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With regard to the above matter, please find attached a manuscript entitled "Total Phenolic, Antioxidant and Antibacterial Activities of Curcumin Extract of Kunci Pepet (*Kaempferia rotunda* L)" to be considered for publication in your esteemed journal.

Thank you for your consideration.

Yours sincerely,

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Date: 3rd March, 2018

Place: Palembang

From: Nura Malahayati
Department of Agricultural Technology,
Faculty of Agriculture,
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To: The Managing Editor

RJPBCS

Sir,

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In reference to the above title, I as a corresponding author, submit the manuscript for publication in Research Journal of Pharmaceutical, Biological and Chemical Sciences. I undertake that animal study (if any) was taken after the prior approval of Country/Institutional Animal Ethics Committee. This manuscript has not been published or considered for publication by any other journal or elsewhere. Kindly consider the manuscript for publication in your journal.

Thank you

Nura Malahayati

Total Phenolic, Antioxidant and Antibacterial Activities of Curcumin Extract of Kunci Pepet (*Kaempferia rotunda* L)

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ABSTRACT

Kunci pepet (*Kaempferia rotunda* L) is plant belonging to the ginger family that grows in Indonesia. The plant has many benefits other than used as a spice, food preservatives and coloring substances is also one of the types of medicinal plants that have many health benefits. *Kunci pepet* contains flavonoid and curcuminoid compounds as antioxidant. The aims of this study was to investigate total phenolic, antioxidant and antibacterial activities of curcumin extract from *kunci pepet* by using n-hexane, ethyl acetate and etanol solvent. Total phenolic was conducted by the Folin-Ciocalteau method. Antioxidant activity was detected by using DPPH (2,2-diphenyl-1-picrylhydrazyl) method with parameter specified was inhibition concentration (IC_{50}). The agar disc-diffusion method was adopted to examine antibacterial activity. The study showed ethanol extracts exhibited the highest curcumin yield (10,36±0.64%), curcumin value (1.92±0.02 μg/mL), total phenolic (5.11±0.01 μg/mL), and antioxidant activity (67.95±0.21 μg/mL). IC_{50} of n-hexane, ethanol and ethyl acetate fractions categorized in active level (IC_{50} 50-100 μg/mL). Ethyl acetate extracts exhibited the maximum zone of inhibition against *Staphyloccocus aureus* (5.32±0.12 mm) and *Escherichia Coli* (5.21±0.01 mm).

Keywords: *Kaempferia rotunda* L, curcumin, total phenolic, antioxidant, antibacterial

INTRODUCTION

Kunci pepet (*Kaempferia rotunda* L) or white turmeric plant, a perennial herb belonging to the ginger family, is cultivated extensively in Indonesia especially in Java and Sumatera islands. The rhizome of this plant is also referred to as the root and is the most useful part of the plant for culinary and medicinal purposes.

This plant is known by the Indonesian community as a traditional medicine because it has many health benefits such as abdominal pain, diarrhea, dysentery, and cold [1]. Recently, the dried powder of *kunci pepet* rhizomas is famous as traditional prevention and treatment of cancer diseases. In vitro tests showed that *kunci pepet* can increase the number of lymphocytes, specific antibodies, and can kill cancer cells [2]. Moreover, results of the research conducted by Atun *et al*. [3] showed that methanol extract from kunci pepet showed antimutagenik activity.

Active component in *kunci pepet* that plays a role is curcumin, an orange–yellow crystalline powder practically insoluble in water. Curcumin as a phenolic group compound contains antioxidants so *kunci pepet* rhizomas is useful as an anti-inflammatory, anti-arthritis, anti-tumor, anti-cancer, and anti-microbial.

Increased public awareness of the negative effects of chemical drugs makes consumer demand for traditional medicine continues to increase. The average turmeric intake of Asians ranges from 0.5-1.5 g/day/person, where this amount of intake does not provide poisoning symptoms [4]. Based on clinical trials in humans showed that curcumin has no toxicity when administered at a dose of 1-8 g/day [5] and 10 g/day [6]. Thus, both turmeric and curcumin have the potential for development of modern medicine.

The fresh and dried powder of *kunci pepet* rhizomes are widely sold in Indonesian traditional medicine market. The common practise of producing traditional medicine is by boiling fresh rhizomes, eating directly dried powder rhizomes, and/or making dried powder rhizomes as a drink by adding hot water. Curcumin, as a liposoluble bioactive component, in its pure form has poor solubility in water, potentially limiting its medicinal use for humans when it is taken orally. Curcumin can be extracted from turmeric root with organic solvents such as ethanol or acetone. As a medicine that has many properties, it is necessary to improve curcumin's low solubility using an extract as a carrier.

There are many research have already done related with the turmeric (*Curcuma longa* Linn.) but research on the genus *Kaempferia* has not been widely reported. So, this study attempted to investigate total phenolic, antioxidant and antimicrobial activities of curcumin extract of *kunci pepet* (*Kaempferia rotunda* L) by using n-hexane, ethyl acetate and etanol solvent.

MATERIALS AND METHODS

Source of Material

Kunci pepet (*Kaempferia rotunda* L) used for this study was purchased from a local market in Jakabaring, Palembang City, South Sumatra Province, Indonesia. The reference curcumin and extraction solvent (n-hexane, ethyl acetate and ethanol) were purchased from Merck Company. *Staphyloccocus aureus* 0047 and *Escherichia Coli* 081were obtained from Food and Nutrition Culture Collection (FNCC) Gajah Mada University, Yogyakarta, Indonesia.

Sampel Preparation

Fresh rhizomes of *kunci pepet* were cleaned, washed with deionised water, sliced in small pieces (2 cm in thickness) and dried at 50°C in a hot air oven for overnight. Dried rhizomes powdered by electronic mill and sieved through a100 mesh (150 μm) sieve.

Chemical Analyses of Sampel

Ash and moisture contents were determined using the AOAC methods 923.03 and 925.10, respectively [7]. Total fat was determined with FOSS Soxtec Automated System 2050 (FOSS, Sweden) which complied with AOAC method 920.85 [7]. Total protein was determined using Kjeldahl method based on AOAC method 920.87 [7]. The carbohydrate content was determined by difference.

Extraction of Curcumin

The extraction of curcumin was performed by maceration that set up with various solvent from non polar to polar. The solvent used were n-hexane (nonpolar), ethyl acetate (semipolar), and ethanol (polar), with a ratio between sample and solvent was 1: 7 (w/v) for 24 hours and then filtered with No. 1 Whatman paper. The filtrate obtained from the three kinds of solvents was concentrated using a rotary evaporator.

The powder *kunci pepet* were macerated three times at room temperature and yield was calculated through the following equation.

$$Curcumin\ yield\ (\%) = \frac{Curcumin\ extracted\ (g)}{Kunci\ pepet\ used}\ x\ 100\%$$

Estimation of Curcumin Value

Kulkarni *et al*. [8] method was used for estimation of curcumin. Preparation of standard curve : a pure curcumin was dissolved into 95% methanol to get concentration of 0.5 ppm, 1 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm. The absorbance was read by using Spectrophotometer at 420 nm and plotted against concentration. The determination of the curcumin level on the extracted sample was done by dissolving 1 mg of the sample into 95% methanol. Then, measured its absorbance by spectrophotometer at 420 nm wavelength. The result of curcumin quantification using spectrophotometer is expressed as total curcumin.

Total Phenolic Compounds Determination

The Folin-Ciocateu method was used for this determination according to procedure given by Septiana *et al.* [9]. The sample (50 mg) was mixed with 2.5 mL of 95% methanol. Centrifuged the homogenate at 10,000 rpm for 10 min. The supernatant (1 mL) dissolved in 1 mL ethanol and 5 mL distilled water, then added 0.5 mL of Folin-Ciocalteau reagent. After 3min, added 1ml of 5% Na2CO3 solution. This was left to stand at room temperature for 20 min and then the absorbance was measured at 725 nm. The extract volume was replaced by distilled water as a control. A standard curve was plotted using gallic acid (0 – 200 μg/mL). Tests were performed in triplicate and the results were expressed as μg/mL.

Antioxidant Activity

The antioxidant activities of *kunci pepet* extract were evaluated according to the DPPH radical-scavenging activity as described by Braca *et al*. [10]. The extracted sample (1 mL) was mixed with 1.2 mL of 0.003% DPPH in methanol at varying concentrations (2.5–80.0 μg/mL). The percentage of DPPH inhibition was calculated using the following equation: % of DPPH inhibition = $[(A_{DPPH} – A_s)/A_{DPPH}] \times 100$

where A_{DPPH} is the absorbance of DPPH in the absence of a sample and A_s is the absorbance of DPPH in the presence of either a sample or the standard. DPPH

scavenging activity is expressed as the concentration of a sample required to decrease DPPH absorbance by 50% (IC_{50}). This value can be graphically determined by plotting the absorbance (the percentage of inhibition of DPPH radicals) against the log concentration of DPPH and determining the slope of the nonlinear regression.

Antimicrobial Activity

The agar disc-diffusion method [11] was used for testing the antimicrobial activity of *kunci pepet* extract. Gram-positive bacteria (*Staphyloccocus aureus*) and gram-negative bacteria (*Escherichia Coli*) were incubated in Nutrient Broth (NB) at 37°C for 24 hr. The inoculums suspension of bacterial strains (10µL) was added to the medium and poured into petri dish. Sterile filter paper discs with a diameter of 5mm were placed on the surface of inoculated mediums and impregnated with 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm of the *kunci pepet* extract dissolved in dimethylsulfoxide (DMSO). The plates were left at ambient temperature for 30 min to allow excess rediffusion of extracts prior to incubation for 24 hr at 37°C. After incubation, the inhibition zone diameters were measured and expressed in mm. The presence of the inhibition zone indicates the activity of the tested samples against bacteria.

RESULTS AND DISCUSSION

The chemical composition of dried, powdered rhizomes of *kunci pepet* (*Kaempferia rotunda* L) was the following: 10.48±0.34% moisture content, 3.78±0.04% ash content, 10.08±0.18% protein content, 2.67±0.09% fat content, and 72.98±0.17% carbohydrate content.

The level of total curcumin or curcumin yield extracted using ethanol, ethyl acetate, and n-hexane solvent were 10.36±0.64%, 5.62±0.36%, and 1.55±0.14%, respectively. Ethanol extract of total curcumin extracted in this study was higher than the findings of Mohanty *et al*. [12], who reported that the total curcumin extracted of *Kaempferia rotunda* L using methanol solvent was 8.5%. Ethanol was the right solvent for extraction of cucumin in *kunci pepet* (*Kaempferia rotunda* L). The result was supported by the findings of previous studies which indicated that ethanol was the most appropriate solvent for the extraction of curcumin of turmeric (*Curcuma Longa* L., Zingaberaceae) [13] and *temulawak* rhizome (*Curcuma xanthorrhiza Roxb*) [14].

Curcumin Value

Curcumin value of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent is shown in Table 1.

Table 1 showed that ethanol extracts exhibited the highest value of curcumin (1,92±0,02 μg/mL). Curcumin is a non-aqueous liposoluble non-polar compound, but soluble in organic solvents, and soluble well in semi-polar alcohol solvents such as ethanol. The result was in good agreement with that of Liu *et al*. [13] who reported that ethanol extracted the highest curcumin value of turmeric (*Curcuma Longa* L., Zingaberaceae).

Total Phenolic and Antioxidant Activity

Total phenolic and antioxidant activity (IC_{50}) of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent is shown in Table 2.

Table 2 showed that ethanol extracts exhibited the highest value of total phenolic (5.11±0.01 μg/mL) and IC_{50} (67.95±0.21 μg/mL). The order of the total phenolic content and IC_{50} in the extract was the ethanol fraction > ethyl acetate fraction> n-hexane fraction. This result was supported by the findings by Suryani and Setyowati [15] who concluded that ethanol extract peoduced the highest total phenolic content for clove flower (Syzygium aromaticum), cinnamon (Cinnamomum verum), and ginger (Zingiber officinale). According to Atun *et al*. [3], three classes of flavonoids in *kunci pepet* (*Kaempferia rotunda* L) extracted on ethanol solvent are 5-hydroxy-7-methoxyflavanone, 7-hydroxy-5-methoxyflavanone, and 5,7-dihydroxyflavanone.

According to Jun *et al*. [16] the level of antioxidant strength is strong (IC_{50} <50 μg/mL), active (IC_{50} 50-100 μg/mL), moderate (IC_{50} 101-250 μg/mL), weak (IC_{50} 250-500 μg/mL), and inactive (IC_{50}> 500 μg/mL). Antioxidant activity of *kunci pepet* (*Kaempferia rotunda* L) using n-hexane, ethanol and ethyl acetate was active (IC50 50-100 μg/mL). The antioxidant mechanism of curcumin is attributed to its unique conjugated structure, which includes two methoxylated phenols and an enol form of *b*-diketone; the structure shows typical radical-trapping ability as a chain-breaking antioxidant [17].

Further studied conducted by Masuda *et al*. [18] stated that the antioxidant mechanism of curcumin using linoleate as an oxidizable polyunsaturated lipid and

proposed that the mechanism involves oxidative coupling reaction at the 3 position of the curcumin with the lipid and a subsequent intramolecular Diels–Alder reaction.

Phenolic compounds act as antioxidants because can bind oxygen so that oxygen is not available for oxidation process, otherwise it can also bind to that metal capable of catalyzing oxidation reactions [19]. Thus, there is a relationship between phenolic compounds or total phenolic with antioxidant activity.

The relationship of total phenolic content to antioxidant activity in this study is shown in Figure 1.

Based on Figure 1, this study confirms that the higher total phenolic of *kunci pepet* (*Kaempferia rotunda* L) obtained the higher of antioxidant activity. These results were also supported by the findings of previous studies which indicated that the higher the total phenolic the more the antioxidant [20, 21, 22,23].

Antimicrobial Activity

The antimicrobial activity of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent are shown in Table 3.

Among the three solvent extract of *kunci pepet* (*Kaempferia rotunda* L), ethyl acetate extract showed maximum zone of inhibition against *Staphyloccocus aureus* and *Escherichia Coli*. A 2000 ppm of ethyl acetate extract was effective in inhibiting *Staphyloccocus aureus* and *Escherichia Coli* with zone of inhibition 5.32±0.12 mm and 5.21±0.01 mm, respectively. The study was in agreement with Kumar *et al*. [24] who reported that ethyl acetate extract of *Kaempferia rotunda* rhizomes has potential antibacterial activity against *Staphyloccocus aureus* (MTCC 1144) among the four solvent extracts tested (n-hexane, methanol, ethyl ecetate and water). This was due to during extraction process, solvents diffuse into the *kunci pepet* (*Kaempferia rotunda* L) powder and soluble compounds of similar polarity. The polarity of solvent effects quantity and composition of secondary metabolite of an extract.

The extract found to be more effective on inhibiting *Staphyloccocus aureus* (gram- positive bacteria) than *Escherichia Coli* (gram-negative bacteria). This result was correlates with Chattapadhyay *et al*. [25] and Mohammed and Habil [26] who reported that gram-positive bacteria was sensitive to curcumin extract.

CONCLUSION

Based on the results of the study it can be concluded that the fraction of ethanol had the highest curcumin yield (10.36±0.64%), curcumin content (1.92±0.02 μg/mL), total phenolic (5.11±0.01 μg/mL), and antioxidant activity (67.95±0.21 μg/mL) of *kunci pepet* (*Kaempferia rotunda* L) among all fractions. The antioxidant activity (IC_{50}) of n-hexane, ethanol and ethyl acetate fractions was active (IC_{50} 50-100 μg/mL). Total phenolic contributed 80% to antioxidant activity. The antibacterial study of *kunci pepet* (*Kaempferia rotunda* L) revealed that ethyl acetate had the highest zone of inhibition against *Staphyloccocus aureus* and *Escherechia Coli*. Curcumin of *kunci pepet* (*Kaempferia rotunda* L) is highly promising as natural antioxidant compound and antimicrobial agent, and have the potential for development of modern medicine.

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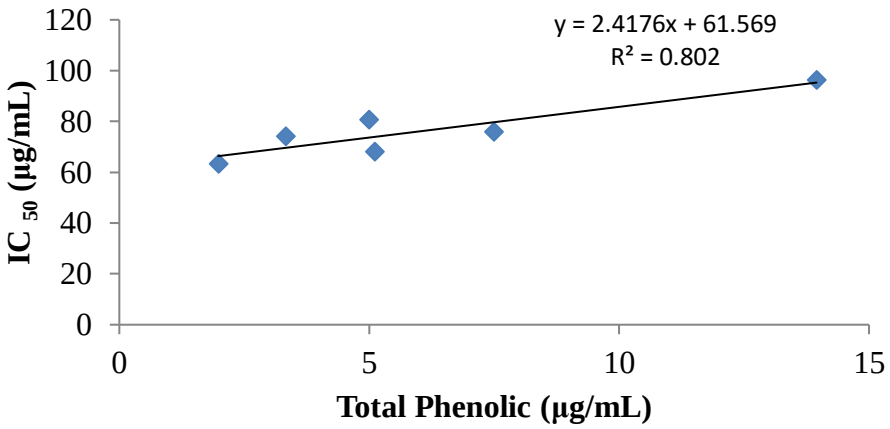

Figure 1. The relationship of total phenolic content to antioxidant activity

Table 1. Curcumin value of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent

Type of solvent	Curcumin value (µg/mL)
n-hexane	0.41±0.03
Ethyl acetate	0.50±0,02
Ethanol	1.92±0,02

Values are means ± standard deviations of triplicate determinations.

Table 2. Total Phenolic and antioxidant activity of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent

Type of solvent	Total Phenolic (µg/mL)	IC_{50} (µg/mL)
n-hexane	1.98±0.01	96.27±0.32
Ethyl acetate	3.33±0.11	75.94±0.35
Ethanol	5.11±0.01	67.95±0.21

Values are means ± standard deviations of triplicate determinations.

Table 3. Antimicrobial activity of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent against *Staphyloccocus aureus* and *Escherichia Coli*

Type of solvent	Zone of inhibition in (mm)				
	Type of bacteria	**500 ppm**	**1000 ppm**	**1500 ppm**	**2000 ppm**
n-hexane	*Staphyloccocus aureus*	3.29±0.03	3.59±0.05	3.76±0.05	4.12±0.03
	Escherichia Coli	2,02±0.06	2.27±0.05	3.72±0.04	3.89±0.03
Ethyl acetate	*Staphyloccocus aureus*	4.51±0.03	4.86±0.06	5.12±0.12	5.32±0.12
	Escherichia Coli	3.85±0.06	4.38±0.03	4.79±0.14	5.21±0.01
Ethanol	*Staphyloccocus aureus*	3.40±0.07	3.60±0.04	3.95±0.03	4.34±0.05
	Escherichia Coli	3.29±0.09	3.54±0.05	3.81±0.06	4.16±0.05

Values are means ± standard deviations of triplicate determinations.

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Total Phenolic, Antioxidant and Antibacterial Activities of Curcumin Extract of Kunci Pepet (*Kaempferia rotunda* L).

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ABSTRACT

Kunci pepet (*Kaempferia rotunda* L) is plant belonging to the ginger family that grows in Indonesia. The plant has many benefits other than used as a spice, food preservatives and coloring substances is also one of the types of medicinal plants that have many health benefits. *Kunci pepet* contains flavonoid and curcuminoid compounds as antioxidant. The aims of this study was to investigate total phenolic, antioxidant and antibacterial activities of curcumin extract from *kunci pepet* by using n-hexane, ethyl acetate and etanol solvent. Total phenolic was conducted by the Folin-Ciocalteau method. Antioxidant activity was detected by using DPPH (2,2-diphenyl-1-picrylhydrazyl) method with parameter specified was inhibition concentration (IC_{50}). The agar disc-diffusion method was adopted to examine antibacterial activity. The study showed ethanol extracts exhibited the highest curcumin yield (10,36±0.64%), curcumin value (1.92±0.02 µg/mL), total phenolic (5.11±0.01 µg/mL), and antioxidant activity (67.95±0.21 µg/mL). IC_{50} of n-hexane, ethanol and ethyl acetate fractions categorized in active level (IC_{50} 50-100 µg/mL). Ethyl acetate extracts exhibited the maximum zone of inhibition against *Staphyloccocus aureus* (5.32±0.12 mm) and *Escherichia Coli* (5.21±0.01 mm).

Keywords: *Kaempferia rotunda* L, curcumin, total phenolic, antioxidant, antibacterial

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INTRODUCTION

Kunci pepet (*Kaempferia rotunda* L) or white turmeric plant, a perennial herb belonging to the ginger family, is cultivated extensively in Indonesia especially in Java and Sumatera islands. The rhizome of this plant is also referred to as the root and is the most useful part of the plant for culinary and medicinal purposes.

This plant is known by the Indonesian community as a traditional medicine because it has many health benefits such as abdominal pain, diarrhea, dysentery, and cold [1]. Recently, the dried powder of *kunci pepet* rhizomas is famous as traditional prevention and treatment of cancer diseases. In vitro tests showed that *kunci pepet* can increase the number of lymphocytes, specific antibodies, and can kill cancer cells [2]. Moreover, results of the research conducted by Atun *et al*. [3] showed that methanol extract from kunci pepet showed antimutagenik activity.

Active component in *kunci pepet* that plays a role is curcumin, an orange–yellow crystalline powder practically insoluble in water. Curcumin as a phenolic group compound contains antioxidants so *kunci pepet* rhizomas is useful as an anti-inflammatory, anti-arthritis, anti-tumor, anti-cancer, and anti-microbial.

Increased public awareness of the negative effects of chemical drugs makes consumer demand for traditional medicine continues to increase. The average turmeric intake of Asians ranges from 0.5-1.5 g/day/person, where this amount of intake does not provide poisoning symptoms [4]. Based on clinical trials in humans showed that curcumin has no toxicity when administered at a dose of 1-8 g/day [5] and 10 g/day [6]. Thus, both turmeric and curcumin have the potential for development of modern medicine

The fresh and dried powder of *kunci pepet* rhizomes are widely sold in Indonesian traditional medicine market. The common practise of producing traditional medicine is by boiling fresh rhizomes, eating directly dried powder rhizomes, and/or making dried powder rhizomes as a drink by adding hot water. Curcumin, as a liposoluble bioactive component, in its pure form has poor solubility in water, potentially limiting its medicinal use for humans when it is taken orally. Curcumin can be extracted from turmeric root with organic solvents such as ethanol or acetone. As a medicine that has many properties, it is necessary to improve curcumin's low solubility using an extract as a carrier.

There are many research have already done related with the turmeric (*Curcuma longa* Linn.) but research on the genus *Kaempferia* has not been widely reported. So, this study attempted to investigate total phenolic, antioxidant and antimicrobial activities of curcumin extract of *kunci pepet* (*Kaempferia rotunda* L) by using n-hexane, ethyl acetate and etanol solvent.

MATERIALS AND METHODS

Source of Material

Kunci pepet (*Kaempferia rotunda* L) used for this study was purchased from a local market in Jakabaring, Palembang City, South Sumatra Province, Indonesia. The reference curcumin and extraction solvent (n-hexane, ethyl acetate and ethanol) were purchased from Merck Company. *Staphyloccocus aureus* 0047 and *Escherichia Coli* 081were obtained from Food and Nutrition Culture Collection (FNCC) Gajah Mada University, Yogyakarta, Indonesia.

Sampel Preparation

Fresh rhizomes of *kunci pepet* were cleaned, washed with deionised water, sliced in small pieces (2 cm in thickness) and dried at 50°C in a hot air oven for overnight. Dried rhizomes powdered by electronic mill and sieved through a100 mesh (150 µm) sieve.

Chemical Analyses of Sampel

Ash and moisture contents were determined using the AOAC methods 923.03 and 925.10, respectively [7]. Total fat was determined with FOSS Soxtec Automated System 2050 (FOSS, Sweden) which

complied with AOAC method 920.85 [7]. Total protein was determined using Kjeldahl method based on AOAC method 920.87 [7]. The carbohydrate content was determined by difference.

Extraction of Curcumin

The extraction of curcumin was performed by maceration that set up with various solvent from non polar to polar. The solvent used were n-hexane (nonpolar), ethyl acetate (semipolar), and ethanol (polar), with a ratio between sample and solvent was 1: 7 (w/v) for 24 hours and then filtered with No. 1 Whatman paper. The filtrate obtained from the three kinds of solvents was concentrated using a rotary evaporator. The powder *kunci pepet* were macerated three times at room temperature and yield was calculated through the following equation.

$$\textit{Curcumin yield (\%)} = \frac{\textit{Curcumin extracted (g)}}{\textit{Kunci pepet used}} \; x\; 100\%$$

Estimation of Curcumin Value

Kulkarni *et al*. [8] method was used for estimation of curcumin. Preparation of standard curve: a pure curcumin was dissolved into 95% methanol to get concentration of 0.5 ppm, 1 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm. The absorbance was read by using Spectrophotometer at 420 nm and plotted against concentration. The determination of the curcumin level on the extracted sample was done by dissolving 1 mg of the sample into 95% methanol. Then, measured its absorbance by spectrophotometer at 420 nm wavelength. The result of curcumin quantification using spectrophotometer is expressed as total curcumin.

Total Phenolic Compounds Determination

The Folin-Ciocateu method was used for this determination according to procedure given by Septiana *et al.* [9]. The sample (50 mg) was mixed with 2.5 mL of 95% methanol. Centrifuged the homogenate at 10000 rpm for 10 min. The supernatant (1 mL) dissolved in 1 mL ethanol and 5 mL distilled water, then added 0.5 mL of Folin-Ciocalteau reagent. After 3min, added 1ml of 5% Na2CO3 solution. This was left to stand at room temperature for 20 min and then the absorbance was measured at 725 nm. The extract volume was replaced by distilled water as a control. A standard curve was plotted using gallic acid (0 – 200 µg/mL). Tests were performed in triplicate and the results were expressed as µg/mL.

Antioxidant Activity

The antioxidant activities of *kunci pepet* extract were evaluated according to the DPPH radical-scavenging activity as described by Braca *et al*. [10]. The extracted sample (1 mL) was mixed with 1.2 mL of 0.003% DPPH in methanol at varying concentrations (2.5–80.0 µg/mL). The percentage of DPPH inhibition was calculated using the following equation:

$$\%\ \text{of DPPH inhibition} = [(A_{DPPH} - A_s)/A_{DPPH}] \times 100$$

where A_{DPPH} is the absorbance of DPPH in the absence of a sample and A_s is the absorbance of DPPH in the presence of either a sample or the standard. DPPH scavenging activity is expressed as the concentration of a sample required to decrease DPPH absorbance by 50% (IC_{50}). This value can be graphically determined by plotting the absorbance (the percentage of inhibition of DPPH radicals) against the log concentration of DPPH and determining the slope of the nonlinear regression.

Antimicrobial Activity

The agar disc-diffusion method [11] was used for testing the antimicrobial activity of *kunci pepet* extract. Gram-positive bacteria (*Staphyloccocus aureus*) and gram-negative bacteria (*Escherichia Coli*) were incubated in Nutrient Broth (NB) at 37°C for 24 hr. The inoculums suspension of bacterial strains (10µL) was added to the medium and poured into petri dish. Sterile filter paper discs with a diameter of 5mm were placed on the surface of inoculated mediums and impregnated with 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm of the *kunci pepet* extract dissolved in dimethylsulfoxide (DMSO). The plates were left at ambient temperature

for 30 min to allow excess rediffusion of extracts prior to incubation for 24 hr at 37°C. After incubation, the inhibition zone diameters were measured and expressed in mm. The presence of the inhibition zone indicates the activity of the tested samples against bacteria.

RESULTS AND DISCUSSION

The chemical composition of dried, powdered rhizomes of *kunci pepet* (*Kaempferia rotunda* L) was the following: 10.48±0.34% moisture content, 3.78±0.04% ash content, 10.08±0.18% protein content, 2.67±0.09% fat content, and 72.98±0.17% carbohydrate content.

The level of total curcumin or curcumin yield extracted using ethanol, ethyl acetate, and n-hexane solvent were 10.36±0,64%, 5.62±0,36%, and 1.55±0.14%, respectively. Ethanol extract of total curcumin extracted in this study was higher than the findings of Mohanty *et al*. [12], who reported that the total curcumin extracted of *Kaempferia rotunda* L using methanol solvent was 8.5%. Ethanol was the right solvent for extraction of cucumin in *kunci pepet* (*Kaempferia rotunda* L). The result was supported by the findings of previous studies which indicated that ethanol was the most appropriate solvent for the extraction of curcumin of turmeric (*Curcuma Longa* L., Zingaberaceae) [13] and *temulawak* rhizome (*Curcuma xanthorrhiza Roxb*) [14].

Curcumin Value

Curcumin value of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent is shown in Table 1.

Table 1: Curcumin value of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent

Type of solvent	Curcumin value (µg/mL)
n-hexane	0.41±0.03
Ethyl acetate	0.50±0,02
Ethanol	1.92±0,02

Values are means ± standard deviations of triplicate determinations.

Table 1 showed that ethanol extracts exhibited the highest value of curcumin (1,92±0,02 µg/mL). Curcumin is a non-aqueous liposoluble non-polar compound, but soluble in organic solvents, and soluble well in semi-polar alcohol solvents such as ethanol. The result was in good agreement with that of Liu *et al*. [13] who reported that ethanol extracted the highest curcumin value of turmeric (*Curcuma Longa* L., Zingaberaceae).

Total Phenolic and Antioxidant Activity

Total phenolic and antioxidant activity (IC_{50}) of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent is shown in Table 2.

Table 2: Total Phenolic and antioxidant activity of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent

Type of solvent	Total Phenolic (µg/mL)		IC_{50} (µg/mL)
n-hexane	1.98±0.01		96.27±0.32
Ethyl acetate	3.33±0.11		75.94±0.35
Ethanol	5.11±0.01		67.95±0.21

Values are means ± standard deviations of triplicate determinations.

Table 2 showed that ethanol extracts exhibited the highest value of total phenolic (5.11±0.01 µg/mL) and IC_{50} (67.95±0.21 µg/mL). The order of the total phenolic content and IC_{50} in the extract was the ethanol fraction > ethyl acetate fraction> n-hexane fraction. This result was supported by the findings by Suryani and Setyowati [15] who concluded that ethanol extract peoduced the highest total phenolic content for clove

flower (Syzygium aromaticum), cinnamon (Cinnamomum verum), and ginger (Zingiber officinale). According to Atun *et al*. [3], three classes of flavonoids in *kunci pepet* (*Kaempferia rotunda* L) extracted on ethanol solvent are 5-hydroxy-7-methoxyflavanone, 7-hydroxy-5-methoxyflavanone, and 5,7-dihydroxyflavanone.

According to Jun *et al*. [16] the level of antioxidant strength is strong (IC_{50} <50 µg/mL), active (IC_{50} 50-100 µg/mL), moderate (IC_{50} 101-250 µg/mL), weak (IC_{50} 250-500 µg/mL), and inactive (IC_{50}> 500 µg/mL). Antioxidant activity of *kunci pepet* (*Kaempferia rotunda* L) using n-hexane, ethanol and ethyl acetate was active (IC50 50-100 µg/mL). The antioxidant mechanism of curcumin is attributed to its unique conjugated structure, which includes two methoxylated phenols and an enol form of *b*-diketone; the structure shows typical radical-trapping ability as a chain-breaking antioxidant [17].

Further studied conducted by Masuda *et al*. [18] stated that the antioxidant mechanism of curcumin using linoleate as an oxidizable polyunsaturated lipid and proposed that the mechanism involves oxidative coupling reaction at the 3 position of the curcumin with the lipid and a subsequent intramolecular Diels–Alder reaction.

Phenolic compounds act as antioxidants because can bind oxygen so that oxygen is not available for oxidation process, otherwise it can also bind to that metal capable of catalyzing oxidation reactions [19]. Thus, there is a relationship between phenolic compounds or total phenolic with antioxidant activity.

The relationship of total phenolic content to antioxidant activity in this study is shown in Figure 1.

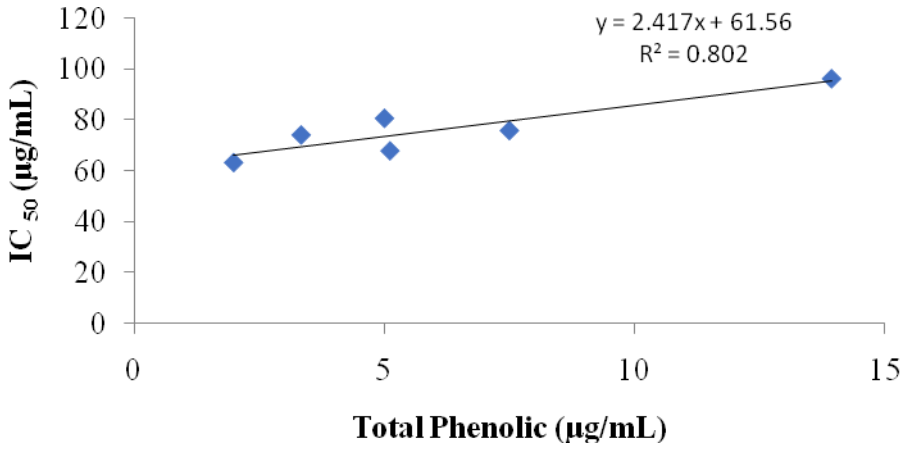

Figure 1: The relationship of total phenolic content to antioxidant activity

Based on Figure 1, this study confirms that the higher total phenolic of *kunci pepet* (*Kaempferia rotunda* L) obtained the higher of antioxidant activity. These results were also supported by the findings of previous studies which indicated that the higher the total phenolic the more the antioxidant [20, 21, 22,23].

Antimicrobial Activity

The antimicrobial activity of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent are shown in Table 3.

Among the three solvent extract of *kunci pepet* (*Kaempferia rotunda* L), ethyl acetate extract showed maximum zone of inhibition against *Staphyloccocus aureus* and *Escherichia Coli*. A 2000 ppm of ethyl acetate extract was effective in inhibiting *Staphyloccocus aureus* and *Escherichia Coli* with zone of inhibition 5.32±0.12 mm and 5.21±0.01 mm, respectively. The study was in agreement with Kumar *et al*. [24] who reported that ethyl acetate extract of *Kaempferia rotunda* rhizomes has potential antibacterial activity against *Staphyloccocus aureus* (MTCC 1144) among the four solvent extracts tested (n-hexane, methanol, ethyl ecetate and water). This was due to during extraction process, solvents diffuse into the *kunci pepet* (*Kaempferia rotunda* L) powder and soluble compounds of similar polarity. The polarity of solvent effects quantity and composition of secondary metabolite of an extract.

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Table 3: Antimicrobial activity of *kunci pepet* (*Kaempferia rotunda* L) extracted by different type of solvent against *Staphyloccocus aureus* and *Escherichia Coli*

Type of solvent	Zone of inhibition in (mm)				
	Type of bacteria	500 ppm	1000 ppm	1500 ppm	2000 ppm
n-hexane	*Staphyloccocus aureus*	3.29±0.03	3.59±0.05	3.76±0.05	4.12±0.03
	Escherichia Coli	2,02±0.06	2.27±0.05	3.72±0.04	3.89±0.03
Ethyl acetate	*Staphyloccocus aureus*	4.51±0.03	4.86±0.06	5.12±0.12	5.32±0.12
	Escherichia Coli	3.85±0.06	4.38±0.03	4.79±0.14	5.21±0.01
Ethanol	*Staphyloccocus aureus*	3.40±0.07	3.60±0.04	3.95±0.03	4.34±0.05
	Escherichia Coli	3.29±0.09	3.54±0.05	3.81±0.06	4.16±0.05

Values are means ± standard deviations of triplicate determinations.

The extract found to be more effective on inhibiting *Staphyloccocus aureus* (gram- positive bacteria) than *Escherichia Coli* (gram-negative bacteria). This result was correlates with Chattapadhyay *et al*. [25] and Mohammed and Habil [26] who reported that gram-positive bacteria was sensitive to curcumin extract.

CONCLUSION

Based on the results of the study it can be concluded that the fraction of ethanol had the highest curcumin yield (10.36±0.64%), curcumin content (1.92±0.02 µg/mL), total phenolic (5.11±0.01 µg/mL), and antioxidant activity (67.95±0.21 µg/mL) of *kunci pepet* (*Kaempferia rotunda* L) among all fractions. The antioxidant activity (IC_{50}) of n-hexane, ethanol and ethyl acetate fractions was active (IC_{50} 50-100 µg/mL). Total phenolic contributed 80% to antioxidant activity. The antibacterial study of *kunci pepet* (*Kaempferia rotunda* L) revealed that ethyl acetate had the highest zone of inhibition against *Staphyloccocus aureus* and *Escherechia Coli*. Curcumin of *kunci pepet* (*Kaempferia rotunda* L) is highly promising as natural antioxidant compound and antimicrobial agent, and have the potential for development of modern medicine.

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