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ANTIBACTERIAL ACTIVITY AND TLC-BIOAUTOGRAPHY ANALYSIS OF THE ACTIVE FRACTIONS OF *Muntingia calabura* L. LEAVES AGAINST *Staphylococcus aureus*

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ABSTRACT

This study aims to determine the antibacterial activity and TLC-bioautography profile of the active fractions of *Muntingia calabura* L. leaves against *Staphylococcus aureus*. *Muntingia calabura* L. leaves were macerated with ethanol 96% then fractionated with n-hexane, ethyl acetate, and methanol solvent, respectively. The antibacterial activity was tested by the Kirby-Bauer method to determine the most active fraction and the lowest concentration that inhibited the growth of Staphylococcus aureus. TLC-bioautography was tested using chloroform: ethyl acetate (2:8) as the mobile phase and silica gel F_{254} as the stationary phase. Antibacterial activity test of n-hexane and ethyl acetate fractions at a concentration of 10% w/v showed activities with inhibition zone diameter of 0.33±0.288 and 9.66±5.77 mm, respectively. At the same time, the methanol fraction showed no activity. The lowest concentration of ethyl acetate fraction which still showed the inhibition zone was 0.312% w/v. The TLC-bioautography profile showed active spots with an Rf value of 0.82 and had an inhibitory zone diameter of 4.013±0.864 mm. It can be concluded that the ethyl acetate fraction was the most active fraction that inhibited the growth of *Staphylococcus aureus* and had one active spot on the bioautography test.

Keywords: antibacterial; *Muntingia calabura* L. leaf; *Staphylococcus aureus*; TLC-bioautography.

INTRODUCTION

Staphylococcus aureus is a commensal bacteria in human skin and mucosae but this bacteria often causes serious infections with high morbidity and mortality (Sakr *et al*., 2018). Besides being frequently associated with skin and soft tissue infections, *Staphylococcus aureus* can also cause a variety of serious invasive infections such as osteomyelitis, necrotizing pneumonia, and bacteremia (Williamson *et al*., 2013). *Staphylococcus aureus* is a serious problem because of the increased resistance of these bacteria to various types of antibiotics (Multi-Drug Resistance). *Staphylococcus aureus* has unique adaptability so that it can be resistant to many antibiotics (Afifurrahman *et al*., 2014). Therefore, it is necessary to explore new antibacterial compounds from various sources such as medicinal plants.

Muntingia calabura L. leaves have been shown to have antibacterial activity against both Gram-positive and Gram-negative bacteria (Arum *et al*., 2012; Handoko *et al*., 2019). Arum *et al*. (2012) reported that ethanol and methanol extracts of *Muntingia calabura* L. leaves have antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*. Research done by Manik *et al*. (2014) showed that ethanol extract of *Muntingia calabura* L. leaves has antibacterial activity against *Staphylococcus*

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aureus where the Minimum Bactericidal Concentration (MBC) value of ethanol extract is 1.25 mg/mL. The antibacterial activity of *Muntingia calabura* L. leaves is presumed to exist because it contains bioactive compositions such as flavonoids, tannins, and saponins (Zebua *et al*., 2019).

The extract still contains a mixture of various compounds so that fractionation is needed to attract the active compound based on their polarity level. One way to detect the active compounds is by using the TLC-bioautography method. TLC-bioautography can show spots from Thin Layer Chromatography (TLC) that can inhibit bacterial growth (Muthadi *et al*., 2012). This study aims to determine the antibacterial activity and TLC-Bioautographic profile of the active fractions of *Muntingia calabura* L. leaves against *Staphylococcus aureus*.

METHODS

Materials and Chemicals

Muntingia calabura L. leaves were collected from Sleman, Yogyakarta, Indonesia. *Staphylococcus aureus* isolates were collected from Center for Health Laboratory Yogyakarta. The other materials and chemicals used were Brain Heart Infusion (Oxoid), Mueller Hinton Agar (Oxoid) NaCl 0.9% (sterile), Mc Farland 0.5 (concentration 1.5 x 10^8 CFU/mL), ethanol 96%, n-hexane, ethyl acetate, methanol, paper disk (Oxoid), and TLC silica gel F_{254} (Merck).

Extraction and Fractionation

Muntingia calabura L. leaves were dried using an oven at 45ºC for four days then mashed by blending. The dry powder obtained was extracted by employing the maceration method. The dry powder of *Muntingia calabura* L. leaves (500 g) was extracted with 4000 mL ethanol 96% (1:8), stirred using a stirrer for 3 hours and then stored in 24 hours. Next, the macerated substance was filtered and concentrated using a rotary evaporator (Sulaiman *et al*., 2017). The extract obtained was then suspended in warm water and fractionated with n-hexane, ethyl acetate, and methanol solvent, respectively using the liquid-liquid partitioning method (Mulyani and Sukandar, 2011).

Media Preparation

Brain Heart Infusion (BHI) media was prepared by dissolving 3.7 g of BHI powder in 100 mL of distilled water while being heated and stirred until homogeneous. BHI media was sterilized using an autoclave at 121°C for 15 minutes (Lolongan *et al*., 2016).

Mueller Hinton Agar (MHA) media was prepared by dissolving 19 g of MHA powder in 500 mL distilled water while being heated and stirred until homogeneous. The media was sterilized using an autoclave at 121°C for 15 minutes and then put in a 20 mL petri dish and allowed to harden (Mahmudah *et al.*, 2017).

Bacterial Preparation

A total of 1 mL of *Staphylococcus aureus* stock was put into 1 mL of BHI media and incubated at 37ºC for 24 hours. The bacteria culture was then taken for as much as 100 µL, put into 1 mL of BHI media, and incubated at 37ºC for 3-5 hours. The bacterial culture was then taken for as much as 100 µL and was diluted using 0.9% NaCl sterile until the turbidity was the same as the Mc Farland 0.5 standard (1.5 x 10^8 CFU/mL).

Antibacterial Activity Test

The determination of the active fraction was carried out using the Kirby-Bauer method. A total of 20 µL of extract and fractions with a concentration of 10% w/v each were dripped on a 6 mm disc paper. The disc paper was then transferred aseptically to the MHA media that had been planted with test bacteria and then incubated using an incubator at 37ºC for 24 hours. The most active fraction is the one that has the largest inhibitory zone (Sylvester *et al*., 2015). The lowest concentration of the most active fraction that inhibited *Staphylococcus aureus* was determined using the same method. The most active fractions with concentrations of 10%, 5%, 2.5%, 1.25%, 0.625%, 0.312 and 0.156% were tested using the Kirby-Bauer method.

TLC-Bioautography

The test to find out the most active fraction was carried out by TLC method using chloroform:ethyl acetate (2:8) as the mobile phase and silica gel F_{254} as the stationary phase. After elution, spots on the TLC plate were then observed under visible light and UV light with wavelengths of 254 and 366 nm. Furthermore, the chromatogram plate was contacted with the surface of MHA media which had been inoculated by *Staphylococcus aureus* for 30 minutes. The plate was removed and the culture was incubated at 37^{o}C for 24 hours (Muthadi *et al*., 2012).

Statistical Analysis

Inhibition zone data in the minimum inhibitory test were tested for their normality and homogeneity. The normality test was done using the Kolmogorov-Smirnov and Lilliefors, while the homogeneity test was done using One Way ANOVA and LSD with a confidence level of 95%. If the distribution is normal and homogeneous, then the correlation test will be done using the Pearson test. If the data are not normally distributed and are not homogeneous, further tests need to be performed using the Kruskal-Wallis and Mann-Whitney while the correlation test is performed using the Spearman test.

RESULTS AND DISCUSSION

In this study, *Muntingia calabura* L. leaves were extracted using the maceration method which obtained 19.07% extract yield. A non-heating extraction method was chosen to avoid damaging heat-resistant compounds that might be contained in the sample. Immersion of *Muntingia calabura* L. leaves in the solvent can make the compounds contained in cells soluble, and because of the differences in concentration between the solution of compounds inside and outside the cell, the compounds from high concentrations in the cell will diffuse out. In a previous study conducted by Puspitasari *et al.*, (2016), *Muntingia calabura* L. leaves extraction using the maceration method obtained a yield of 26.58%. Yield extraction results can be influenced by several factors, including biological factors and chemical factors. The biological factors include harvesting time, location of growth, plant species and plant parts used while the chemical factors include extraction methods, size, hardness, dryness of the material, type of solvent used, and types and levels of active compounds contained in plant material (Prastiwi *et al*., 2017).

The extract was fractionated successively using n-hexane, ethyl acetate and methanol solvents which aimed to separate the compounds based on their degree of polarity. The results of fractionation in this study showed that the yield of n-hexane, ethyl acetate, and methanol was 38.36%, 13.00%, and 7.70%, respectively. Non-polar compounds such as oil, carotenoids, steroids, and triterpenoids will tend to dissolve in non-polar solvents such as n-hexane. Semi-polar compounds such as aglycone flavonoids will tend to dissolve in semi-polar solvents such as ethyl acetate. Polar compounds such as flavonoids glycosides will tend to dissolve in polar solvents, such as methanol, according to the principle of 'like dissolves like' (Suryanto, 2012).

The most active fraction that inhibited the growth of *Staphylococcus aureus* was determined through antibacterial activity testing of each fraction using the Kirby-Bauer method. As a comparison, ethanol extract, negative control (96% ethanol) and positive control (Vancomycin Antimicrobial Susceptibility Disks 30 μg) were also tested. The results showed that the ethyl acetate fraction was the most active fraction associated with the formation of the largest inhibition zone diameter compared to the inhibition zone of the related fraction presented in (Figure 1). The inhibition zone of ethanol extract, n-hexane and ethyl acetate fractions in 3 replications were 3.33 ± 0.577, 0.33 ± 0.288 and 9.66 ± 0.577 mm while the methanol fraction did not show inhibitory activity, as shown in (Table 1). The category of antibacterial activity can be distinguished based on its strength according to Rahmawati *et al*. (2014): if the diameter of antibacterial inhibition zone is more than 20 mm, the activity is classified into very strong; if it is between 11-20 mm, it is classified into

moderate; and if it is less than 5 mm, it is classified into weak. Based on this classification, the ethyl acetate which produced a clear zone of 9.66 ± 0.577 mm was classified into medium category.

The lowest concentration of ethyl acetate fraction that still showed the zone of inhibition against *Staphylococcus aureus* was determined using the Kirby-Bauer disk diffusion method. The results showed that the ethyl acetate fraction could still inhibit the growth of *Staphylococcus aureus* bacteria at the lowest concentration of 0.312% (Figure 2) with the inhibition zone of 0.33 ± 0.288 (Table 2).

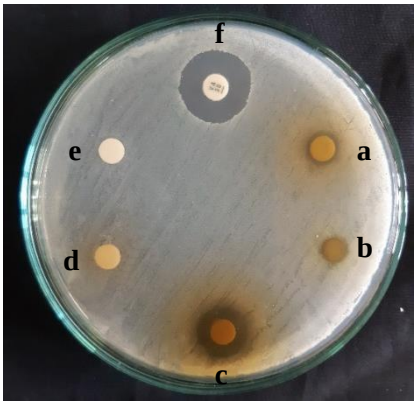

Figure 1. Screening of The Active Fractions; (a) 10% ethanol extract, (b) 10% n-hexane fraction, (c) 10% ethyl acetate fraction, (d) 10% methanol fraction, (e) negative control and (f) positive control.

Table 1. Inhibition zone diameters of the extract and fractions against *Staphylococcus aureus.*

Extract and Fractions	Inhibition Zone (mm)	Category
Ethanol Extract (10 % w/v)	3.33±0.577	Weak
n-hexane Fraction (10 % w/v)	0.33±0.288	Weak
Ethyl Acetate Fraction (10 % w/v)	9.66±0.577	Medium
Methanol Fraction (10 % w/v)	0.00±0.00	-
Negative Control (Ethanol 96%)	0.00±0.00	-
Positive Control (Vancomycin 30 µg)	12.33±0.577	Strong

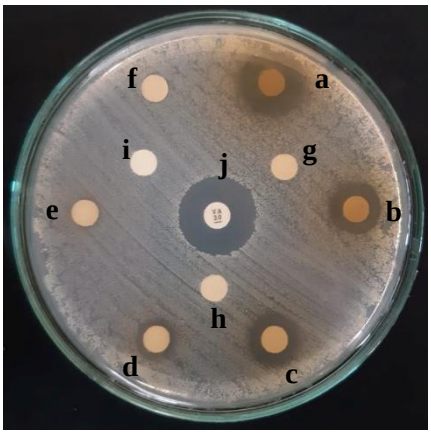

Figure 2. Testing the minimum inhibitory level of the ethyl acetate fraction with a concentration of: (a) 10%, (b) 5%, (c) 2.5%, (d) 1.25%, (e) 0.625%, (f) 0.312%, (g) 0.156% , (h) = 0.078%, (i) = negative control, (j) = positive control.

Table 2. Diameters of the inhibition zone of ethyl acetate fraction against *Staphylococcus aureus.*

Ethyl acetate fraction (% b/v)	Inhibition zone (mm)	Category
10	9.83±0.28	Medium
5	5.66±0.57	Medium
2.5	4.16±0.288	Weak
1.25	2.5±0.50	Weak
0.625	1.83±0.288	Weak
0.312	0.33±0.288	Weak
0.156	0.00±0.00	-
0.078	0.00±0.00	-
Negative Control (Ethanol 96%)	0.00±0.00	-
Positive Control (Vancomycin 30 µg)	12.33±0.577	Strong

Statistical analysis showed that the inhibition zone data at the lowest level of ethyl acetate fraction testing was not normally distributed and not homogeneous. It was based on the significance value of <0.05 in Kolmogorov-Smirnov and Lilliefors test and the significance value of 0.019 <0.05 in One Way ANOVA and LSD test. Therefore, further testing was done using the Kruskal-Wallis test and the Mann-Whitney test. In the Kruskal-Wallis test, the result showed the significance of 0.002 <0.05. This shows that the treatment of the addition of the ethyl acetate fraction had a significant influence on the inhibition zones that emerged. Furthermore, the Mann-Whitney test showed that all concentration groups had significant differences. Therefore, it can be said that each concentration of ethyl acetate fraction- which consisted of 0.072%, 0.156%, 0.312%, 0.625%, 0.125%, 0.25%, 0.5% and 10% w / v- had different inhibitory zones. In addition, the Spearman correlation test showed the correlation coefficient value of 0.98. The value falls between range 0.81-1.00 and therefore it can be concluded that there was a very strong positive correlation where the higher the concentration of ethyl acetate fraction was, the greater the inhibitory zone that appeared.

Furthermore, to separate the content of compounds in the ethyl acetate fraction, Thin Layer Chromatography (TLC) technique was used. TLC was done by bottling 5 µL of ethyl acetate 10% onto the TLC plate. The results of the elution profile using mobile phase chloroform:ethyl acetate (2:8) and the stationary silica gel F_{254} phase can be seen in figure 3. By viewing through visible light and UV light, there were eight spots detected during the observation (Table 3).

Table 3. The Rf value of the ethyl acetate fraction detected on the Thin Layer Chromatogram using chloroform:ethyl acetate (2:8) as the mobile phase.

No.	Rf	Visible Light	UV 254	UV 366
1	0.00	Brown	Brown	Black
2	0.21	Brown	Brown	Black
3	0.44	Green	Blue	Red Fluorescence
4	0.50	-	-	Blue Fluorescence
5	0.69	-	-	Blue Fluorescence
6	0.73	Yellow	Yellow	Black
7	0.82	Yellow	Yellow	Black
8	0.93	Green	Yellow	Red Fluorescence

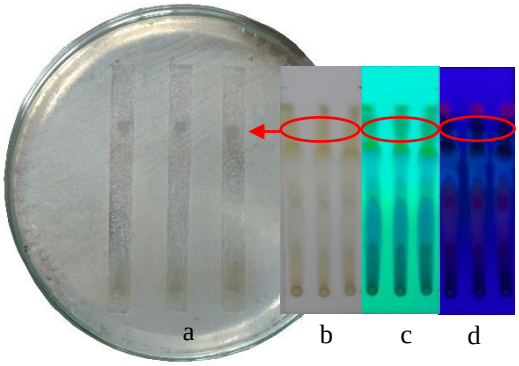

Figure 3. TLC-bioautography of ethyl acetate fraction, (a) TLC-bioautography of ethyl acetate fraction against *Staphylococcus aureus* by three replications; (b) TLC results viewed with visible light; (c) TLC results viewed with UV light 254 nm, (d) TLC results viewed with UV light 366 nm.

The bioautography test aims to determine which compound spots on TLC chromatograms have antibacterial activity. The active spots are marked by the formation of clear zones. The Rf value of the clear zone is calculated and matched to the Rf value of the chromatogram plate. TLC-Bioautographic test results of ethyl acetate fraction with three replications showed one active spot with an Rf value of 0.82 and had an inhibition zone diameter of 4.013 ± 0.864 mm as presented in figure 3. The inhibition zone formed was caused by the presence of active compounds from the chromatogram spot which diffused into the media and caused inhibition of bacterial growth in the diffusion site of the active compound (Muthadi *et al*., 2012).

CONCLUSION

Ethyl acetate fraction is the most active fraction which inhibits the growth of *Staphylococcus aureus* bacteria and has the lowest levels of 0.312% w / v which still shows the inhibitory zone. Based on TLC-Bioautography testing using mobile phase of chloroform with ethyl acetate (2:8) and silica gel F_{254} as a stationary phase, ethyl acetate fraction has one active spot with an Rf value of 0.82.

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ASSOCIATION BETWEEN SMOKING BEHAVIOUR AND GLYCOHEMOGLOBINE LEVELS AMONG ADULT JAVANESE INDONESIAN SMOKERS

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ABSTRACT

Nicotine, the active compound in cigarettes, can cause impaired glucose metabolism by increasing insulin resistance as well as decreasing insulin secretion in β cell pancreas. This condition can increase the risk of type 2 diabetes in human. This study aims to evaluate the effect of smoking behaviour, determined by Cigarette per Day (CPD) and smoking duration, on glychohemoglobine (HbA1c) levels of Javanese Indonesian smokers. 30 smokers were studied consisting of 7 smokers with <10 CPD, 19 smokers with 11-20 CPD and 4 smokers with 21-30 CPD. They had been smoking for more than 10 years. The whole blood sample was used to examine the HbA1c levels. The HbA1c levels were tested at Bethesda Hospital's clinic laboratories using Architect 600 instrument. The results showed that CPD and smoking duration significantly influenced HbA1c, in which F count was > F table (370.541> 3.354) with significance < 0.05 (2.35. 10-20 < 0.05) and multiple correlation coefficient (R) of 0.982. Therefore, based on this research finding, it was concluded that longer smoking duration and higher CPD caused higher smoker's Hba1c level.

Keywords: CPD; HbA1c; smoking behaviour; smoking duration.

INTRODUCTION

Diabetes is one of the largest global health emergencies in the 21st century. The International Diabetes Federation has predicted that there will be an increasing prevalence of diabetics in 2033 by 2.8% over the past 20 years. In addition, the number of people affected by the disease will also increase by 57% from 382 to nearly 600 million. The prevalence of diabetes has increased in Asian countries and it has contributed to more than 60% of the world's diabetic population. Indonesia ranks the seventh highest prevalence rate of diabetes in the world after China, India, United States of America, Brazil, Mexico and Russian Federation (IDF, 2015). WHO estimates the number of patients with type 2 diabetes (T2DM) in Indonesia will increase significantly to 21.3 million in 2030. The estimated number of deaths due to diabetes among adults aged 45-54 years in urban areas is 14.7% while in rural areas is 5.8% (R.I., 2009).

T2DM has been the most prevalent form of diabetes and has increased alongside cultural and societal changes. Smoking has been believed to be one of the factors that can increase the risk of diabetes. The increasing risk of diabetes to smokers has been reported in several studies. Smokers possess 45% higher risk of diabetes than nonsmokers (Willi *et al*., 2007). Even though empirical data have shown that smoking may cause serious health problems in some countries, production and consumption of tobacco products in several Asian countries such as China, India and Indonesia still exist until now. The prevalence of smoking among Indonesian men was 67.4%, which was the highest smoking rate

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within ASEAN regions (Lian *et al.*, 2014; Tandilittin *et al.*, 2013). According to the Ministry of Health (2017), the percentage of Indonesian population with T2DM risk factor due to smoking activity over the age of 10 is 24.3%.

Several diabetes experts, namely the International Committee of Experts, the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD), have recommended the use of HbA1c to diagnose diabetes. HbA1c can be used to reflect average blood glucose levels over 3-4 months, providing a useful longer-term measurement of blood glucose control. Prior to the test of HbA1c, fasting was not needed. Therefore, meal issues are not an urgent factor. Currently, HbA1c test is preferable because it can be done at any time, the value can reduce daily variation of sugar content and it is more reproducible. In 2011, ADA and WHO used HbA1c as diabetes diagnostic criteria with the following propositions; HbA1c under normal circumstances was 3.5% -5% and diabetes was confirmed if HbA1c $\geq$ 6.5%. If HbA1c levels are between 6.0 and 6.5%, it was at particularly high risk and might be considered for diabetic prevention interventions (ADA, 2011; WHO, 2011).

Nicotine, the active compound in cigarettes, is the most responsible compound for increasing blood sugar levels (Bajaj, 2012; Borowitz *et al.*, 2008; Xie *et al.*, 2009). Nicotine can cause impaired glucose metabolism and increase insulin resistance, which may lead to an increasing risk of developing type 2 diabetes (T2DM) (Houston *et al*., 2006; Willi *et al*., 2007). This study aims to investigate the effect of smoking behaviour, indicated by Cigarette per Day (CPD) and smoking duration, on HbA1c levels among Javanese Indonesian smokers. This research was conducted primarily in order to prevent the increase of T2DM in Indonesia.

METHODS

Materials

All chemicals, reagents, and solvents used during this study were analytical grade and highly pure. Ethanol 70% were purchased from Sigma (St. Louis, MO, USA) and we used sterilized water for injection from Ikaphamindo as a solvent.

Instrumentations

The HbA1c measurements were carried out using Arcitec 600 at Bethesda Hospital's clinic laboratories.

Methods

The study had been accomplished in 2017 and had been approved by the Ethics Committees of Medical Research Duta Wacana University, Yogyakarta, Indonesia with clearance number of 424/C.16/FK/2017. A total of 30 male healthy Javanese Indonesian smokers were recruited from Sanata Dharma University staffs who had been involved in previous studies of CYP2A6 *1, CYP2A6 *4, and CYP2A6 *9 genotypes. Research subjects had smoked for minimum 10 years, were not currently planning to stop smoking, were between 20 and 45 years-old, had body weight between 46 and 75 kg with height varying between 150 and 170 cm, took no concurrent medications, and had no illnesses requiring investigation or treatment. Smoking status was collected based on questionnaires which requested information on the number of cigarettes smoked per day (CPD), the age at which the subject started smoking, and other tobacco products used. All of smoking subjects were cigarette smokers. Smokers with a family history of diabetes were excluded from this study.

Venous blood samples from all the subjects were collected in vacutainer tubes. The tubes were labelled and used to determine the HbA1c levels. The subjects had been given informed consent prior to participating in this study. The HbA1c examination was performed at Bethesda Hospital's clinic laboratories using Architect 600 instrument.

The data were analyzed using Microsoft Excel 2016. We conducted t-test and ANOVA test to examine the effect of smoking behaviour, determined by CPD and smoking duration, on HbA1c levels. The multi-liner regression analysis was used to examine correlation between CPD and smoking

duration with HbA1c. P values <0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Diabetes mellitus is one of serious problems on public health where many people are unaware to suffer from this disease. Diabetes in Indonesia, mostly T2DM caused by lifestyle, was growing precipitously. Smoking, another public health problem encountered by Indonesia, is one of lifestyle factors causing diabetes. The subjects involved in this study were active smokers who had smoked cigarettes for a minimum of 10 years with CPD between 8-30 cigarettes per day. According to Fagerstrom Test for Nicotine Dependence (FTND), the number of smoker's CPD is categorized into four criteria; 10 or less, 11-20, 21-30 and 31 or more cigarettes. Based on these criteria, the subjects The subjects involved in this study were active smokers who had smoked cigarettes for a minimum of 10 years with CPD between 8-30 cigarettes per day. According to Fagerstrom Test for Nicotine Dependence (FTND), the number of smoker's CPD is categorized into four criteria; 10 or less, 11-20, 21-30 and 31 or more cigarettes. Based on these criteria, the subjects involved in this research were 30 smokers consisting of 7 smokers with CPD <10 cigarettes, 19 smokers with CPD between 11-20 cigarettes and 4 smokers with CPD between 21-30 cigarettes.

In another CYP2A6 genotype study, it was found not only active form CYP2A6*1 but also a non-active allele, CYP2A6*4 and CYP2A6*9 alleles in the population. The alleles frequencies among the subjects were 48.5% (CYP2A6*1), 48.5% (CYP2A6*4) and 3% for CYP2A6*9 respectively (Patramurti and Fenty, 2019). All subjects were categorized as slow metabolizers with 28 subjects had a CYP2A6*1/*4 genotype and two subjects had CYP2A6*1/*4/*9 genotype. CYP2A6 is an enzyme responsible for nicotine metabolism, and it transforms nicotine into inactive form, i.e., cotinine and 3-hydroxycotinine (Figure 1).

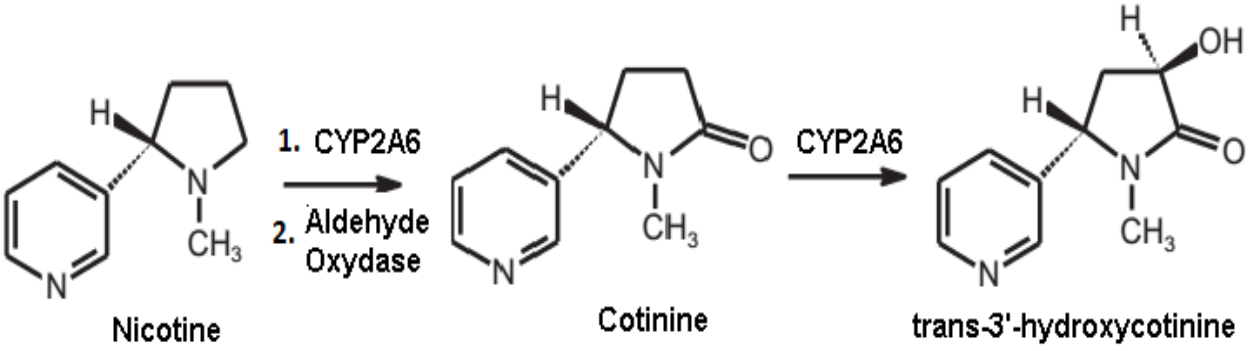

Figure 1. The main metabolism of nicotine mediated by CYP2A6

Table 1. Characteristics and distribution of HbA1 content test subjects

Distribution	Characteristics		
	CPD	Smoking Duration (year)	HbA_{1c} (%)
Mean ± SD	14 ± 1	23,57 ± 1,22	5,28 ± 0,082
Range	6 - 30	14 - 36	4,83 - 7,16
Median	12	23,5	5,28

The polymorphic form of CYP2A6 in the subjects will lead to a decreasing CYP2A6 activity in metabolizing nicotine. Slow metabolizers of nicotine will decrease nicotine metabolism. Furthermore, increasing exposure to nicotine often leads to increasing nicotine levels in the blood and creates increasing blood sugar levels. According to Liu *et al*. (2011), smokers categorized as slow metabolizers or poor metabolizers (having alleles * 4 and * 9) are more susceptible to T2DM than smokers categorized as normal metabolizers or intermediate metabolizers.

The risk of T2DM in this study was expressed by HbA1c parameters. According to ADA (2014) HbA1c levels are classified into 3 groups, i.e. normal (<5.7%), prediabetes (5.7 - 6.4%) and diabetes (≥ 6.5). The smokers' HbA1c levels will be higher than those nonsmokers (Jyothirmayi *et al.*, 2013; Nilsson, *et al*., 2004; Padhy *et al.*, 2015; Vlassopoulos, Lean *et al.*, 2013). The characteristics of the subjects covering CPD, duration of smoking, and distribution of HbA1c levels are shown in table 1.

From table 1, it can be seen that the average CPD is 14 cigarettes per day. All subjects had been smoking for more than 10 years, meaning that each subject had been exposed to nicotine for a long time. The average HbA1c levels in all the 30 subjects were still in normal conditions with mean scores 5.28 ± 0.082 %, respectively. The range of HbA1c levels was 4.83-7.16. It showed that there were some subjects with diabetic condition. In the measurement of HbA1c levels, it was revealed that HbA1c levels of 25 test subjects were still normalized within range of 4.83-5.56%; 4 subjects were identified in prediabetic condition within range of 5.7-5.97% and 1 subject had diabetes with HbA1c level of 7.16%. These conditions indicate that smoking behaviour affects HbA1c levels which can lead to the occurrence of T2DM disease. This finding is in line with several studies which reveal the association between active smoking and T2DM incidence worldwide (Hang, 2011; Jee *et al*., 2010; Kowall *et al*., 2010; Peter *et al*., 2014; Saeed, 2012). In addition, several other studies have also shown that exposure to environmental tobacco smoke (ETS) which contain nicotine towards passive smokers can also increase the risk of T2DM (Wang *et al*., 2013; Yeh *et al*., 2010).

Smoking behaviour in this study was observed from two elements namely CPD and duration of smoking. The effect of CPD and duration of smoking on HbA1c levels was partially analyzed using t test. In t test conducted on each variable, the t value of CPD was > t table (3.689> -2.0480) with significance <0.05 (0.001 <0.05). Likewise, the t value of duration of smoking was > t table (8.586> -2.048) with significance <0.05 (0.000 <0.05). Based the t test on each variable, it can be concluded that both CPD and duration of smoking can partially influence HbA1c levels. Due to these findings, the analysis was continued using ANOVA test which would observe the effect of CPD and duration of smoking on HbA1c levels simultaneously. Anova test shows that F value was > F table (370.541> 3.354) with significance <0.05 (2.35.10-20 <0.05). This suggests that CPD and long smoking have a

simultaneously significant effect on HbA1c levels.

The relationship between smoking behaviour, e.g. CPD and smoking duration, and HbA1c levels in this research was analyzed by multiple correlations using correlation coefficient parameter R. There was a significant correlation between CPD and smoking duration with HbA1c ($R=0.982$, $P < 0.001$). This result indicated that there was a statistically close relationship of CPD and smoking duration with HbA1c levels. The R value in this study was positive which means that CPD, smoking duration, and smokers' HbA1c levels have positive correlation. These results suggest that the risk of T2DM indicated by HbA1c will increase with interaction of cigarettes smoked and smoking duration. Higher risk of T2DM had been observed in smokers who started smoking at age 18 years old or younger. Starting smoking at a younger age might be associated with greater dependence and heavy smoking patterns (Kawakami *et al*., 1997). These results also support many other previous studies which state that the more cigarettes a smoker smokes, the higher the T2DM risk is (Foy *et al*., 2005; Liu *et al*., 2011; Nakanishi *et al*, 2000; Will *et al*., 2001). The R square for the regression analysis was 0.963. This means that the percentage contribution of CPD and smoking duration to HbA1c levels was 96.3% while the rest 3.7% was related to other variables which were not included in this study.

The relationship between smoking behaviour, which is indicated by CPD and smoking duration, and HbA1c levels was formulated by the following equation: $Y = 0.106\ X2 + 0.152\ X1$, where: Y is HbA1c, X1 is CPD, and X2 is the duration of smoking. Based on this equation, it can be seen that the magnitude of the influence of CPD and smoking duration on HbA1c levels is for as much as 0.106 and 0.152 respectively. Thus, if it is assumed that the duration of smoking is constant, each cigarrete smoked will cause an increase on HbA1c levels for as much as 0.106%. On the other hand, if CPD does not change, each year of smoking duration will contribute to the increase of HbA1c level for as much as 0.152%. The relationship curve between CPD and duration of smoking with HbA1c levels is illustrated in the following figure 2. Based on those equations, pre-diabetic condition of a smoker will occur at least if the number of cigarettes smoked per day is as many as 20 cigarettes with a minimum smoking duration of 25 years. Meanwhile, diabetic condition of a smoker will occur at least if the number of cigarettes smoked per day is as many as 20 cigarettes with a minimum smoking duration of 29 years. Based on these results, chronic cigarette smokers will have a high risk of T2DM. Therefore, it is possible for smokers who smoke less than 20 cigarettes per day to be exposed to T2DM if they never stop their smoking habit.

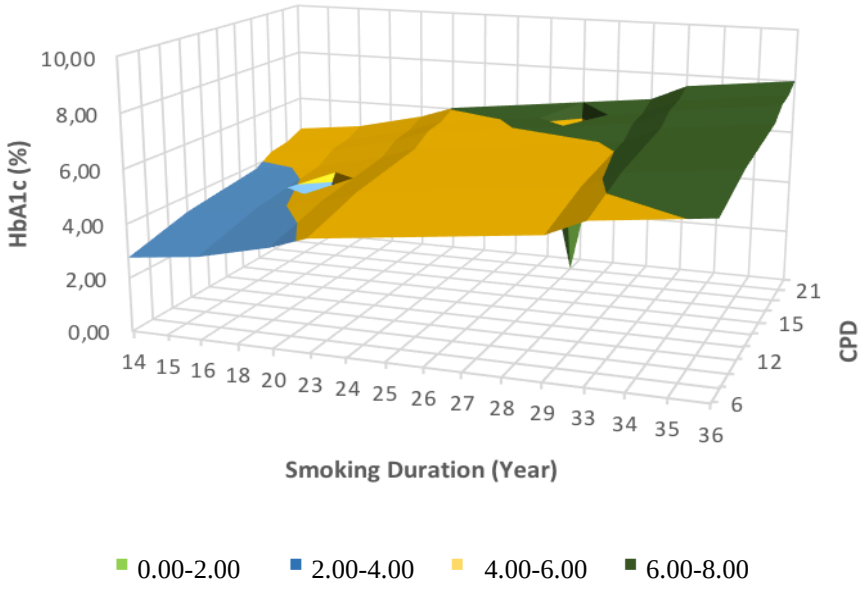

Figure 2. Relationship curve between CPD and duration of smoking with HbA1c levels. The colors show predicted HbA1c value caused by interaction of smoking duration and CPD.

Nicotine, the active compound in cigarette, could decrease insulin sensitivity. In human skeletal muscle and muscle cell cultures, nicotine could interact with nicotinic acetylcholine α1 receptors. Due to this interaction, mammalian target of rapamycin (mTOR/p70S6) and insulin receptor substrate-1(IRS-1) Ser636 phosphorylation will be activated and it causes insulin resistance or impairment of pancreatic β cells which lead to induced insulin resistance inhibit and inhibit insulin secretion insulin (Bajaj, 2012; Bergman *et al*., 2012). Once insulin resistance occurs, the absorption of glucose in the tissue will be disrupted and the blood glucose levels will increase. Other than this, nicotine can also inhibit the secretion of insulin. The effect of nicotine on insulin secretion is caused by an interaction of nicotine to nicotinic acetylcholine receptors on β-cells and it will increase apoptosis of islet β-cells (Xie *et al*., 2009). Due to this condition, the occurrence of long-lasting exposure to nicotine in a smoker will increase the risk of T2DM disease.

Some meta-analysis studies have shown that nicotine exposure over long periods will lead to increased metabolic syndrome (Chang, 2012; Harris *et al.*, 2016; Sun *et al.*, 2012). Smoking is known to reduce body weight; it is linked with central obesity. Stadler *et al*. (2014) find that higher triglycerides and lower High Density Lipoprotein (HDL) cholesterol which play role as major determinants in the development of T2DM in predisposed individuals can be triggered by chronic smoking. Nicotine can rise the concentration of free fatty acids in a smoker's blood by triggering an increasement in the breakdown of fats (lipolysis). This chronic rise in the concentration of fatty acids unfavorably affects insulin sensitivity and insulin secretion through direct effects on liver, pancreas and muscle. It has also been recommended that the distribution of a smoker's body fat has been directly influenced by chronic tobacco smoking (Targher, 2005; Loria *et al*., 2013). Further studies are needed to explore the role of association between triglycerides and HDL levels with HbA1c among Javanese Indonesian smokers.

Based on these results, we concluded that an important aspect to avoid T2DM is a prevention of smoking in early life. According to Bergman *et al*. (2012), insulin resistance and sensitivity caused by nicotine are reversible with smoking cessation. Therefore, to prevent the occurrence of diabetes, a smoker must stop smoking activities. Some other studies suggest that it is important to motivate smokers particularly with diabetes to stop smoking than general smoker population (Reinhard *et al*., 2006; Cho *et al*., 2009;

Nilsson *et al*., 2014; Luo *et al*., 2013). The form of CYP2A6 allele owned by a person will affect whether it is easy for a smoker to quit smoking. Several studies conducted on adult smokers show that smokers with inactive allele types (CYP2A6*4 or *9) have less number of CPD and lower tendency to cigarette dependence compared to smokers who have active allele type (CYP2A6 * 1) (Chenoweth *et al*., 2013; O'Loughlin, 2004; Schoedel *et al*., 2004). In addition, smokers with inactive alleles have a tendency to quit smoking more easily (Ando *et al*., 2003; Ariyoshi *et al*., 2002; Fujieda *et al*., 2004; Minematsu *et al*., 2006; Rao *et al*., 2000). According to Padmawati *et al*. (2009), as much as 65% of subjects tested T2DM in Yogyakarta had a history of smoking before they were diagnosed with T2DM, even some patients with T2DM still smoked regardless of the risks. This was due to the absence of integrated effort from related parties to seek program to stop smoking. In Indonesia, where there are a high consumption rate of cigarette and a rapidly increasing prevalence of diabetes, we conclude that reducing the burden of T2DM can be done by cigarette cessation strategies which might pose some advantages. The burden illness caused by smoking will be reduced if health professionals encourage smoking cessation. Future study is required to further explain the relation between smoking cessation and prevalence of T2DM.

There are several important limitations in this study. The confounding factors like obesity, physical activity, and dietary factors have not been investigated. Further study is needed to investigate clinical significance of the result of this research. These results support other studies stating that smoking leads to increasing blood glucose levels and HbA1c levels which may lead to T2DM if smoking is not controlled.

CONCLUSION

In conclusion, our study suggests that interaction between the number of cigarettes smoked and smoking duration is strongly and independently associated with the risk of T2DM in smokers indicated by HbA1c. Chronic smokers, either light or intermediate smokers, who have been classified as slow and poor metabolizer genotypes will have high risk to suffer from T2DM. There are several limitations in this study in which several possible contributing variables have not yet been included. Those variables might be confounding to our results, for example obesity, physical activity, dietary factors and biochemical variation such as plasma nicotine levels or total urinary nicotine equivalents.

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CORRELATION AMONG SLEEP DURATION, BLOOD PRESSURE, AND BLOOD GLUCOSE LEVEL OF MORANGAN PEOPLE, SINDUMARTANI, NGEMPLAK, SLEMAN

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ABSTRACT

Sleep deprivation is a risk factor for cardiovascular and metabolic diseases. There was a high prevalence of high blood pressure and type 2 diabetes in Morangan, Yogyakarta Province. This study aims at studying the correlation between sleep duration, systolic blood pressure and fasting blood glucose levels of people in Morangan. This study was a cross-sectional study conducted in cooperation with a public health care program for people in Morangan. Data collection was done using a questionnaire and health screening procedure during the public health care program. Collected data were covering aspects of systolic blood pressure, fasting blood glucose, sleep duration, and sleep habit. There was a high prevalence of high systolic blood pressure (>130mmHg; 66.04%) and high fasting blood glucose level (>100mg/dL; 39.62%) in Morangan people. There was a significantly positive correlation between sleep duration and systolic blood pressure (p:0.024; r:0.31) but no significant correlation between sleep duration and fasting blood glucose level. The major contributing habits towards sleep deprivation were caffeine consumption and medium pre-sleep routine. The result of this research will provide help in designing an education program for people of Morangan in preventing and treating high blood pressure and type 2 diabetes.

Keywords: blood glucose; blood pressure; Morangan; sleep duration.

INTRODUCTION

Sleeping is a physiological process important to human which in recent years, its duration and quality are declining. This decline in sleep duration and quality causes an increase in sleep deprivation related diseases. Sleep deprivation is a risk factor for cardiovascular and metabolic diseases (Becker *et al*., 2015; Wang *et al*., 2015). A meta-analysis study shows that there is a strong connection between sleep deprivation and hypertension (Wang *et al*., 2015). A previous study also shows a fact that the majority of type 2 Diabetes patients in *puskesmas* (healthcare center) of Ngesrep Semarang also suffers from sleep deprivation (Simanjutak *et al*., 2018).

Healthy sleep is composed of several components which include duration, quality, timing and lack of sleep disorder. Disturbing factors towards healthy sleep include consumption of caffeine prior to sleep, lack of physical activity, exposure to artificial light and screens at night and inconsistent bedtime routine. Sleep deprivation is generally associated with fatigue, sleepiness, and other health and safety problems (Chaput *et al*., 2018).

While lack of sleep is commonly associated with increasing health risks, excess of sleep in the elderly is associated with

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mortality and morbidity (Hirshkowitz *et al*., 2015). The ideal sleep duration varies from person to person and among different age groups. The National Sleep Foundation suggests that for an adult of 26-64 years of age, the ideal sleep duration is between 7-9 hours while for an elderly person above 65 years old, the ideal sleep duration is between 7-8 hours daily (Chaput *et al*., 2018).

In a research conducted in 2018 by the Ministry of Health of Indonesia, the finding revealed an increasing prevalence in both type 2 DM and cardiovascular disease since 2013 in the province of Yogyakarta (DIY) *(Badan Penelitian dan Pengembangan Kesehatan Kementrian Kesehatan RI*, 2018). This result agrees with the previous research which reports a high prevalence of type 2 DM and hypertension (10.4% and 39.8% respectively) in Sleman (Suhadi *et al*., 2017). One of the villages used for sampling was Morangan village, Ngemplak, Sleman, DIY. The majority of Morangan people consisted of elderly yet they lacked proper knowledge towards the treatment and prevention of type 2 DM and hypertension.

Morangan village was the subject of United Board Project Grant in 2019-2020 entitled Inter-Professional Collaboration to Enhance Student Collaboration and Public Health Care. This project provided an opportunity for research and collaboration to study the correlation between sleep duration, blood pressure and blood glucose level of Morangan people. The result of this study was expected to be used to provide better health care towards people of Morangan.

METHODS

This study was a cross-sectional study conducted in Morangan, Ngemplak, Sleman, DIY. Data were collected on 10th of August, 2019 during the United Board Inter-Professional Collaboration to Enhance Student Collaboration and Public Health Care program. The subjects were chosen by purposive sampling method and data were collected from 53 respondents. This study had been granted an ethical clearance by the Faculty of Medicine, Duta Wacana Christian University Ethical Committee with clearance number of 1077/C.16/FK/2019.

Respondents were asked to fill out an informed consent form before data collection began. Systolic blood pressure was measured using an Omron® automatic blood pressure monitor. Fasting blood glucose level was measured using Easytouch® blood glucose monitor. Information regarding sleep duration and sleep habits was collected using a validated questionnaire. The questionnaire used in this study was derived from the Sleep Quality Questionnaire by Girschk *et al*., (2012) and modified to ensure its validity to be used in Morangan context (Girschik *et al*., 2012). Data collection was done by a group of trained medical personnel.

Data on sleep duration gathered from the questionnaire were then divided into ideal and non-ideal categories based on the age groups. The collected data were then analyzed using SPSS for their descriptive characteristics and for the correlation among sleep duration, systolic blood pressure, and blood glucose level. The analysis was conducted using an independent T-test for the internal difference in systolic blood pressure and fasting blood glucose level followed by a correlation test between groups using Pearson correlation.

RESULTS AND DISCUSSION

As can be seen in table 1, the majority of the respondents was predominantly female compared to male (67.69% to 32.31% respectively). Most respondents were less than 65 years old (83.02%). More than half of them had an ideal sleep duration (52.83%). The majority of respondents in this study had systolic blood pressure above 130mmHg (66.04%) and fasting blood glucose less than 100mg/dL (60.28%). Systolic blood pressure and fasting blood glucose measurement conducted in this study served as both data collection and screening processes for high blood pressure and type 2 diabetes. There was an increasing prevalence of high systolic blood pressure (from 39.8% to 66.04%) and high fasting blood glucose (from 10.4% to 39.62%) compared to a previous study conducted towards the same population in 2017 (Suhadi

et al., 2017). Although this measurement was only done as a screening, this result showed that people in Morangan village was in dire need of treatment and education for high blood pressure and type 2 diabetes.

As can be seen in table 2, statistical analysis of systolic blood pressure shows a significant increase (p=0.024) in people without an ideal sleeping duration (147.8 ± 27.55mmHg) compared to people with an ideal sleeping duration (142.79 ± 25.9 mmHg). This result supports the notion that there is a positive correlation (p= 0.024; r=0.31) between non-ideal sleep duration with an increase in systolic blood pressure as stated in table 3. This result is reflective of previous finding which states that there is an increasing risk of developing hypertension in insomniac patients (St-Onge *et al*., 2016). Ideal sleep duration is essential in maintaining cardiovascular health. A study by Aggarwal (2013) states that lack of sleep is associated with increasing prevalence of stroke, myocardial infarct, and congestive heart failure while the excess of sleep, on the other hand, is associated with an increasing prevalence of coronary artery disease and angina (Aggarwal *et al*., 2013).

Table 1. Characteristics of Morangan people

		N	Percentage
Gender	Male	16	30.19%
	Female	37	69.81%
Age	≤65 years old	44	83.02%
	>65 years old	9	16.98%
Sleep Duration	Ideal	28	52.83%
	Non-Ideal	25	47.17%
Systolic Blood Pressure	≤130 mmHg	18	33.96%
	>130 mmHg	35	66.04%
Fasting Blood Glucose	≤100 mg/dl	32	60.38%
	>100 mg/dl	21	39.62%

Table 2. Comparison between ideal and non-ideal sleep duration

	Ideal Sleep Duration	Non-Ideal Sleep Duration	P
Systolic Blood Pressure (mmHg)	142.79 ± 25.9	147.8 ± 27.55	0.024
Fasting Blood Glucose (mg/dL)	97.5 ± 16.39	99.4 ± 17.33	0.835

Table 3. Correlation among sleep duration, systolic blood pressure, and fasting blood glucose

Parameter	r	p
Sleep Duration and Systolic Blood Pressure	0.31	0.024
Sleep Duration and Fasting Blood Glucose	-0.029	0.835
Systolic Blood Pressure and Fasting Blood Glucose	0.291	0.034

Statistical analysis of fasting blood glucose displays no significant correlation between fasting blood glucose and sleeping duration (p=0.835; r=-0.029) as stated in table 3. This result was in contrast with the result of previous study which showed that there was an additive effect between losses of sleep and impaired fasting blood glucose level (Lou *et al*., 2014). Lack of sleep causes a dysregulation of leptin and ghrelin which produces higher appetite and fasting blood glucose (Morselli *et al*., 2010). This lack of correlation can be partially attributed to a lack of difference (p=0.835) between people with ideal sleep duration (97.5 ± 16.39mg/dL) and the ones with non-ideal sleep duration (99.4 ±

17.33mg/dL) as can be seen in table 2. Even though the people of Morangan showed a high prevalence of high fasting blood glucose (39.62%), the majority of them (60.28%) showed fasting blood glucose less than 100mg/dL.

A positive correlation (p= 0.034; r=0.291) is also found between systolic blood pressure and fasting blood glucose level as stated in table 3. Hypertension and type 2 diabetes share several pathophysiological mechanisms including obesity which causes oxidative stress and chronic low-grade inflammation leading to inappropriate activation of renin-angiotensin-aldosterone system and insulin resistance (Lastra *et al*., 2014). The sleep habits of Morangan people can be seen in table 4. The majority of the respondents slept for less than 8 hours daily and woke up earlier than 6 o'clock in the morning without any noticeable difference between workday and holiday. Their activities before sleeping was almost equally divided between light activities (43.40%) and moderate activities (56.60%). The majority of the respondents used bed for sleeping (75.47%). More than half of the population consumed caffeinated drink prior to sleeping (58.49%) with the most common beverages of tea and coffee. There was only a minuscule amount of the population who smoked as a pre-sleep routine and no one routinely consumed alcohol. Most of the respondents slept with lights off and without any disturbing noise. The majority slept in the bedroom with their companion as their sleep habit and most did not have a television in their bedroom.

Table 4. Sleep habits of Morangan people

		N	Percentage
Sleep duration on a working day	<8 Hours	37	69.81%
	>8 Hours	16	30.19%
Sleep duration on holiday	<8 Hours	38	71.70%
	>8 Hours	15	28.30%
Waking schedule on a working day	<06.00 a.m	51	96.23%
	>06.00 a.m	2	3.77%
Waking schedule on holiday	<06.00 a.m	51	96.23%
	>06.00 a.m	2	3.77%
Sleep schedule on a working day	<22.00 p.m	39	73.58%
	>22.00 p.m	14	26.42%
Sleep schedule on holiday	<22.00 p.m	39	73.58%
	>22.00 p.m	14	26.42%
Activity before sleeping	Light activity, reading, praying, listening to music	23	43.40%
	Watching movie, studying, having a conversation	30	56.60%
Activity on bed	Sleeping	40	75.47%
	Others	13	24.53%
Caffeine consumption before sleeping	Yes	31	58.49%
	No	22	41.51%

		N	Percentage
Smoking before sleeping	Yes	3	5.66%
	No	50	94.34%
Alcohol consumption	Yes	0	0.00%
	No	53	100.00%
Disturbing noises before sleeping	Yes	20	37.74%
	No	33	62.26%
Lights off during sleeping	Yes	18	33.96%
	No	35	66.04%
Sleeping in bedroom	Yes	47	88.68%
	No	6	11.32%
Sleeping companion	Yes	40	75.47%
	No	13	24.53%
Television in bedroom	Yes	9	16.98%
	No	44	83.02%

CONCLUSION

There was a high prevalence of high blood pressure and high blood glucose level in people of Morangan. There was a significantly positive correlation between systolic blood pressure and sleep duration, but there was no significant correlation between fasting blood glucose and sleeping duration. There was also a significantly positive correlation between systolic blood pressure and fasting blood glucose. The major disturbing sleep habits of Morangan people were caffeine consumption and heavy bedtime activities. This information will help to form a fitting education program towards Morangan people in treating and preventing hypertension and type 2 diabetes.

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OPTIMIZATION OF OLIVE OIL, TWEEN 80, AND PROPYLENE GLYCOL OF SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM OF ZINC OXIDE BY D-OPTIMAL METHOD

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ABSTRACT

The incidence of skin cancer in Indonesia reaches 6-8%, so the skin needs effective protection. Zinc Oxide (ZnO) is a sunscreen with Sun Protecting Factor (SPF) 50 which is able to reduce exposure to Ultraviolet rays. ZnO is difficult to dissolve in water making an obstacle if dispersed in a hydro gel matrix, so it is formulated into a Self-Nanoemulsifying Drug Delivery System (SNEDDS) preparation. SNEDDS formula was made using tween 80 as surfactant, propylene glycol as a co-surfactant, and olive oil. The optimum proportion of the three components was optimized with the D-Optimal method using Design Expert Stat-Ease 9 Trial software. Software obtained 16 formulas which were tested for physical stability response: transmittance value (%) and pH value. SNEDDS optimum formula was compared with the D-Optimal prediction formula using the student's t-test statistical analysis ($p>0.05$), the loading dose of ZnO, Particle Size Analysis, and Zeta Potential. The optimum proportion of propylene glycol, tween 80, and olive oil making up SNEDDS were 9.9%: 81%: 9.1% respectively. The result of the percent transmittance response was 92.30% and the pH value was 7.20. Software prediction results: transmittance value was 92.59% and pH value was 7.37. Statistical analysis of one sample t-test showed no difference between observations and D-Optimal predictions. SNEDDS was able to load 2.0 mg ZnO/gram SNEDDS with a particle size of 150.2 nm; polydispersity index of 0.54 and zeta potential of -28.50 mV. The SPF value of SNEDDS ZnO was 16.

Keywords: D-Optimal; SNEDDS; UV protective; ZnO.

INTRODUCTION

Indonesia is a tropical country that is crossed by the equator line. This situation causes the region of Indonesia to be always exposed to sunlight with high intensity, where the sunlight contains ultraviolet (UV). UV light A (320-400 nm) can penetrate the deeper layers of the skin to the dermis and could cause aging, pigmentation, erythema, tanning, and DNA damage due to the presence of reactive oxygen compounds or ROS (Reactive Oxygen Species). UV B (290-320 nm) could penetrate into top surface layer of the skin and cause DNA damage. Whereas UV C (100-290 nm) could be filtered by the atmosphere and it could not penetrate to the surface of the earth (Cefali *et al*., 2016; Kulkarni *et al*., 2014). Human's skin, when getting too much exposure of UV rays for a long period, will be susceptible to cancer, sunburn, eye damage such as cataract and melanoma, premature skin aging, pigmentation, erythema, and also immune system damage (Lolo *et al*., 2017; Kockler *et al*., 2012).

World Health Organization (WHO) in 2015 estimated that the incidence of non-melanoma cancer increased by 300.000 cases due to ozone depletion. The incidence of skin cancer in Indonesia reaches 6-8% (Suharyanto and Prasetyo, 2004). Therefore, skin needs

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protection against UV light. ZnO is one of the materials which are able to absorb the sun's spectrum with light quantum better than TiO2 which has an SPF value of 45-50 (Hutabarat, 2012). The SPF (Sun Protecting Factor) value has a range between 2 to 60. ZnO has an SPF value of 50 (Cosmetics Formulary, 2012). Dermatologists recommend using sunscreen at least at SPF 15-30 (Charisma, 2012) to protect skin from UV light. ZnO has a physical action by reflecting UV light.

ZnO powder is water insoluble and has a cloudy white physical appearance which is a problem if dispersed into a hydro gel matrix. Colloidal dispersion of ZnO is one of resolves to disperse ZnO into hydro gel matrix to form transparent appearance, and reduce the side effects of skin irritation when using ZnO with a concentration of more than 10% (Martorano, 2010). SNEDDS (*Self-Nanoemulsifying Drug Delivery System*) is a technique to reduce the particle size of water insoluble material such as ZnO. SNEDDS is an isotropic mixture of oil, surfactant and co-surfactant that would form nanoemulsion of oil in water without stirring. The SNEDDS formula consist of olive oil, tween 80 as surfactant, and propylene glycol as co-surfactant. The oil requirement in the SNEDDS is medium-long chain oil such as candlenut oil, coconut oil and olive oil. Tween 80 is hydrophilic with Hydrophil Lipophil Balance (HLB) value of 15 and to get the result of percent transmittance value of SNEDDS preparations more than 90% (Diba *et al*., 2014). The higer concentration of surfactant could decrease the particle size of ZnO. Propylene glycol helps the surfactant to reduce the surface tension between oil and water so able to reduce the ZnO particle size.

The proportions of composition of olive oil, tween 80, and propylene glycol in SNEDDS formula are not yet known with certainty so optimization is performed. SNEDDS of ZnO formula optimization in this study was carried out using a D-Optimal mixture design (D-Optimal mixture design). D-Optimal is a method that is widely used in formulations, especially in cosmetics, pharmaceuticals, and food. The advantage of using the D-Optimal method is that it could reduce the number of experimental treatments (Borhan *et al*., 2014). In addition, the costs used are lesser due to the small number of experimental treatments (Zen *et al*., 2015).

METHODS

Materials

ZnO cosmetics grade (Zochem, Canada), distilled water, olive oil (Maroco), propylene glycol (DOW, New York), Tween 80 (Hercules).

Compatibility test of ZnO with SNEDDS components

Amount of 10.0 mg of ZnO was added to Eppendorf which contained 5.0 grams of total system consisting of olive oil, tween 80, and propylene glycol. The mixture was vortexed for 10 minutes then incubated in a shaker incubator at 45±2°C for 15 minutes. Insoluble ZnO was separated by centrifuge at 5000 rpm for 20 minutes. The supernatant formed was filtered and dissolved with ethanol. The test was carried out with replication of 3 times and analyzed with spectrophotometer UV-VIS (Khan *et al*., 2015). A solution that was visually clear and had a transmittance value of more than 80% was a compiler component of SNEDDS that was compatible with ZnO. Distilled water was used as blank.

Experimental design

Determination of the upper and lower limits was done by comparison of oil: surfactant: co-surfactant starting from 1: 1: 1 to 1: 9: 1 with percent transmittance value as clarity parameters. The SNEDDS formula design was performed using Design Expert Stat-Ease 9 Trial software with the D-Optimal method. The main components of SNEDDS were oils (Olive oil), surfactants (Tween 80), and co-surfactants (Propylene glycol). The three criteria for free variables were set as the lower limit (low) and the upper limit (high) described in table 1. The responses tested including percent transmittance value (%) and SPF values entered in the D-Optimal method using DX software.

Table 1. Upper and lower limit value of SNEDDS formula entered in DX software

Materials	Lower limit (%)	Upper limit (%)
Olive oil	9.09	11.11
Tween 80	77.77	81.81
Propylene Glycol	9.09	11.11

Optimization of SNEDDS formula using D-Optimal method

Software Design Expert Stat-Ease 9 Trial would design 16 formulas after determining the upper and lower limits of the components of the SNEDDS formula. All ingredients were weighed (Table 2), then ZnO with the prescribed dose was dissolved into the SNEDDS formula into flacon disk. The mixture was homogeneous with vortex for 3 minutes and sonicated for 10 minutes. This sonication method was carried out to help reduce the size of the emulsion droplets. The SNEDDS ZnO mixture was then incubated using water bath at temperature of 45°C for 15 minutes until homogeneous. SNEDDS was stored at room temperature during the characterization process (Savale, 2015; Ke *et al*., 2015).

Physical responses of SNEDDS ZnO

Transmittance Test (%): amount of 1.0 mL SNEDDS was dissolved with redistilled water ad 50 mL at room temperature, then vortex for 3 minutes until homogeneous. Percent transmittance was measured using Spectrophotometer UV-VIS at maximum wavelength of λ 650 nm with the redistilled water as a blank (Winarti *et al*., 2016). The pH Value Test: amount of 1.0 mL SNEDDS was dissolved with 9.0 mL redistilled water then the pH value was checked using pH meter.

Loading dose of ZnO into SNEDDS formula

The loading dose was determined by varying the amount of ZnO starting from 2 mg; 3 mg; 5 mg; 8 mg; and 10 mg per gram of SNEDDS (total weight of SNEDDS formula was 5.0 g) then dissolved at 50 mL redistilled water into disk at room temperature, then vortex for 3 minutes until homogeneous. Percent transmittance was measured using Spectrophotometer UV-VIS at maximum wavelength of λ 650 nm with the redistilled water as a blank (Winarti *et al*., 2016). Mixture formula with high percent transmittance value was chosen as optimum loading dose of ZnO into SNEDDS.

Optimum formula of SNEDDS ZnO

The optimum formula of SNEDDS was determined using Design Expert Stat-Ease 9 Trial software with D-Optimal method. The SNEDDS formula consist of oil, surfactants and co-surfactants were evaluated by percent transmittance value (%) and pH values. ANOVA statistical analysis with p-value $\leq$ 0.05 (Winarti *et al*., 2016; Gohel *et al*., 2016) for analysis and included into software, then characterization of optimum formula with software prediction was verified using one sample t-test analysis with IBM SPSS Statistics 22 software. The particle size distribution for the optimum formula of SNEDDS ZnO was also checked using a particle size analyzer (PSA) in the Nanotechnology Department of the Faculty of Mathematics and Natural Sciences, Islamic University of Indonesia, Yogyakarta. Amount of 1.0 mL SNEDDS ZnO was dissolved with redistilled water ad 50 mL then vortexed for 3 minutes until homogeneous. The emulsion formed was taken by 3.0 mL and was put into a cuvette for analysis. Replication was carried out 3 times for each test (Jevana and Sreelaksmi, 2011). The optimum SNEDDS ZnO was observed under SEM (Scanning Microscope Electron) to determine the distribution of ZnO nano particles into SNEDDS.

RESULTS AND DISCUSSION

Optimization of composition of olive oil: tween 80: propylene glycol was carried out to obtain the optimal proportion that was able to form colloidal dispersion so as to reduce the size of ZnO particles. The upper and lower limits of the concentration of each component were determined based on a preliminary test in

order to obtain the optimum percentage range with the D-Optimal method using the Design Expert Stat-Ease 9 Trial software according to the desired criteria. Three components in determining the composition of the SNEDDS formula consisting of olive oil (A), tween 80 (B) surfactant, and propylene glycol (C) co-surfactant were determined as independent variables with percent transmittance values (%) and pH value as response variables. The independent variables that had been carried out in the previous orientation had lower limits and upper limits range of 9.09-11.11% (A), 77.77-81.81% (B), and 9.09-11.11% (C). The use of oil and co-surfactants to produce a good SNEDDS formula was less than 20% and surfactants reached 60% (Cerpnjak *et al*., 2013). The results obtained from D-Optimal were 16 run formulas with different compositions of oil; surfactant; co-surfactant where the total of each component was 100% and total weight of SNEDDS formula was 5.0 grams.

The loading dose was carried out to determine the maximum amount of ZnO that could be dispersed into SNEDDS. ZnO loading dose test results that could be completely dispersed was at a dose of 2.0 mg/g SNEDDS. ZnO was perfectly dispersed when it has clear visual appearance, homogeneous, and there are no deposits in SNEDDS. Propylene glycol is a co-surfactant that is often used in cosmetic preparations where the use of co-surfactants could reduce the flexibility of surface tension so that it has enough flexibility to form nanoemulsions with large compositions (Senapati *et al*., 2016).

Physical characteristics parameters include the percent transmittance value, where the higher value of percent transmittance which is close to the water transmittance value (100%) indicates a smaller particle size (Ahmad *et al*., 2014). The results of percent transmittance value from 16 run formulas ranged from 54.75% to 95.02%. The higher transmittance value indicated the smaller particle size, so the solution appearance was clear (Table 2). Clear visual appearance with transmittance value of more than 80% could be categorized as nanoemulsion (Fratter, 2016; Winarti *et al*., 2016). The pH value determines the chemical stability of the preparation and the suitability of the formula for the pH of the skin as to not to cause allergic reactions. The range of pH value from 16 run formulas were 4.0-7.5 indicating that SNEDDS ZnO pH was suitable for the human skin (Gurning, 2016).

Experimental designs were often used in research designs because they provide maximum information with only requiring a small amount of experimentation. D-Optimal is a method that was used to optimize the proportion of components formula. The amount of olive oil (A), surfactant: tween 80 (B), and co-surfactant: propylene glycol (C) were chosen as the independent factor. The mixture profile was determined by D-Optimal based on the Bolton equation, where Y was the response, ABC were the proportion of the components, and α was the coefficient:

$$Y = \alpha 1\,(A) + \alpha 2\,(B) + \alpha 3\,(C) + \alpha 12\,(A)\,(B) + \alpha 13\,(A)\,(C) + A23\,(B)\,(C) + \alpha 123\,(A)\,(B)\,(C) \ldots. \quad (1)$$

Table 2. Results of formula analysis with D-Optimal method using DX Stat-Ease 9 Trial software that produced 16 run formulas and response parameters of physical properties: percent transmittance and pH value

Run	Olive Oil (%)	Tween 80 (%)	Propylene glycol (%)	Transmittance (%)	pH
1	10.11	80.81	9.09	68.11	7.34
2	10.10	78.79	11.11	66.59	7.33
3	9.09	79.80	11.11	95.02	7.27
4	9.09	80.81	10.11	94.28	7.53
5	11.11	79.80	9.09	69.05	7.42
6	11.11	78.79	10.10	62.28	7.40

Run	Olive Oil (%)	Tween 80 (%)	Propylene glycol (%)	Transmittance (%)	pH
7	9.50	81.00	9.50	90.44	7.29
8	11.11	77.78	11.11	81.70	7.54
9	11.11	77.78	11.11	81.81	7.55
10	10.00	79.50	10.51	63.82	6.82
11	11.11	79.80	9.09	69.29	7.61
12	9.09	81.81	9.10	89.56	7.58
13	11.11	78.79	10.10	63.89	7.41
14	9.90	80.20	9.90	54.75	6.91
15	9.09	79.80	11.11	82.87	7.43
16	9.09	81.81	9.10	89.71	7.68

*****SNEDDS system with 5.0 grams amount

Anova is a statistical analysis that explains about the percent transmittance response with cubic models and pH with quadratic models. The model shows the effect of using the composition of oil phase, surfactant, and co-surfactant of each formula which has significant differences that could be known from the ANOVA analysis contained in the software ($p>0.05$). Table 3 showed the mixture of oil, surfactants and co-surfactants to the transmittance response parameters and pH value of SNEDDS, where there was an interaction between olive oil, tween 80 and propylene glycol which the highest value was propylene glycol with the value of -4513.94, it means that concentration of propylene glycol could decrease the transmittance value. The combination of the three components in mixture formula also provided a response to decrease the transmittance value. This was possible because ZnO component can refract the light during the transmittance test using the spectrophotometry UV-VIS method. The pH value response parameter has a quadratic model where there was an interaction between two components in the mixture with the highest response value was oil that valued of +27.56, it means that concentration of oil would increase the pH value.

The numbers in the triangle show the composition of oil, surfactants and co-surfactants in the modeling. The highest response is shown in the red area; the lower response is shown in the yellow area and the lower one is in the green and blue area (Figures 1 and 2). The solution chosen was the one with the highest desirability value that was closed to 1.0, result of software analysis based on the chosen criteria was 0.84 meaning that the formula would produce physical characteristics which was optimal according to the desired target. The proportion of the optimum composition of oil, surfactant and co-surfactant was 9.1%: 81%: 9.9%.

Table 3. Results of software analysis into mathematics model based on physical properties and ANOVA statistical analysis

SNEDDS response of physical properties	Mathematics Equation	Mathematics Model	*p-value* [ANOVA]
Percent transmittance (%)	-3652.73(A)-111.78(B)-4513.94(C)+52.39(A)(B)+523.42(A)(C)+63.31(B)(C)-6.78(A)(B)(C)	*Cubic*	0.053
pH	27.56(A)+0.70(B)+14.70(C)-0.35(A)(B)-0.45(A)(B)-0.18(B)(C)	*quadratic*	0.077

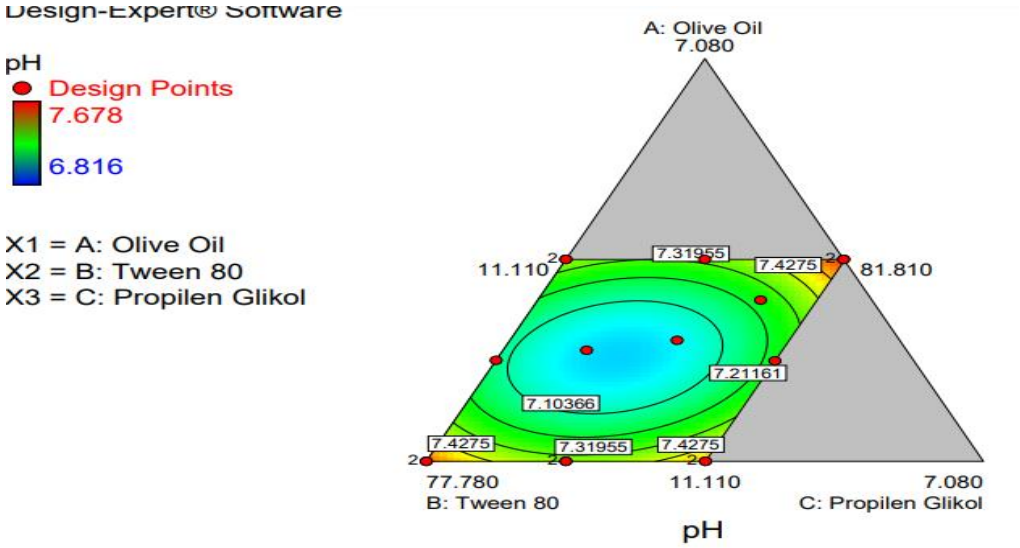

Figure 1. Results of Contour plot diagram showed that the optimum pH response parameter area was in the range of 6.82-7.68 with red color area

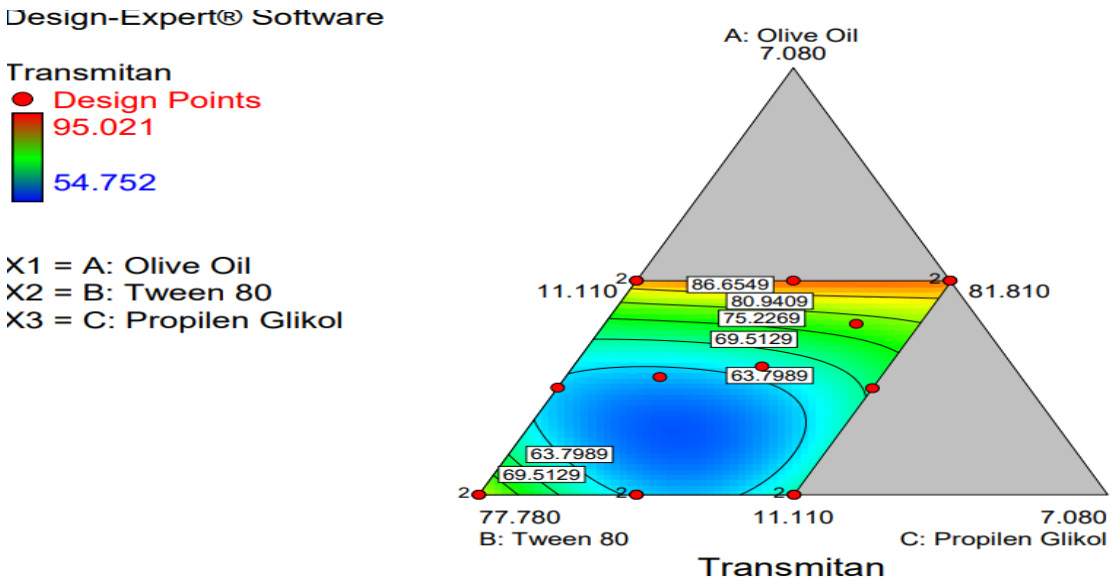

Figure 2. Results of Contour plot diagram showed that optimum percent transmittance response parameter area was almost 95.02% with red color area

Table 4. Results of observation value of SNEDDS ZnO optimum formula compared with Software Prediction value

Respons of physical properties	D-opt prediction	Observation results	*Sig-value*
Transmittance (%)	92.59	92.30	0.053
pH	7.37	7.20	0.077

The software predicted value which was obtained from the D-Optimal method shows that the confidence level of prediction interval of 95% if compared with the response value of the observations for the optimal formula using statistical analysis. The criteria that were given were maximized for percent transmittance value and target for pH value. Based on the test results as presented in table 4, the response percentage of transmittance (clarity)

and pH value from the observation of the optimal formula did not differ significantly from the predicted values given by the Design Expert Stat-Ease 9 Trial software (p-value>0.05).

Measurement of particle size and zeta potential of SNEDDS ZnO optimum formula

The nanoemulsion droplet size of ZnO into SNEDDS was 150.2 nm with a polydispersity index value of 0.54. The recommended PI value requirements indicate that the particles in the SNEDDS formula are stable and reduce the possibility of deposition because brown motion of ZnO particles are rapidly. PI values that meet the observed PI value was quite good and still met the good PI standard value.

PI in particle measurement was used to describe the homogeneity of nanoemulsion particles which have a range of 0.0 to 1.0 (Pratiwi *et al*., 2016). The small particle size could increase the surface area of the particle so it could increase absorption of the drug when it was applied on skin surface area. Nano size in the droplets would reduce the time of emulsification (Bandyopadhyay *et al*., 2012).

Low polydispersity index value shows a narrow particle size distribution meaning that the particle size in the SNEDDS was uniform (Avachat and Patel, 2014). Uniform particle size could increase homogeneity of ZnO when dispersed into SNEDDS and would be also absorbed faster with relatively the same speed (Balakumar *et al*., 2013). The potential zeta value obtained from SNNEDS ZnO was -28.5 mV. SNEDDS with the potential zeta value in range of more than +30 mV and less than -30 mV would produce relatively stable preparations.

This negative potential zeta value indicates that the SNEDDS formula has a negative charge and it was sufficient to counteract the repulsive force so that it would produce a stable preparation (Dash *et al*., 2015). The range of potential zeta values to maintain stability was less than -30 mV or more than +30.

Sun protective factor (SPF) value and scanning electron microscope (SEM) of SNEDDS ZnO optimum formula

SEM results at 100 times magnification showed that ZnO powder had an irregular surface shape and formed an aggregate, whereas on SNEDDS ZnO showed smaller particle sizes and uneven agglomerated in SNEDDS. The smaller the particle size would increase the specific surface area of the particle, thereby increasing the particle distribution (Figure 3). The SNEDDS formula (olive oil: tween 80: propylene glycol) has an SPF value of 5.0 while SNEDDS ZnO has an SPF value of 16. Research Idaho (2008) stated that sunscreen was recommended at least 15 minutes that a person has natural resistance to sunlight for 30 minutes (Moore, 1982).

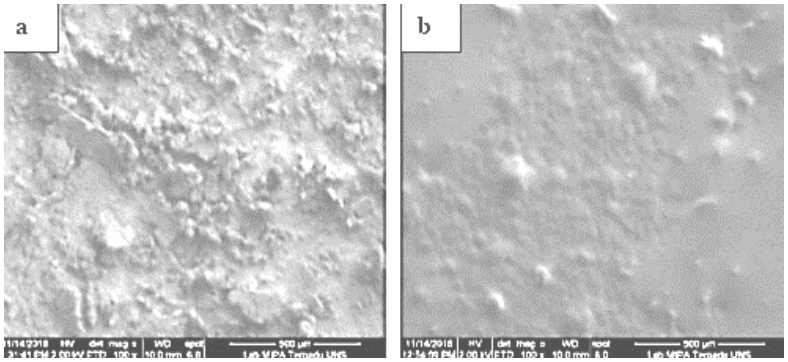

Figure 3. Results of SEM analysis using carbon coating. ZnO powders (a); SNEDDS ZnO (b) at 100 times magnification showed that ZnO powder had an irregular surface shape and formed an aggregate, whereas on SNEDDS ZnO showed smaller particle sizes and uneven agglomerated in SNEDDS

CONCLUSION

The optimum proportion of composition based on D-Optimal method with physical characteristics of transmittance and pH value was olive oil: tween 80: propylene glycol of 9.1%: 81%: 9.9% where the interaction of olive oil and propylene glycol would increase the transmittance value but reducing the pH value. SNEDDS ZnO optimum formula result of the percent transmittance value was 92.30% and pH value of 7.2. D-Optimal prediction value for percent transmittance value was 92.59% and pH value of 7.37. The results of the one sample t-test statistical analysis showed that there was no difference between the observations and D-Optimal predictions value. SNEDDS optimum formula was capable load of 10 mg ZnO particle with SPF value of 16. The particle size of SNEDDS ZnO was 150.2 nm; polydispersity index of 0.54; zeta potential of -28.50 mV; and SPF value of 16. As further studies, SNEDDS ZnO would be dispersed into the hydrogel for topical preparations that is used as UV Protection.

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CHOLESTEROL LOWERING EFFECT OF CHITOSAN NANOPARTICLES USING PARIJOTO FRUITS EXTRACT

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ABSTRACT

Parijoto (*Medinilla speciosa* Reinw. ex Blum) fruit is known to have pharmacological activity as cholesterol lowering levels. Its activity needs to be increased with nanoparticle system so that the active substance can bind 100% to the action target. This study aims to determine the formation of nanoparticles from parijoto fruit (NEBP) and activity test as a decrease in cholesterol levels. The formation of nanoparticles used variations of concentration and volume of chitosan and NaTPP. Anti-cholesterol testing is based on the amount of free cholesterol in the sample that reacted with Lieberman-Burchard into complex green compounds. The best formation of NEBP was 0.2% chitosan, 0.1% NaTPP and volume ratio 5:1. The particle size showed an average size of 269.3 nm (10-1000 nm). The result of the percent transmittance and polydispersity index were 99,379 (close to 100%) and 0.378 (PDI <0.5). The functional group-specific of NEBP was –OH, N-H, PO_3. The morphology was round and non-uniform particles. NEBP can decrease 50% cholesterol levels with a smaller EC_{50} value was 89.08 compared to the extract (EC_{50} 259.98 ppm). Nanoparticles of parijoto fruit is a potential candidate for anti-cholesterol drug.

Keywords: anti-cholesterol; fruit; *Medinilla speciosa* Reinw. ex Blum; nanoparticles.

INTRODUCTION

The consumption level of fat intake in Indonesian society is increasing. The increase of the level of fat intake is related to the incidence of cardiovascular disease. In 2030, there will be an estimated 23.6 million people who die of cardiovascular disease (Bastien *et al.*, 2014; Kemenkes RI, 2018). Parijoto fruit is one of the plants preferred by researchers related to its health benefits, especially in degenerative diseases. Parijoto fruit extract is proven to have anti-diabetic, anti-oxidant, anti-bacterial and anti-cholesterol activities (Wachidah, 2013; Sa'adah *et al.*, 2017; Sugiarti *et al.*, 2017; Vifta *et al.*, 2018).

Bioactive compounds contained in parijoto fruit have low weaknesses. That compounds are very sensitive to processing factors, thus increasing the amount of absorbed active substances. The activity of parijoto fruit metabolites needs to be improved by the application of nanotechnology so that the economic value of Parijoto fruit can be increased (Fathinatullabibat *et al.*, 2014; Irawati *et al.*, 2018).

Nanotechnology can be the main solution for active substances with low bioavailability. Nano allocation particles have a very large surface area that makes it easier to use more effectively and easily in passing through the intestinal wall (Elgadir *et al.*, 2015). The nano method that is easy to implement uses polymeric ionic gelation. The basic principle for this method is the presence of electrostatic attraction between oppositely charged molecules. This force can occur between bioactive components and nanocapsule material (Rizvi *et al.*, 2018).

Chitosan or α (1-4) 2-amino 2-deoxy a β-D-glucane is a form of deacetylation of chitin,

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a biopolymer contained in the exoskeleton of crustaceans and insects. Chitosan has a sensitivity to pH because it is easily soluble at acidic pH (pH<6.5). Chitosan does not dissolve at a higher pH range. The advantages of chitosan as an encapsulate ingredient that chitosan can prolong the duration of drug activity, improve therapeutic efficiency and reduce side effects (Kleine-brueggeney *et al.*, 2015).

Based on the description above, it is necessary to research the increasing parijoto fruit extract activity through the encapsulation process used chitosan and evaluate its activities as anti-cholesterol. This research is important to provide further results regarding herbal plants in the field of phytopharmaceutical, specifically in herbal plants as anti-cholesterol candidates.

METHODS

Materials

Dried parijoto fruit, chemicals used include ethanol 96%, ethanol pa, glacial acetic acid pa, Liebermann Burchard reagent, concentrated sulfuric acid, anhydrous acetic acid (Merck), chitosan powder (92% acetylation degree) from Zhejiang Golden-Shell Pharmaceuticals, NaTPP (Brataco) powder, distilled and redistilled water (Ikapharmindo Putra Mas), chloroform pa, cholesterol standard (Sigma).

Instrumentations

Maceration tools, filter paper, laboratory glassware, rotary evaporator (RE 100-Pro), water bath (Memmert), analytic balance (OHAUS), magnetic stirrer (Thermo Scientif Cimarec), a set of centrifugation devices (PLC Series), UV-Vis spectrophotometer (Shimadzu UV Mini 1240), Particle Size Analyzer (Malvern), Fourier Transform Infrared/FTIR spectrophotometer (Perkin Elmer Spectrum 100) and Scanning Electron Microscopy (Phenom Pro-X).

Determination of plants

Parijoto fruit was obtained from Colo Village, Kudus Regency, Central Java at the beginning of March 2019 with the specification of purplish-pink fruit with a sour taste. The sample was determined at the UPT. Herbal Materia Medica Laboratory, Batu, Malang Health Office to ensure the authenticity of plants used and avoided mistakes in plant selection.

Extraction

Parijoto was macerated by soaking 200 grams of parijoto dried powder which had been mashed with 2 L ethanol 96% (1:10). The maceration was carried out for 2 days (48 hours) and followed by re-maceration. Macerate was evaporated by a rotary evaporator at 80°C and it was concentrated using water bath at 80°C. The concentrated extract was calculated as percent yield and percent moisture content.

Nano extract procedure

NEBP making procedure consists of 2 steps, namely (1) optimization of the concentration of chitosan:NaTPP, and (2) optimization of the volume of chitosan : NaTPP. The extract was weighed 100 mg and dissolved in 100 mL of ethanol, mixed with 15 mL of redistilled water. The liquid extract was taken by 10 mL then 50 mL of chitosan solution was added with various concentrations (0.1%; 0.2%; 0.3% w/v). In the next step, the solution was stirred at 400 rpm for 20 minutes. Then, gradually added 10 mL NaTPP with varying concentrations (0.1%; 0.2% w/v). After that, it was homogenized using a magnetic stirrer to form nanoparticles at 400 rpm for 20 minutes and the mixture entered centrifugation process at 3000 rpm for 15 minutes. The obtained supertanant was characterized by particle size, particle distribution (polydispersity index) and transmittance percent.

The best nanoparticle formation result was based on variations in the concentration of chitosan: NaTPP, then it proceeded to search for the formation of the best nanoparticles based on variations in the volume of chitosan: NaTPP. The volume ratio of chitosan: NaTPP used was 2:1, 5:1, 8:1, and 10:1. The best formation of nanoparticles was characterized by particle size, percent transmittance, specific functional groups, and shape morphology.

Test cholesterol reduction activity and data analysis

The in vitro assay of anti-cholesterol activity was based on research procedures from Anggraini *et al*. (2018). The nano extract and crude extract (25, 50, 75, 100, 125 and 150 ppm) were taken 4 mL, transferred to a test tube with a lid, then added with 1 mL of 1000 ppm cholesterol stock solution. They were mixed until homogeneous then added 2 mL of anhydrous acetic acid and 0.1 mL concentrated H_2SO_4. The solution was incubated for 15 minutes in a dark place (the container was covered with aluminum foil) until the color changed to green. The study was replicated 3 times and the color results obtained were read with a UV-Vis spectrophotometer at a maximum wavelength of 623.20 nm. The blank reagent used was 5 mL chloroform plus 2 ml anhydrous acetic acid and 0.1 ml concentrated H_2SO_4. The negative control used was 1 ml of 1000 ppm cholesterol solution in 5 ml of chloroform, plus 2 ml of anhydrous acetic acid and 0.1 ml concentrated H_2SO_4.

The parameter for decreasing cholesterol levels is a decrease in color intensity (green). The green color is a reaction between the Liebermann-Burchard reagent and ergocalciferol (cholesterol); a decrease in color intensity is due to the reaction between cholesterol and secondary metabolites (flavonoids) in the parijoto fruit nano-extract. Percent reduction in cholesterol levels used absorbance value data obtained from measurements of NEBP and parijoto fruit extract compared to standard cholesterol solutions.

The percentage formula for decreasing cholesterol levels = (Cholesterol Solution Absorbance - Sample Absorbance) / (Cholesterol Solution Absorbance) x 100%. The statistical test used data of the percentage reduction in cholesterol levels from each concentration treated. The application used SPSS version 24 with the One-Way ANOVA post hoc Tukey HSD to determine the differences in each sample treatment. The EC_{50} value was obtained based on a linear regression calculation of the reduction in cholesterol levels from each concentration. The EC_{50} value was the concentration of the sample which can inhibit cholesterol levels by 50%. The purpose of determining the EC_{50} is to determine the concentration of the dosage which is expected to produce an effect of reducing cholesterol levels by 50%. The result of the equation y = a + bx can be calculated EC_{50} using the formula:

Y = a + bx

50 = a + bx

(x) EC_{50} = (50-a) / b

RESULTS AND DISCUSSION

Determination

Plant determination was carried out at the UPT. Materia Medica Batu, Batu City, Malang, East Java. Plant identification key of parijoto is 1b-2b-3b-4b-12b-13a-14bb-17b-18b-19b-20b-21b-22b-22b-23b-24b-25b-26b-27a-28b-29b-30b-31a-32b-33a-34a-35a-36b-1b-4b-6b-9b-10b-14b-15b-16a-17b-18b-20b-23b-24b-25b-27b-1b-3a-4a.

Parijoto is a shrub (1-2 meters high) typical of the Muria Mountains, Kudus. The fruit is similar to kersen, purplish red, rounded, and has a distinctive hemispherical section attached to the petals, 5-8 mm in diameter. The seeds are round, larges, mall, and white (Figure 1).

Figure 1. Parijoto fruit

Characterization of Parijoto Fruit Extract

The yield of extract was 10.48% w/v. The percentage of the extract's water content was 4.07%. The identification of moisture content in the extract was supposed to know the minimum limit or range of the amount of water content in the material (extract). The higher the water content is, the easier it is to grow fungi and molds, so that they can reduce the biological activity of extracts in the retention period (Mohammed *et al.*, 2017).

Characterization of nano parijoto fruit extract (NEBP)

The nanoparticles were made broadly into 2, i.e. top-down and bottom-up. Nano extract in this study used the bottom-up method. It arranges atoms or molecules and combines them through chemical reactions to form nanostructures. Nano parijoto fruit extract (NEBP) is made by the ionic gelation method, which was based on the principle of crosslinking between chitosan cation groups and polyanion in NaTPP.

The NEBP formulation used variations in the concentration of chitosan and NaTPP. The concentration of chitosan used in the formula was obtained by conducting a literature study. The concentration of chitosan was 0.1%, 0.2% and 0.3% w/v, the NaTPP concentration was 0.1% and 0.2% w/v (Sulistyawati *et al.*, 2017). The results of the formation of nanoparticles based on variations in the concentration of chitosan: NaTPP are presented in table 1**.**

Table 1. The results of NEBP formulation based on concentration variation of Chitosan:NaTPP

The concentration of Chitosan: NaTPP	Particle size (nm)	PDI	%T
0,1:0,1	292,4	0,426	99,269
0,2:0,1	269,3	0,372	99,379
0,3:0,1	1097	0,549	96,618
0,1:0,2	315	0,332	99,109
0,2:0,2	777,9	0,513	98,274
0,3:0,2	1171	0,476	98,025

PDI : Polydispersity index
%T : Percent transmittance

The formation of NEBP 0.2% w/v chitosan and 0.1% w/v NaTPP produced the best nanoparticle characteristics. These results follow the study about the synthesis of red mangosteen peel extract which the concentration of 0.2% chitosan and 0.1% NaTPP gave the best physical and functional properties with a particle size of 214.4 nm in Garcinia forbesii extract nanoparticles and 285.2 nm in Garcinia extract mangosten (Mardliyanti *et al.*, 2012).

Prevention of particle formation at a micro size, chitosan must use concentrations below 0.3%. If the concentration of chitosan used is too small, it will produce small and easily aggregated particle sizes, and it causes a larger particle size. Increasing the concentration of NaTPP with the same concentration of chitosan will also cause a larger particle size. This is because the high concentration of NaTPP results in an increase in the availability of amine groups to combine small particles into larger particles (Ningsih *et al.*, 2017). The small size of nano extract can increase the absorption by the mechanism of diffusion through the intestinal wall and reaches the blood compared to large particles (Shah *et al.*, 2016).

Results of variations in chitosan volume:NaTPP based on particle size, polydispersity index, and transmittance value showed that the best formula obtained was chitosan 0.2% w/v and NaTPP 0.1% w/v with a volume ratio of 5:1 (Table 2). The volume

ratio greatly influences particle size, polydispersity index, and percent transmittance. Nano parijoto fruit extract made with a volume ratio of 5:1 has the smallest particle size and polydispersity index of 269.3 nm and 0.372. Increasing the ratio of chitosan and NaTPP will produce nanoparticles with smaller particle size, smaller polydispersity index, and larger percent transmittance.

The polydispersity index value is used to estimate the range of particle size distributions that exist in a sample and finds out whether there is aggregation (Bunglavan *et al.*, 2014). The smaller the polydispersity index value is the more homogeneous the particle size. The polydispersity index value (Table 1 and 2) ranges from 0.3 to 0.7 showed wide particle size distribution. Non-uniform particle size was caused by the tendency of particles to agglomerate to form larger particle aggregates. Factors that can influence particle size and polydispersion index are the concentration of chitosan and crosslinker, the ratio of volume and mass between chitosan solution and crosslinker, stirring speed and stirring time (10). Percent transmittance (% T) is used to measure the clarity of a solution or dispersion system. The percent transmittance value ranges from 98.025-99.379%. Transmittance values close to 100% show clear and transparent dispersion.

Characterization of functional groups nano parijoto fruit extract

The interaction between parijoto fruit extract and chitosan-NaTPP is needed to determine the ability of coating. One method that can be used to determine the presence of extract is Fourier Transform Infra-Red (FTIR). FTIR in this study used 4000-400 cm^{-1} wavenumbers for extract samples, NaTPP, and chitosan. Parijoto fruit extract used wavenumbers of 4000-600 cm^{-1}. FTIR results can be seen in figure 2.

Table 2. The results of NEBP formulation based on volume variation of Chitosan: NaTPP

Volume Chitosan: NaTPP	Particle size (nm)	PDI	%T
2:1	2.168 x 10^4	0.441	98.643
5:1	269.3	0.372	99.379
8:1	315.7	0.493	99.127
10:1	346.2	0.612	98.997

PDI : Polydispersity index
%T : Percent transmittance

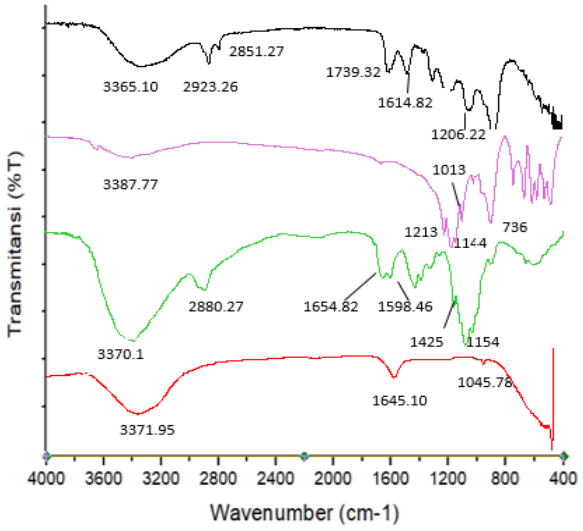

Figure 2. Graph of FTIR results of Ethanol extract of Parijoto fruit (black), NaTPP (purple), Chitosan (green) and Nano Parijoto Fruit Extract (red)

The transmittance graph of FTIR results on nano parijoto fruit extract (*Medinilla speciosa* Reinw. Ex Blume) shows that there was an interaction between chitosan, NaTPP, and nano parijoto fruit extract which was indicated by the shift of wavenumber in the group (-OH) from 3370 cm^{-1} (chitosan), 3387 cm^{-1} (NaTPP) and 3365 cm^{-1} (parijoto ethanol extract) to 3371 at nano parijoto fruit extract (Napsah *et al.*, 2014).

At N-H uptake also experienced a shift in wavenumber from twin uptake 1654 and 1598 cm^{-1} (chitosan) to 1645 cm^{-1} at nano parijoto fruit extract. This change showed the deformation of the N-H group to be one peak because of the cross-linking process (Dewandari *et al.*, *2014*). The stretching of the PO_3 group at wavenumber of 1045 cm^{-1} showed the formation of cross bonds between amino groups from chitosan and anionic groups in NaTPP (Lusiana *et al.*, 2018).

Nano morphology of parijoto fruit extract

Physical particle characterization was carried out by Scanning Electron Microscopy (SEM) to observe the morphology and determine the particle size. This method is an efficient way to obtain images of the specimen's surface. Data obtained from SEM is a two-dimensional photo that displays the specimen's surface and particle size. The morphological results of the NEBP were round and non-uniform with particle sizes ranging from 100-1000 nm (Figure 3). This is shown by the graph of particle size ranges obtained from measurements using a particle size analyzer.

Morphology of NEBP based on SEM photos had a round shape and was not uniform. Measurement of particles applied SEM to obtain particles with sizes ranging from 100-1000 nm. The SEM measurement results have followed the graph of particle size ranges obtained from measurements using particle size analyzers in the range of 100-1000 nm.

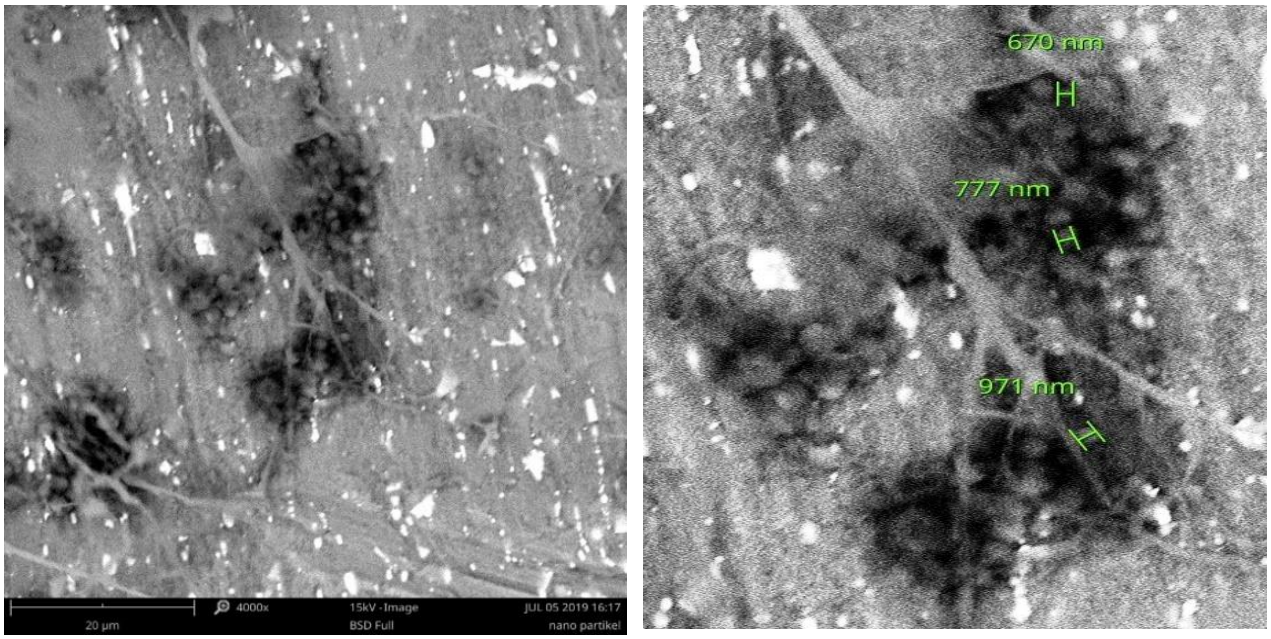

Figure 3. Results of scanning electron microscopy nano Parijoto fruit extract in 4000x zoom

Determination of maximum waves and operating time

Determination of maximum wavelength and operating time using the 100 ppm cholesterol which was reacted with anhydrous acetic acid and concentrated H_2SO_4 produced the following results: the maximum wavelength of 623.20 and 15th-minute operating time. Determination of the value of the operating time is used to determine the timing of the reaction perfectly and stably.

Reduction of the cholesterol level in the Liebermann-Burchard method

The Lieberman-Burchard method was used to determine the amount of free cholesterol found in samples that reacted to green compounds that could be measured using UV-Vis spectrophotometers. The more concentrated form of green from the solution shows the high free cholesterol contained in the sample solution. The Liebermann-Burchard reagent consists of a mixture of anhydrous acetic acid with a small amount of concentrated sulfuric acid. Concentrated sulfuric acid was useful for cutting the hydroxyl group on cholesterol and then oxidized to 3.5 *kolekalsiterol* (3.5 cholestadienes), resulting in a green color. Anhydrous acetic acid to form acetyl was derived from steroids.

The reaction carried out in this method must be free of water, because the reaction was very sensitive and unstable to water. The presence of water can affect the reaction process and make the compounds formed become unstable. The addition of anhydrous acetic acid is useful for removing water content and ensuring the system to be free of water to form derivative products of acetyl from steroids. The removal of the water content by anhydrous acetic acid is carried out by binding to the OH and H. Water groups makes the anhydrous acetic acid turn into acetic acid which will not react with concentrated cholesterol and sulfuric acid (Suptijah *et al.*, 2011).

The percentage of cholesterol decrease levels in the concentration of NEBP was greater than the extract (Table 3). The result of EC_{50} values was inversely proportional to the level of activity of compounds in the sample in reducing cholesterol levels. The smaller the EC_{50} value is, the stronger the activity of decreasing cholesterol levels.

Table 3. Results of the percentage of inhibition of cholesterol and EC_{50} value

Concentration (ppm)	Percentage of Inhibitory Cholesterol Levels (%)	
	NEBP	**Extract**
25	14,61	6,82
50	25,22	14,29
75	41,88	20,78
100	62,98	24,35
125	71,75	27,60
150	78,38	28,25
EC_{50}	89,08	259,98

The ability of NEBP to reduce cholesterol levels by as much as 50% only requires a small concentration of 89.08 ppm. NEBP can increase the effectiveness of parijoto fruit extract in reducing cholesterol levels which is equal to 3 times greater than the extract. Statistical analysis using Tukey HSD as well as variations in the concentration of nano extract (150-75 ppm) resulted in a reduction in cholesterol levels which differed significantly from the activity of decreasing cholesterol extract (p-value 0,000). Modification of nanoparticles will help increase the absorption of compounds contained in the extract by increasing the surface area so that the amount of the absorbed substance will increase or, in other words, also increase the effectiveness of the extract (Ferreira *et al.,* 2018). Anti-cholesterol activity in the NEBP concentration of 25 ppm was comparable with the extract concentration of 50 ppm (p-value 1,000). The smallest concentration of nano extract can reduce the dose as much as 2 times compared to the extract, although, based on statistical results, showed no significant difference between the two treatments.

The content of the active ingredient which is considered to have cholesterol-lowering activity in parijoto fruit is flavonoids. Flavonoids are biologically polar and soluble in water, so they are absorbed poorly due to

the large particle size. The particle size of these metabolites makes it difficult to absorb through the mechanism of passive diffusion since the lipid solubility is not good so that the ability of compounds to penetrate or penetration to lipid membranes is limited (Anggraini *et al.*, 2017). Nanotechnology is recommended because there are some side effects on the formula that is already available in the market; one of which is influenced by the factor of non-compliant patient because the formulations use large doses and are less effective, there is no clear target specificity.

Nanotechnology can affect the bioavailability and the increase of absorption of active ingredients due to an increase in particle surface area and solubility and has a longer residence time because it is trapped by intestinal mucosa. The increased surface area is due to the smaller particle size, so that the residence time in the intestine will be longer and ensnared by the intestinal mucosa (Napsah *et al.*, 2014). Increasing the effectiveness of parijoto fruit extracts using the ionic gelation nanoparticle technology method can increase effectiveness by using lower doses.

CONCLUSION

Parijoto fruit extract nanoparticles were successfully synthesized with 0.2% chitosan and 0.1% NaTPP. The best volume ratio of chitosan:NaTPP was 5:1. Chitosan-encapsulated parijoto fruit extract based on the percentage reduction in levels and EC_{50} values had a greater cholesterol-lowering activity compared to parijoto fruit extract ($p<0.05$). Nano parijoto fruit extract can be used as a potential candidate for anti-cholesterol drugs. It is necessary to find out the optimum dosage of nano extract in vivo test assay.

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EFFECTS OF INTERPROFESSIONAL COLLABORATION PROGRAM IN COMMUNITY ON THE PERCEPTION OF PHARMACY AND MEDICAL STUDENTS

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ABSTRACT

Cardiovascular disease is the highest cause of mortality in Indonesia. One of the factors that causes the disease is low level of health awareness, including healthy lifestyle and health control. Improving public health awareness can be done by providing health education in collaboration with interprofessional health services. A collaboration involving students from various health-related disciplines aims to build good collaboration in the future after the corresponding students become health workers. This study is quasi-experimental. Students from health-related disciplines, especially pharmacy and medical students, were authorized to work directly in health screening and to provide education to the community. Student's perceptions on the Interprofessional Collaboration (IPC) program were explored through Student Perceptions of Physician-Pharmacist Interprofessional Clinical Education (SPICE) questionnaire, which were given before and after the community project. Among 87 SPICE questionnaires, 78 questionnaires filled in completely were collected from pharmacy and medical students. The results of the questionnaire showed good perceptions before and after activities in the community with mean scores of 4.46 (0.35) and 4.5 (0.37) respectively. Wilcoxon test results found no significant difference on the total SPICE scores before and after the activity (P> .05). One item from the SPICE questionnaire, which was the seventh item related to understanding the role of other professionals in the interdisciplinary team, had a significant increase (P <.05).

Keywords: Interprofessional Collaboration (IPC); Interprofessional Education (IPE); medical student; pharmacy student.

INTRODUCTION

Cardiovascular disease is still the highest threat of mortality in Indonesia. The dominating cause suffered by Indonesian population is hypertension. The prevalence of hypertension among subjects in Sleman Regency was almost 50% and had a very low adherence to therapy. Adherence to therapy was found in only 13.5% of hypertension subjects (Suhadi *et al*., 2015). The prevalence of hyperglycemia was 9.9%, dyslipidemia was 17.6%, and overweight and obesity was 56.3%, which indicates low level of health and public awareness about health (Suhadi *et al*., 2017, 2015). In addition, cardiovascular risk factors such as smoking, lack of physical activity, hypertension, hyperlipidemia, and diabetes induce the development of atherosclerosis. Exposure to these risk factors accelerates atherosclerosis and the process of complex plaque formation, narrowing of blood vessels, and blockage of blood flow in vital organs covering the kidneys, heart and brain (Douglas *et al.*, 2014).

Literatures show that other obstacles identified in blood pressure management are high drug prices, forgetting to take medication, high trust in alternative medicine, lack of

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understanding of the treatment received, and poor doctor-patient relationships (Perera *et al*., 2019). Another dominant factor that adversely affects blood pressure is smoking (Mitra *et al.*, 2019). Result from cross-sectional survey undertaken with diabetic patients in India, show slow health awareness about diabetes, from 207 diabetic patients only 37 (18%) were aware of having diabetes (Tripathy *et al*., 2017). This fact shows great potential for developing cardiovascular occurrences in this population. Besides, hypertension and diabetes are closely related to body composition, and overweight individuals tend to suffer from this disease. People living in rural communities tend to be more easily overweight compared to those living in urban communities. So, there is a need for counseling, examinations, and interventions that target this population (Patterson *et al*., 2002).

Interprofessional collaboration is considered essential for the provision of safe, effective, and efficient medical services. This collaboration serves as a response to medication errors that cause serious adverse effects and potentially cause a fatal risk of disease. Medical error ranks fifth in the top ten causes of death in the United States according to the Joint Commission on Accreditation of Healthcare Organizations. The root of the problem is poor collaboration between health workers that causes treatment delays and fatal errors in surgery (Hakiman *et al*., 2016).

Interprofessional Education (IPE) is an educational activity between two or more students from health-related disciplines that aims to improve the understanding on the competencies of each profession that ultimately creates collaboration or ready to work together. IPE can occur in community-based experience programs. Community-based experience shows how Inter-professional Collaborations (IPC) provides services to patients and how the environment and availability of resources have impact on health status (Bridges *et al*., 2011). Students majoring in health-related disciplines, as prospective professionals, can be trained to provide education independently in collaboration with other health professional students. This collaboration can create a collaborative environment that is expected to be applied until they become health professionals (Figure 1). Because of the reasons, it is necessary to examine student perceptions in working together to educate the public to control the risk of cardiovascular disease.

Figure 1. A Framework for Interprofessional Education

METHODS

This research is quasi-experimental. Student perceptions about teamwork, roles, and responsibilities through the Student Perceptions of Physician-Pharmacist Interprofessional Clinical Education (SPICE) questionnaire were investigated. The ethical clearance of this study, No. 1077/C.16/FK/2019, was issued by the Ethics Committee from Duta Wacana Christian University. The program was carried by the Faculty of Pharmacy Universitas Sanata Dharma and the Medical Faculty of Universitas Kristen Duta Wacana by involving 48 pharmacy students in semester 7 from Universitas Sanata Dharma and 39 medical students in semester 7 from Universitas Kristen Duta Wacana. The participants were divided into four groups. Each group performed educating process with the following details. Group 1 provided education on the topic of hypertension in the first month, group 2 provided education on the topic of diabetes mellitus in the second month, group 3 provided education on the topic of dyslipidemia in the third month, and group 4 provided education on the topic of obesity in the fourth month. Students' individual tasks were divided by their specialties with medical student focused on physical examination and pharmacy students focused on education of healthy life and adherence to medication. In addition, the students had to monitor public health including blood pressure checking, body mass index (BMI), blood sugar, blood cholesterol, uric acid, and other vital signs every month. The monthly activities were supervised by teaching staff from the Faculty of Pharmacy and Faculty of Medicine.

Before educating and examining the residents, students had undergone training to conduct counseling and peripheral blood examinations provided by the teaching staff of the Faculty of Pharmacy, Universitas Sanata Dharma and Faculty of Medicine, Universitas Kristen Duta Wacana. Materials provided during the training included how to deliver educational material to the community, healthy lifestyle, goals of pharmacological and non-pharmacological therapy, how to take blood pressure, BMI, and blood sampling (blood sugar, blood cholesterol, uric acid). The education module was created collaboratively by lecturers from both universities. The created module contained materials about cardiovascular risk, hypertension, diabetes, dyslipidemia, and adherence to the therapy. The Student Perceptions of Physician-Pharmacist Interprofessional Clinical Education (SPICE) questionnaire was given before the training session and after the students conducted counseling and health examination on residents. The questionnaire consisted of 10 questions regarding teamwork, roles and responsibilities, and patient outcomes. Each statement was given different scales from 5 (strongly agree), 4 (agree), 3 (neutral), 2 (disagree), to 1 (strongly disagree). The questionnaire results were processed with statistical assistance from the Wilcoxon test because the data were not normally distributed to discover differences on students' perceptions on interprofessional collaboration before training and after the counseling and health examination on residents.

RESULTS AND DISCUSSION

Table 1 shows that the average number of students from pharmacy was slightly higher. A total of 87 questionnaires were collected from students with the exclusion of 9 questionnaires because they were not filled out completely (Figure 2).

Table 1. The number of students in each counseling and examination session (n=87)

Group	Pharmacy Student	Medical Student	Total
Group 1 (first month)	12	10	22
Group 2 (second month)	12	10	22
Group 3 (third month)	12	9	21
Group 4 (fourth month)	12	10	22
Total	48	39	87

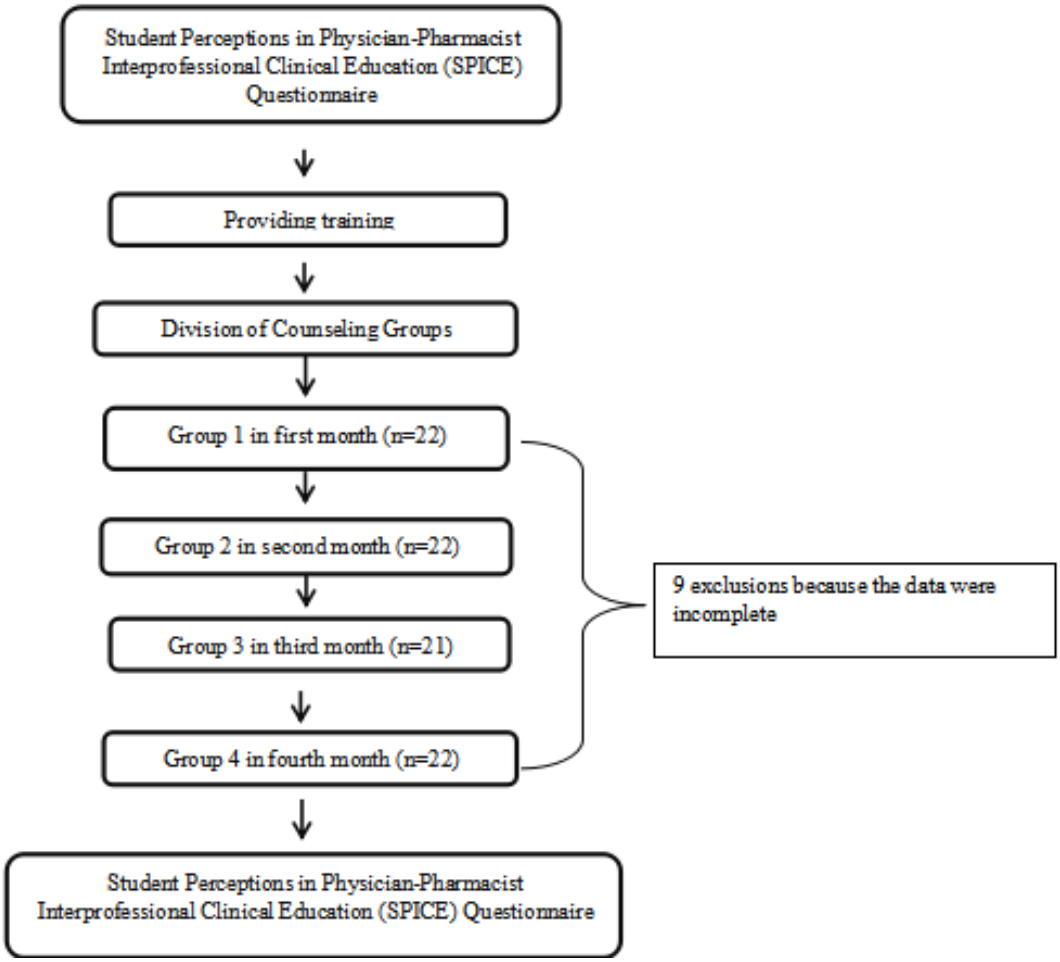

Figure 2. Research flow and student placement schedule

Table 2 shows that mean total scores of SPICE in pre-activity were already superior and mean scores in post-activity remained superior, i.e. 4.46 out of 5 and 4.5 out of 5 respectively. The results of statistical tests with Wilcoxon showed no significant difference between total pre and post SPICE scores (P> .05). SPICE scores on interprofessional teamwork and team-based practice (statements 1, 5, 6, and 8-10) showed no significant difference between pre- and post-activity (P> .05). SPICE scores regarding roles/responsibilities in collaborative practice (statements 2 and 7) showed no significant difference between pre- and post-activity scores (P> .05). SPICE scores regarding patient outcomes from collaborative practice (statements 3 and 4) showed no significant difference between pre-education and post-education scores (P> .05). The results imply that the students have already had a high perception on interprofessional collaboration as those students had been exposed to interprofessional education materials from their lecturer while in college. A study that targeted 837 fourth-year students from numerous disciplines (i.e., nursing, medicine, dentistry, pharmacy, social welfare, food and nutrition, environmental engineering, medical engineering, public health, clinical pathology, physical therapy, occupational therapy, radiology, and dental hygiene) suggests that it is important for students from various majors to have an open mind, accept different views, recognize their own limits, respect other majors, and cooperate with each other to enhance their learning experience (Kim *et al*., 2019). It shows the congruence in which interprofessional education must be carried out as part of formal learning to enhance their competence in collaborating with individuals from other health-related professions.

Table 2. SPICE score according to descriptors (n=78)

Factor	Pre Median (Min-max) Mean (SD)	Post Median (Min-max) Mean (SD)	P-value*
Working with another discipline of students enhances my education (Item 1)	5 (4-5) 4.59 (0.49)	5 (3-5) 4.55 (0.52)	.57
My role within the interdisciplinary team is clearly defined (Item 2)	4 (3-5) 4.13 (0.63)	4 (2-5) 4.17 (0.65)	.69
Health outcomes are improved when patients are treated by a team of professionals from different disciplines (Item 3)	5 (3-5) 4.6 (0.51)	5 (3-5) 4.58 (0.52)	.73
Patient satisfaction is improved when patients are treated by a team of professionals from different disciplines (Item 4)	4 (3-5) 4.4 (0.56)	5 (3-5) 4.45 (0.65)	.50
Participating in educational experiences with another discipline of students enhances my future ability to work on an interdisciplinary team (Item 5)	5 (4-5) 4.63 (0.48)	5 (3-5) 4.55 (0.55)	.28
All health professions students should be educated to establish collaborative relationships with members from other disciplines (Item 6)	5 (3-5) 4.65 (0.5)	5 (4-5) 4.64 (0.48)	.85
I understand the roles of other professionals within the interdisciplinary team (Item 7)	4 (3-5) 4 (0.52)	4 (3-5) 4.23 (0.6)	.01
Clinical rotations are the ideal place within their respective curricula for medical and pharmacy students to interact (Item 8)	4 (3-5) 4.41 (0.59)	5 (3-5) 4.46 (0.59)	.53
Physicians and pharmacists should collaborate in teams (Item 9)	5 (4-5) 4.69 (0.46)	5 (4-5) 4.77 (0.42)	.18
During their education, medical and pharmacy students should be involved in teamwork in order to understand their respective roles (Item 10)	5 (3-5) 4.58 (0.54)	5 (3-5) 4.65 (0.5)	.20
Interprofessional Teamwork and Team-Based Practice (Items 1, 5, 6, & 8-10)	4.67 (3.83-5) 4.59 (0.37)	4.67 (3.67-5) 4.6 (0.38)	.99
Roles/Responsibilities for Collaborative Practice (Items 2 & 7)	4 (3-5) 4.07 (0.47)	4 (2.5-5) 4.19 (0.51)	.07
Patient Outcomes from Collaborative Practice (Items 3 & 4)	4.5 (3-5) 4.5 (0.45)	4.5 (3.5-5) 4.51 (0.54)	.70
Total	4.6 (3.7-5) 4.46 (0.35)	4.6 (3.7-5) 4.5 (0.37)	.50

*Wilcoxon test

Statement 7 from SPICE questionnaire regarding understanding the roles of other professionals within the interdisciplinary team had a significant increase between before and after activity (P< .05). This result is in line with research conducted by Riskiyana (2018) revealing that Inter-professional Collaborations (IPC) learning can improve student understanding and performance on inter-professional collaboration. This collaboration improves the quality of service by increasing the behavior of the health service team in collaborating between professionals (Riskiyana *et al*., 2018). A study that compares group of internal medicine residents, nurse practitioners trainees, psychology trainees, and pharmacy students who participated in a Centers of Excellence in Primary Care Education (CoEPCE) initiative and those who did not participate in CoEPCE initiative. These two different groups created one working group each to give educational activities to patients. Results showed that the CoEPCE initiative was associated with modest improvements in quality of care and gave improvements in patient outcomes (Edwards *et al*., 2019).

The obstacle in conducting collaborative practice is difficulty in uniting the opinions of each student. A study shows that some health care professionals still have an ego where they feel that their opinions are the most correct (Rachma Sari *et al*., 2018). Health professionals, such as nurses and midwives, report that they often find obstacles in participating in decision making, especially during ward rounds. They only serve to convey information and answer doctors' questions about the patient's condition and are not involved in providing input for decision making (Lestari *et al*., 2016). Another obstacle that can hamper collaborative practice is that there are many areas of overlapping responsibility among health workers (Setiadi *et al*., 2017). This can be prevented by learning IPC which can increase understanding on the role of other health workers in serving the community. Other important things are interventions in IPC practice namely education, daily communication, collaborative team visits, case presentations, and treatment reconciliation to improve communication, team care, outcomes for subgroups of high-risk patients, and productivity of health care providers (Nagelkerk *et al*., 2018).

The interprofessional collaboration learning activity showed little change in interprofessional perception according to SPICE instruments because participants had a high perception at the beginning of the study with a slight improvement at the end of the study. The results indicate that the students had been exposed to interprofessional education from their universities. Interprofessional collaboration learning is recommended to enter formal curriculum for students of health-related majors to improve their competence and to prepare the students for collaborating with other health workers to deliver excellent health services.

CONCLUSION

Inter-professional Collaborations (IPC) learning provides new experiences for students. Health education can be provided collaboratively by health professionals who need cooperation from various health professions. Good collaboration requires trust from professional colleagues, high competence from each profession, effective communication, and knowledge of competencies among other health professions. Suggestions for this research need to be measured from the perspective of community members who got education and examination regarding IPC activities that have been carried out.

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IDENTIFICATION OF THE SPREAD OF BORAX USE IN MEATBALL SKEWERS IN BANTUL DISTRICT, SPECIAL REGION OF YOGYAKARTA

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ABSTRACT

Borax is an additive that is often added to some foods such as meatball skewers. The government has banned this ingredient from being added to food because it is harmful to health when consumed for a long time. This study aims to identify the use of borax in *Bakso Tusuk* or meatball skewers sold in Bantul area. Samples were drawn from 17 sub-districts in Bantul, Special Region of Yogyakarta. Two samples were taken from each sub-district, so the number of total samples was 34. The speed of decay test was carried out by observing the appearance of fungus, maggots, and consistency of the surface of meatball skewers. The presence of borax in the meatball skewers was done by turmeric paper and flame test, while the quantitative analysis utilized the acid-base titration method. Based on the results of the study, all samples tested positive for borax with concentrations between 0.06% - 5.15%, and the decay rate test showed that the speed of decay was independent of the level of borax.

Keywords: borax; identification; meatball skewers.

INTRODUCTION

Food additives are frequently added during food processing in order to give impressions that the food has a good shape, delicious taste, tempting smell, and is also long-lasting. Constitution has postulated regulation on which food additives are safe to be used in food processing. The goal of using food additives is to make the food favorable, to promote excellent quality, and to ensure its safety to be consumed by society.

Natrium Tetraborat ($NaB4O_7.10H_2O$), or more commonly known as borax, is shaped in white crystal, does not have any smell, and is stable at room temperature. Borax is usually used to produce detergent and antiseptic. When consumed, borax will gradually create sediment and will cumulatively be absorbed by consumers' bodies, which eventually leads to a negative effect (Tubagus *et al*., 2013).

According to Asteriani *et al*. (2008), the use of borax in foods must be anticipated, for it may cause a negative effect on the body cumulatively. Consuming 5 grams or more may lead to death for children and infants, while the deadly dosage of consuming borax for adults is 10 to 20 grams (Asteriani *et al*., 2008).

One of the currently popular and favored foods in our society is *Bakso Tusuk* or meatball skewers. Not only is it highly affordable, but meatball skewers are also easily found everywhere. The food is highly favored by the community, especially by school-age children, for being sold at a relatively low price and is commonly sold in the school area. The fact that meatball skewers sellers display their food on the street sides should also be a concern. The sellers of meatball skewers usually press the production

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cost by producing meatballs in a large number and by adding preservatives to be able to store the meatballs for more extended periods and, at the same time, make them last longer. Sellers usually use borax as a preservative because it can easily be obtained at an affordable price from shops that sell baking supplies. The use of borax is banned by the Ministry of Health of the Republic of Indonesia through its issued regulation No.772/Menkes/Per/IX/88 and No.1168/Menkes/Per/X/1999.

Regarding the harm of borax added in food, many researchers carried out research on the identification of borax in food. The analysis of qualitative and quantitative was reported. Turmeric paper and flame tests were employed during the qualitative analysis method (Asteriana *et al*., 2008; Tubagus *et al*., 2013) while the quantitative one was reported using a spectrophotometer or acid-base titration (Asterina *et al*., 2008; Tubagus *et al*., 2013; Aryani *et al.*, 2018, Harimurti *et al*., 2019). Acid-base titration for borax analysis is very simple without specific instruments required. The preserving nature of borax is also the target of research carried out by observing the resistance of the meatballs to storage time (Wibowo, 2005). Based on the previous explanation, this paper will report the identification of borax in meatball skewers sold in Bantul district to educate the public about the dangers of borax consumption for an extended period.

METHODS

Instruments and materials

Instruments used in this research were mortar, stamper, burette (Pyrex®), clamp, stative, measuring pipette (Pyrex®), funnel (Herma®), measuring glass (Pyrex®), drop pipette, beaker glass (Iwaki Pyrex®), Erlenmeyer (Pyrex®), volumetric flask (Iwaki Pyrex®), porcelain cup, stainless spoon, filter paper, electric stove (Maspion®), digital scale (Mettler Toledo®), flasks, zip-lock plastic bag, and wooden matches.

Materials used in this research were turmeric (*Curcuma longa Linn. Syn. Curcuma domestica Val*.), all samples of meatball skewers from Bantul district, distilled water (Brataco®), CO_2-free water, 37% concentrated hydrochloric acid (HCl) (Brataco®), oxalic acid ($H_2C_2H_4$) (Brataco®), sulfuric acid (H_2SO_4) (Brataco®), ammonia (Brataco®), sodium hydroxide (NaOH) (Brataco®), borax, phenolphthalein indicator (pp), methyl orange indicator (mo), and methanol (Brataco®).

Sample

The samples used in this research were meatball skewers obtained from all sellers in every sub-district of Bantul. The samples were taken by considering a particular area or population, commonly called cluster sampling (Nursalam, 2013).

Sampling technique

Meatball skewers obtained from the sellers were put inside a previously labeled zip-lock plastic bag. It was then put into a flask containing ice cubes to preserve its condition, and was then kept in a freezer in the research laboratory of UMY. Further, a test to identify the content of borax was done in the research and biochemical laboratory of UMY.

Sample preparation

The sample of meatball skewers was taken as a whole and was weighed meticulously to measure its weight before being added with 50 ml of CO_2-free water. The sample was then mashed using a mortar and stamper and later was filtered using filter paper. The filtrate of the meatball skewers was used in titration test analysis, turmeric test, and flame test. Especially in the flame test, the filtrate must be heated using an electric stove (Maspion®) until it turned into powder.

The sample used in the decomposition test was one whole meatball skewer, which was rested for three days at room temperature. Its color, smell, and whether or not it collected maggot and fungus, along with its texture, were observed.

Decomposition test

The meatball skewer was put on a baking paper and was coded. It was left for three days at room temperature and was being observed

regularly at the same exact time when it was initially prepared (Rahmat *et al*., 2017).

Flame test

The samples which had been prepared were then added with five drops of concentrated H_2SO_4 and 5 ml of methanol in a porcelain cup and were later burned using wooden matches. Samples that were positive to contain borax would create a green-colored flame (Clarke, 2004; Roth *et al.*, 1988).

Turmeric paper test

a. The Production of Turmeric Paper

A total of 4 sheets of filter paper were cut into rectangle with a size of 2 x 4 cm. 250 g of turmeric was grated, and its water was filtered. The filter papers were dipped into the turmeric orange water and later were dried. The end product of such a process was called turmeric paper.

b. Qualitative Test using Turmeric Paper

A positive control was made by putting one spoon of borax into a beaker glass (Pyrex®), which was added with distilled water, and was stirred. The solution was dropped onto a turmeric paper. The color created through this process was used as a positive control.

The sample solution was prepared and dropped onto turmeric paper. If it produced the same color as the positive control, the sample was positive to contain borax. The same indication also applied if the sample turned into dark green-blue when being steamed up with ammonia (Roth *et al.*, 1988).

Quantitative analysis

a. The Standardization of NaOH Using Oxalic Acid Primary Standard Solution

1) The Preparation of Oxalic Acid

Oxalic acid was used as the primary standard solution. Oxalic acid was made by weighing 0.63 grams of oxalic acid and dissolving it with distilled water inside a volumetric flask until its volume reached 100 ml. Subsequently, 25 ml was taken from the solution and was put into Erlenmeyer.

2) The Preparation of NaOH solution

Forty grams of NaOH were weighed accurately, put into a volumetric flask, and was dissolved with CO_2-free water until its volume reached 1000 ml. NaOH is a strong base that can quickly react to CO_2 so that CO_2-free water is required to dissolve it.

3) The Standardization of NaOH using Oxalic Acid

The prepared oxalic acid solution was added with the phenolphthalein indicator. When the indicator was added, the solution remained clear. However, after being titrated with oxalic acid primary raw solution, the color turned into pink. The titration solution was used as the primary stock solution of NaOH to standardize HCl, which would be used to measure the concentration of borax in meatball skewers in Bantul district.

b. The Preparation of HCl for Titration

The titration process used HCI because HCl can create salt that is easily dissolved in water (Day *et al.*, 1996).

1) The Preparation of HCl

As much as 9.90 ml of concentrated HCl 37% was dissolved in distilled water until it reached 1000 ml in a volumetric flask to prepare 1.1 M of HCl.

c. The Standardization of HCl

Twenty five ml of a secondary standard solution of HCl was put into Erlenmeyer and was then added with phenolphthalein indicator. It was later being titrated with a primary stock solution of NaOH 1 N, which had been standardized with oxalic acid. Titration was stopped right after the solution turned into pink, and the volume was recorded. Replication was done three times. The HCI normality was then measured using the following equation 1.

$$V_1 . N_1 = V_2 . N_2 \quad \text{..... (1)}$$

with the following details;

V_1: NaOH volume
N_1: NaOH normality
V_2: HCl volume
N_2: HCl normality

d. The Measurement of Borax Concentration on the Sample

Ten ml was taken from the sample solution and was then put into Erlenmeyer. It was further added with five drops of methyl orange indicator and was titrated with HCl until the color changed into pink. The titration test was replicated three times. The average volume of titration used in each of the samples was measured. Afterward, the concentration of the borax was measured using the following equation 2.

$$\text{Borax Concentration (\%)} = \frac{5xVxNxBEquivalent\ weight}{Sample\ weight\ (mg)} \text{ x } 100\% \quad (2)$$

with the following details:
V: Sample volume
N: HCl normality
BE: Equivalent weight

RESULTS AND DISCUSSION

Qualitative analysis of borax content in meatball Skewers in Bantul district

In this study, qualitative analysis was carried out using three methods, namely decomposition test, turmeric paper test, and flame test. Particularly in this research, the decay test was carried out to study the speed of spoilage of the meatballs considering that borax is often added for preservative purposes. Observations were accomplished by observing the changes that occurred over time. The changes observed were changes in color and odor, the presence or absence of maggots, and the presence or absence of mold. Two qualitative methods that were implemented were the turmeric paper test and flame test. Both tests were carried out to identify the initial presence of borax in the meatball skewer. Two kinds of the test were carried out to confirm each other.

a. Decomposition test results

The decomposition test results, as shown in table 1, indicate that the average samples experienced significant changes on the second day, but 14 samples with sample codes of BT 02, 09, 10, 11, 15, 17, 18, 19, 20, 27, 30, 31, and 33 indicate rather significant changes on the first day. All samples experienced considerable transformations, turning brownish, black beige, or black, producing a bad smell and growing fungi.

Table 1. The qualitative inspection results of Borax in *Bakso Tusuk* in Bantul district

Sample	Sample Code	Decay Test			Turmeric Test	Flame Test
		Day 1	Day 2	Day 3		
Bambang lipuro 1	BT.01	Brownish beige, slightly wet, maggots -, fungi -	Brownish beige, undamaged, maggots -, fungi -, smelly	Brownish beige, undamaged, maggots -, fungi -, smelly	+	+
Bambang lipuro 2	BT.02	Brownish, undamaged, maggot -, fungi +	Color began to turn black, watery, maggots -, fungi ++	Blackish, watery, soft, maggots -, fungi ++++, yellow-colored fungi, bad odor	+	+
Bangunta pan 1	BT.03	Brownish beige, undamaged, maggots -, fungi -	Brownish beige, dry, hard, ant +, maggot -, fungi -	Dark brown, dry, hard, maggots -, fungi +, smelly	+	+
Bangunta pan 2	BT.04	Brown beige, undamaged, maggots -, fungi -	Brownish, dry, hard, maggots -, fungi -	Color began to turn black, dry, hard, maggots -, fungi +, bad odor	+	+
Bantul 1	BT.05	Beige, maggots -, fungi -	Brownish beige, undamaged, maggots -, fungi -, smelly	Brownish beige, undamaged, maggots -, fungi -, smelly	+	+

Bantul 2	BT.06	Beige, ants +, maggots -, fungi -	Brownish beige, undamaged, slightly watery, maggots -, fungi -, smelly	Brownish beige, undamaged, watery, maggots -, fungi -, bad odor	+	+
Dlingo 1	BT.07	Brownish beige, undamaged, ants ++++, maggots -, fungi -	Dark brown, hard, ants +++, maggots -, fungi -	Dark brown, hard, ants +++, maggots -, fungi -, bad odor	+	+
Dlingo 2	BT.08	Dark brown, undamaged, maggots -, fungi -	Grayish, hard, ants +, maggots -, fungi -	Color began to turn black, hard, ants ++, maggots -, fungi -, began to damage	+	+
Imogiri 1	BT.09	Brownish beige, undamaged, ants +, maggot -, fungi +	Brownish beige, ants +, maggots -, fungi ++, smelly	Brownish beige, undamaged, maggots -, fungi +++, bad odor	+	TD
Imogiri 2	BT.10	Brownish beige, undamaged, maggot -, fungi +	Color began to turn black, partly dry, maggots -, fungi +, smelly	Black, hard, dry, maggots -, fungi ++, bad odor	+	+
Jetis 1	BT.11	Brownish beige, undamaged, maggot -, fungi +	Color began to turn black, rather soft, maggots -, fungi +, smelly	Blackish, soft, watery, maggots -, fungi +++, bad odor	+	+
Jetis 2	BT.12	Brownish beige, undamaged, maggots -, fungi -	Brown, slightly hard, maggots -, fungi +	Blackish brown, rather hard, maggots -, fungi ++, bad odor	+	TD
Kasihan 1	BT.13	Creamy-colored, undamaged, maggots -, fungi -	Grayish beige, rather dry, slightly hard, maggots -, fungi -	Grayish, dry, hard, maggots -, fungi -, bad odor	+	TD
Kasihan 2	BT.14	Brownish beige, maggots -, fungi -	Grayish brown, maggots -, fungi -, smelly	Grayish, undamaged, maggots -, fungi -, bad odor	+	+
Kretek 1	BT.15	Brownish beige, maggots -, fungi +	Dark brown, soft, maggots, fungi ++, smelly	Blackish brown, soft, maggots, fungi ++, bad odor	+	+
Kretek 2	BT.16	Brownish beige, maggots -, fungi -	Dark brown, soft, maggots, fungi -, smelly	Blackish brown, soft, maggots -, fungi -, bad odor	+	+
Pajangan 1	BT.17	Brown, maggots -, fungi +	Dark brown, maggots -, fungi +, smell	Blackish, undamaged, maggots -, fungi ++, bad odor	+	TD
Pajangan 2	BT.18	Brown beige, ants +, maggots -, fungi +	Brown, undamaged, maggots -, fungi +, smelly	Dark brown, undamaged, maggots -, fungi ++, smelly	+	TD

Pandak 1	BT.19	Brownish beige, undamaged, maggots -, fungi +	Gray, slightly hard, maggots -, fungi +, smelly	Gray, hard, maggots -, fungi ++, smelly	+	+
Pandak 2	BT.20	Brownish beige, undamaged, maggot -, fungi +	Dark brown, hard, maggots -, fungi +, smelly	Blackish brown, hard, maggots -, fungi ++, bad odor	+	+
Pleret 1	BT.21	Grayish, undamaged, ants +, maggots -, fungi -	Grayish, hard, ants +, maggots -, fungi -	Blackish, dry, hard, maggots -, fungi +, bad odor	+	TD
Pleret 2	BT.22	Blackish beige, undamaged, maggots -, fungi -	Grayish, hard, maggots -, fungi -	Gray, dry, hard, maggots -, fungi -, smelly	+	TD
Piyungan 1	BT.23	Blackish brown, undamaged, maggots -, fungi -	Blackish brown, hard, ants +, maggot -, fungi -	Blackish, dry, hard, maggots -, fungi -, smelly	+	+
Piyungan 2	BT.24	Brownish beige, undamaged, maggots -, fungi -	Brown, hard, dry, ants +, maggots -, fungi -	Dark brown, dry, hard, maggots -, fungi +, smelly	+	+
Pundong 1	BT.25	Brownish beige, undamaged, maggots -, fungi -	Brown, slightly soft, maggots -, fungi -, smelly	Dark brown, soft, maggots - , fungi -, bad odor	+	+
Pundong 2	BT.26	Brownish beige, undamaged, maggots -, fungi -	Brown color, slightly soft, maggots -, fungi -, smelly	Dark brown, soft, maggots -, fungi -, bad odor	+	+
Sanden 1	BT.27	Brownish beige, maggots -, fungi +	Blackish brown, undamaged, maggots -, fungi +, smelly	Blackish, undamaged, maggots -, fungi +, bad odor	+	+
Sanden 2	BT.28	White to beige, undamaged, maggots -, fungi -	Light beige, slightly hard, slightly dry, maggots -, fungi +, smelly	Beige, hard, dry, maggots -, fungi +, bad odor	+	+
Sedayu 1	BT.29	Brownish beige, undamaged, maggots -, fungi +	Blackish brown, slightly soft, maggots -, fungi ++, smelly	Blackish, watery, soft, maggots -, fungi +++, bad odor	+	+
Sedayu 2	BT.30	Brownish beige, undamaged, maggots -, fungi +	Blackish brown, slightly soft, maggots -, fungi +, smelly	Blackish, watery, soft, maggots -, fungi +, bad odor	+	+
Sewon 1	BT.31	Brownish beige, undamaged, maggots -, fungi +	Blackish brown, slightly soft, maggots -, fungi +, smelly	Blackish brown, soft, watery, maggots -, fungi +, bad odor	+	+
Sewon 2	BT.32	Brownish beige, undamaged, maggots -, fungi -	Blackish brown, soft, maggots -, fungi -, smelly	Blackish, soft, maggots -, fungi -, bad odor	+	+

Srandakan 1	BT.33	Brownish beige, dry and hard, maggots -, fungi +	Blackish brown, dry, hard, maggots -, fungi +, smelly	Blackish, dry, hard, maggots -, fungi +, bad odor	+	TD
Srandakan 2	BT.34	Brown, maggots -, fungi -	Dark brown, maggots -, fungi -	Dark brown, undamaged, maggots -, fungi -, smelly	+	+

Source: Primary Data 2016-2017

Information:

Decay Test
- = No Fungi, Maggots
+ = No Fungi Detected
++ = ½ Funged Surface
+++ = ¾ Funged Surface

Flame Test
+ = Positive on the Flame Test
TD = No Flame Test Detected

Turmeric Paper Test
- = Negative against the Turmeric Paper Test
+ = Positive against the Turmeric Paper Test

According to Wibowo (2005), the factors that influence the decomposition of meatball skewers are the various amount and kinds of meat, and the preservative added, which cannot strengthen the researcher' hypothesis so that another more specific testing to analyze borax content in the samples needs to be conducted.

b. Turmeric paper test results

The results of the turmeric paper test for meatball skewers samples are presented in table 1. The findings indicate that all 34 meatball skewers samples positively contained borax since they showed the same color as the positive control. The samples were stated to be positive if they showed the same color as the positive control, and they were stated to be negative if the color was the same as the negative control. The principle of this test is that the turmeric extract attached to the filter paper contains curcumin. Curcumin has two forms of tautomer, namely ketone, and enol. The ketone and hydroxyl groups interact with boric acid to produce a red compound called rososianin. The curcumin and borate complex can be seen in figure 1.

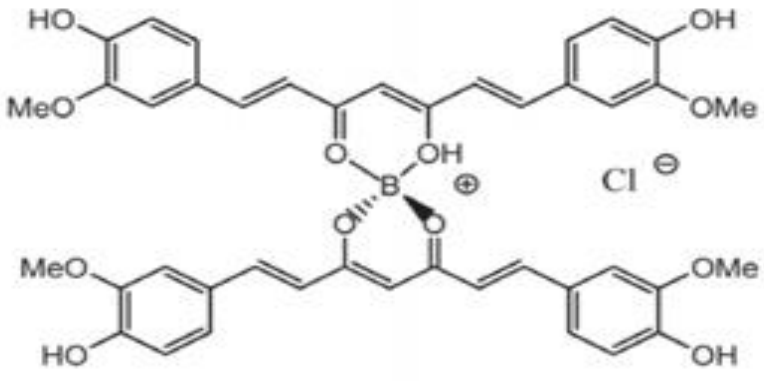

Figure 1. Curcumin complex with borate forming rososianin (Grynkiewicz *et al.*, 2012)

c. Flame test result

The research result (Table 1) using flame indicates that 8 out of 34 samples were not detected for borax content, and the 26 samples were detected for borax content. This is shown from the same color between the flame and the positive control. The samples that were not detected by green flame were caused by several factors such as the existence of obstructing compounds in meatball skewers samples that generated yellowish-red blue flame. This was because the samples did not go through the process of removing obstructing compounds during the pollination process.

Quantitative analysis with acid-base titration

The titration volume was known from the titrated samples. The content calculation was conducted, and its result is presented in table 2. The result of the titration indicates that all samples positively contained borax with various dosages. The average number of the samples was calculated, added, and deduced for the highest and lowest content of meatball skewers in Bantul District, Special Region of Yogyakarta. The highest content of borax in meatball skewers in Bantul District, Special Region of Yogyakarta was 5.15% (Pandak 2), the lowest content was 0.06% (Banguntapan 2), and the average content was 1.64%. This research indicates that all samples contained borax with various amounts.

Table 2. The results of acid-based titration test of borax in Meatball Skewers samples in Bantul district.

Sample	Sample Code	Weight of 1 ball of meatball skewers (g)	HCl (ml)			Average HCl (ml)	Borax content (%)
			Rep. I	Rep. II	Rep. III		
Bambanglipuro 1	BT.01	10.684	9.40	9.10	9.10	9.20±0.14	3.53±0.05
Bambanglipuro 2	BT.02	13.249	6.30	6.10	6.40	6.27±0.12	1.94±0.04
Banguntapan 1	BT.03	13.968	7.70	7.60	7.40	7.57±0.12	2.22±0.04
Banguntapan 2	BT.04	4.683	0.20	0.00	0.00	0.07±0.09	0.06±0.08
Bantul 1	BT.05	10.522	3.60	3.50	3.40	3.50±0.08	1.36±0.03
Bantul 2	BT.06	4.642	1.30	1.40	1.50	1.40±0.08	1.24±0.07
Dlingo 1	BT.07	14.744	0.90	0.70	0.60	0.73±0.12	0.20±0.03
Dlingo 2	BT.08	4.888	1.20	1.00	1.20	1.13±0.09	0.95±0.08
Imogiri 1	BT.09	10.057	2.70	2.70	2.70	2.70±0.00	1.10±0.00
Imogiri 2	BT.10	13.39	6.70	6.60	6.50	6.60±0.08	2.02±0.02
Jetis 1	BT.11	16.106	4.00	4.00	4.00	4.00±0.00	1.02±0.00
Jetis 2	BT.12	9.569	3.60	3.90	3.70	3.73±0.12	1.59±0.05
Kasihan 1	BT.13	3.747	2.00	1.80	2.10	1.97±0.12	2.15±0.15
Kasihan 2	BT.14	9.562	4.30	4.00	3.90	4.07±0.17	1.74±0.07
Kretek 1	BT.15	11.527	7.10	6.80	6.90	6.93±0.12	2.46±0.04
Kretek 2	BT.16	4.956	9.40	9.10	9.10	4.97±0.12	4.11±0.10
Pajangan 1	BT.17	11.493	6.30	6.10	6.40	2.63±0.09	0.94±0.03
Pajangan 2	BT.18	10.862	7.70	7.60	7.40	3.53±0.12	1.33±0.05
Pandak 1	BT.19	17.444	0.20	0.00	0.00	4.37±0.09	1.03±0.02
Pandak 2	BT.20	3.476	3.60	3.50	3.40	4.37±0.19	5.15±0.22
Pleret 1	BT.21	18.783	1.30	1.40	1.50	3.03±0.05	0.66±0.01
Pleret 2	BT.22	7.111	0.90	0.70	0.60	2.20±0.14	1.27±0.08
Piyungan 1	BT.23	6.181	1.20	1.00	1.20	4.10±0.16	2.72±0.11
Piyungan 2	BT.24	22.681	2.70	2.70	2.70	3.90±0.21	0.70±0.04
Pundong 1	BT.25	9.754	6.70	6.60	6.50	4.20±0.16	1.76±0.07
Pundong 2	BT.26	10.203	4.00	4.00	4.00	3.80±0.14	1.53±0.06
Sanden 1	BT.27	10.702	3.60	3.90	3.70	3.47±0.12	1.32±0.05
Sanden 2	BT.28	3.852	2.00	1.80	2.10	2.67±0.12	2.84±0.13

Sedayu 1	BT.29	17.329	4.30	4.00	3.90	3.43±0.17	0.91±0.04
Sedayu 2	BT.30	7.602	7.10	6.80	6.90	1.87±0.19	0.10±0.01
Sewon 1	BT.31	9.821	9.40	9.10	9.10	4.27±0.21	1.83±0.09
Sewon 2	BT.32	14.666	6.30	6.10	6.40	4.30±0.16	1.19±0.05
Srandakan 1	BT.33	13.399	7.70	7.60	7.40	3.17±0.09	0.97±0.03
Srandakan 2	BT.34	15.612	7.70	7.80	7.80	7.77±0.05	2.04±0.01
Total							55.76
Average							1.64
Highest value							5.15
Lowest value							0.06

Source: Primary Data 2016-2017

Map borax distribution in meatball Skewers in Bantul district

Bantul District is a district with 50,685 Ha in width and has 17 sub-districts namely Bambanglipuro, Banguntapan, Bantul, Dlingo, Imogiri, Jetis, Kasihan, Kretek, Pajangan, Pandak, Pleret, Piyungan, Pundong, Sanden, Sedayu, Sewon, and Srandakan (BPS Kabupaten Bantul, 2015). Figure 1 below illustrates that the samples from all sub-districts positively contained borax with the highest content of 5.15% (Pandak 2), the lowest content of 0.06% (Banguntapan 2), and average content of 1.64%.

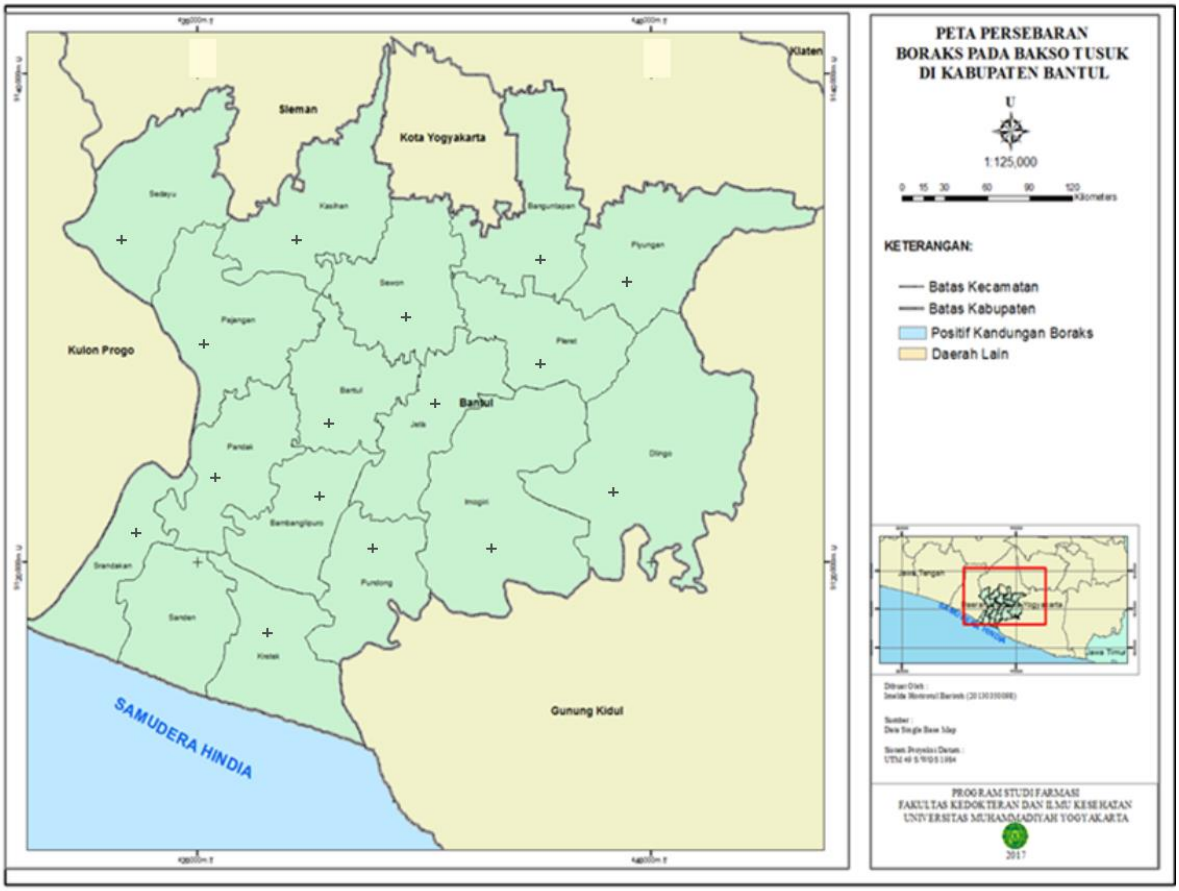

Figure 2. The map of distribution of meatball Skewers sample points containing borax in Bantul district (Source: SHP *Single Base Map* Data)

Borax use has been prohibited by the Minister of Health through a regulation statement issued called as SK (Decree) No. 722/MENKES/PER/IX/88. Winarno (1997) states that borax toxicity stated in acute LD_{50} (lethal dose) is 4.5-4.98 g/kg of mice body weight. Consumed borax will be absorbed by the body and stored cumulatively in the liver, brain, and testicles (Winarno, 1997). A high dose of borax in the body can cause nausea, throw up, diarrhea, stomach cramp, and the acute lethal dose for an adult is 10-20 grams or more (US Department of Health and Human Services, 2010).

Based on Etimine USA, Inc., Safety Data Sheet, oral LD_{50} is 3500 mg/kg- 4100 mg/kg of mouse weight. Dermal LD is 2000 mg/kg of rabbit weight. Inhale LC_{50} (lethal concentration) of borax acid is > 2.03 mg/L of mouse weight for 4 hours. Meanwhile, Saparinto *et al.* (2006) state that the highest borax dosage is 10 g/kg of BW-20 g/kg adult BW and 5 g/kg of children BW, which causes poisoning and death. Based on the toxicity data, the research result is still far from toxicity dosage. However, the fact that meatball skewer is an inexpensive and frequently consumed food raises concern that it is accumulated in the body and is dangerous. There should be strong supervision from various parties such as the government, people, and vendors who do not use the dangerously addictive substance so that the foods sold have good quality and are worth consuming.

CONCLUSION

Based on the survey analysis with 34 samples taken in 17 sub-districts, all meatball skewers sold in Bantul District, Special Region of Yogyakarta contain borax with concentration ranged between 0.06% - 5.15%.

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