the antioxidant value with a concentration of 10ppm ranged from 9.54-40%. The antioxidant value with a concentration of 20 ppm ranged from 27.38-52%. The antioxidant value with a concentration of 30 ppm ranged from 48.31 to 66.46%. The antioxidant value with a concentration of 40 ppm ranged from 68.92-78.77%, and the antioxidant value with a concentration of 20 ppm ranged from 86.15-94.46%. For the antioxidant value of vitamin C as a comparison, the concentration is calculated between 4-12 ppm with antioxidant values ranging from 45.23-73.54%. The value of the percentage of antioxidants from each treatment is then made a line equation curve that will

be used to calculate the value of the antioxidant activity of IC50. The line equation curve for each treatment is shown in Figure 1.

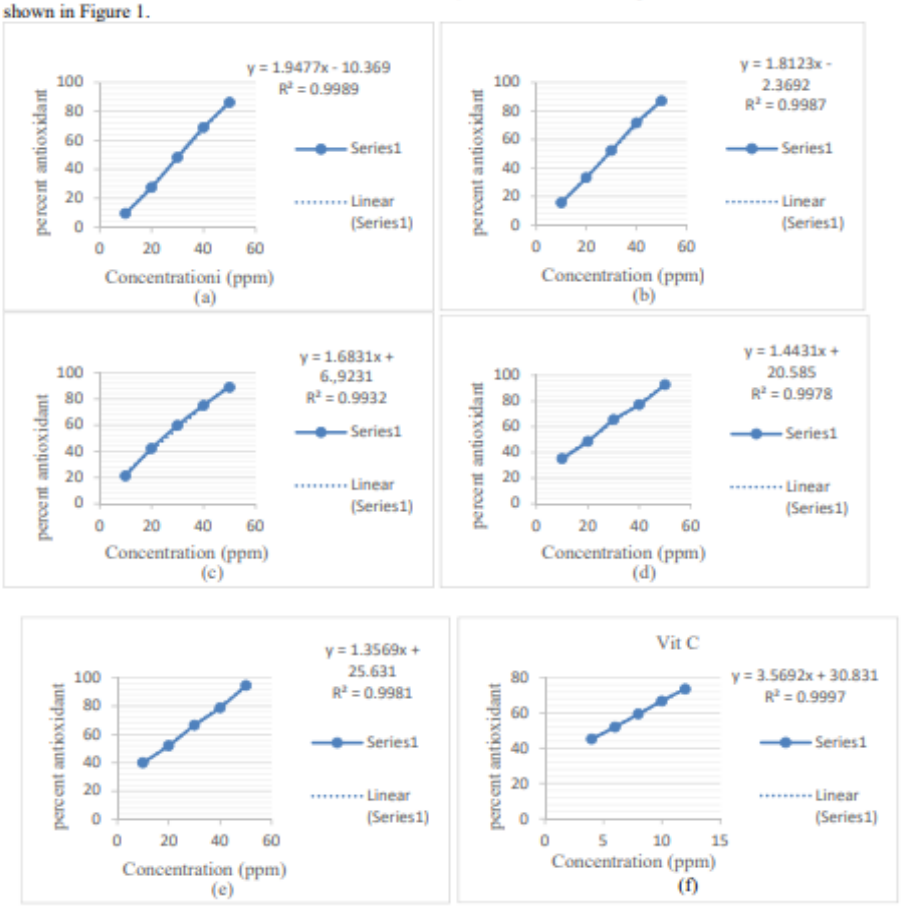

FIGURE 1. Linear regression analysis curve equation of gambier leaf tea line with the addition of red ginger

Figure 1 shows the linear equation curve of each treatment with vitamin C as a comparison. The line equation obtained is then used as a formula to find the value of antioxidant activity IC50. The value of the antioxidant activity of IC50 gambier tea with the addition of red ginger powder is shown in Table 3.

TABLE 3. Antioxidant levels of IC50 gambier tea with the addition of red ginger powder

Treatment	Equation of line	a	b	y	x (ppm)
A	y = 1.9477x - 10.369	1.9477	-10,369	50	31.00 a
B	y = 1.8123x - 2.3692	1.8123	-2.3692	50	28.90 b
C	y = 1.6831x + 6.9231	1.6831	6.9231	50	25.59 c
D	y = 1.4431x + 20.585	1.4431	20.585	50	20.38 d
E	y = 1.3569x + 25.631	1.3569	25.631	50	18.00 e
Vitamin C	y = 3.5692x + 30.831	3.5692	30.831	50	5.37 f

Note: The numbers in the same column are followed by different lowercase letters, indicating a very significant difference according to the DNMRT test at the 5% level.

Based on the data in Table 3, it can be seen the IC_{50} antioxidant value of gambier tea with the addition of red ginger powder. The antioxidant value of IC_{50} gambier tea with the addition of red ginger powder ranged from 31-18 ppm. The strongest IC_{50} antioxidant activity value was found in treatment E with the addition of 20 grams of red ginger powder. The strongest IC_{50} antioxidant activity value was found in treatment E with the addition of 20 grams of red ginger powder with a value of 18 ppm. The lowest IC_{50} antioxidant activity value was found in treatment A with no addition of red ginger powder. The antioxidant activity value of IC_{50} vitamin C is 5.37 ppm.

The smaller the IC_{50} value, the higher the antioxidant activity. IC_{50} values <50-100 indicate very active antioxidants, IC_{50} values <101-250 indicate moderate antioxidant activity, and IC_{50} values <250-500 indicate weak antioxidants, and IC_{50} values <500 are less active as antioxidants[15]. The data above shows an increase in the strength of the antioxidant activity of gambier tea with the addition of red ginger powder, the more ginger powder is added, the stronger the antioxidant activity of the gambier IC_{50}. The addition of red ginger powder to the gambier increases the antioxidant value and belongs to the very active category. The increase in antioxidant levels is due to red ginger contains antioxidant compounds and phenolic compounds. Research result Manju *at al.* (2005 [16] stated that the natural antioxidant compounds in ginger are quite high and very efficient in inhibiting superoxide and hydroxyl free radicals. Red ginger has higher oleoresin than white ginger and emprit ginger[17]. The content of essential oils and solubility determines the number of antioxidants and total phenols contained in ginger[18].

CONCLUSION

From the research that has been done, it can be concluded as follows:

1. The addition of red ginger powder to the by-product of gambir leaf tea is significantly different from the antioxidant value. Its is puzzling test has met the requirements set by SNI.
2. The correct formulation of red ginger powder were in treatment E (40% red ginger powder concentration).

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CERTIFICATE

No: 20830/UN.25/TU/ICALS/2021

This is to certify that

Dr.Ir. I Ketut Budaraga.MSi

has attended as participant

at the 5th International Conference on Agriculture and Life Sciences
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held at University of Jember - Indonesia, November 3-4, 2021

Rector,

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This is to certify that

Dr. Ir. I Ketut Budaraga, MSi.

has attended as oral presenter following paper entitled:

Antioxidant Study of the Gambier Leaves By Product in to Tea with Red Ginger Powder Addition (Zingiber officinale Var. Rubrum)

at the 5th International Conference on Agriculture and Life Sciences
"Accelerating Transformation in Industrial Agriculture Through Sciences Implementation"
held at University of Jember - Indonesia, November 3-4, 2021

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