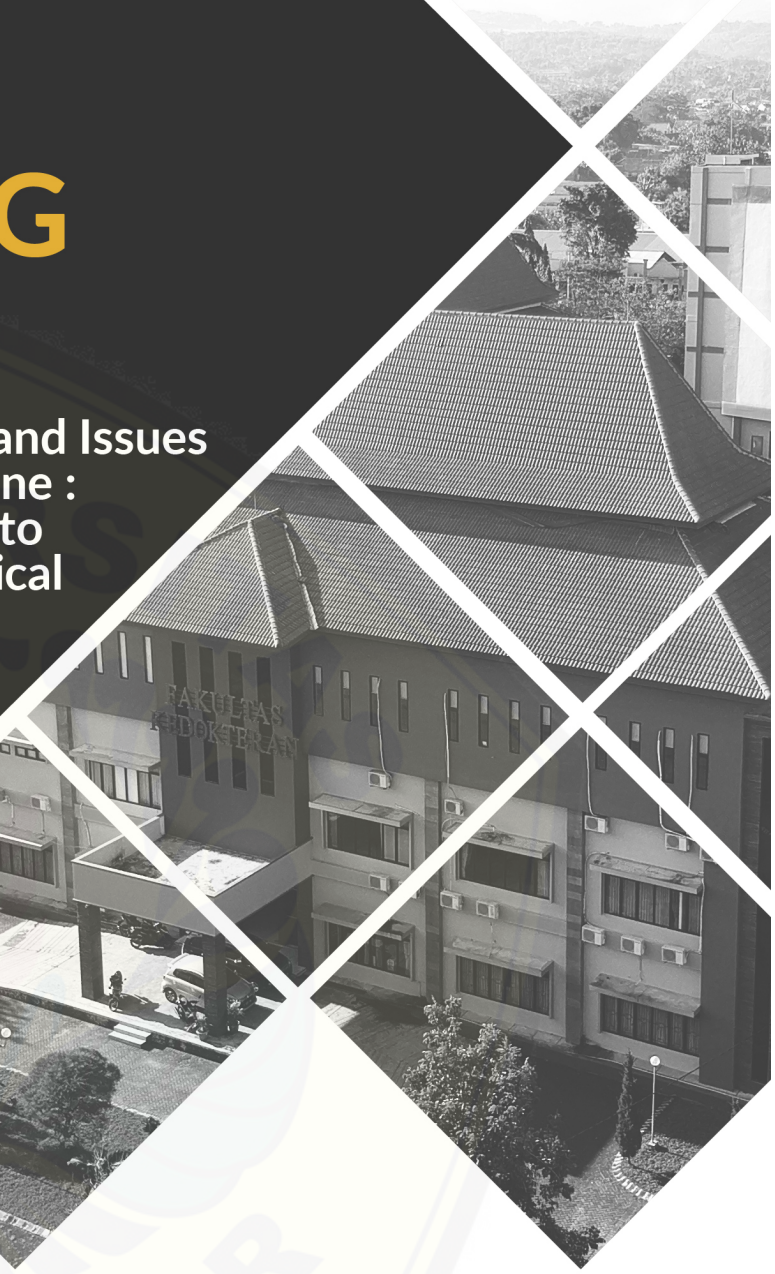
PROCEEDING BOOK

Current Trends, Challenges, and Issues in Agricultural Health Medicine : From Rural to Urban, Ocean to Island, and Molecular to Clinical



The 2nd International Conference of Agromedicine and Tropical Diseases

FACULTY OF MEDICINE
UNIVERSITY OF JEMBER

PROCEEDING

2nd INTERNATIONAL CONFERENCE ON AGROMEDICINE AND TROPICAL DISEASE

"Current Trends, Challenges, and Issues in Agricultural Health Medicine: From Rural to Urban, Ocean to Island and Molecular to Clinical"

Aston Jember Hotel and Conference Center, Jember, East Java
October, 20th – 21, 2018

UPTM PENERBITAN
UNIVERSITAS JEMBER

2nd INTERNATIONAL CONFERENCE ON AGROMEDICINE AND TROPICAL DISEASE

"Current Trends, Challenges, and Issues in Agricultural Health Medicine: From Rural to Urban, Ocean to Island and Molecular to Clinical"

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GREETING MESSAGE

Assalamualaikum Wr. Wb.
Good morning and best wishes

The Honorable, Rector of University of Jember
The Honorable, Dean Faculty of Medicine, University of Jember
The Honorable, All Speaker of The Conference
The Honorable, Guest
The Honorable, Conference Committee
Dear All, All Participants of The Conference

The Faculty of Medicine, University of Jember organize **The International Conference on Agromedicine and Tropical Diseases (ICATD)** that has been held biannually at different venues. The first time, ICATD held in The Faculty of Medicine, University of Jember on 2016 and now we organize the second ICATD at Aston Jember Hotel and Conference Center on October 20th -21st , 2018.

Agromedicine is needed to provide occupational and environmental health safety in agriculture. In the simple definition, agromedicine is a study of human health related to agriculture. The needs for agromedicine research for the improvements on occupational and environmental health and safety in agriculture are growing. The challenges in tropical disease are also increasing that requires a global solution for prevention and elimination. This event would facilitate dissemination of research on this topic, and surely, it will be an outstanding place for networking opportunities to discuss interesting ideas and develop the fruitful project in the future. As a major goal of this event, we hope that it can be an excellent chance for coordinating new partnerships which advance collaboration in the research field as well as the career of all participants. The researchers, practitioners and students from universities, health institutes, hospitals, chemists, government and non-government agencies in health sciences could actively participate on this conference.

Researches in agromedicine and tropical disease field have to be encouraged. One of the way to encourage the development of agromedicine and tropical disease research in Indonesia, and in all over the world, is to promote scientific forums, where scientists can share their experiences, publish their results, and get new insight/idea for the improvement of their research.

Jember is a city full of wonder located in the East Java province, Indonesia. Jember area is dominated by farming, plantation, and fishing, on the other hand Jember has a beautiful view of mountains and beaches. It is a great privilege for me to serve as the chair of the 2nd ICATD and it is my hope that this international event will expand our horizon in agromedicine and tropical diseases, particularly in current trends, challenges and issues in Agricultural Health medicine.

Yunita Armiyanti
Chair of the 2nd ICATD Organizing Committe

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LIST OF ORAL PRESENTATION

LIST OF POSTER PRESENTATION

PROGRAM
2nd INTERNATIONAL CONFERENCE ON AGROMEDICINE AND TROPICAL DISEASE
"Current Trends, Challenges, and Issues in Agricultural Health Medicine: From Rural to Urban, Ocean to Island and Molecular to Clinical"

DAY 1 : 20 October 2018

07.30 – 08.30	Registration Day 1	
08.30 – 09.00	Opening Ceremony	Opening Ceremony
		Traditional Dance
		Speech:
		1. Chairwoman of organizing (DR. dr. Yunita Armiyanti, M.Kes)
		2. Dean of Faculty of Medicine University of Jember (dr. Supangat, M.Kes, PhD, SP.BA)
09.00 – 11.30	Plenary Session	Keynote Speaker I (Prof. DR. dr. Nasronudin,Sp.PD.,K-PTI FINASIM)
		Keynote Speaker II (Prof. Drs. Bambang Kuswandi, M.Sc.,Ph.D)
		Keynote Speaker III (Prof. Susan Alison Brumby)
		Discussion
11.30 – 13.00	Lunch (ISHOMA)	
13.00 – 15.00	Paralel Session	Oral & Poster presentation
15.00 – 15.30	Coffee break	
15.30 – 17.00	Bussiness Meeting	Establishment of Konsorsium Agromedis Indonesia (invitation only)

DAY 2 : 21 October 2018

07.30 – 08.00	Registration Day 2	
08.00 – 10.40	Plenary Session	Keynote Speaker IV (Prof. Chihaya Koriyama, MD, Ph.D)
		Keynote Speaker V (Dr. Vickneshwaran Muthu)
		Keynote Speaker VI (Dr.rer.nat. Anna Artati, M.Sc., M.Si)
		Discussion
10.40 – 12.40	Parallel Session	Oral & Poster presentation
12.40 – 13.30	Lunch	
13.30 – 14.00	Closing ceremony	

ORAL PRESENTATION

2nd INTERNATIONAL CONFERENCE ON AGROMEDICINE AND TROPICAL DISEASE

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IDENTIFICATION AND IN SILICO ANALYSIS OF ANTI INFLAMMATION AND ANTIOXIDANT POTENTIALS OF POLYPHENOL COMPOUNDS IN METHANOL EXTRACT OF *Tamarindus indica* SEEDS

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Abstract

Indonesia has abundant stock of *Tamarindus indica* seeds, but it is not yet utilized maximally, especially in medical field. *Tamarindus indica* seeds have high content of polyphenols compound. No reseacrh is supported by in silico data on polyphenol compound in *Tamarindus indica* seeds extract. Polyphenols compound can be utilized as a neuroprotective agent. This research aims to measure polyphenols concentration in methanol *Tamarindus indica* seeds extract and determine the anti inflammation and antioxidant potential of each polyphenol compound in methanol *Tamarindus indica* seeds extract by in silico method. The extraction of *Tamarindus seeds* used maceration method and methanol as solvent. Identification and measurement of polyphenols compound applied HLPC-MS. PASS tool was used for in silico analysis. Extract recidu was obtained from methanol *Tamarindus indica* seeds as much as 12%w/v. HPLC-MS anaysis mentioned that levels of procyanidin B2, myricentin and caffeic acid were respectively 38.850 mg/kg, 5.845 mg/kg and 260 mg/kg. The highest anti inflammatory potential was owned by myricentin than caffeic acid, while the lowest potential in procyanidin B2. Furthermore, the highest antioxidant potential was sequentially in myricentin, procyanidin B2 and caffeic acid. It is very possible to utilize methanol *Tamarindus indica* seeds extract for preventing neurodegenerative diseases since its pathogenesis involves inflammatory and stress oxidative process.

Keywords : *Tamarindus indica*, myricetin, procyanidin B2, caffeic acid, anti inflammatory, antioxidant

1. Introduction

In traditional medicine, parts of *Tamarindus indica* plant, including leaves, flowers, fruits, skin and roots, have been widely used as a therapy for various diseases such as inflammation, tumors, snake bites, wound healing, fractures and fever [1,2]. *Tamarindus indica* is an endemic plant in the tropic areas [3]. Indonesia has abundance of *Tamarindus indica* seeds, but they have not been utilized optimally, especially in medical field sector [4].

Research by Chunglok *et al*. [5], shows that *Tamarindus indica* seeds contain higher levels of polyphenols than those of other plants. Research by Razali *et al.* [6], reveals that polyphenol compounds in Tamarindus indica seed extract contain procyanidin B2, caffeic acid and myricetin. The active ingredient of polyphenol compounds in plants is believed to provide neuroprotective benefits, including through anti-inflammatory and antioxidant effects [7,8]. Inflammation and oxidative stress are important in the pathogenesis of neurodegenerative diseases such as Parkinson's, Alzheimer's and Hutington's disease [9,10].

There has been no research on the identification of polyphenol compounds in *Tamarindus indica* seed extract that is supported by in silico data about the anti-inflammatory and antioxidant potentials. This study aimed to measure the levels of polyphenol compounds in methanol extract

of *Tamarindus indica* seeds and to determine the anti-inflammatory and antioxidant potentials of each polyphenol compound in silico.

2. Methods

Tamarindus indica seeds were obtained directly from trees in Jember region in the period of June 2017. Species identification was carried out at Purwodadi Botanical Garden Plant Conservation Center, LIPI with identification number 0876/IPH.06/HM/VII/2017.

Standard procyanidin B2 and caffeic acid (ChemFaces PRC), myrecitin (Sigma USA). Mobile phase acetic acid 2%, double distillate water and acetonitrile.

The seeds were separated from the flesh, then washed with distilled water. Drying was conducted at room temperature for 7 days, then continued with grinding.

Tamarindus indica seeds powder (100gr) were dissolved in methanol (500ml) for 72 hours at room temperature. After that, it was filtered and the solution was evaporated with a water bath to obtain the extract residue, and store at a temperature of -20^{0}C [11].

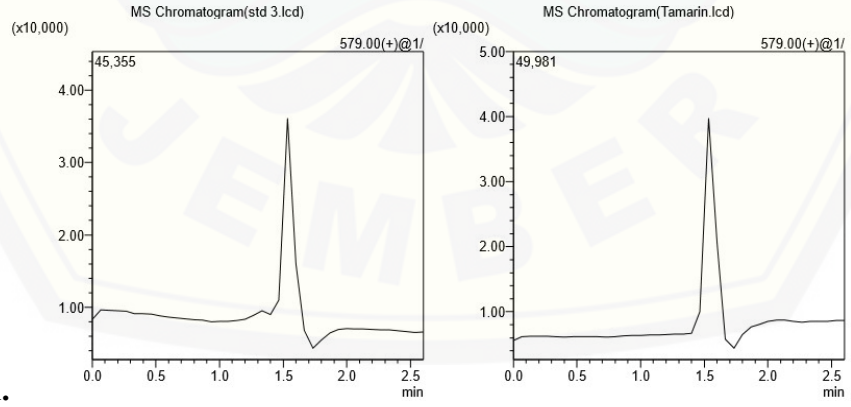
HPLC-MS tool (Shimadzu LC-MS 2020) with Colom SunFire C18 (5µm, 4.6x150mm) was set according to the desired conditions (separation process for 13 minutes, flow rate 1 ml / minute), then the samples were placed on the SIL 20AC auto sample (Shimadzu) Mobile phase, solution A with acetic acid 2% in double distillate water, B acetonitrile solution with a 5% to 100% gradient in 10 minutes, then maintained 100% for 3 minutes until complete. After the separation process was complete, a graph would be generated, showing the compounds undergoing separation according to their retention time and molecular weight, compared to the results with the standard.

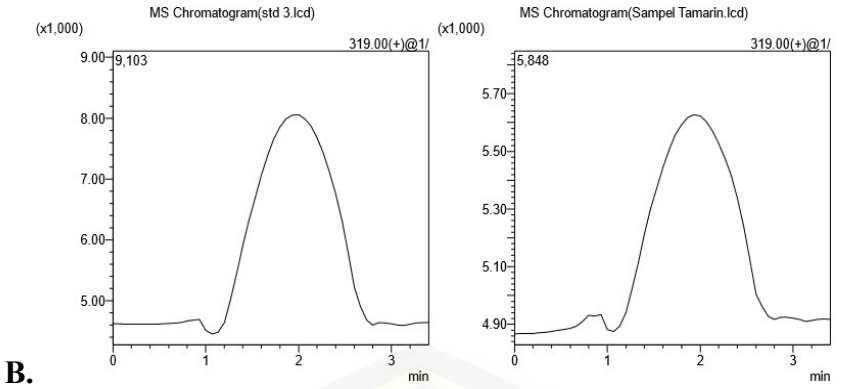
Biological activity analysis was based on the Probability activity (Pa) value with a range of values from 0 to 1, using PASS tool.

3. Results

Methanol extract of *Tamarindus indica* seeds was obtained as much as 12% w/v.The results of HPLC-MS analysis of levels of procyanidin B2 were respectively myricetin and caffeic acid were 38,850 mg/kg (3.89%), 5,845 (0.58%) mg/kg and 260 mg/kg (0.03%). Curve levels of each compound can be seen in Figure 1.

A.





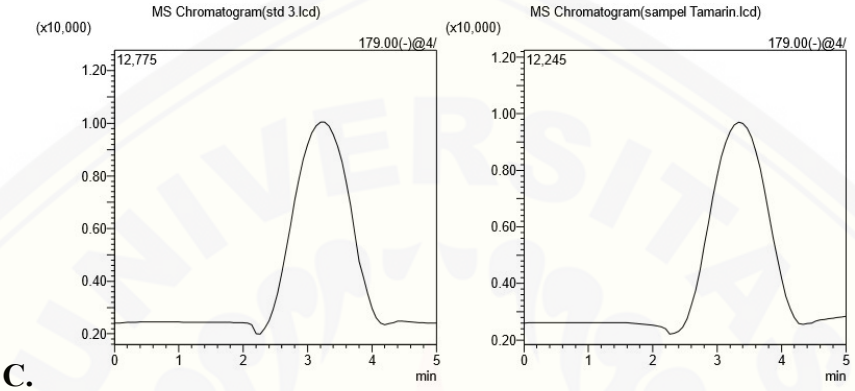


Figure 1. Standard chromatogram and *Tamarindus indica* seed extract samples.
A. Chromatogram procyanidin B2 & sampel. B. Chromatogram myricetin & sampel. C. Chromatogram caffeic acid & sampel.

The results of the in silico anti-inflammatory and antioxidant potential of the three compunds of procyanidin B2, myricetin and caffeic acid can be seen in Figure 2. The highest antioxidant potential is owned by myricetin, then procyanidin B2 and the lowest is caffeic acid. The highest anti-inflammatory potency in myricetin is owned by caffeic acid and the lowest is owned by procyanidin B2.

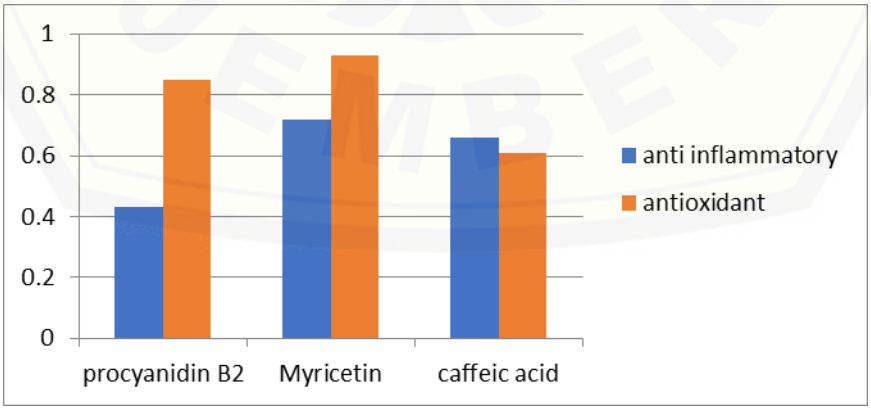


Figure 2. Potential anti inflammatory and antioxidant polyphenol compounds in *Tamarindus indica* seed extract.

4. Discussion

The results of this study showed that based on analysis with HPLC-MS levels of compounds in the extraction of methanol from *Tamarindus indica* seeds, procyanidin B2 is the highest (the level of procyanidin B2 is large enough, almost 4%) compared to myricetin, and the lowest is in caffeic acid. This is different from the study by Razali, *et al*.[6], with 24-hour soaking time using UHPLC, the biggest compounds are procyanidin B2 (0.06%) and caffeic acid, and myricetin is the smallest among the three. This is possible because of differences in the place of origin of the samples and the length of time for soaking the *Tamarindus indica* seeds. The longer extraction time will produce more extract residues and higher potential biological activity [12,13].

Based on the value of the activity probability of in silico test, if Pa value >0.7, the laboratory estimate will be the same as that of the computational test. This shows that myricetin has the highest anti-inflammatory and antioxidant potential compared to the three other compounds; procyanidin B2 has a relatively high antioxidant potential but low in anti-inflammatory potential, whereas caffeic acid has comparable anti-inflammatory and antioxidant potential but with Pa value <0.7. The computational results are in line with some of the existing research results. The research of Zhao et al. [14] showed the presence of anti-inflammatory and antioxidant potential of myricetin compounds characterized by a decrease in MDA, a decrease in IL1β and IL6 in rats with ulcerative colitis. Anti-inflammatory and antioxidant potential are owned by procyanidin B2, indicated by a decrease in IL6 and PGE2 levels in culture exposed to LPS. Increased prooxidant enzymes and decreased MDA in rat liver exposed to CCl_4 [15,16]. Caffeic acid has anti-inflammatory and antioxidant potential shown by a decrease in malondialdehide (MDA) and nitric oxide (NO) in rat brain tissue [17]. Decrease in IL1β and TNFα occurs in mice of DM models [18].

Pathogenesis of neurodegenerative diseases occurs due to inflammation and oxidative stress [19], so that the material that has anti-inflammatory and antioxidative potential is very useful as a neuroprotective, for the prevention of neurodegenerative diseases [20,21]. it is very possible to use *Tamaridus indica* seed extract as a preventative source for neurodegenerative diseases including Alzheimer's and Parkinson's. It can be concluded that there are procyanidin B2, myricetin and caffeic acid compounds in methanol extract of *Tamarindus indica* seeds, and the three have the potential of anti-inflammatory and neuroprotective antioxidants.

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FACTORS AFFECTING UTILIZATION OF NATIONAL HEALTH INSURANCE (JKN) IN DENTAL HEALTH AMONG COASTAL COMMUNITY IN WATU ULO, JEMBER

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Abstract

The objectives of this study are to identify factors associated with national health insurance (JKN) utilization in dental health at primary health cares among PBI participants in a coastal community in Watu Ulo, Jember. A survey was conducted among 104 adults enrolled incapable polis insurance (PBI JKN) living in Watu Ulo in February 2018. A descriptive statistic was done to analyze the socioeconomic characteristics, knowledge of dental insurance coverage and utilization of the insurance in dental health at primary health cares. Multiple regression was performed to examine the determinants of dental insurance utilization. The mean score of knowledge on dental health coverage was 2.28 (SD=1.16) with the minimum score at 0 and maximum at 5. More than 86% of the respondents never use their insurance in any dental services provided at primary health centers. The knowledge on dental insurance coverage was strongly associated with utilization of the insurance ($p = 0.001$), while four other factors, age, sex, education, and occupation were not associated with utilization of dental insurance. The knowledge of insurance coverage influenced the utilization of dental health insurance. It is required more efforts on dissemination knowledge on the coverage of national health insurance on dental health to achieve equity on health among the coastal community.

Keywords: dental insurance, dental care utilization, coastal community.

1.Introduction

Indonesia is one of the largest archipelagic countries in the world and has a long coastline of 81,000 km. Around 60% of the Indonesian population lives around the coastal area [1]. Coastal communities are communities that closely connected to the coastal systems [2]. This community's livelihood depends on the coast, from the natural resources to services in the marine system. Nowadays, Indonesia aims to develop a strong and independent maritime country by increasing the efforts for developing marine and coastal resources [3,4]. Development of human resources is important to achieve this national goal. Consequently, better health care needs to be improved for quality human resources.

Dental health plays an important role for general health to develop quality human resources in Indonesia. Nonetheless, there are an increasing proportion of dental health problems in Indonesia. Data of National Basic Health Research (RISKESDAS) showed dental health problems were reported by 25.9% people in 2013, a 2.7% increase over 2007 [5]. This increasing number showed the need for dental health insurance to ensure equity health services across this nation.

Indonesia started a new health insurance system named JKN (Jaminan Kesehatan Nasional) in January 2014. This national health insurance is managed by BPJS (Badan Penyelenggara Jaminan Sosial) as mandated by law. Enrolment scheme of JKN consisted of two types polis holders. These are including incapable polis insurance (PBI) which paid by the Indonesian government and capable polis insurance (non-PBI) [6]. JKN covers medical and nonmedical benefits. Medical benefits of JKN are health services which include promotive, preventive, curative, rehabilitative. Non-medical benefits are services in the form of accommodation benefits and ambulance [7]. The insurance

benefits of JKN also include dental treatments.

Dental health benefits of JKN are some primary care and advanced care. However, this national health insurance doesn't provide all kinds of dental treatments and exclude some clinical and pharmacy treatments [8]. General practitioners provide primary dental care services at primary health centers (Puskesmas) or private clinics and private practices. Moreover, advanced dental services are provided by dental specialists at hospitals [7].

Watu Ulo is a coastal area in Jember, East Java which well-known for its beautiful beaches. Maduranese dominates the communities. A study found that human resources were below the average among 60% of the communities in this region [9]. In 2017, there were 810 JKN participants in this region [10]. However, data obtained from Puskesmas Sabrang, where this region is one of the service areas, showed only around 40 patients visited dental health centers in a month. This is a small number considering there were in total 14,598 JKN participants in the Sabrang health center [10]. Therefore, this study aims to identify factors associated with national health insurance (JKN) utilization in dental health at primary health cares among PBI JKN participants in a coastal community in Watu Ulo, Jember and assess their knowledge of dental insurance coverage.

2.Methods

This is a quantitative study with observational methods using a cross-sectional approach. Coastal communities are those who live within 20 km of the coast [11]. The location of this study was coastal communities in Watu Ulo, Sumberejo village, subdistrict Ambulu, Jember. The samples are 104 of PBI JKN participants aged 15 to 64 years in these communities. Data was collected in February 2018. The ethical clearance was approved by the Ethical Committee of Faculty of Dentistry, University of Jember.

Independent variables of this study are sex, age, education, occupation and knowledge of dental insurance coverage. The dependent variable is the utilization of national health insurance in dental health. Questionaries of knowledge on dental insurance coverage consisted of 9 items whereas the utilization of dental insurance also consisted of 9 questions. The questioners were multiply closed-ended questions. The correct answers for knowledge questioner coded as '1' and wrong answers ceded as '0'. Scoring method for utilization questioner was based on the frequency of dental service utilization which coded '0' for never, '1' for sometimes, '2' for always.

The questioners were made based on official service guideline of JKN dental coverage in primary cares. The JKN service coverage in the primary oral health is as follows:

a. service administration which includes administration fees for participant registration, provision and provision of referral for treatment to advanced health facilities if first-level health facilities can not handle the patient's illness;

b. Examination, treatment, and consultation with medical experts;

c. Premedication, post-extraction drugs;

d. oro-dental emergency;

e. extraction of primary teeth (with topical anesthesia/infiltration);

f. removal of permanent teeth without complications;

g. Dental filling with composite lift / GIC;

h. Tooth cleaning / dental scaling (once a year);

i. denture prosthesis [7].

The correct answers for knowledge questioner coded as '1' and wrong answers ceded as '0'.

Data of the respondent characteristics and questioner of the knowledge and utilization was analyzed in the descriptive table. Multivariate analysis was performed using a multiple regression model on the factors related to the

utilization of national dental health insurance in primary cares. This model included sex, age, education, occupation, knowledge as predictors. All analyses were performed using SPSS version 19. A statistical significance level of 0.05 was used in the analysis

3.Results

This study identified the factors associated with the utilization of JKN in dental health among coastal communities whose their insurance paid by governments (PBI). As shown in Table 1, in total there were 104 participants which mostly female (53.8%).

Table 1. Characteristic of the respondents (N=104)

Variables	N	%
Sex		
- Male	48	46.2
- Female	56	53.8
Age		
- 15-24	32	30.8
- 25-44	26	25.0
- 45-60	30	28.8
- < 60	16	15.4
Education		
- No education	17	16.3
- Primary education	55	52.9
- High schools	32	30.8
Occupation		
- Fishermen	44	42.3
- Housewives	36	34.6
- Student	20	19.2
- Others (farmers&shopkeepers)	4	3.9

Mean age of the samples was 38.84, and about 30.8% of the participants were age 15-24 years and only 15.4% were age 60 years and older. The samples mostly had low education that 52.9% of participants were in primary education and 16.3% had no education. In this study, 42.3% of the samples were fishermen, and 34.6 % were housewives.

Regarding the knowledge on the insurance coverage in dental health, the mean score of knowledge was 2.28 (SD=1.16) with the minimum score at 0 and maximum at 5. Majority of the participants answer drug prescriptions correctly after dental treatments were included on the insurance services (51%). As presented in Table 2, this service was the highest percentage of the correct answers among other dental treatments. Tooth extraction was the second highest percentage which about 48% of the respondent knew that this treatment was covered in the insurance. The following treatments that less popular among this community was premedication (37.5%) and dental emergencies (33.7%). Less than a quarter respondents knew that dental filling was included in the insurance services. This study found very few samples had information that dental cleaning (7.7%) and denture (5.8%) were covered in JKN.

Table 2. Knowledge of insurance (JKN) coverage in primary dental health (N=104).

Dental services	N	%
Post-treatment drugs	53	51.0
Tooth extraction	50	48.1
Premedication	39	37.5
Dental emergencies	35	33.7
Dental filling	25	24.0
Dental cleaning/scaling	8	7.7
Denture	6	5.8

Among 104 PBI JKN holders, the mean score of JKN utilization in dental health was 0.70 (SD=1.56) with the

minimum score at 0 and maximum at 6. In general, the utilization of JKN insurance in dental treatment was very low. As shown in Table 3, the majority of the respondents never use their JKN for dental treatments. Only 14.4% of respondents reported always use their JKN insurance for drugs prescription whenever they had oral health problems. In a condition of emergencies related to oral health, 11.5% of responded reported had ever use JKN but not always. The utilization of JKN for tooth extraction was reported by ten respondents (9.6%), whereas scaling only six respondents (5.8%). A similar figure was found for JKN utilization in routine oral health examination and dental filling which reported by four people (3.8%). All respondents of this study never had denture treatment using their JKN in health centers.

Table 3. Utilization of JKN in dental services at primary health centers (N=104).

Dental services	**Never**		**Sometimes**		**Always**	
	N	**%**	**N**	**%**	**N**	**%**
Prescription drugs	87	83.7	2	1.9	15	14.4
Dental emergencies	92	88.5	12	11.5	0	0
Tooth extraction	94	90.4	10	9.6	0	0
Tooth cleaning (scaling)	98	94.2	6	5.8	0	0
Routine oral health examination	100	96.2	4	3.8	0	0
Dental filling	100	96.2	4	3.8	0	0
Dentures	104	100	0	0	0	0

As presented in Table 4, this study found that the independent variable which related to utilization was the knowledge of insurance coverage. This positive association indicates the higher the knowledge on the insurance, people are more likely use the insurance for dental treatments (p=0.002).

Table 4. Multiple linear regression of determinants of JKN dental insurance utilization (N=104)

Variables	**B**	**Sig**
Sex		
- Male (Ref)		
- Female	-0.230	0.390
Age	0.024	0.861
Education		
- No education(Ref)		
- Primary education		
- High schools	0.027	0.856
	0.015	0.940
Occupation		
- Fishermen (Ref)		
- Housewives		
- Students&others	0.219	0.431
	0.307	0.195
Knowledge	0.338	0.001

4.Discussion

This study aimed to examine determinants of the utilization of national health insurance (JKN) in dental health among PBI participants in coastal communities of Watu Ulo, Jember. This study found very low utilization of JKN insurance in dental health. PBI JKN participants consisted of people who categorized as poor and disadvantaged [6]. The income of the most family in this community depends on the sea as fishermen who are not a routine income. Therefore, it requires contributions from the government, especially for the majority of fishers who cannot afford the regular payment of the JKN [12].

In this study, knowledge of the JKN coverage of dental health was very low. Majority of PBI JKN holders had no information on dental service provided by the insurance. This may be related to the low educational level of the respondents. It is known that poor educational level may lead to low income, unemployment, and poor occupational status. Financial costs and a low level of information about the importance of oral health could be a hindrance to dental care [13].

Utilization of JKN in dental health was very low. This study found that individual knowledge on dental insurance coverage is the only predictor that statistically significant for dental insurance utilization. An increase of knowledge on the insurance coverage is associated with an increase in utilization. This finding was consistent with prior studies in some region in Indonesia. A study in Rowosari primary health center, Semarang found a significant relationship between knowledge level (p=0,00) of JKN participants to the utilization of health care services in the primary health center [14]. The other study among JKN participants in Jumpadang Baru health centers, Makassar, Sulawesi showed a relationship between knowledge, attitude and family support with JKN Health Care Service utilization [15]. The better the level of community knowledge, the better public awareness of the use of health facilities. This awareness can increase the utilization of health services which can improve the community health status [16].

5.Conclusion

The results showed the majority of the PBI participants in coastal communities never use JKN for dental treatments. In this study, the knowledge of the insurance coverage played an important role in the utilization of the insurance.

People with less knowledge are also less likely to use the insurance for seeking dental treatment. Given the low level of education in this community, it poses a particular challenge for policymakers to find ways to effectively reach those people to give a better understanding on the JKN scheme.

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HEALTH SEEKING BEHAVIOUR AMONG TENGGERESE SOCIETY: AN UPDATE

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Abstract

Indonesia is a country with cultural diversity. This diversity makes Indonesia rich in local wisdom including the use of plants for various purposes, like in health sector. This study aims to explore the health seeking behavior of the Tenggerese. This study uses a qualitative approach with a type of descriptive research. The study was conducted in June 2017 until August 2018 with the Tenggerese as informants in three districts: Lumajang, Probolinggo, and Pasuruan. The results of the study show that currently the Tenggerese uses three approaches to overcome their health problems. The approaches include: using plants that are believed to have medicinal properties, go to the traditional healers named "Dukun" who are using spell (mantra) or "suwuk" as the media, and go to the health workers / health facilities. Recently, the use of plants that are believed to be efficacious for medicine is very rarely used for various reasons. The plant is only used to treat mild health problems. Plants that are still used today are: *binahong* (to heal itching and acne), *dringu*(to heal fever), *jeruk nipis* (to heal cough), *tebu ireng* (to heal cough), and *tepung otot* (to heal muscle pain). The fact that local of the use of plants believed to have medicinal properties by the Tenggerese is almost extinction, shows the importance of educating for the Tenggerese about conservation and utilization of plants. In addition, the role of researchers is also needed to conduct ethno-pharmacology studies to explore the efficacy and how to use these plants.

Keywords : Tenggerese, Health seeking behaviour, Traditional Medicine, Plants.

1. Introduction

Indonesia is a country with cultural diversity. The Indonesian Central Bureau of Statistics in 2013 recorded at least 633 large ethnic groups in Indonesia. If it broken down to the sub-tribe, the number of it becomes 1331 groups [2] (BPS, 2015). This diversity makes Indonesia rich in local wisdom including in the health seeking behaviour which is involving the traditional medicine.

Based on National Socio-economic Cencus, the population using traditional medicine increased by 15,05% in 1999 to 30,67% in 2003, 32.87% in 2004, 35.25% in 2005, and 38.30% in 2006. In 2010, the use of traditional medicine increased to 45.17% and 49.53% in 2011 [5] (Rahayu, 2012)

In addition, the use of traditional health service is also large. So that the government want to provide the service which is in accordance to the religious and cultural norms and it is believed could heal the disease or illness.

The Ministry of Health made it happen in the 2015-2019 strategic plan which was launched in 2015. it is expected that 15% of the puskesmas have carried out traditional health services. From Indonesia's health profile data in 2015, there were 1,532 health centers (Puskesmas) that had traditional health services, or 15.7% of 9,754 health centers in 34 provinces in Indonesia. The highest number of puskesmas that gave traditional health services was the province of South Sulawesi with 135 health centers. Followed by the province of North Sumatra with 99 puskesmas, and Banten with 92 puskesmas. The provinces of West Papua, Jambi, and South Kalimantan are provinces with the least number of traditional health services with 7, 9 and 10 puskesmas [3] (Kemenkes, 2015).

Although medical treatment develops and advances rapidly, it does not make traditional medicine recede from the flow of medical technology, because traditional medicine has been recognized as a means of healing diseases that are well known to the public.

A person's response to stimuli or objects related to health-illness, illness,

and factors that affect healthy-sickess is a health behavior (healthy behavior) [4] (Notoatmodjo, 2005). The behavior of people who are sick or have been exposed to health problems, to get healing or problem-solving. This behavior is called health-seeking behavior. This behavior includes actions taken by someone to get healing or regardless of the health problems they suffer. Health services sought are modern health facilities (hospitals, health centers, polyclinics and so on) and traditional (shaman, sinshe, paranormal), as well as modern or professional medicine (hospitals, health centers, polyclinics, etc.)

Based on the results of research conducted by researchers it is known that there are 3 patterns of treatment seeking in the Tengger tribe, diseases that can be cured only by treating themselves using traditional medicine (plants), diseases that will be cured if treated with traditional medicine (shaman) who use the mantra media " suwuk ", the third disease that can only be cured by going to the health worker or health facility (midwife).

The Tengger tribe, which is located in East Java, is one of the tribes in Indonesia that still maintains its culture and tradition well. Tengger tribes are spread in four districts in East Java, namely Lumajang, Malang, Probolinggo and Pasuruhan. Argosari Village, Senduro District is one of the two Tengger traditional villages in Lumajang district. Argosari village is a village in the highlands with access to uphill and winding roads, which requires considerable driving ability. Argosari village consists of four hamlets namely gedog, pusung duwur, bakalan and argosari. Argosari Hamlet has the most population of 4 hamlets namely, 1,045 people, almost 100% of the population is Hindu and native of the Tengger tribe, this is due to an agreement between the residents and the village to limit and prohibit outsiders from the Tengger tribe to enter and settle in Argosari village. The prohibition or agreement made has a purpose, so that the existing customs of the ancestors will not quickly become extinct due to the modernization of the age brought by outsiders of the Tengger tribe.

Based on the background that has been described, the authors are interested in researching to explore the current health seeking behavior of the Tengger.

2. Methods

Research Setting

The study was conducted in June 2017 to August 2018 in highlands of three districts: Lumajang, Probolinggo, and Pasuruan. These three districts is located in East Java. These locations were chosen where the Tenggerese as the subject of this research are clustered. The Tenggerese residing in these remote mountain territories focus on practicing farming techniques and subsistence agriculture

Research Design

This study used a qualitative approach with a type of descriptive research. The informants of this research were: Public Figure (Wive of *kades*, Village Staf, Village Elders), *Dukun*, Tengger Village Midwive, ordinary Tenggerese. Data was collected through in-depth interviews with informants. Data from various sources were validated using triangulation method.

3. Result and Discussion

The age of participants is 19-62 years, living in the Argosari hamlet, Senduro District, Lumajang Regency. Participants consisted of 15 people, namely 6 women and 9 men. Having a job background as a midwife, health promotion officer, village staff, village elder, mother of the village head, dukun, former dukun, 2 people as a teacher, 2 people as traditional birth attendants, and 4 people as farmers.

The results of the study show the following discussion:

1) The attitude that is often done by the Tengger tribe if a relative or a member of familily is sick

The attitude of all respondents when have family members got mildly ill, such as cold, fever, itching, acne, and others,

generally doing self-medication using traditional ingredients. However, if giving traditional medicine does not give good side effects, the respondent goes to the dukun and ask a "suwuk" mantra, and comes to the nearest health worker or health facility (midwife). The respondents explain:

"If the pain is mild and we think it can be cured by medicinal plant around here, so we will use self medication by taking the medicinal plant. However, if the pain is severe, we will go to the medical doctor and shaman" (Informant 10)

"(if we sick) we will take medicine. Here, in Tengger community, we have three ways of healing: the first one is self-medication using medicinal plantation. However nowadays, using medicinal plantation has rarely practiced. The second one is asking herbs or water and spells to the shaman. And the last is go to the midwife or doctor" (Informant 11).

" we are treated either with traditional medicines made by ourselves or going to the doctor, then go to the shaman too. The Tengger people more often go to the doctor to seek treatment because we only got back pain due to working in the fields" (Informant 12)

From the statement of the informant, it can be seen that informants generally do their own treatment in dealing with health problems, although this self-medication is carried out in different ways, depending on the knowledge of the community. A person will give a different response or reaction in a state of pain which is based on several things such as the level of seriousness or severity of the illness, an experience of pain, alternative drug selection and so on (Tinendung, 2009).

This shows that the informant chose to use the traditional treatment process because it is more efficient, free, available in nature, close to home, and has been hereditary from the ancestors. Then the reason the informant went to the dukun rama to cure the disease because it was related to belief, went to the dukun rama in order to find out whether there was a disease due to witchcraft or not and was given a "suwuk" spell, the spell was of course equipped with water, salt, incense and frankincense. If it is known that the illness suffered is not a spell disease, the informants come to the nearest health worker or health facility to get help. This was done by several communities due to an appeal from the government for treatment to the nearest health worker or health facility

2) Beliefs, values , and teachings that underlie the Tengger tribe community using plants for treatment and lastly used.

Respondents' trust in treatment varied, there were those who believed more in medicine, some believed in traditional medicine, and some believed both. The use of traditional medicine for the healing process has long been abandoned about 3 generations ago around the 1980s, like the following statement:

"from ancestors, but now there are many who do not know about medicinal plants, I also do not know what types of plants to treat, the last medicinal plants used around 30-35 years ago" (Talked in Informal Bahasa Indonesia)
(Informant 8)

"If you talk about belief, the Tengger tribe community is a bit convinced that this is because the level of public trust is transferred to medical personnel. Then the knowledge of medicinal plants in the community is also very low " (Talked in Informal Bahasa Indonesia)
(Informant 9)

"If the ceremony, all the many plants that are used are almost all ceremonies using plants, the use of plants for medicine has a good effect, some are not giving effect. If the administration of

medicinal plants for 2-3 days does not give a good effect directly taken to the doctor, sometimes it is also brought to the shaman if the characteristics of Uses are known." (Talked in Informal Bahasa Indonesia)
(Informant 10)

From the results of interviews with respondents, it is known that the level of respondents' trust in treatment services is different, this is certainly influenced by the experience of each individual in seeking treatment services. According to Agoes A. and T. Jacob (2006), a fact shows that anywhere or in any country with a low or high level of education, including Indonesia, health services are usually given for two types of medical and non-medical treatments. Although medical treatment has proven its existence as a successful treatment, there are still many sick people who seek non-medical treatment. The treatment is carried out both traditionally by utilizing the power of traditional healers (dukun, datu, and physician) as well as healing of diseases carried out in a modern way by utilizing medical personnel and using the modern medical equipment. Both types of ways are different and irreconcilable and until now these two methods are still needed by the community, both in urban and rural areas. This depends on how the treatment search pattern is understood by the individual and who develops in the surrounding environment (Tinendung, 2009).

3) Knowledge of informants about medicinal plants that are still used and their properties.

Information that has been presented by informants has many similarities related to the delivery of medicinal plants and their properties. The informants knew well about traditional medicine and plant species, but the number of medicinal plants believed to have healing properties could not be clearly stated. This conclusion is obtained from the following statement:

" 1. Sri Pandak = to treat a fever
2. Laos = to treat a fever
3. Puyang = to treat a fever
4. Turmeric = to treat a fever
5. Blocking = to treat a fever
6. Lime = to treat a fever
7. Onions = to treat a fever
8. Banana sap = to clog or stop the bleeding caused by sores
9. Dringu Plant = to treat fever or bloating by being finely ground after it is limped
10. Ciplukan Leaf = to treat high blood pressure by means of boiled leaves and then drink
11. Black sugarcane = to treat a cough, by means of sugar cane is burned first and then immediately eaten.
12. Tepung otot = to heal muscle pain"

(Informant 9)

"there are plants that are used for medicine, such as binahong to treat itching, strokes, and pimples, by means of mashing and then applying it to the place that complains of itching, the dringu plant is also the same as binahong, then there is muscle flour used to reduce sore on muscles " (Talked in Informal Bahasa Indonesia)
(Informant 10)

"If what I often use I don't know, if it's still used today there are dringu, black sugar cane, lime, spinach for breastfeeding mothers, babah guava, puse (wild plants), and binahong to treat itching" (Talked in Informal Bahasa Indonesia)
(Informant 11)

From the interview results, it can be seen that more informants mentioned that the plants that are often used to date are binahong for itching and acne, dringu for fever, lime for coughing, black cane for coughing, muscle flour for aches. The informants also understood about traditional medicine and the type of traditional medicine, but the Tengger tribe community still did not know how to cultivate good and true medicinal plants, so it was necessary to educate the Tengger tribe about conservation and drug use

efforts. These results are consistent with the WHO's understanding of traditional medicine and herbal medicine, namely traditional medicine is defined as the total number of all knowledge and good practices that can be explained or not used in diagnosis, prevention and elimination of physical and mental imbalances relying only on practical experience and observations from generation to generation. While herbal remedies can also be defined as finished products that contain active ingredients contained in plants or other ingredients either raw or in a processed condition

4. Conclution

Tenggeree uses three approaches to overcome their health problems. The approaches include: using plants that are believed to have medicinal properties, go to the traditional healers named “Dukun” who are using spell (mantra) or "suwuk" as the media, and go to the health workers / health facilities.

Recently, the use of plants that are believed to be efficacious for medicine is very rarely used for various reasons. The plant is only used to treat mild health problems.

Plants that are still used today are: *binahong* (to heal itching and acne), *dringu* (to heal fever), *jeruk nipis* (to heal cough), *tebu ireng* (to heal cough), and *tepung otot* (to heal muscle pain).

5. Suggestion

The fact that local of the use of plants believed to have medicinal properties by the Tenggerese is almost extinction, shows the importance of educating for the Tenggerese about conservation and utilization of plants. In addition, the role of researchers is also needed to conduct ethno-pharmacology studies to explore the efficacy and how to use these plants.

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VISCOSITY AGENT VARIATION CONCENTRATION EFFECT TO IRRITATION AND PHYSICAL PROPERTIES KATUK LEAF *(Sauropus androgynus* (L) Merr*)* SHAMPOO

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Abstract

Katuk Leaf (Sauropus androgynus (L) Merr) had strong antioxidant activity with IC50 value of 80.81 ppm. This research formulated shampoo with concentration variation of HPMC 0.5% (F1); 1%(F2), and 1.5% (F3). The result showed that organoleptic assessment of three formulas had same odor which is slightly jasmine. All formulas had different form and colour. Higher HPMC concentration in the three formulas showed higher viscosity and dark green color. The result of viscosity analysis showed that F3 had the highest viscosity of 8 dpas. The result of pH measurement showed that three formulas had pH value of 6 and met the requirement of SNI specification. Statistical analysis showed that concentration variation of HPMC affected amount of foam and viscosity of the shampoo, but did not affect to pH. Skin irritation assay was applicated to rabbits in 72 hours. The result of three formulation of skin irritation showed that there was no eritema and edema effects occurred on skin rabbits. The conclucion of skin irritation assay was concentration variation of HPMC had no irritation effect.

Keywords: HPMC, Shampoo, Katuk leaf (Sauropus androgynus (L) Merr)

1.Introduction

The preliminary screening results of the katuk methanol extract reported that katuk leaves contain flavonoids. Flavonoids as antioxidants have the ability to reduce free radicals [1]. The LC50 value obtained was 80.81 which indicated that the flavonoids from katuk leaves (Sauropus androgynus (L) Merr) have the ability as a strong antioxidant. One of the preferred hair care cosmetics is shampoo. In the formulation of shampoo preparations, the katuk leaf extract used viscosity agent, namely Hydroxy Propyl Methyl Cellulose (HPMC) which serves to increase the physical stability of shampoo preparations and thicken the texture so that the shampoo is easy to use. HPMC is a cellulose derivative that can stabilize foams so that it enhances the aesthetic and psychological value of consumers [2]. The use of a viscosity agent at excessive concentration can cause skin irritation problems and cause discomfort due to sticky texture during use [3,4]. Irritation is a symptom of inflammation that occurs in the skin or mucous membranes due to prolonged or repeated use of chemicals. Common symptoms that can occur due to irritation include heat which is caused by blood vessel dilatation due to irritants that can be seen with the appearance of redness on the skin area (erythema). In addition, edema can also occur, it can be observed by the enlargement of plasma that freezes in the injured area and accelerated by the presence of fibrous tissue that covers the area. Based on the explanation above, it is necessary to do an irritation test before use in humans to prevent hypersensitivity reactions. The presence of irritation signs on the skin of experimental animals allows irritation to human skin. Based on the description above, it is necessary to conduct a study to determine the irritation and physical properties of the preparation of katuk leaf extract shampoo (Sauropus *androgynus* (L) merr) with various concentrations of viscosity agent HPMC.

2.Methods

2.1Research design

The type of research conducted was laboratory experimental research (true experimental).

2.2Research Tools and Materials

Tools

Blender, sieve of 30 mesh, rotary evaporator, desiccator, glassware laboratorium.

Materials

Katuk leaves (*Sauropus androgynus* (L) Merr), 96% ethanol, chloroform, aquadest, FeCl3, Sodium lauryl sulfate, Cocamide DEA, BHA, HPMC, EDTA Na, Sodium benzoate, Dimeticone, Citric Acid, Menthol, DNC Green # 5

2.3 Research Material Preparation

The sample investigated was katuk leaves (*Sauropus androgynus* (L) Merr) from Jember district, East Java. Fresh katuk leaves were weighed and washed thoroughly with water then dried in the air (without being exposed to direct sunlight). The dried katuk leaves were then mashed using a blender to become a powder, then weighed and sieved with a 30 mesh sieve to obtain a fine powder.

2.4 Preparation of ethanol extract of katuk leaves

A total of 100 grams of katuk leaf powder that has been dried and mashed with a certain degree of fineness in maceration for 1 hour using 96% ethanol solvent as much as 800 mL, allowed to stand overnight then filtered and separated from the pulp and filtrate. The pulp is re-macerated (re-maceration is carried out 3x). The obtained filtrate was collected and the extract mixture was concentrated with a rotary evaporator and evaporated using a 60^{o}C temperature until a constant weight was obtained. The results are then weighed and stored in a desiccator (Arista, 2013).

2.5 Phytochemical Screening Assay

Qualitative preliminary test was performed through color reaction, that is by strongly shaking the katuk leaf extract using chloroform and distilled water was added to form two layers. The water layer is divided into 3 (three) parts:

a) The first filtrate was added 2 drops of FeCl3 1% and resulted in a black color which indicates the presence of flavonoids.
b) The first filtrate was added with 2 drops of 10% NaOH resulted in a bluish green color which indicates the presence of flavonoids (Zuhra *et al*, 2008).

Table 1. Formulation of Katuk Leaf Extract Shampoo Preparations

Function	Material	F1 (%)	F2 (%)	F3 (%)
Active ingredient	Katuk leaf extract	0.5	0.5	0.5
Non-ionic detergent	Sodium lauryl sulfate	10	10	10
Emollient	Cocamide DEA	4	4	4
Antioxidant	BHA	0.02	0.02	0.02
Viscosity agent	HPMC	0.5	1	1.5
Preservative	Sodium benzoate	0.15	0.15	0.15
Stabilizer	EDTA Na	0.1	0.1	0.1
Anti foaming	Dimeticone	0,05	0,05	0,05
Buffering agent	Citric acid	0.1	0.1	0.1
Corigen	Menthol	0.25	0.25	0.25
Dye	DNC Green #5	4	4	4
Solvent	Ethanol	5	5	5

Solvent	Aquadest	Ad 100 mL	Ad 100 mL	Ad 100 mL

2.6 Shampoo preparations

A dispersion of viscosity agent HPMC was prepared. Cocoamide was mixed with BHT, EDTA Na then stirred with the homogenizer for 5 minutes then added sodium lauryl sulfate and stirred for 1 minute (here in after referred to as mixture b). Katuk leaf extract and sodium benzoate were dissolved in distilled water then added with HPMC dispersion and stirred until homogeneous (mixture c). D & C green # 5 was dissolved in water then mixed in mixture c and stirred until dissolved. Menthol, oleum jasmin royal in alcohol was dissolved until homogeneous. Mixture B was added with the mixture c, then added dimeticone and stirred until homogeneous then added menthol solution and stirred until homogeneous. The remaining distilled water was added to the preparation to the desired extent and homogenized with a homogenizer with a speed of 1000 rpm for 10 minutes. Acidity was adjusted by adding citric acid.

2.7 Evaluation of Shampoo Preparations Physical Properties Analysis of Shampoo Preparations

Physical properties of shampoo preparations were observed which included organoleptic, foam, viscosity and pH measurements. The steps taken in the analysis of physical properties are as follows:

2.8 Organoleptic Assessment

Organoleptic assessment was carried out by observing changes in texture, odor, and color of shampoo preparations containing various concentrations of katuk leaf extract.

2.9 Foam Amount Measurement

A total of 2% of anti-dandruff shampoo containing various concentrations of katuk leaf extract was dissolved in 500 ml of water. The solution was then put into a measuring flask with a capacity of 1L. The solution was filled with 50 ml of tested solution and placed under a measuring flask. A total of 500 ml of the tested solution from the flask was passed to each measuring cup containing 50 ml of the tested solution until it runs out. The amount of foam formed was observed after 0.5, 3.5 and 7 minutes.

2.10 Viscosity Measurement

Viscosity measurement was performed using the Brookfield Viscometer. The trick is to place an anti-dandruff shampoo preparation to be examined in a glass beaker (± 200 mL), then was put under the Brookfield model DV-E viscometer using the appropriate spindle. The spindle was inserted into the preparation until it was submerged and the viscosity could be measured.

2.11 pH Measurement

PH measurement was carried out using the pH indicator

2.12 Irritation Assay of Shampoo Preparations on Rabbit Skin

Irritation assay of shampoo preparation was carried out using experimental animal, namely male rabbits with the Draize method. Rabbits were divided into 4 groups of treeatment: groups 1, 2, 3, 4 which were applied with shampoo containing viscosity agent HPMC with a concentration of 0.5%, 1%; 1.5% and control. On the back of the rabbit, a rectangular pattern with a size of 3 x 2 cm was made with a distance of 2 cm between parts, then the pattern that has been made was smooth shaving (not to scuff) using scissors and razor blades, then cleaned and smeared with 95% ethanol and was given treatment. The control was a group that did not given treatment. Each 0.5 g irritant

sample was applied to the shaved back of the rabbit, then covered with sterile gauze then tied with tape for 24 hours. After 24 hours, the tape and bandage were opened and left for 1 hour, then observed. After being observed, the part was resealed with the same tape and re-observed after 72 hours. Furthermore, each skin condition was given a value based on the presence or absence of erythema.

The irritation index was calculated by adding up the value of each rabbit after 24 hours and 72 hours of giving the sample then divided. The assessment of irrigation is as follows:

- 0.00 – 0.03 = Not irritating
- 0.04 – 0.99 = A little irritating
- 1.00 – 2.99 = Mild irritation
- 3.00 – 5.99 = Moderate irritation
- 6.00 – 8.00 = Severe irritation

2.13 Data analysis

The organoleptic data and homogeneity obtained would be compared visually, while the viscosity, pH, the amount of foam will be analyzed using the Kolmogorov-Smirnov method then further analyzed with one way ANOVA.

3.Results and Discussion

3.1 Results of Screening Assay

In order to determine the presence of flavonoid compounds found in katuk leaves (*Sauropus androgynus* (L) Merr), a preliminary test (phytochemical screening) was carried out. Qualitative preliminary test with the addition of 1% FeCl3 resulted in black color, which indicates the presence of flavonoid compounds. The addition of 10% NaOH to the extract resulted in a bluish green color. This shows that katuk leaf extract contains flavonoids [5].

.Figure 1 The result of phytochemical screening assay

3.2 Results of physical properties Analysis of shampoo preparations

Organoleptic Assessment

Organoleptic assessment was carried out by observing changes in texture, odor, and color of shampoo preparations of katuk leaf extract with various concentrations of viscosity agent HPMC.

Table 2 Results of organoleptic assessment

Type of Formula	Organoleptic Criteria		
	Texture	Odor	Color
Formula 1	A little thick	Slightly Jasmine	Light green
Formula 2	Thick	Slightly Jasmine	Light green
Formula 3	Thick	Slightly Jasmine	Dark green

This study used 3 different respondents. Organoleptic assessment also aims to see the physical appearance of the preparation by observing the shape, smell, and color of the katuk leaf extract shampoo that has been made [6]. Based on the results of the organoleptic assessment obtained, the three formulas had the same odor that was slightly jasmine yet have different textures and colors. The higher the concentration of HPMC used, the shampoo preparations

have thicker texture [7]. Shampoo preparations with HPMC concentrations 0.5% and 1% had light green color while 1.5% HPMC concentration had a dark sgreen color.

Foam Measurement Results

Anti-dandruff shampoo preparations containing various concentrations of HPMC were prepared and foam formed was observed after 0.5, 3.5, and 7 minutes. Foam measurement results can be seen in Table 3.

Table 3. Foam measurement results

Type of Formula	Amount of Foam (cm)			
	0 minute	3 minutes	5 minutes	7 minutes
Formula 1	14	12	11.5	11.5
Formula 2	13	11	10.5	10.5
Formula 3	15	13.5	13.5	13.5

Based on the results of foam measurements, it is known that the higher the concentration of viscosity agent used in the formula, the higher the foam produced. All three formulas met the expected amount of foam, which was approximately 1.3-22 cm. Statistical Analysis of Product Services Solution (SPSS) version 16 showed a significance value of 0.200 (p> 0.05) with normality tests performed using the Kolmogorov-Smirnov test. The results of the measurement of foam amount were then analyzed using the one way ANOVA and it was obtained a significance value of 0,000 (p <0.05) which indicated that the physical properties of foam in the three formulas had a significant difference.

3.3 Results of Viscosity Analysis

Physical properties analysis of viscosity from gel preparations aims to determine the viscosity of gel preparations which subsequently affects topical drug use [8]. Viscosity of gel preparation was measured using a brookfield VT-04F viscometer with spindle No. 3. Viscosity measurement results can be seen in Table 4.

Table 4 Results of viscosity measurement of shampoo preparations

Type of Formula	Viscosity (dpas)		
	Replication 1	Replication 2	Replication 3
Formula 1	0.6	0.6	0.6
Formula 2	3	3	3
Formula 3	8	8	8

The results of viscosity analysis on shampoo preparations showed that the highest viscosity was obtained by formula 3 with concentration of viscosity agent HPMC of 1.5%. The higher the concentration of HMPC used in the shampoo formulation, the higher the viscosity produced. HPMC is one of the cellulose derivative semisynthetic polymers that have a stable viscosity in long-term storage [3]. HPMC has strong active substance binding properties compared to carbopol [9]. There is no limit to the range of viscosity in shampoos, yet the viscosity of shampoo is very important so that shampoo can be poured properly.

Statistical Product Services Solution (SPSS) analysis using one way ANOVA showed a significance value of 0,000 (p <0.05) which indicated that the physical properties of viscosity in all three formulas had significant differences.

3.4 Results of pH Measurement

PH measurement aims to determine the safety of a preparation, especially for topical preparations. Ideally, topical preparations have the same pH value as the

pH of the skin so as not to irritate the skin surface (Afianti and Murrukmihadi, 2015). PH measurement results can be seen in Table 5.

Table 5 Results of pH measurement shampo

Type of Formula	pH		
	Replication 1	Replication 2	Replication 3
Formula 1	6	6	6
Formula 2	6	6	6
Formula 3	6	6	6

Based on the table above, it is known that all three formulas have a pH of 6. This is because HPMC has a stable pH ranging from 3-11[3,10].

Measurement of physical properties showed that the shampoo preparation met the range of pH requirements in accordance with SNI, namely 5.0-9.0. Based on the pH range obtained, it is expected that shampoo preparations do not irritate the scalp because shampoo preparations that tend to be acidic can irritate the skin while preparations of shampoos that tend to be alkaline can make the skin dry [11].

3.5 Irritation Assay

The irritation assay of shampoo preparation was carried out using experimental animal that is male rabbits. Each sample of irritant was applied as much as 0.5 g on the back of the rabbit that had been shaved, then covered with sterile gauze then tied with tape for 24 hours. After 24 hours, the tape and bandage are opened and left for 1 hour, then observed. After being observed, the part was resealed with the same tape and re-observed after 72 hours.

Table 6. Results of irritation assay of shampoo on rabbits

Type of Formula	24 Hours		48 Hours		72 Hours	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
Control	0	0	0	0	0	0
Formula 1	0	0	0	0	0	0
Formula 2	0	0	0	0	0	0
Formula 3	0	0	0	0	0	0

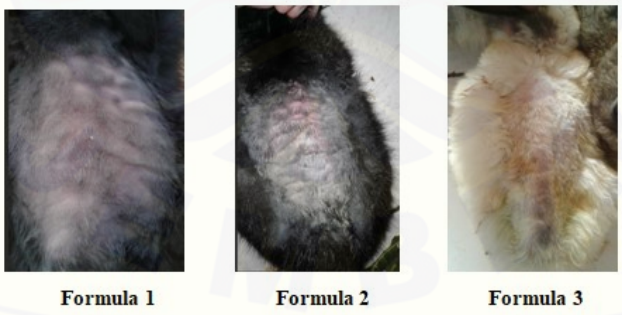

Figure 5.2 The results of irritation assay of Katu leaf extract shampoo on male rabbits

Shampoo formulation with various concentrations of viscosity agent HPMC did not cause erythema or edema in rabbits. Thus, shampoo with viscosity agent HPMC is safe to use because it does not cause irritation. According to previous research, the use of HPMC at concentrations above 5% can cause skin irritation and cause discomfort such us sticky texture during use [3,4].

4. Conclusion

1. There is an effect of various HPMC concentrations on organoleptic physical properties, amount of foam, viscosity of katuk leaf

(*Sauropus androgynus* (L) Merr) extract shampoo.

2. There is no effect of various HPMC concentrations on physical properties in the form of pH from the extract of katuk leaf extract (*Sauropus androgynus* (L) Merr).
3. Katuk leaf (*Sauropus androgynus* (L) Merr) extract shampoo with various concentrations of HPMC does not cause irritation to rabbit skin.

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TOTAL PHENOLS CONTENT OF ANTIHIPERTENSIVE MEDICINAL PLANTS USED BY THE VILLAGERS OF A JAVANESE COMMUNITY IN PONOROGO EAST JAVA, INDONESIA

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Indonesia has high biodiversity, and some of them has been used for traditional medicine. The villagers of Javanese community in Ponorogo East Java use several plants for hypertension therapy, such as bayam duri (*Amaranthus spinosus*), temu hitam (*Curcuma aeruginosa*), kumis kucing (*Orthosiphon aristatus*), cincau hitam (*Mesona chinensis*), and semangka (*Citrullus lanatus*). However, the mechanism of the plants as anti-hypertension is still limited. The pathophysiology of hypertension can be stimulated by oxidative stress that can lead damage to various tissues and implicate in cardiovascular disease. Phenols have a great impact as antioxidants in protecting the cardiovascular system. This research aims to examine the total phenols of extract of bayam duri (*Amaranthus spinosus*), temu hitam (*Curcuma aeruginosa*), kumis kucing (*Orthosiphon aristatus*), cincau hitam (*Mesona chinensis*), dan semangka (*Citrullus lanatus*). The fruits were dried and extracted using methanol absolut, then dried with rotary evaporator. The content of total phenols of extracts were analyzed by using a spectrophotometric method in neutral aqueous solutions react with diazotized sulfanilic acid. The total phenols on bayam duri (*Amaranthus spinosus*), temu hitam (*Curcuma aeruginosa*), kumis kucing (*Orthosiphon aristatus*), cincau hitam (*Mesona chinensis*), and semangka (*Citrullus lanatus*) were 363.27 ± 1.44, 335.71 ± 1.44, 599.49 ± 3.61, 409.69 ± 2.16; and 441.33 ± 3.61 respectively.This study suggested that the five plants contain phenols and kumis kucing (*Orthosiphon aristatus*) contents the highest of total phenols.

Keywords:
Total Phenols Content, Antihipertensive Medicinal Plants, Javanese community, East Java Indonesia

1. Introduction

Indonesia is a tropical country with high potential in providing medicinal plants. About 30,000 species of plants from the 40,000 species in the world are in Indonesia, 9,600 of which have been proven to have medicinal properties, and about 300 of them have been used as traditoinal medicines [1]. Some medicinal plants include hypertension therapy. There are sources stating that there are several compounds contained in plants that can play a dual role, namely as angiotensin converting enzyme inhibitors (one type of antihypertensive drug) and as antioxidants [2, 3]. Hypertension is caused by many factors, one of which is the presence of *oxydative stress* [4]. *Oxydative stress* is a chronic imbalance between the antioxidant ability of biological systems and the production of *reactive oxygen species* (ROS). Several experimental and clinical studies indicate that hypertension occurs after the biological system of the body is exposed to *oxydative stress* and an increase in production $\cdot O_2^-$ and H_2O_2 are found in *salt-sensitive and angiotensin II-induced hypertension* cases [5].

Antioxidants are compounds that are important in maintaining a healthy body. These antioxidant compounds work break the chain reaction of free radicals found in the body [6]. The activity of these antioxidant compounds comes from compounds of flavonoids, terpenoids, alkaloids, proanthocyanidin, hydrolyzed, tannins, fatty acids, peptides, xanthones [2] , and phenols [3] . The use of antioxidant compounds is now increasingly

widespread due to the increasing public knowledge of antioxidant functions that can inhibit degenerative diseases such as heart disease,hypertension, and arterisclerosis, cancer and symptoms of aging [6].

Bayam duri (*Amaranthus spinosus*), Temu Hitam (*Curcuma aeru-ginosa*), Kumis Kucing (*Orthosiphon aristatus*), Cincau Hitam (*Mesona chi-nensis*), dan Semangka (*Citrullus lanatus*) are plants that are widely used in Indonesian society, especially Ponorogo as antihypertensive herbs.

From the background above, researchers are interested in comparing phenol levels in both traditional herbal plants which are often used by people around East Java as antihypertensive therapy.

2. Methods & Material

Plant Material

Bayam duri (*Amaranthus spinosus*), Temu Hitam (*Curcuma aeru-ginosa*), Kumis Kucing (*Orthosiphon aristatus*), Cincau Hitam (*Mesona chi-nensis*), dan Semangka (*Citrullus lanatus*) were obtained from the Materia Media Center and around Batu, East Java. Plant determination was carried out at Batu Materia Medica Center.

Plant Extraction

The extraction process requires 1 liter of Absolute Methanol and 100 grams of plant simplicia powder. The method used for extraction is Maseration, where Maseration is done by soaking the simplicia powder in the liquid of the dancer. Sample extraction is done in the Materia Medica Center Laboratory. Each sample is macerated for 24 hours at room temperature avoiding sunlight, then repeating or re-macerating with the same solvent so as to maximize the extraction of the compound's compounds. The extract is then concentrated at temperature 68ºC uses a rotary evaporator to get residues consisting of *crude extract* . All extracts are stored in a closed container at 4ºC until used.

Qualitative Analysis of Phenolate Compounds

Each extract (0.5 g) was dissolved in chloroform and distilled water (1: 1). The mixture is shaken in a test tube and left for a moment until it forms two layers. The water layer above is used for qualitative examination of phenol compounds. A layer of water is inserted into the drop plate and $AlCl_3$ reagent is added . Positive reaction is indicated by the formation of blue-purple color.

Total Phenolics Content

The tools used in measuring phenol levels are analytic scales, sonicators, centrifuges, closed glass bottles, spectrophotometers, micropipettes. The steps in measuring total phenol levels are: sample solution is added with 1 mL of reagent A (mixture of 7.64% sulfuric acid, H2SO4, 4.8% NaNO2, with a ratio of 5: 1: 5), plus 0.5 mL of reagent B (8% NaOH). The mixture between sample and reagent solution was incubated at 100 ^{0} C for 30-40 minutes, then absorbance was measured by UV-Vis spectropometer at a wavelength of 360 nm. The sample measurement results are compared with phenol as a standard solution. Standard solutions are made using phenol solution with a concentration of 1, 2, 3, 4 ppm with the same treatment, then absorbance is measured.

3. Result and Discussion

Collection of Test Plants

Tabel 1. List of 5 Test Plants

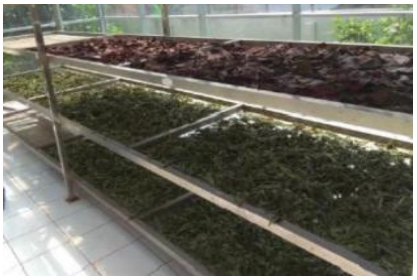
No	Name of Test Plants	Plant Parts Used	Plant Weight (Kg)
1	Bayam duri (*Amaranthus spinosus*)	Leaf	5
2	Temu Hitam (*Curcuma aeruginosa*)	Bulb	5
3	Kumis Kucing (*Orthosiphon aristatus*)	Leaf	5
4	Cincau Hitam (*Mesona chinensis*)	Leaf	5
5	Semangka (*Citrullus lanatus*)	Flesh of Fruit	10

Picture 1. Materia Medica Center (Sample Place Colletion)

The sample collection was carried out starting on May 25-28 2018 at the Materia Medica enter, Batu, Malang, East Java. The materials that will be used in the test are listed in table 1.

Drying of Samples and Simplisia Powder Making

Five samples that have been collected, then cut into smaller pieces to facilitate the drying process. The drying process is carried out for 3-10 days to eliminate the water content in the plant. This makes it easy to make plants into powder. Drying takes place by placing the plant in the sun, so that it takes longer than using a dryer. The drying process is carried out at Materia Medica Center, Batu, Malang, East Java.



Picture 2. Drying Process

After the plants are dried, a simplicia powder is made using a grinding machine. This pollination is intended to expand the contact surface of the simplicia with the mixture so that the process of extracting the compound can be optimal, because the extraction will run more optimally if the powder surface that comes into contact with the liquid of the dancer becomes wider, that is, with relatively small powder. However, too thin a reduction can cause the cell wall to rupture so that solutes are not removed.

Picture 3. Pollination and Powder Results One Simplisia (Temu Hitam)

Sample Extraction

Extraction of medicinal plants using maceration method, a method of extraction with the principle of diffusion of osmosis. The maceration method is used because this method is simple compared to other extraction methods. Maseration is done by soaking the simplicia powder in the liquid of the dancer. Soaking will help infiltrate (penetrate) the liquid of the dancer and soften the cell so that it is easily absorbed. The liquid dancer will penetrate the cell

wall of the plant powder and enter the cell cavity containing the active substance. Withdrawal of the active substance due to differences in concentration between the solution of the active substance in the cell and those outside the cell where the concentration inside is more concentrated than outside the cell so that the liquid with the same polarity as the dancer will dissolve in the penyari then move towards the outside of the cell have a lower concentration. The event occurs repeatedly so that there is a balance of concentration between the solution outside and inside the cell. Maseration is done at room temperature to prevent the loss of volatile substances due to heating. During the maceration process stirring is carried out. This serves to optimize the wetting of the powder so that the whole powder is completely submerged in the dancer. Stirring can also function to prevent the balance of concentration inside and outside the cell so that the diffusion process can continue. The flatter all the submerged parts will be the better because the dancer can enter into all parts of the powder so that the withdrawal of active substances in each cell can be optimized. Then the vessel is closed so that the solvent does not evaporate and mechanical contaminants cannot enter.

After 24 hours, then the maserat is separated by the pulp with the fig filtered and placed in a separate place. Then remaseration is done to get optimal results. After obtaining the mass then concentrating / making thick extracts using a rotary evaporator.

Extraction is done using methanol. The ratio between powder and solvent is 1: 10, where 100 g of simplicia is dissolved in 1 liter of solvent methanol. Making this thick extract uses absolute methanol solvents because it has several advantages over water, which does not cause cell swelling, inhibits enzyme action, and improves the stability of dissolved drug ingredients. Methanol solvents can be used to detect substances with relatively high

polarity until relatively low because methanol is a universal solvent.

Picture 3. Extraction Sample Process

After obtaining the maceration results then concentrating / making thick extracts is carried out. Making thick extracts using a rotary evaporator.

Picture 4. Thick Extract Process

Qualitative Analysis of Phenolate Compounds

Analysis was carried out to determine the qualitative compounds of the presence of phenolic compounds in a sample of Semangka, Kumis Kucing, Bayam Duri, Temu Hitam dan Cincau Hitam. In the 5 samples of methanol extract, the positive results showed that there were phenolic compounds. It can be assumed that the phenolic phenolics are soluble in polar and non-polar solvents. After the qualitative test is carried out, it will be followed by a quantitative test.

Table 2. List of Rendemen 5 Test Plants

No	Plant Name	Simplicia Weight (Gram)	Extration Weight (Gram)	% Rendement
1	Semangka	100	9,82	9,82
2	Kumis Kucing	100	8,39	8,39
3	Bayam Duri	100	8,52	8,52
4	Temu Hitam	100	9,67	9,67
5	Cincau Hitam	100	6,58	6,58

Table 4. Quantitative Tests With Spectrophotometric Methods

No	Plant Name	Parameter	Reagent	Analysis Result	Level of analysis results (mg/L)
1	Semangka	Fenol	Asam Sulfinat	Spektrofotometri	441,33 ± 3,61
2	Kumis Kucing	Fenol	Asam Sulfinat	Spektrofotometri	599,49 ± 3,61
3	Bayam Duri	Fenol	Asam Sulfinat	Spektrofotometri	363,27 ± 1,44
4	Temu Hitam	Fenol	Asam Sulfinat	Spektrofotometri	335,71 ± 1,44
5	Cincau Hitam	Fenol	Asam Sulfinat	Spektrofotometri	409,69 ± 2,16

Quantitative Total Phenol Analysis

Determination of total phenol content in Semangka, Kumis Kucing, Bayam Duri, Temu Hitam dan Cincau Hitam methanol extract were carried out by fig dissolving the sample and then adding 1 mL of reagent A (mixture of sulfuric acid 7.64%, H2SO4, NaNO2 4.8%, with a ratio of 5: 1: 5), plus 0, 5 mL of reagent B (8% NaOH). The mixture between sample and reagent solution was incubated at 100 ^{0} C for 30-40 minutes, then absorbance was measured by UV-Vis spectropometer at a wavelength of 360 nm. The sample measurement results are compared with phenol as a standard solution. Standard solutions are made using phenol solution with a concentration of 1, 2, 3, 4 ppm with the same treatment, then absorbance is measured. From the results of the measurements The total phenols on bayam duri (*Amaranthus spinosus*), temu hitam (*Curcuma aeruginosa*), kumis kucing (*Orthosiphon aristatus*), cincau hitam (*Mesona chinensis*), and semangka (*Citrullus lanatus*) were 363.27 ± 1.44, 335.71 ± 1.44, 599.49 ± 3.61, 409.69 ± 2.16; and 441.33 ± 3.61 respectively. This study suggested that the five plants contain phenols and kumis kucing (*Orthosiphon aristatus*) contents the highest of total phenols.

Phenolic compounds are thought to contribute to antioxidant activity in this plant, but have different levels of each extract [7]. This is due to the influence of the plant's growing place, which is supported by the climate and soil elements, causing the total phenolics contained in different plants.

4. Conclusion

From the results of the measurements The total phenols on bayam duri

(*Amaranthus spinosus*), temu hitam (*Curcuma aeruginosa*), kumis kucing (*Orthosiphon aristatus*), cincau hitam (*Mesona chinensis*), and semangka (*Citrullus lanatus*) were 363.27 ± 1.44, 335.71 ± 1.44, 599.49 ± 3.61, 409.69 ± 2.16; and 441.33 ± 3.61 respectively. This study suggested that the five plants contain phenols and kumis kucing (*Orthosiphon aristatus*) contents the highest of total phenols.

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ISOLATION AND CHARACTERIZATION OF ENDOPHITIC ACTINOMYCETES FROM ANTITUBERCULOSIS MEDICINAL PLANTS

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ABSTRACT

Tuberculosis diseases (TB) prevalency from sensitive and resistant strain has been hight in Indonesia. The finding of bioactive compounds should be developed to solve this problem. Endophitic Actinomycetes specially from *Streptomyces* sp was reported to have the same bioactive compound with its host plant. The aims of this research was to obtain Endophitic Actinomycetes from some medical plants which have antituberculosis effect. Medicinal plants used in this study were *Kaemferia galanga, Hibiscus sabdariffa* and *Piper cronatum*. Actinomycetes was isolated using two different media namely Humic Acid Vitamin (HV) agar and HV with plant extract, and purified on Yeast Malt Agar (YMA) medium. Morphological characterization of Actinomycetes obtained was observed including aerial mycelium and substrate mycelium, pigmentation, and colony surface. A total of 5 Endophitic Actinomycetes of *Streptomyces* genus were obtained from medicinal plant. Isolates K.J3A, K.J3B, K.J4 and K.J11 were endophitic *Streptomyces* from *Kaemferia galanga* radix, S.MJ1 from *Piper cronatum* folium. Actinomycetes was not found from *Hibiscus sabdariffa* calyx. Each isolate had variety of characteristics which could be seen in aerial mass color, substrate mass color, and pigmentation.
Keywods: *Antituberculosis*, endophitic actinomycetes, *Kaemferia galanga, Hibiscus sabdariffa.*

Introduction

Tuberculosis (TB) is a deadly infectious disease caused by *Mycobacterium* sp., especially *M. tuberculosis*. The main infection occurs in the lungs [1]. WHO reports that Indonesia has the largest TB prevalence in the world. Thus, the number of TB patients in Indonesia is greater than in the previous year. This is also further exacerbated by the increase in the number of drug-resistant TB cases [2]. The increase in cases of drug-resistant TB in Indonesia is also quite high in the world, particularly in Multidrug Resistant Tuberculosis/MDR-TB. TB drug resistance is not only caused by treatment failure but also because of the emergence of bacteria with resistant strains transmitted by MDR-TB patients [3]. Therefore, efforts to find renewable bioactive compounds from natural ingredients are con-tinuously being investigated to improve government programs relat-ed to effective and efficient TB treatment.

Medicinal plants which are efficacious as antituberculosis agents in inhibiting *Mycobacterium* spp., are *A. calamus*, *B. Frutescens*, *F. chlamydocarpa*, *F. cordata*, *F. Deltoidea*, *H. Maximum*, *K. galanga*, *S.grandiflora*, *S. pinnata*, S. *rhombifolia, Z. Aromaticum*, *H. sabdariffa* L, and *P. Cronatum* [4, 5, 6, 7, 8, 9, 10]. Bioactive compounds in plants include secondary metabolites such as alkaloids, flavonoids, tannins, xanthines, triterpenes, quinones that act as antituberculosis [11].

Problem that may arises from the use of herbal medicine is method of increasing the medicine pro-duction, while raw materials are limited because raw materials for herbal medicine are taken from the host plant. In fact, most of the raw materials for herbal medicines produced in Indonesia have begun to be imported [12]. In other cases, bioactive compounds are also isolated from endangered plants. This raises concerns, particularly in the conservation of biological resources, although tissue culture methods have been carried out yet production costs are still very high [1].

Exploration of endophytic microbes that can produce bioactive compounds similar to their host plants is one of the development of solutions for higher bioactive production without reducing plant biodiversity. Endophytic microbes

are microbes that live in plant tissues without endangering their hosts, such as fungi and bacteria including actinomycetes. Bioactive compounds that have been produced by endophytic microbes from host plants include anticancer, antifungal, antimalarial, and antituberculosis commonly produced by Actinomy-cetes [13]. Each plant may contain various endophytic microbes that are capable of producing the same bioactive compounds. This is thought to be the result of genetic transfer from host plants to endophytic microbes [14].

Bioactive production from endophytic microbes is relatively easier and more efficient. Further-more, biological molecular diversity is also higher to support renewable bioactive discoveries than epiphytic microbes. In addition, the symbiotic nature that shows that bioactive compounds from endophytic microb-es tend to have low toxicity. This is important for the medical field as a potential drug that does not adversely affect human cells [1].

According to a study conducted by [10] it was reported that *Hibiscus sabdariffa* L, *Kaempferia galanga* and *Piper cronatum* were able to inhibit the growth of *M. tuberculosis* strain MDR. [15] also reported that *Streptomyces* sp. isolated from *Zingiberaceae* have potential as antimicrobials. Therefore, the aim of this study was to isolate endophytic actinomycete especially *Strepto-myces* from *Hibiscus sabdariffa* L, *Kaempferia galanga* L, and *Piper cronatum*.

Methods

Time and place

This research was conducted from April to July 2018. The research was conducted at Micro-biology Laboratory University of Jember and Material Medika Batu in Malang.

Sampling and determination of medicinal plants

Medicinal plants used in this study include *kencur*, red betel and red roselle. These plants were taken from the Rambipuji area of Jember Regency, East Java, which was located in the garden of the Toge plant cultivation owned by the people of Rambipuji. Determination was carried out on the three plants in Malang Medika Batu Material (MMB) to obtain data on the classification of medicinal plants to the species level.

Isolation of endophytic Actino-mycetes from medicinal plants

The medicinal plants taken are healthy plants where *kencur* was taken on the radix, red betel were taken in the folium and red rosella was taken in the calyx. The parts of the plants were cleaned and cut. Surface sterilization was carried out by soaking in 70% alcohol for 1 minute, then proceed with soaking in 1% sodium hypochlorite (NaOCl) for 5 minutes and alcohol 70% for 1 minute. The last step, the samples were rinsed 3 times with sterile distilled water.

Isolation of endophytic actinomycetes was carried out using multilevel dilution method. A total of 25g of crushed plants were dissolved into 225ml phosphate buffer pH 7 and homogenized for 30 minutes. Dilution technique was carried out in 10^{-1} to 10^{-3}. A total of 100 µl of each resulted dilution was planted into HV media. The isolation medium was added with antibiotics nystatin (100 µg/ml) and nalidixic acid (100 µg/ml) and incubated for 2-4 weeks at room temperature [16]. Dilution technique was carried out in 10^{-1} to 10^{-3}. A total of 100 µl of each resulted dilution was planted into HV media. The isolation medium was added with antibiotics nystatin (100 µg/ml) and nalidixic acid (100 µg/ml) and incubated for 2-4 weeks at room temperature [16].

Purification and characterization morphology of Actinomycetes

Isolated actinomycete isolates were re-cultured on Yeast Malt Agar (YMA) or ISP2 agar media. Re-cultured isolates were incubated for 12 days at room temperature. Macroscopic morphological charac-terization includes shape, elevation, aerial mycelium color and pigmen-tation in the media. Microscopic morphological observation includes cell shape and

morphology of spores known by Gram staining [17]. Furthermore, spore types from each endophytic actinomycetes isolate were observed using a light microscope with a magnification of 4000x.

Results and Discussion

Medicinal plants identity

A total of three medicinal plants namely kencur, red betel and red roselle were obtained from Jember. Based on the results of identification in Material Medika Batu in Malang, Kencur belongs to the genus *Kempferia* specificly *Kempferia galanga* L., Red betel belongs to the genus *Piper* specificly *Piper crocatum* Ruiz & Pav., and red rosella belongs to the genus *Hibiscus* specificly *Hibiscus sabdariffa* var sabdariffa race rubber L.

Isolation and characterization of endophytic Actinomycetes derived from medicinal plants

Isolation aims to obtain the actinomycete isolates contained in the medicinal plants. There are two different media used for isolation, namely Humic Acid plus Vitamin B complex and Humic Acid plus Vitamin B complex containing extracts of each plant. Each medium was added with antibiotics, namely nalidixic acid and nystatin. Humic acid serves as a carbon source for the growth of actinomycetes. Vitamin B functions to induce the growth of actinomycete was found on red roselle calyx (Table 1).

According to [18] the population of endophytic microbes is mostly found in the root area and decreases in the stem and folium areas. Endophytic microbes initially enter the plant tissue through lateral roots and then spread into the intercellular space and vascular bundles to the top. Like-wise, the results of this study showed that no endophytic actinomycetes from red roselle calyx were found due to the fact that the actinomycetes population in the upper part of the folium. A total of 4 isolates were isolated from *K. galanga* radix, 1 isolate from red betel folium and no roselle plant was indeed even less. This is also supported by the fact that the endophytic actinomycetes po-

spores in Streptomycetes. Nalidixic acid serves to prevent the growth of Gram negative bacteria, while nystatin serves to prevent fungal growth on HV media during the isolation of actinomycetes. So, it is expected that the isolation results will avoid the contamination of Gram-negative bacteria and fungi, this is because actinomycetes belongs to Gram-positive bacteria. this is because actinomycetes belongs to Gram-positive bacteria.

Resulted actinomycete isolates showed that there were 5 endophytic actinomycete isolates from medicinal plants derived from *K. galanga* radix and red betel

Table 1. Morphological characteristics of endophytic actinomycetes

Isolate Code	Aerial Mycelium	Substrate mycelium	Pigmentation	Surface	Gram	Spore Type
KJ.3A	Gray	Yellow	Light brown	Arises	Positive	Rectiflexibiles
KJ.3B	Gray	Light brown	Red brick	Arises	Positive	Rectiflexibiles
KJ.4	Brownish gray	Black	Black	Arises	Positive	Rectiflexibiles
KJ.11	Brownish pink	Dark brown	Pink	Flat and runny	Positive	Rectiflexibiles
SMJ.1	Brownish gray	Yellowish brown	Dark brown	Arises	Positive	Rectiflexibiles

Description: K: Kencur; SM: Red betel; A: Jember

pulation from *K. galanga* radix was higher than red betel folium. Because the *K. galanga* radix is in the soil which causes the majority of endophytic microbial populations found in such area.

Actinomycete isolates were then purified in solid ISP 2 media in order to characterize the colony morphology and Gram staining was also carried out in order to determine the shape of the cells and spores. The results of the observation show varying colony and cell morphology (Figure 1). The aerial color of the mycelium of each isolate also varied such as us gray, brownish gray, and brownish pink. Furthermore, the color of the mycelium substrate also varied as yellow, light brown, light brown, yellowish brown and black.

All actinomycetes isolates isolated from kencur and red betel plants indicate the presence of pigments in various colors, namely light brown, dark brown, brick red, pink and black (Figure 2). The presence of these pigmentation shows that the actinomycetes produce secondary metabolites which are excreted into the growth media. Surface isolates generally occur, except for KJ.11 isolates which are flat and runny.

Gram staining results showed that all isolates were Gram positive and had rectiflexibile spores. According to [19], rectiflexibiles are long chain spores found in Streptomyces sp. The results of endophytic actinomycetes exploration are expected to produce important secondary metabolites that have properties similar to the metabolites produced by the host plant in further studies.

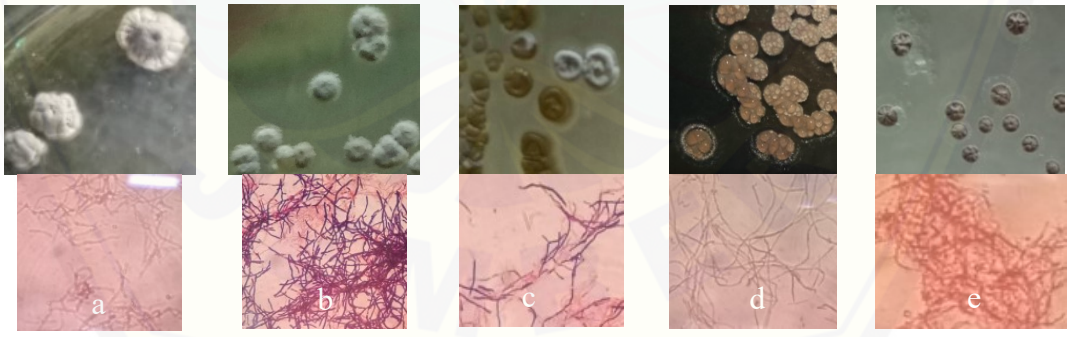

Figure .1. Observed actinomycete colonies (above) in ISP 2 so that after 2 weeks of incubation and actinomycete cells, genus *Streptomycetes* isolated from medicinal plants (bottom) Gram staining with a magnification of 4000x . A) KJ.3A, b) KJ.3B, c) KJ.4, d) KJ.11, e) SMJ.1

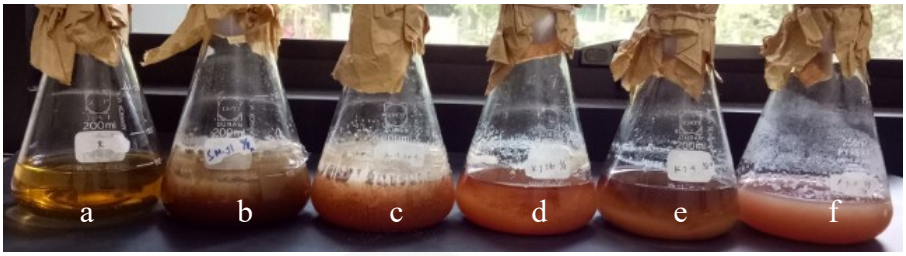

Figure 2. The growth of *Streptomyces* liquid culture in ISP 2 liquid medium (YMB) after 2 weeks incubation. a) control, b) KJ.3A, c) KJ.3B, d) KJ.4, e) KJ.11, f). SMJ.1

Conclusion

A total of 5 endophytic actinomycetes isolates were success-fully isolated from kencur radix and red betel folium yet no actinomycete isolate was found on red Rosella calyx. Macroscopic and microscopic observations showed that 4 actiono-mycetes isolates, namely KJ.3A, KJ.3B, KJ.4, and KJ.11 belongs to the endophytic *Streptomyces* from *K. galanga* radix. In addition, isolate SMJ.1 also belongs to the endophytic *Streptomyces* from *Piper cronatum* folium.

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REFLECTION OF MATERNAL FEEDING PATTERNS ON TODDLERS TOWARDS STUNTING IN JEMBER AGROINDUSTRY COMMUNITY

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Abstract

Impairment in the golden period of children's growth and development, may result in unintelligent kids, short figure, physical and mental susceptability, which refer to the symptoms of stunting. Different socioeconomic factors, including parental feeding patterns, parent's or caretaker's knowledge, habit, etc, have been associated with this increasing malnutrition condition which also leads to the mechanisme of stunting. This research aims to discover the relevance of toddler mother's parental feeding patterns (knowledge, attitude, and eating habit) towards the stunting prevalence in Jember agroindustry community. This research's method uses questionnaires to ask respondents i.e mothers who have toddlers (children under five years old). Respondents were chosen through purposive sampling, namely the respondents who lived in the surrounding public health centre linked with University of Jember, which met the inclusion criteria. The data were then analized by using Spearman's test. The research showed that the majority (86%) of mother's had enough knowledge and most of them (82%) have fair attitude on maternal feeding patterns. It unfortunately also showed that knowledge and attitude on maternal feeding pattern were not related with the occurrence of stunting ($p>0.05$). A fair mother's knowledge and attitude on maternal feeding pattern may not be enough to prevent the occurrence of stunting on toddlers. Another possible cause is genetic factor may have influenced this result. It needs intensive prevention intervention in order to suppress the occurrence of stunting. Research should be done further to see consumption pattern that can identify unmet essential nutrition and exclude the genetic factor.

Keywords: stunting, maternal feeding pattern, agroindustry community

1. Introduction

Different socioeconomic factors include parenting, parental or caregiver knowledge, customs etc. has been linked to an increase in the incidence of these nutritional disorders which can also lead to the onset of stunting, malnutrition and even indirectly lead to death in children. This has triggered many extensive studies and searches to find nutritional health promotion, prevention and therapy formulas through nutritional modification. According to Khumaidi [1], the pattern of habit of feeding children under five including diversification of food consumption is a good cause of nutritional status of children under five. So that the problem of food and nutrition as well as the low quality of food consumption can be found not only in the middle and lower economic strata but also in families who are economically capable but lack care in their parenting.

Sternin and Marsh [2] from the Save The Children Institute wrote about differences in behavior in the same low-income families but with different nutritional status of toddlers, namely mothers of well-fed toddlers providing additional shrimp and crab food from the river as well as sweet potato leaves which is rich in protein. The adoption of adoption behavior from the experience has affected two-thirds of children in the Vietnam region to gain weight and after two years 85% of children no longer experience malnutrition. There are differences in the power of poor families from other poor families [3]. The ability of families to use the existing potential optimally in meeting the nutritional needs of children under five indicates that the contribution of appropriate technology training to the poor population is a necessity, especially to

address the problem of malnutrition in children under five.

Data from the Jember Region Health Office in 2015 [4] showed that there were still many nutritional problems in Jember agroindustry community, including: pregnant women who had nutritional anaemia, who suffered from chronic lack of energy, stunting (short children) and malnutrition. This requires intensive handling to overcome the nutritional status because achieving quality human resources is determined by the quality of nutritional status from diverse, balanced, safe and including halal food consumption. Efforts to improve the quality of nutrition for food insecurity groups can be carried out with a holistic approach ranging from primordial prevention measures, primary, secondary to tertiary. Among them is the provision of nutrition knowledge and awareness (through devices, lectures and multimedia information), production, diversification, and consumption of nutritious foodstuffs.

Therefore, this research is needed as a preliminary study to map and analyse parenting styles and knowledge of parents or caregivers of malnourished and stunting children in the Jember agroindustry community as a recommendation for right approach in the future.

2. Methods

This study used a cross-sectional analytic observational research design. The population of this study were under five years old children in Jember Regency, which amounted to 180,645 people based on the latest BPS update data 31st January 2018 [5]. The sample was chosen using purposive sampling in determining the district area based on the location of the public health centre linked with University of Jember, which met the inclusion criteria, so that a minimum of 30% of the population location was achieved.

The sample in this study were infants aged 0-59 months who met the following inclusion criteria: 1) Respondents who reside in the study area; 2) Parents or caregivers are willing to be respondents in this study. The exclusion criteria in this study were respondents who moved or resigned before the study ended. Primary data acquisition techniques were carried out by interviews and anthropometric measurements [6]. Secondary data were obtained from the public health centre linked with University of Jember and from the Jember District Health Office.

The questionnaire instrument consists of 20 questions on mother's knowledge assessment and 24 questions on maternal feeding pattern attitude assessment. There were also 18 questions structured interviews of mother's eating habits, as an additional information. The respondents' knowledge interval is as follows: (20-14) good knowledge, (13-7) fair knowledge and (6-0) less knowledge. While the attitude on maternal feeding pattern interval is as follows: (72-48) good feeding pattern, (47-24) fair feeding pattern and (23-0) less feeding pattern. The data were then analysed further by using Spearman's test on SPSS software. All method in this study had received the approval of the ethics committee of Medical Faculty, University of Jember.

3. Results and Discussion

3.1. Respondents Characteristics

Majority of the respondents were a family of one child under five years old with household income less than 600.000 IDR/month (equal to 39.4 USD). According to one of the BPS poor household criteria [7] is when the source of income for the head of the household is: farmers with an area of 500 m^2, farm laborers, fishermen, construction workers, plantation workers and or other jobs with income below 600,000 IDR/month. Therefore, in Table 1, economic status was described as poor and not poor according to household income.

Table 1. Respondents Characteristics

Characteristic	Non-Stunting	Short	Very Short	Total

Child age:				
< 24 months	12	22	20	54
24-59 months	11	63	35	99
Number of family member:				
2 members	0	2	2	4
3 members	19	34	26	79
4 members	16	21	15	52
5 members	6	6	8	20
6 members	0	3	4	7
7 members	1	0	0	1
Number of children under five:				
1 child	36	65	51	152
2 children	6	1	4	11
Economic status:				
Poor	18	35	32	85
Not poor	20	29	23	72
Unknown	4	2	0	6
Mother education:				
Uneducated	0	0	0	0
Some elementary school	2	3	8	13
Completed elementary school	22	29	21	72
Completed junior high school	9	20	17	46
Completed senior high school	6	9	6	21
Completed college	3	3	2	8
Unknown	0	2	1	3
Father education:				
Uneducated	1	0	1	2
Some elementary school	4	0	5	9
Completed elementary school	18	31	23	72
Completed junior high school	7	21	13	41
Completed senior high school	7	6	8	21
Completed college	2	4	0	6
Unknown	3	4	5	12

Stunting problems occur more in toddlers age 24-59 months. This might happen because its manifestations are more visible at the age of 24-59 months even though the stunting caused occurs before the toddler reaching 24-59 months. Mother and father education were distributed equally among groups (Table 1) and this showed that education may not relate to stature status. Other studies also state that there is no relation between mother's education level with stunting on toddler [8, 9].

3.2. Mother's Knowledge

Assessment of respondent knowledge about feeding pattern in this study was conducted by using questionnaires in the form of question with the answer in the form of multiple choice. Results of research on mother's knowledge can be seen in Table 2.

The result showed that most of all respondent knowledge in all categories was fair. This probably causing the Spearmen's test had no significant different (p=0.533). The knowledge to be investigated in this study, was all information about the feeding pattern in under five because to ensure a person behaves well is not enough with education alone, needed a basic knowledge and understanding of why

something must be done to arise motivation to willing to do [10].

A good or fair of mother's knowledge is not a guarantee to have a toddler with normal stature status. Mother who has good knowledge is expected to be able to apply possessed knowledge in her daily life. However, behavior not only influenced by the level of knowledge but it also influenced by other factors, such as socio-economic, socio-cultural, and the environment [11].

Table 2. Crosstabulation data inter-variables

	Non-Stunting	Short	Very Short	Total
Knowledge:				
Less	6	7	6	**19 (11.7%)**
Fair	34	58	48	**140 (85.9%)**
Good	2	1	1	**4 (2.5%)**
Feeding Pattern:				
Less	0	0	0	**0 (0%)**
Fair	34	54	45	**133 (81,6%)**
Good	8	12	10	**30 (18,4%)**

The largest number of wrong answers were on questions number 3, 14, 16 and 19. Many of the respondents do not know that sources of energy are carbohydrate, protein and fat. Also, about what vitamin will be lost when boiling too long on vegetable and about how to minimize the loss of vitamin on cooking process. Less to know also, about what mineral that help the growth of bones and teeth. This result showed that there were important materials that were not yet known. Therefore, the education about nutrition is recommended to be given [12].

3.3. Attitude on Maternal Feeding Pattern

Assessment of respondent attitude on maternal feeding pattern in this research is done by using questionnaire in the form of statement with answer of A (Always), O (Often), S (Sometime) and N (Never) with value of 0, 1, 2, 3 in negative statement at number 3, 9, 10, 12, 13, 18, 21 and 22 and value of 3, 2, 1, 0 in the positive statements at number 1, 2, 4, 5, 6, 7, 8, 11, 14, 15, 16, 17, 19, 20, 23 and 24. Results of research on maternal feeding pattern can be seen in Table 2.

The result also showed that most of all respondent attitude in all categories was fair, with Spearmen's test showed no significant result (p=0.901). But when we related knowledge and attitude it showed a significant result (p=0.003). An attitude has not been automatically manifested in an action [10]. Action is a rule that is carried out to make rules to overcome something or the act of a close relationship between attitudes and actions supported by the notion of attitude states that the attitude is a tendency to act. So that to prevent the occurrence of stunting is not enough just by fair and good knowledge and attitude of the respondents.

Based on the evaluation of respondents answered on maternal feeding pattern attitude questionnaire, almost all statements were responded positively, showed by good attitude (18,4%) and fair attitude (81,6%). However, when viewed in detail from each statement there were still respondents that responded negatively. Negative attitudes of 63.8% and 73.6% of the total 163 respondents shown in statements number 11 and 14 about serving food by giving decoration to foods and using cute eye-catching cutlery.

Maternal feeding pattern has a role in the incident of stunting in toddlers due to intake food for toddlers is fully regulated by her/his mother. Mothers with good

attitude tend to be have toddlers with nutritional status better than mothers with fair or less attitude. However, in this study the mothers with good attitude don't necessarily have toddlers with stunting problems less than the mothers with fair or less attitude. This matter could be because even though attitude on maternal feeding pattern is good, in poor families there are limitations to meet daily needs so the attitude on maternal feeding pattern does not affect the occurrence of stunting.

3.4. Mother's Eating Habit

Structured interviews of mother's eating habits give information about daily consumption in the Jember agroindustry family. Most of all of them eat (staple food, vegetable and side dishes) three times a day together with family members at the dining table. They get food mostly from market or shop and processing side dishes by frying (only a few by boiling or grilling). A frequent and preferred foods consumed by respondents can be seen in Fig. 1.

What is interesting here, they don't think that eat fruit and drinking milk is that necessary for nutrition. It is showed by only a few respondents (about 14%) included fruit and milk as their daily food consumption. Based on preference, meat is more preferable than egg as an animal protein source but in fact they consume egg more than meat, equal to fish as the most favourite animal protein source (1-6 times per week). This is probably because the price of egg is more reasonable than the price of meat.

There were also some foods that respondents dislike the most such as bitter melon (52.1%) for vegetable, egg (28.8%) for animal protein and sapodilla (32.5%) for fruit. There were also some respondents that answered no fruit to be disliked, in contrast with the daily consumption statement that there was no fruit included in it. This perhaps also related to affordability of the family to consume foods. In the context of food choices, food consumption requires not only money expenditures for purchasing food but also time expenditures for purchasing, preparing, and consuming food and for cleaning up after preparation and consumption. Therefore, according to household production theory the full price of consumption is the sum of the direct and indirect prices for food, where the direct price is the purchase cost, and the indirect price is the value of the time requirements [13, 14].

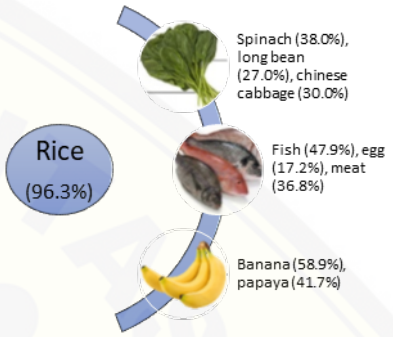

Fig. 1 Foods consume by respondents based on preference

The pattern of feeding habits in toddlers including the diversification of food consumption is a good causal of good nutritional status of children [1]. Food and nutritional problems and low quality of food consumption can be found not only in the middle and lower economic layers but also in economically capable families who are less concern in the care of their toddlers. In addition to household level food availability, it could be that the stunting condition is influenced by other factors, such as a history of infectious diseases [9, 15].

Stunting is a picture of deprivation nutrition for toddlers in a relative long period of time. The height/age index describes toddlers past nutritional status. In general, there is no relationship between maternal education level, mother's nutrition knowledge level or attitude on maternal feeding pattern with stunting problems in toddlers. There are many factors that influence the occurrence of stunting

problems beyond these factors, including the nutritional status of mothers when pregnant. Pregnant women who experience lack of nutrition will result in the fetus also experiencing nutritional deficiencies. Deficiency nutrients in pregnancy that occur continuously will give birth to children who experience lack of nutrition. This condition if it takes place in a period of a relatively long time will cause children experience failure in growth (stunting). In addition, short mothers are also at risk for giving birth to a short child.

4. Conclusion

Based on the result, we concluded that a fair mother's knowledge and maternal feeding pattern may not be enough to prevent the occurrence of stunting on toddlers. Another possible cause is the genetic factor may have influenced in this result. It needs intensive prevention intervention in order to suppress the occurrence of stunting. Research should be done further to see consumption pattern that can identify unmet essential nutrition and exclude the genetic factor that may interfere with the result.

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ANTIBACTERIAL ACTIVITY OF THAGURI LEAF EXTRACT AGAINST *S. AUREUS AND E.COLI*

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Abstract

Thaguri has a potential as a bioactive compounds. This study aimed to investigate the antibacterial activity of crude ethanolic Thaguri leaf extract against *S.aureus and E. coli*. The leaf extract was obtained from an extraction method using ethanol. Antibacterial assay was conducted by using agar disc diffusion method at concentrations of 1%, 10%, 20%, 30% and 40% based on the measurement of inhibition zone for each concentration. The greatest inhibition against *S.aureus* and *E.coli* was demonstrated at a concentration of 40%. It was concluded that the ethanolic Thaguri leaf extract was effective against *S.aureus* and *E.coli.*

Keywords: thaguri, phytochemical screening, antibacterial activity, *S.aureus, E.coli*

1. Introduction

The Bangkalan Health Profile Data revealed that the most commonly occurring diseases in the area were tuberculosis, pneumonia, diarrhea, leprosy, dengue bloody fever, and malaria. Diarrhea, in particular, has been reported to present with a significant incidence. Figure 1 showed that the incidence of diarrhea was highest in 2014 with 37,112 people affected. This data were of significant concern as this incidence fell way above the target of around 20,268 patients. Among the causes of diarrhea, infectious agents including the bacteria *Escherichia coli* and *Staphylococcus aureus* were among the most common causes. The diarrhea caused by these bacteria has been reported to potentially lead to death. In addition, most of these bacteria have been reported to be resistant to antibiotics [1].

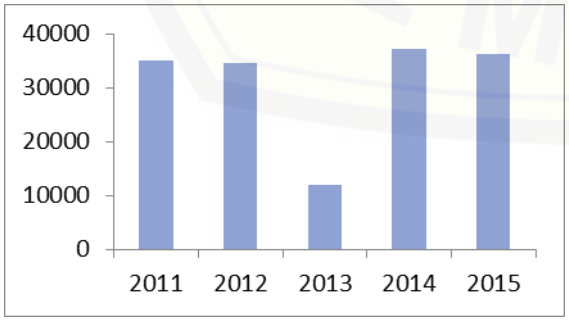

Figure 1 The incidence of diarrhea in Bangkalan

Herbal plants have always had great significance in medicine, culture and nutrition in many societies in the world. Various types of plants have been utilized in herbalism and some of these plants have been reported to have medical benefits. These plants contain many types of ingredients that might be of use as an alternative medicine. Currently, the use of alternative medicines has become a part of lifestyle that might play significant role in maintaining health [2]. Herbal sources have been the most utilized source for the alternative medicine. Assurance of the quality, efficacy and safety of herbal plants and herbal products has now become an important issue both in developing and industrialized countries [3].

Indonesia is a country with a rich plant and animal biodiversity. Plants, in particular [4], have provided a significant support to our daily lives, both as food ingredients as well as ingredients for medicine. The use of plants as medicines has been increasingly popular within the community. The increasingly high prices of medicines has urged people to look for other alternatives for treatment, including by utilizing medicinal plants [5]. Madurese people have been using herbs and this use has been mostly based on habits that have been established empirically. This might be of concern as the use of herbs with incorrect preparation might reduce the ability of the medicine to work effectively (Muharni et al, 2017). Thaguri is one of the herbs that is often used by the Madurese. Thaguri (*Sida*

rhombifolia L) has been empirically used as a natural medicine by the community [6]. Flavonoids contained in thaguri leaf extract in vitro have demonstrated the effect of xanthine oxidase inhibitors which is to reduce excess uric acid production [7]. In addition, it has a diuretic effect that facilitates the excretion of the excessive of uric acid through the urine. This plant has been used in traditional medicine and has several therapeutic activities. Ref. [8] with its in vitro study reported that the petroleum ether, chloroform and ethyl acetate extracts of the thaguri stem had a significant antibacterial activity against *Bacillus substilis*, *Sarcinia lutea* and *Shigella shiga*. However, no study have made reports on the antibacterial activity of the leaves, especially in Madura. This research was therefore conducted.

2. Materials and Methods

2.1 Plant material

Leaves of Thaguri (*Sida* rhombifolia) were collected from Gili Timur district, Kamal, Bangkalan, Madura. The leaves were then crushed and put into glass container and saved until the extraction procedure was performed in the laboratory.

2.2 Chemicals

Aquadest, ethanol 96%, acetone p.a, chloroform, MHA media, sulfuric acid, sterile cotton stick, NaCl, reagents for phytochemical tests, *E.coli, S.aureus* and Whatman filter paper.

2.3 Preparation of extracts

Thaguri leaves were properly dried and grinded to form a grainy powder. Each 50 g of the powder was soaked into 500 ml of 96% ethanol, and kept for three days. After three days of being dissolved, the mixture was filtered. The filtrate was subsequently evaporated using rotary evaporator.

2.4 Bacterial suspension

E. coli and *S. aureus* colonies (24 hours) were taken with oasis wire and then suspended into sterile aquadest by applying bacterial colonies to the test tube walls. The tubes were then stirred with distilled water to form turbidity or density.

2.5 Antibacterial activity

The antibacterial activity was assessed with the Kirby Bauer method (using a disc). The media used 19 g of Mueller-Hinton Agar (MHA) dissolved in 0.5 L distilled water, then sterilized in an autoclave at 121^0C for 15 minutes. Each petri dish is filled with 15-20 mL of MHA media and left for a while until it solidifies. In the solid media, 0.1 mL bacterial suspension was applied using spreader rods. The first MHA media was divided into 7 parts. Each part was then provided with a specified treatment including a positive control (tetracycline), a negative control (DMSO), and ethanolic extract of thaguri leaves with different concentrations (1%, 10%, 20%, 30% and 40%).The dishes were then incubated at 37° C for 24 hours and the bacterial growth was observed. The diameter of the inhibitory zone formed was measured by a ruler and would be expressed in millimeters.

3. Results and Discussions

The present study aimed to conduct a preliminary evaluation of the phytochemical and antibacterial activity of Thaguri ethanolic extract against *E. coli* and *S. aureus*. The previous screening of phytochemical constituents of Thaguri had revealed the presence of steroids, alkaloids, tanins, and flavonoids (Table 1). Further phytochemical screening was then conducted to identify the secondary metabolites [9].

Previous studies have indicated that there were variations in the phytochemical constituents of Thaguri. The leaves were found to contain alkaloids, calcium oxalate, tannins, saponins, phenols, amino acids, and volatile oil, while the stem contained calcium oxalate and tannins. In addition, alkaloids, steroid, and efedrine have been found in thaguri roots [10]. Another study added that the root extract from contained alkaloids, flavonoids, steroids and phenolics [11]. The presence

of secondary metabolites in thaguri might play role in its potential bioactivity [12].

Numerous studies that investigated various organic extracts of thaguri have shown the antinociceptive, anti inflammatory, cytotoxicity, antibacterial and larvicidal activities [6], [13], [14]. The results of the antibacterial activity of the ethanolic extract from thaguri leaves in Gili Timur are shown in Table 2.

Table 1 Phytochemical constituents of ethanolic extract of thaguri leaves

Chemicals Constituents	**Ethanol extract**
Alkaloids	-
Saponins	+
Flavonoids	+
Tannins	+
Terpenoids	-
Steroids	+

Table 2. Antibacterial activity of the ethanolic extract of thaguri leaves

No.	**Extract Concentration (%)**	**Inhibitory zone (mm)**	
		S. aureus	***E. coli***
1	1	1,17	0
2	10	1,66	1,18
3	20	2,20	1,63
4	30	2,49	1,85
5	40	3,17	2,38
6	Control (+)	3,73	2,92
7	Control (-)	0	0

The results presented in Table 2 demonstrated the antibacterial activity of various concentrations of thaguri leaf extract against *S.aureus* and *E.coli* growth. This is further confirmed with the formation of an inhibitory zone around the disc (Figure 2). This result was in concordance with another research of [15] that showed the potential antibiotic activity of thaguri. However, the absence of inhibition zone on *E.coli* growth at 1 week observation showed that at concentrations of 1% with three repetitions, no antibacterial activity against *E.coli* was found. This might be because the resulting ethanol extract of thaguri leaves was only crude extract [16]. It is likely that the low concentration of thaguri within the 1% extract was not able to damage the cell wall of *E. coli* [17].

Table 2 and Figure 2 (a) present the inhibitory test results of ethanol extract of Thaguri leaves on *S. aureus* growth. Different concentrations seemed to show different inhibitory capacity as well. Other factors that might play role for the differences of the antibacterial activity were the chemical structure and species of the bacteria [18]. This antibacterial activity was caused by the presence of secondary metabolites in thaguri leaves including saponins, flavonoids, tannins and steroids [10].

The antibacterial activity of secondary metabolites against microorganisms had also been reported by [18,19]. In addition, r**ef. [21] reported that t**annin found in agricultural products influenced the antimicrobial and antioxidant activities. Other studies **[11,22–29] have also demonstrated** that saponins, steroids, tannins and flavonoids present potentials as antimicrobials and antioxidants.

4. Conclusion

The ethanolic extract of thaguri leaves showed an antibacterial activity againts *S. aureus and E.coli*. Further in vivo and toxicity studies are required to follow up the results of this study.

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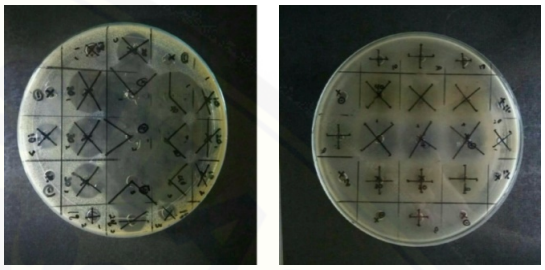

Figure 2. Inhibition zone of extract Thaguri leaves

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RANDOMIZED CONTROLLED TRIAL OF TRANEXAMIC ACID'S EFFECT ON THE DURATION OF BLEEDING: A STUDY ON DMPA USERS WITH ABNORMAL UTERINE BLEEDING

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Abstract

Objective: To determine the effect of tranexamic acid on the duration of bleeding among DMPA users who received oral contraceptive pills (OCP)
Methods: Forty-four subjects were divided into two groups, twenty-two subjects each. Group 1 received 250 mg tranexamic acid four times a day for 5 days and OCP once a day for 28 days, while Group 2 received placebo four times a day for 5 days and OCP once a day for 28 days. Both groups were evaluated for bleeding pattern, episodes and duration outside the menstrual period.
Results: There was no significant difference of risk factors (age, education, body mass index (BMI), and number of previous pregnancies) between the two groups ($p>0.05$). The median duration of bleeding was 4.5 days and 8 days in Group 1 and 2, respectively. The duration of bleeding duration was significantly different between the two groups ($p=0.018$). However, side effects including dizziness and nausea were not significantly different between groups ($p=0.185$).
Conclusion: The administration of tranexamic acid significantly reduced the duration of bleeding among DMPA users who used OCP. Confounding factors, such as age, education, BMI, and number of previous pregnancies, did not affect the duration of bleeding.

Keywords: duration of bleeding, depomedroxy progesterone acetate, oral contraceptive pill, tranexamic acid

1. Introduction

Exponential population growth has been a threat to human's life, hence the necessity of improving the community's awareness towards fertility control [1]. Establishing a contraception program in countries with fast-growing population has been found to decrease maternal mortality of 32% and neonatal mortality of 10%, leading to the achievement of Sustainable Developmental Goals (SDG) [2][3]. To date, more than 214 million women worldwide have expressed their wish to prevent pregnancies. However, neither the women nor their partners use contraception. Productive couples who want to delay pregnancy or do not want to be pregnant anymore but do not use contraception (unmet need) comprised 9% of Indonesian population in 2012. Reasons for not using contraception include their unavailability or limited supply, concerns about side effects, the client state of health, and ignorance of the presence of methods and use of contraception [4].

Good contraception should combine aspects of safety and effectiveness with the convenience of use. Furthermore, it should also provide additional health benefits. For the last 30 years, progesterone only contraceptive (POC) has been used worldwide and proven as a safe and effective method of contraception [4][5]. In 2012, sixty-two percent of married women aged 15-49 years old in Indonesia use contraception, and, most of them used the modern methods, with contraceptive injection being the most common (32%) [5]. Among contraceptive injections, depomedroxy progesterone acetate (DMPA) is the most effective method with a pregnancy rate of 0.3 pregnancies in 100 women annually. Additional benefits of this method include its maintained efficacy upon combination with other drugs,

independence from weight gain, convenient dosing of only 4 injections per year, no effect on lactation, as well as its capacity to prevent anemia, pelvic inflammatory disease, ectopic pregnancy, and dysmenorrhea due to endometriosis [6][7][8][9]. However, several side effects such as breakthrough bleeding (BTB) or iatrogenic abnormal uterus bleeding (I-AUB) have been reported. Approximately 10% of DMPA users experience regular bleeding monthly, eventually leading to discontinuation of the contraception [10].

The mechanism of the bleeding related to use of DMPA is not fully understood [4][6][7]. The proposed mechanism is the continuous endometrial exposure to constant dose of progesterone. The activity of matrix metalloproteinase (MMP) increases in DMPA users, especially MMP-9, while the activity of Tissue Inhibitory Metalloproteinase (TIMP) decreases. These events lead to weakened supportive tissue around the vascular and under the epithelial, hence the fragility of the endometrial layer and the vascular vulnerability to damages that eventually results in bleeding [4]. Strategies for managing this bleeding include counseling, supplemental estrogen, non-steroidal anti-inflammatory drugs (NSAID), and combined oral contraceptive pills (OCP) [8][9].

Reference [11] have demonstrated that levonorgestrel IUD (LNG IUD) was statistically superior than low dose OCP in reducing idiopathic menorrhagia, with reduction of mean blood loss of 87% and 34,9%, respectively. Considering that the use of IUD as method for managing AUB has not been popular, OCP is preferable. Based on this finding, this study was then conducted to explore the effectiveness of tranexamic acid in combination with low dose OCP in the management of irregular bleeding among DMPA users. Tranexamic acid is widely known as antifibrinolytic, which is expected to increase the effectiveness of low dose OCP in managing the bleeding.

2. Materials and Methods

This double-blind randomized controlled trial was conducted in several clinical practices, public health center and dr. Kariadi General Hospital in Semarang from October 2016 to August 2017. This study was approved by the Ethical Committee of our institution. The subjects were DMPA users aged 20-45 years old. Subjects with gynecological disorders, bleeding disorders, allergy to tranexamic acid, contraindication of OCP, and liver and/or renal disorders were excluded. A total of 44 subjects were then divided into two groups, each consisted of twenty-two subjects. Before the study, each subject was asked to read and sign the informed consent. The treatment for both groups were packed in capsules with the same color. To ensure the blinding, we asked people outside the author and co-author to choose a closed envelope containing which group the subjects would be assigned to. Both authors and subjects were blinded for the administered drugs.

Tranexamic acid with the dose of 250 mg was given to the first group, while placebo capsule was given to the second group. Both tranexamic acid and placebo were administered four times a day for 5 days. Both groups also received low dose OCP containing 30 mcg ethinylestradiol and 150 mg levonorgestrel (Microgynon®, Bayer Schering Pharma, Berlin, Germany) administered once a day for 28 days. Each subject received a card for documenting vaginal bleeding other than the menstrual bleeding. Data were collected to determine the pattern, episodes, and the end of the bleeding. The obtained data were then analyzed. Parametric data were analyzed with unpaired T-test, while non-parametric data were analyzed using Mann-Whitney test. The data were considered significant if p value <0.05.

3. Results

Characteristics of the subjects are presented in Table 1. No significant difference was found for age, education, BMI, and number of previous pregnancies) between the two groups. However,

Table 1. Characteristics of subjects in both groups

Characteristics	Group		P
	Group 1 (n = 22)	Group 2 (n = 22)	
Age (mean±SD; median; range)	31.1±5.,84;31.20-43	27.7±6.21;26;20-42	0.086[a]
Education			0.237[b]
Elementary School (n (%))	1 (4.5)	1 (4.5)	
Junior High School (n (%))	4 (18.2)	10 (45.5)	
Senior High School (n (%))	14 (63.6)	10 (45.5)	
Colleague (n (%))	3 (13.6)	1 (4.5)	
Body Mass Index (mean±SD; median; range)	21.78±4.600;19.92; 14.88-31.63	20.95±1.843;20.59; 18.17-24.97	0.716[a]
Number of previous pregnancies			0.067[b]
1 (n (%))	7 (31.8)	15 (68.2)	
2 (n (%))	11 (50)	5 (22.7)	
3 (n (%))	2 (9.1)	2 (9.1)	
4 (n (%))	2 (9.1)	0 (0)	

SD = Standard Deviation
a) Mann Whitney; b) χ^2

the mean duration of bleeding was significantly different between group 1 (5.2±3.62 days; range 1-11 days) and group 2 (9.2±6.16 days; range 0.2-23 days) (p=0.018).The side effects reported in this study were dizziness and nausea. Five subjects in group 1 experienced side effects, while only one subject in group 2 experienced them. However, these side effects were not significantly different between the two groups (p = 0.185).

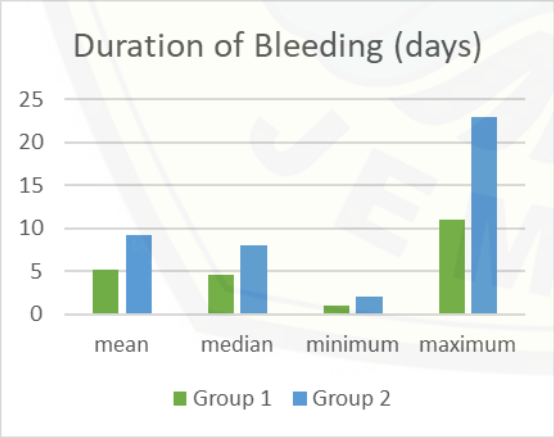

Figure 1. Duration of bleeding in both groups

4. Discussions

This study demonstrated that the duration of bleeding was shorter in Group 1 (tranexamic acid and low dose OCP treatment). Several studies have showed similar results, especially for cases of irregular bleeding. Reference [12] found significant effect of the antifibrinolytic drugs in Norplant users who experienced irregular bleeding. Treatment with 500 mg tranexamic acid twice daily for 5 days was able stop the bleeding in less than 7 days in the treatment group. Reference [13] have demonstrated similar results in their study of tranexamic acid effect among DMPA users. The number of patients with shorter duration of bleeding (less than 1 week) was significantly higher in treatment group (88% vs 8.2%). The mean of days without bleeding was also higher in the treatment group (20.6 days) compared to the control group (7.5 days).

Tranexamic acid can reversibly block lysin (the binding site of plasminogen), leading to inhibition of interaction between plasmin and fibrin polymers. It also promotes fibrin degradation, clot stabilization and bleeding reduction. This mechanism is therefore beneficial for the management of irregular bleeding caused by DMPA [3]. The fibrinolytic enzymes in

endometrial layer play a significant role in the homeostasis of menstrual bleeding. In dysfunctional bleeding, the activity of plasmin and plasminogen activator increase due to increased fibrinolysis. Tranexamic acid treatment would exert its antifibrinolytic effects and reduce hypermenorrhea by 50% [2]. Mild side effects including dizziness and nausea were experienced by the subjects in group 1. Similar side effects were also reported by subjects treated with tranexamic acid in the study by Ref. [13].

Risk factors including age, number of previous pregnancies and BMI, were not significantly different between the two groups, hence their potentially minimum effect on the duration of bleeding. Reference [6] have also reported that the mean ages (29 and 34 years old in treatment and control group, respectively) did not significantly affect the duration of bleeding. Similar results were demonstrated by reference [13] with mean ages of 27 and 29 years old in the treatment and control group, respectively. Furthermore, both studies did not find significant effect of BMI and number of previous pregnancies on the duration of bleeding.

This study has several limitations. First, this study was conducted in a relatively short duration. In addition, data for the level of hormones that might affect the duration of bleeding and the duration of bleeding before treatment were not measured, hence the impossibility to establish an explanation for the mechanism of the effect of tranexamic acid on the duration of bleeding. In conclusion, this study showed that the duration of bleeding was reduced among DMPA users who were treated with tranexamic acid. Further studies should be conducted with longer duration (follow-up in 3 months) as well as examination of progesterone and estrogen levels.

Conflict of Interest

The authors declare no potential conflict of interest.

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THE EFFECT OF BANANA PEEL EXTRACT (*Musa* acuminata) ON TOTAL CHOLESTEROL, HDL AND LDL LEVEL IN HYPERCHOLESTEROLEMIA-INDUCED RATS

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Abstract

Hypercholesterolemia, characterized primarily by high level of LDL, is an important factor in the pathogenesis of atherosclerosis. Some people have used traditional herbs for healing remedies, including using them for atherosclerotic causes of hypercholesterolemia. Banana peel (*Musa acuminata*) have been generally regarded as a waste. However, banana peel extract contains antioxidants that are able to inhibit fat oxidation activity and are used as a therapy for hypercholesterolemia. The present investigation was undertaken to determine the effect of oral administration of an alcoholic bark extract of *Musa acuminata* on hypercholesterolemia-induced rats. Hypercholesterolemia rat model were induced by feeding the rats with cholesterol containing food. The rats were then divided into six experimental groups. For two weeks, each group received different types of feeding: basal diet (N), cholesterol (C), simvastatin (CS), and three different doses of banana peel extract (300 mg for CB1, 600 mg for CB2 and 1200 mg for CB3). The results showed that the total cholesterol level was lower than control ($P<0.05$). HDL level showed that no significant difference with control ($P>0.05$) but LDL level decreased with banana peel extract ($P<0.05$).

Keywords: hypercholesterolemia, *Musa acuminata*, cholesterol, LDL, HDL

2. Introduction

Cholesterol is the most abundant sterol in the body. This compound generally exists in the blood and lymph as the fatty acid-bound cholesteryl-ester or as free form cholesterol in the blood cells of muscle, liver, and other tissues.

Cholesterol is an important element required to regulate various chemical processes in the body. However, high cholesterol level might cause atherosclerosis. If atherosclerosis occurs in the blood vessels of the heart, it will cause coronary heart disease (CHD). Blood clots mixed with fat attached to the blood vessels might lead to the occurrence of a heart attack. A clear correlation has been established between the coronary artery disease caused by atherosclerosis and the blood cholesterol level, which primarily reflects the cholesterol content of LDL (LDL cholesterol) [1].

Antioxidants have been widely used to prevent and treat degenerative diseases including atherosclerosis. Many traditional medicines contain antioxidants. Flavonoids are phenolic compounds (phenolic hydroxyl) capable of acting as antioxidants. Flavonoids can be found in many fruits and vegetables. One of the fruits that contains flavonoids is banana. The fruit can be consumed directly or processed into several products. Banana peel waste represents about 30% of the fruit. This waste might become an environmental problem as it contains large amounts of nitrogen, phosphorus and high moisture content that might promote the growth of unwanted microorganisms [2]. The total flavonoid content found in banana peels is about 0.9 to 3.0 g / 100 g dry waste (DW) [3, 4]. Catehin is the most abundant flavonoid identified in the banana peel. In our previous study, the catechin content of the banana peel extract was about 0.8 to 1.0 g / 100g extract.

The objective of this study was to examine the antioxidant activity of the banana peel. This was conducted by studying the effect of orally administered banana peel extract on the total cholesterol, HDL and LDL levels on hypercholesterolemia induced rats.

2. Materials and Methods

2.1 Materials

Bananas (*Musa Canvendish*) sample from Lumajang, Indonesian, were obtained from a local market. The plant was identified, confirmed and authenticated by *Balai Konservasi Tumbuhan* of The Purwodadi Botanical Garden Purwodadi, Pasuruan. Catechin was obtained from E. Merck (Darmstadt, Germany) and the rat polyclonal antibody VEGF was from Sigma Chemical Co. (St. Louis, USA). All other reagents were of high analytical grade from E. Merck (Darmstadt, Germany).

2.2 Preparation of alcoholic extract of banana peel (EBP)

The banana peels were separated manually, cut into small pieces (±2mm), dried and turned into powder. The dried banana peel (50 g) was extracted with 250 ml 70% ethanol at the room temperature for 5 days. The extract was then concentrated using a rotary evaporator. The crude extract was weighted and kept in dark glass bottle in the freezer dryer for further use.

2.3 Animal hypercholesterolemia model

Thirty Wistar rats were provided with the hypercholesterolemic solution for 14 days. The solution consists of 300 grams of pork fat and 200 grams of duck egg yolks in 100 mL of aquadest and 0.5% CMC [5].

2.4 Treatments for the hypercholesterolemia rat model

Thirty Wistar rats (150-200 g) were divided randomly into six groups: the normal group (N), the hypercholesterolemic control (C), the simvastatin group (CS), and three other groups provided with banana peel extract of different concentrations (300 mg/kgBW, 600 mg/kgBW and 1200 mg/kgBW per day). The normal group (N) was fed with the basal diet, while the other five groups (C, CS, CB1, CB2, and CB3) were given basal diet added with 3 g cholesterol/day. The hypercholesterolemic diet was provided for 4 weeks. After 4 weeks of inducing hypercholesterolemia, levels of plasma total choles-terol, LDL and HDL level were measured using a Biolyzer machine (Analiticon). Hypochlesterolemia was defined as a plasma total cholesterol level of more than 54 mg/dL. Following this hypercholeste-rolemia induction, the hypercholeste-rolemic diet was continued with the addition of different type of treatments for each group. All animals were kept in individual stainless steel metabolic cages at a temperature of 27^0C ± 10C with a 12 hour light and dark cycle. The diet was prepared once daily at 10.30 AM. The treatment continued for two weeks. About 1 ml of blood was obtained from the ocular vein at the end of the treatment.

2.5 Statistical Analysis

Data were statistically evaluated using ANOVAs and expressed as mean ± S.D. Statistical analyses were performed using SPSS version 22.0 software package (SPSS® Inc., USA). The signifi-cance of differences among means was separated by the LSD test. Significance was set at $P < 0.05$.

4. Results and Discussions

3.1 Antioxidant activities of the banana peel extract

The evaporated banana peel extract was dissolved with ethanol to a concentration of 160 mg/L, then diluted into four different concentra-tions of 80, 90.4, 104.3 and 125.22 mg/L. the goal is to determine the antioxidant activity by creating the IC50 curve. IC50 represented a banana peel extract value of 70.41 mg/L. Based on the classification of antioxidant activity, the IC50 extract of banana peel demonstrated a strong antioxidant activity in 50-100 mg/L level [6].

3.2 Total cholesterol, HDL and LDL levels

The means of total cholesterol, HDL, and LDL levels of the control and experimental groups are depicted in Fig. 1. The total cholesterol level significantly decreased with the administration of the banana peel extract ($P < 0.05$). This

decrease of the total cholesterol level was more significant with increased dose of banana peel extract. The total cholesterol level of the control (C), simvastatin (CS), and the 1200 mg/kgBW (CB3) treatment groups also demonstrated decreases of 9.22%, 17.95%, and 17.09%, repectively. This decrease in total cholesterol level was similar to the decrease showed by the simvastatin group (CS).

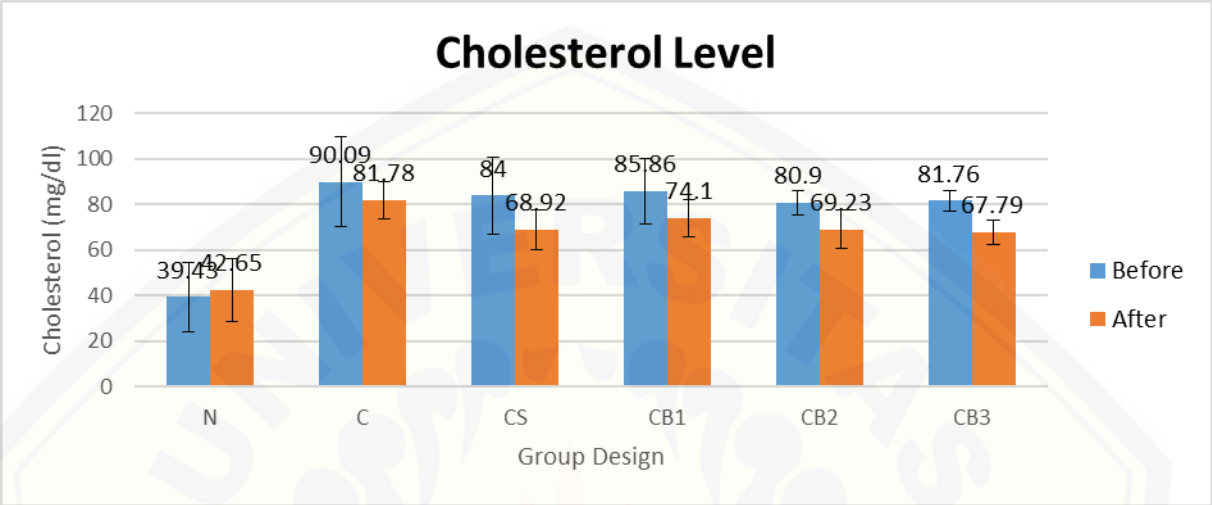
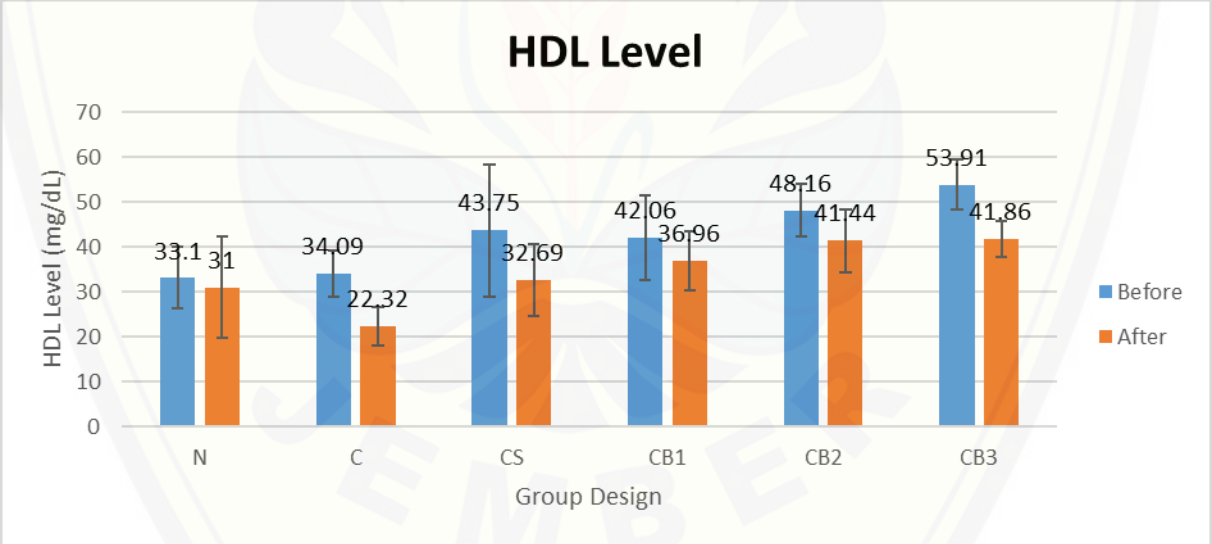


Figure 1 : Total cholesterol levels before and after treatment. The mean total cholesterol levels showed



significant differences among the treatment groups ($P < 0.05$)

Figure 2: HDL levels before and after treatment. The mean HDL levels showed no significant differences among the treatment groups ($P > 0.05$)

Figure 2 demonstrated that the HDL level decreased after treatment but no statistically significant differences were found between the control and treatment groups ($P > 0.05$). In contrast, Figure 3 showed that the LDL level decreased with the administration of oral banana peel extract ($P < 0.05$). The decrease of total cholesterol level was more significant with the adminitration of increased dose of banana peel extract.

The total cholesterol levels also decreased by 6.11% in the control group (C), by 27.60% in the simvastatin (CS) group and by 38.79 in the banana peel etract group with the dose of 1200

mg/kgBW (CB3). This demonstratd a more significant decrease in LDL level than the decrease induced by simvastatin (CS).

The decrease in the cholesterol levels in treatment groups might be caused by antioxidant content of the banana peel. This includes pectin, tannin, saponin, and flavonoids [7]. Pectin binds the cholesterol in the digestive system, preventing it from being absorbed into the bloodstream. Use of pectin for two week can decrease the serum cholesterol level by 13%. The total cholesterol level could be significantly lowered by taking pectin at least 6 grams/day [8].

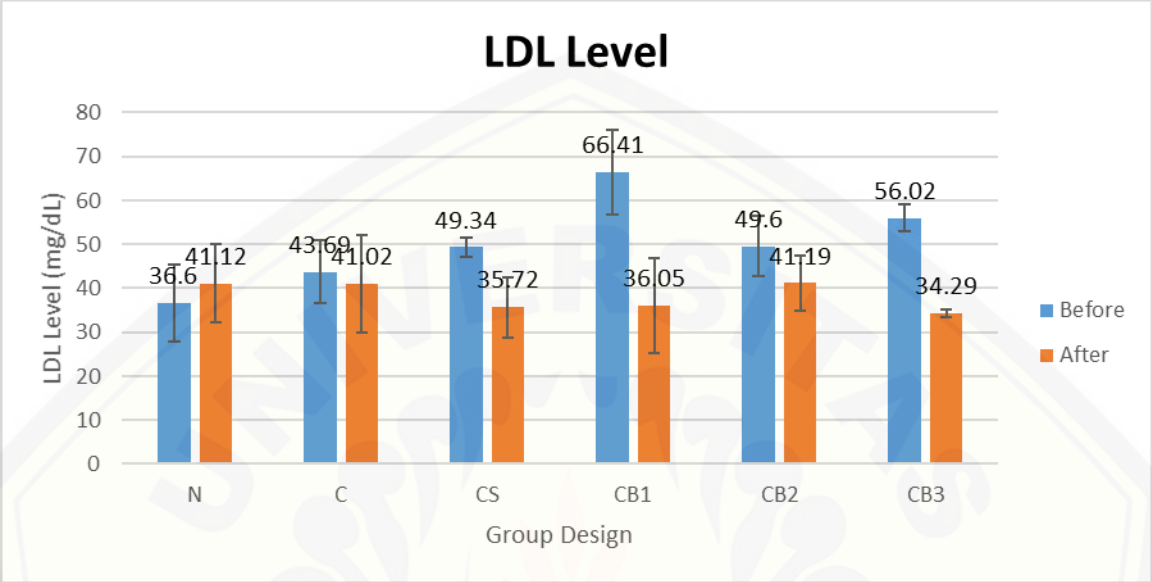


Figure 3: LDL levels before and after treatment. The mean HDL levels showed significant differences among the treatment groups ($P < 0.05$)

Tannin might also play role in this decrease as this compound inhibits the biosynthesis of cholesterol that would subsequently inhibit its absorption. In result, the total cholesterol levels will decrease [9]. Saponin also binds the cholesterol in the intestinal lumen which subsequently prevents its absorption. In addition, saponin binds to bile acids to decrease the enterohepatic bile acids cycle and increase the excretion of cholesterol [10][11][12]. Flavonoids protect the body from free radicals by preventing lipid peroxidation [7]. Flavonoids decreases the plasma total cholesterol level by inhibiting the action of 3-hydroxy 3-methylglutaril coenzyme A reductase (HMG Co-A reductase) [13].

4. Conclusion

Oral administration of banana (*Musa acuminata*) peel extract was proven to lower the levels of total cholesterol, HDL and LDL in the hypercholesterolemia induced rats.

Competing Interests

The authors have declared that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

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PERSONAL PROTECTIVE EQUIPMENTS (PPE) USAGE REDUCE IMPAIRED SKIN INTEGRITY ON FARMERS

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Abstract

Farmers as their activities in fields and its exposure with many dangerous chemicals, such as pesticides becoming risky to have many health problems. One of the health problem due to farming activities is a skin integrity disruption. Occupational skin disease is a skin disease caused by exposure to chemical substances in the workplace environment. This study aimed to analyze the relationship between Personal Protective Equipment (PPE) with impaired skin integrity to farmers. This study was a cross-sectional study with 93 respondents obtained by cluster sampling. Data was gathered using two questionnaires. Data analyzed by using chi-square with 95% CI. The result showed that there was a relationship between usage of PPE with skin integrity disorder of farmers (p: 0,001; α: 0.05). Therefore, it is concluded that usage of PPE can reduce the risk of skin integrity in farmers. It is important to educate farmers to obey with their risk during work at the field and how to reduce it by using PPE.

Keywords: Agriculture, Personal Protective Equipment, Skin Integrity

1. Introduction

The agricultural sector in Indonesia plays an important role, considering that more than 40% of the workforce depends on this sector. Data from the International Labor Organization (ILO), around 1.3 million people work in agriculture around the world (half of the total number of workers). Moreover, 60% of them work in developing countries and unfortunately, fatal accident rates in developing countries are four times higher than in industrialized countries [1].

Agriculture is one of the sectors that raise the entire spectrum of occupational safety and the risk of accidents. The risk of health hazards depends on the health status of the farmers and the pesticides used. Emerging health hazards in agriculture are related to the equipment and fertilizer used, both to eradicate pests and fertilize plants [2]. One of the health problems due to agricultural activities is a skin problem.

Occupational skin diseases are skin diseases caused by exposure to chemical substances in the workplace environment [3]. Occupational skin diseases have a typical appearance like itching, lesions that can become scratching, or infection if not treated properly which results in skin integrity disorders in workers [4]. Clinically, occupational skin diseases can be divided into two groups, contact dermatitis and non-eczema dermatitis [5]. Although it does not cause death, skin diseases that cause skin integrity disorders in workers are crucial factors for a decrease in work productivity and increasing rates of sick leave.

The results of the preliminary study held in one of Public health center showed that there were 557 cases of contact dermatitis per year, the most cases being workers aged 19-60 years. The results of interviews with farmers in said that that almost all farmers did not use Personal Protective Equipment (PPE) while working in the fields properly. Many farmers complained of itching on the feet and hands due to not using PPE such as boots and gloves. The lack use of PPE can increase the risk of being exposed to many chemical agents that can make impaired skin integrity, especially contact dermatitis.

2. Methods

This research was a cross-sectional research design. The sampling technique

used cluster sampling involving 93 respondents. This research was conducted in the working area of one Health Center in Jember Regency, during March until May 2016. Data collection techniques in this study used questionnaires to respondents. Data analysis using univariate and bivariate analysis, the bivariate analysis in the form of chi-square test with confidence interval ρ = <0.001 (α = 0.05). The research ethics applied were Autonomy, confidentially, Anonymity, Justice, and Veracity.

3. Results and Discussion

3.1 Characteristics of Respondents

Table 1. showed that most farmers are male (89/95.7 %), within the education level in elementary school (basic) school education as many as 53 people (57.0%) it can be seen from the table that most of the farmers still have low education levels. Furthermore, family income per month of them mostly less than 500,000 as many as 49 people (52.7%), and only 8 people (8,6 %) who got income as standard that is 1,450,000.

Table 1. Farmers Characteristics (n=93)

Variable	Mean	Med	Min-max
Old (year)	39,48	38,00	26-56
variable	**n**	**%**	
Gender			
a. male	89	95,7	
b. Female	4	4,3	
Total	**93**	**100**	
Education			
a. Elementary	53	57,0	
b. Junior high school	24	25,8	
c. Senior high school	16	17,2	
Total	**93**	**100**	
Family income			
a. Less than 500.000	49	52,7	
b. 500.000-1.450.000	36	38,7	
c. More than 1.450.000	8	8,6	
Total	**93**	**100**	

The results showed that the average age of farmers was 39.48 years, the youngest age was 26 years old and the oldest was 56 years. Age is one of the factors that can describe a person's maturity both physically, psychologically, and socially, thus helping someone in his knowledge. Increasing age will also increase the wisdom of one's ability to make decisions, think rationally, control emotions, and tolerate the views of others, thus influencing their motivation.

The results of the analysis of distribution by sex showed that the majority were male, as many as 89 people (95.7). Gender would affect the occurrence of skin integrity disorders experienced by farmers because male farmers have a higher risk to get skin integrity problems due to their heavier and longer daily activities than female in agriculture land.

The other results showed that most farmers have the last or low level of education at the elementary school level as many as 53 people (57.0. Education can influence health behavior and help prevent disease. Low education can lead to a lack of knowledge of farmers about disorders of skin integrity disorders, so farmers who experience skin integrity disorders do not understand the adverse effects and ways of prevention, and ultimately affect the low usage of PPE to prevent skin integrity disorders.

The income level in farmers was mostly in the range of less than Rp. 500,000 for 49 people (52.7), which is under the Minimum District Wage. According to Friedman (1998) income is part of the family's economic status. Economic status is part of the social class component which refers to the level of family income and family income sources. The low income makes basic needs not met, so farmers prefer to fulfill their family needs first than buy PPE to protect them during work in the land.

Table 2. Distribution of Personal Protective Equipment (PPE) Usage (n = 93)

No.	PPE Usage	n	%
1.	poor	51	49,5
2.	good	42	45,2
	Total	**93**	**100**

3.2 Personal Protective Equipment (PPE) Usage of Farmers

The results from table 2 showed that most farmers did not use PPE as many as 51 people (54.8), and the rest were good at using PPE as many as 42 people (45.2).

The usage of PPE is an important behavior in maintaining the health and safety of farmers. From the results of the study, it was found that the farmers did not know the importance of maintaining health and safety while working. They assumed that the work they were doing was a mild risk and there was no need to fear dangerous things. These were especially in disorders of skin integrity and presumption if there was a disturbance in skin integrity according to the farmers are at risk in working. During interviews with farmers, it was found that farmers did not PPE because they felt uncomfortable, The low usage of PPE is also caused by economic factors, farmers are unable to buy types of PPE.

PPE is a set of tools used by workers to protect part or all of their body from the potential hazards of work accidents. Based on the reality in the field, it appears that workers who use PPE well are still fewer than those who are not good at wearing PPE. This shows that the use of PPE by workers is still not good. There are still many workers who remove PPE while working. If this is done then the skin becomes unprotected and the skin becomes more easily exposed to irritants and allergens.

PPE is a tool used by farmers to protect the exposure that results in the integrity of the skin to farmers. In addition, the use of PPE can support the productivity of farmers, but there are still many farmers who have not used PPE while working on agricultural land.

3.3 Impaired skin integrity in farmers

Impaired skin integrity is a nursing diagnosis that accepted by the North American Nursing Diagnosis Association (NANDA), defined as an alteration in the epidermis and/or dermis. The skin is subject to injury from a variety of external and internal factors. Extremes of heat and cold; pressure, shearing, and other mechanical forces; allergens; chemicals; radiation; and excretions and secretions such as those from an ostomy or a draining wound are all potentially damaging conditions and substances that exist in the external environment. Internal factors include emaciation, drugs, altered circulation and impaired oxygen transport, altered metabolic state, and infections. In Agricultural activities, impaired skin integrity the external factors that

Table 3. Distribution of Impaired skin integrity on farmers

No.	Impaired Skin Integrity	N	%
1.	No	46	49,5
2.	Yes	47	50,5
	Total	**93**	**100**

The results of impaired skin integrity distribution on farmers showed that most of them experienced 47 skin disorders (50.5), and the rest did not experience 46 skin integrity disorders (49.5) (Table 3). Impaired skin integrity is a change in the epidermis and dermis tissue; skin damage occurs due to various external and internal factors. External factors include extreme hot and cold weather, pressure, friction, mechanical equipment, allergens, chemicals, radiation, or all potentially damaging conditions and substances in the external environment. From the results of the study, it was found that the farmers did not know what caused the integrity of the skin disorder was not using PPE. When interviewed farmers, it was found that

farmers did not realize that the cause of skin disorders related to the usage of PPE and due to the lack of interest and willingness of farmers' to use PPE.

Impaired skin integrity is skin disorders characterized by the presence of superficial inflammatory processes in the skin and morphologically changes in acute and chronic dermatitis changes. Efforts that can be done to overcome the problem of skin integrity damage due to work are to do the three main steps. The first step is the introduction or identification of hazards that can arise in the workplace environment; this is done by observation around the workplace environment and the problems perceived by workers. This step is a basic step to determine the next step.

The second stage, evaluation of the work environment is an advanced stage of the identification phase of problems that arise in the workplace environment. This stage is the stage of assessing the characteristics and magnitude of potential hazards that may arise. The final result of this stage can determine priorities in overcoming problems. The third step, control of the conditions in the work environment, the final goal of this stage is to reduce or eliminate exposure to substances or substances that are harmful in the work environment. Final results of this stage can control all exposures of substances or substances that can endanger workers.

After the researcher distributed questionnaires, further analyzing showed the relationship between PPE usage in farmers with the incidence of skin integrity with a percentage of 45.2% at good category. It means the awareness of farmers in the use of PPE is good, while the percentage of not good 54.8% means that more than half of farmers do not have good awareness about the importance of using PPE.

This study assessed several types of PPE that farmers use when working to reduce the risk of skin integrity disorders, namely work clothes that function to protect the body from extreme temperatures, bad weather, splashes of chemicals or molten metals, bursts of leaky pressure, penetration of sharp objects and dust contamination (ILO, 2011). Kurniawidjaja (2012), mentioning that heat stress exceeds adaptation, can cause heat cramp, heat exhaustion, and heat stroke. Skin disorders in the work environment heat stress or cold pressure can arise due to extreme temperature exposure originating from certain equipment or work locations. So that work clothes are needed to reduce the risk of health problems, especially disorders of skin integrity.

3.4 The Association between the Use of Personal Protective Equipment (PPE) and impaired skin integrity in farmers

The data collected to analyze the association between the use of PPE and impaired skin integrity incidence that figured in table 4. It is showed that farmers who are not good at using PPE and do not experience skin integrity disturbances as many as 11 respondents, who are not good at using PPE and experience skin integrity disorders as many as 40 respondents, who use PPE well and did not experience skin integrity disturbances as many as 35 respondents and who used PPE properly and experienced skin integrity disorders as many as 7 respondents.

In the above analysis, it can also be seen that there was a relationship between the use of personal protective equipment (PPE) and skin integrity disorders in farmers (p: 0,01, α 0.05. In addition, it is can also be seen from the value of Odds Ratio (OR) 18,182, which means that farmers who do not use PPE have a greater chance of impaired skin integrity by 18.182 times compared to farmers who use PPE properly. PPE usage reduces the risk of getting skin disorders in farmers. Alfian (2004) said that the habit of changing clothes in farmers that is not good increase the suffering from skin diseases by 88% (p:0.021) that is mean there was a relationship between habits change clothes with skin diseases.

Table 4. The Relationship between Personal Protective Equipment (PPE) Usage and Impaired Skin Integrity

PPE Using	Impaired Skin Integrity				
	No	Yes	Total	*OR*	ρ
Poor	11	40	51	18,18	<0,001
Good	35	7	42		
Total	**46**	**47**	**93**		

Impaired skin integrity is a process caused by dermatitis eczema which morphologically signs and symptoms characterized by the presence of superficial inflammatory processes in the skin and morphologically changes in acute and chronic dermatitis changes are specific and recognizable. Impaired skin integrity is a change in the epidermis and dermis tissue, skin damage occurs due to various external and internal factors [19]. Health problems caused by various factors in work and work environment can be avoided if workers use PPE to prevent it. The proper use of PPE can reduce the occurrence of direct contact through chemical exposure including; pesticides, insecticides, herbicides and also avoid environmental factors such as wind-blown dust containing pesticides.

4. Conclusions

Based on the results it can be concluded that there were more farmers that do not wear PPE than those who wear PPE properly during they work in the land. That behavior increases the risk of getting health problem related to farm activities, especially impaired skin integrity. This study convinces that there is a relationship between the use of PPE and skin integrity disorders in farmers. Therefore, it is important for health profession (nurse) to give education for farmers how to use PPE during their activities in farm and how to prevent health problem that related to agricultural activities through health promotion program, the activities can be established through joint activities and community organizing farmer groups involving farmers that are really working in the fields. For Farmers, it is expected that they have adherence to wearing PPE during doing activities in their fields.

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VALORIZATION OF COFFEE PULP WASTE BY ESTERIFICATION REACTION AS ANTICANCER: QSAR STUDY

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Abstract

Indonesia is the fourth coffee producer in the world. Coffee pulps arise as the biggest waste products (40%-45%) through the production of coffee seeds from coffee fruits. This work mainly focuses on the investigation of potential cancer activity of ferulic acid, a bioactive compound contained in coffee pulp waste. However, the anticancer activity in coffee pulp waste is still weak. One suggestion on how to enhance anticancer activity is structural modification by esterification on ferulic acid. Quantitative Structure and Activity Relationship (QSAR) analysis has been studied for ferulic acid derivatives from esterification products. The research subject is experimental data on average inhibitory levels (IC_{50}) as the dependent variable and the fitting result of charge atom data, dipole moment data, and partition coefficient of four ferulic derivatives. QSAR analysis was obtained from multilinear regression calculations of compounds and showing the effect of dipole moments and logarithm of partition coefficients on anticancer activity. The results are the QSAR equation as follows: Log ($1/IC_{50}$) = 45.367 + 235.555C_2 + 0.111 log P; N = 4; adjusted R^2 = 0.999; sig = 0.014. This work suggests that the esterification reaction is one structural modification that can significantly increase cancer activity on coffee pulp which might contribute to enhancing utilization of this waste in agromedicine sector.

Keywords: Coffee pulp waste, esterification, anticancer activity, ferulic acid derivatives

1. Introduction

Indonesia is the world's fourth-largest coffee producer which the production reached up to 668.70 thousand tons in 2017 [1]. The processing of coffee cherry into coffee beans generates by-product as pulp coffee which is the main residue of this process (40%-45%). Recently, several studies focused on the composition of pulp coffee and their secondary application. The coffee pulp contains phenolic acids like hydroxycinnamic acids. Phenolic acids have been widely reported of their activity as antioxidants [2, 3, 4]. Other application of phenolic acids includes anticancer [5], antiviral [6], antitumor [7], antidiabetic [8], antihypertensive [9], etc. Hydroxycinnamic acid like ferulic acid, caffeic acid, and chlorogenic acid are the natural compound in coffee pulp [10, 11].

Cancer is the second leading cause of mortality worldwide, counting 9.6 million estimated to die from cancer in 2018. Prostate, lung, colorectal, stomach and liver cancer are the most common types of cancer occurred in men. Besides, breast, colorectal, lung, cervix and thyroid cancer are the most common types among women [12]. Ferulic acid has anticancer activity. Ferulic acid scavenges the free radical, regulates cell growth and proliferation and inhibits cytotoxic systems [13].

Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) is biosynthesized from amino acid phenylalanine through the shikmic acid pathway. Ferulic acid has shallow anticancer activity. Ferulic acids possess free carboxylic acid with gastric irritation as an inevitable adverse effect when consumed orally. So, its ester derivatives of ferulic acid are favorable in used as drugs. Modification of carboxylic group with different alkyl group of ferulic acid affected the anticancer activity [16].

Moreover, the value of an anticancer activity is an important parameter in drug design. The anticancer activity might measure both experimental and theoretical. In this research, we study about Quantitative structure-activity relationship (QSAR) of four ferulic ester derivatives to establish the relationship between structural characteristics of the molecule and its properties.

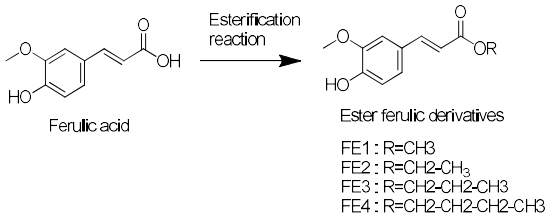

Figure 1. Synthesis mechanism of ferulic derivatives [16]

2. Methods

2.1 Research Material

The research materials are four ferulic acid derivative compounds as shown in Table 1, with secondary data are average inhibitory level (IC_{50}) data from an analog series of structures and activities [14, 15]. The mechanism for the reaction of ferulic acid derivatives formation is shown in Figure 1.

2.2 Equipment

This research used Intel (R) Celeron (C) processor with 204 MB RAM as computer hardware. Hyperchem 7.02 computational chemistry software for computational chemical calculations and SPSS 16.020 for statistical analysis, and Chemdraw Ultra 8.0 to create 2D molecular structures [14].

Table 1. The physical properties and anticancer activity of ferulic ester compounds, FE [16]

Compound	R'	Yield %	IC_{50}	Log ($1/IC_{50}$)
FE1	$-CH_3$	89	92	-1.96
FE2	$-CH_2CH_3$	85	70	-1.85
FE3	$-CH_2CH_2CH3$	75	64	-1.81
FE4	$-CH_2CH_2CH_2CH3$	78	61	-1.79

2.3 Research Procedure

a. Collection of Descriptors

Descriptor determination is important for determining the best QSAR equation. Descriptors used in this study are the atomic charge, dipole moment (μ) and the logarithm of the partition coefficient (log P). The data can be seen in Table 2. For electronic descriptors, calculations are carried out covering the stages of computational chemistry modeling with the geometry optimization procedure of each compound structure. Each compound used in this study was made into a two-dimensional structure model using the Chemdraw Ultra 8.0 application. After that, the model is equipped with hydrogen atoms on each atom and is formed into a three-dimensional (3D) structure with the Build (Add H and Model Build) menu [14].

Table 2. List of descriptors and how to optimize them

No	Symbol	Descriptor	Unit	Calculation Method
1	qC_1, qC_2, qC_3, qC_4, qC_5, qC_{12}	The atomic charge of C_1, C_2, C_3, C_4, and C_{12}	Coulomb	Semiempirical method of PM3, Hyperchem, compound optimization
2	μ	dipole moment	Debye	Semiempirical method of PM3, Hyperchem, compound optimization
3	Log P	Partition coefficient of n-octanol/ water	-	QSAR Properties, Semiempirical method of PM3, Hyperchem, compound optimization

The next process is to perform structural geometry optimization by minimizing molecular energy to obtain the most stable molecular conformation. The calculation used the semiempirical method of PM3 with the convergence limit was 0.001 kcal / Å.mol. The optimization method was based on the Polak-Ribiere algorithm. The data obtained were atomic net charge and dipole moment. The atomic charge is chosen only at atoms adjacent to the esterification target group (C_1, C_2, C_3, C_4, and C_{12}) (Figure 2) [14].

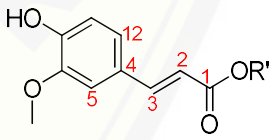

Figure 2. The structure of esterified ferulic

Log P descriptor is also used as a representation of the compounds lipofility in this study. The descriptor value is obtained from the QSAR properties calculation in the Hyperchem program. Log P calculation is determined theoretically based on a fragmental approach [14].

b. QSAR Study

The best QSAR equation used to predict IC_{50} was determined by multilinear regression statistical analysis. The analysis was performed using SPSS application with the backward method. In this study, the original data using four fitting compounds were optimized by select the independent and dependent variables that influence the QSAR equation. Log (1 / IC_{50}) data was set as an independent variable and combination value between dipole moment, atomic charge, and partition coefficient as independent variables. In general, the final relationship of the QSAR approach is expressed by the regression equation as follows:

$$\text{Log } (1/IC_{50}) = k_1 \log P + k_2 qC_1 + k_3 qC_2 + k_4 qC_3 + k_5 qC_4 + k_6 qC_5 + k_7 qC_{12} + k_8 \mu + k_9$$

Multilinear regression analysis is performed on the output data by statistical parameters as correlation coefficient r, r2, standard deviation (SE) and significance value. The model is chosen based on the consideration of the statistical results obtained [14].

c. Determination of the final QSAR equation

Determination of the final equation is obtained from the combination of independent variables involved in the best model obtained from regression analysis and testing on the data of fitting compounds. Regression analysis was carried out on a total of 4 ferulic acid derivative compounds using the SPSS program with the enter method, which uses the independent variables that have been selected based on the previous analysis [14].

3. Result and Discussion

3.1 The result of descriptors calculation

This research was performed by PM3 semi-empirical method for optimizing the structure of four ferulic derivatives. The four compounds are FE1 which attached methyl group on carboxylic moiety in ferulic structure. FE2 has ethyl group attached in carboxylic moiety and FE3 has n-propyl group bind to the carboxylic moiety in the ferulic structure. Besides, incorporating n-butyl group on carboxylic moiety in ferulic structure named as FE4 which is shown in Figure 1.

QSAR between dependent variable cytotoxicity data (see Table 1) and eight independent descriptors was studied in this research. The cytotoxicity ferulic esters were expressed as IC_{50} (µg/ml) which is the concentration of the compound that inhibited proliferation rate of the Hela (Cervical Cancer Cell Lines) by 50% as compared to the control untreated cells which were reported on the previous research [16]. The descriptors are the atomic net charge, dipole, and log P (coefficient partition n-octanol/water). We analyze the atomic net charge of carbon number 1, 2, 3, 4, 5, 12 which have high probability affect in the different attachment of alkyl group. The position of the carbon is shown in Figure 2. Atomic net charge is determined by comparing the number of the proton (positive charge) and electron (negative charge) in an atom. If an atom gains electron then it has more electrons, which give it a negative charge, and vice versa. Atomic charge indicates the density of electron in an atom. Dipole describes the polarity of molecules. Besides, log P represents hydrophobicity properties of a molecule.

The result of calculation of atomic net charge, dipole, and log P of four ester ferulic derivatives are shown in Table 3. Substitution of a different chain of alkyl group influenced the carbon net charge. The net atomic charge of C_1 in ester ferulic derivatives slightly decreased in relative to that of atomic charge of C_1 in ferulic acid. This tendency is similar for net atomic charge of C_2, C_3, C_5, and C_{12}. This indicates incorporating different alkyl chain can induce atoms in the adjacent position. Furthermore, the dipole of ferulic ester declined compare to a dipole of ferulic acid. Substitution of alkyl enhanced the value of log P. The longer carbon chain

establishes more non-polar compounds, so the solubility of the compound in lipid is greater. Ferulic acid has a lower log P of -0.63. On the other hand, FE4 which incorporating butyl has higher log P of 0.61.

3.2 Analysis of QSAR

The relationship between chemical structure and biological activity (cytotoxicity) was conducted by statistical calculation using SPSS program. The analytical data used eight dependent variables (C_1, C_2, C_3, C_4, C_5, C_{12}, atomic net charge, dipole, log P). Besides, the dependent variable is log ($1/IC_{50}$). The multilinear regression was employed to correlate between biological activity and physical chemistry parameter. The validity of linear equation in QSAR Hansch model was determined by R, R^2, F, and SE. Based on the calculation, the best correlation is presented in Table 4.

Table 3. Descriptor data of atomic net charge, dipole, and log P as independent variables

No	Compound	Atomic Net Charge (Coulomb)						Dipole (deybe)	Log P
		C_1	C_2	C_3	C_4	C_5	C_{12}		
1	FE1	0.410535	-0.20065	0.004779	-0.08484	-0.097802	-0.071531	4.3340	-0.60
2	FE2	0.414901	-0.20032	0.003293	-0.08413	-0.09809	-0.07191	4.3540	-0.25
3	FE3	0.415019	-0.20036	0.003415	-0.08422	-0.09804	-0.07189	4.3530	0.21
4	FE4	0.414959	-0.20047	0.003572	-0.08425	-0.09805	-0.07185	4.3560	0.61
5	Ferulic acid	0.419850	-0.19856	0.011135	-0.087716	-0.096573	-0.069798	4.491	-0.63

The descriptors which have a strong correlation with cytotoxicity are log P and the net charge in C_2. The prolongation of alkyl group might increase nonpolar properties followed by enhancing of log P value. SE is standard error of the estimated which explain error value about the calculation. It is seen that the SE value of this calculation is small. Because of using multi-regression, we used adjusted R^2 which has a value of 0.999. This value represents that the calculation result can explain the descriptors of the response data around its mean. It is seen that the equation is excluded of several descriptors which are qC_1, qC_3, qC_4, qC_5, qC_{12}, and dipole. This doesn't indicate that those descriptors don't influence the cytotoxicity.

Table 4. The result of the best correlation between cytotoxicity and descriptors

Equation	Y=0.111LogP+4.979 C_2+45.367
R	1
adjusted R^2	0.999
SE	0.00190116
Sig	0.14
F	2.638E3

However, the influences of those descriptors are lower than those of log P and C_2. The prolongation of the alkyl chain of ferulic derivatives showed higher log P followed by higher cytotoxicity activity. This work suggests esterification with long alkyl chain is favorable to give higher cytotoxicity activity of ferulic derivatives.

Table 5 showed that the residual errors between the experimental log ($1/IC_{50}$) and calculated Log ($1/IC_{50}$) were so low. We were employed PRESS (predicted residual error sum of square) as cross-validation of this calculation. PRESS statistic is calculated as the sum of the squares of all the resulting prediction errors. The calculation Log ($1/IC_{50}$) has lowest PRESS value that indicates the calculation of calculated log ($1/IC_{50}$) using QSAR Hansch Model for ester ferulic derivatives has excellent agreement with experimental data of Log ($1/IC_{50}$).

Table 5. Experimental Log ($1/IC_{50}$), Calculated Log ($1/IC_{50}$), and PRESS Value

Experimental Log ($1/IC_{50}$)	Calculated Log ($1/IC_{50}$)	Residual error	[Residual error]^2
-1.96379	-1.96395	0.00015848	2.51152E-08
-1.8451	-1.84642	0.00132290	1.75005E-06
-1.80618	-1.80525	-0.00092573	8.56974E-07
-1.78533	-1.78677	0.00143546	2.06055E-06
	PRESS		4.69269E-06

4. Conclusion

Ferulic derivatives have been analyzed experimentally to produce ester compounds with their anticancer activity. The C_2 atomic charge and partition coefficient has been estimated and shown an influence on the activity of compounds as anticancer for ferulic acid derivatives. The best QSAR equation for ferulic acid derivative compounds is: Log ($1/IC_{50}$) = $45.367 + 235.555C_2 + 0.111$ log P; N = 4; adjusted $R^2 = 0.999$; sig = 0.014.

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POTENCY WET-THERAPY REDUCE APO-B AND TOTAL CHOLESTEROL IN HYPERCHOLESTEROLEMIA PATIENTS

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Abstract

Hypercholesterolemia is a high level of cholesterol in the blood. Patients must take anti-cholesterol drugs for a long time, so they are at risk of experiencing side effects from the drug. Apo-B and total cholesterol are indicators of cholesterol levels in the blood. Wet cupping therapy is a method of excreting metabolic waste in the blood through the surface of the skin. The study aims to prove the potential of wet cupping therapy as a complementary therapy to reduce Apo-B and total cholesterol. Method: This research is Quasy experimental research using humans as research subjects. The dependent variable is Apo-B, and total cholesterol gave wet cupping treatment. Cupping is done twice, 7 points, using a G21 needle. A large sample of 32 people with hypercholesterolemia divided into treatment groups and control groups. Apo-B measurement using ELISA sandwich method, Elabscience® reagent, in units of ng/ml. Total cholesterol uses the enzymatic colorimetry method, Diasys® reagent, in mg/dl units. Data analysis was carried out with the Wilcoxon Signed Ranks Test with a significance level of 5% ($\alpha = 0.05$), the pre-data compared with the post data. Results: A significant reduction in Apo-B measurements with p-value 0.000 ($\alpha < 0.05$), SD 42. A significant reduction also occurred in the total cholesterol group. Obtained p-value 0.005 ($\alpha < 0.05$) SD 0.23. Conclusion: Intervention of wet cupping therapy can reduce Apo-B levels and total cholesterol in the blood. Further research needs to be done to measure the potential for prevention of atherosclerosis.

Keywords: wet cupping, Apo-B, total cholesterol, blood

1.Introduction

Cupping has been used in medicine since ancient times. Even Hippocrates uses cupping in cases of internal disease [1]. The duration of this cupping history proves that cupping done correctly is safe and effective. There is a misperception in interpreting wet cupping. The needle depth of the skin is only 0.05mm. The wound with the needle does not cause blood to bleed. New blood comes out after being withdrawn with a 200mmHg negative power pump [2]. Cupping is not an act of removing blood but removing metabolic waste called causative pathological substances [3]. In other words, wet cupping does not reduce circulating blood volume. The blood coming out of the wound is "bloodlike" which trashes cholesterol metabolism, old erythrocytes, etc. The amount of cupping blood done correctly does not reduce hemoglobin [4]. The study aims to prove the potential of wet cupping therapy as a complementary therapy to reduce Apo-B and total cholesterol.

Cholesterol is present in tissues and plasma lipoproteins in the form of free cholesterol or a combination of long chain fatty acids as cholesterol esters. Cholesterol is synthesized in many tissues from acetyl co-A and is removed from the body in the bile as a cholesterol salt. Cholesterol esters are a form of cholesterol storage in almost all body tissues. The main source of cholesterol comes from the synthesis in the body itself, namely endogenous cholesterol and from foods known as exogenous cholesterol. Acetyl CoA is the source of all carbon atoms in cholesterol [5].

Cholesterol is not soluble in blood fluids, for it to be sent to the whole body needs to be packaged with proteins into particles called lipoproteins, which can be considered as carriers of cholesterol in the blood. The main proteins that

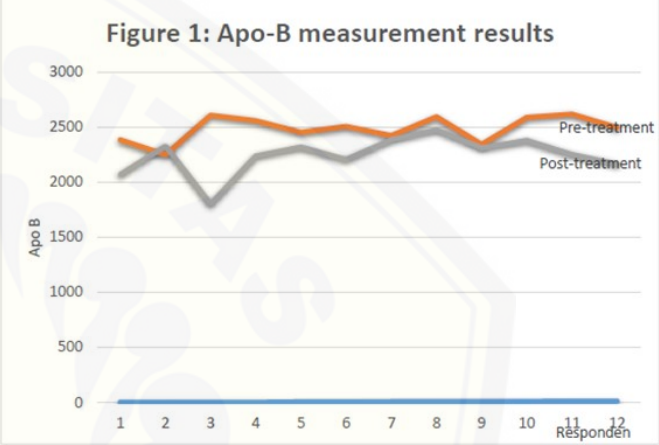
makeup LDL are Apo-B (Apolipoprotein- B) [6]. In contrast, HDL in its operation clears excess cholesterol from the walls of blood vessels by transporting it back to the liver. The main protein that forms HDL is Apo-a (Apolipoprotein-A). The involvement of HDL cholesterol in reserve cholesterol transport is a mechanism to protect the endothelium against the risk of atherosclerosis. HDL has anti-inflammatory, antioxidant, antithrombotic properties. HDL is also antiatherogenic [7].

2. Methods

This research is Quasy experimental research using humans as research subjects. The independent variables were wet cupping therapy, seven coats in the back area, negative pump 5 minutes then wound with a G21 needle as many as 15 punctures with a depth of 0.05mm. The dependent variable is Apo-B and total cholesterol. Measurements are carried out twice, pre and post. A large sample of 33 people with hypercholesterolemia was divided into treatment groups and control groups. The research subjects were selected based on sample inclusion criteria, aged 45-55 years, not suffering from chronic diseases, total cholesterol >200mg. After 12 hours of fasting and still taking statin anti-cholesterol drugs, blood was taken through 5ml of the brachial vein. Put into a 2ml purple tube containing EDTA the rest was inserted in a red container. Apo-B measurement using ELISA sandwich method, Elabscience® reagent, Biopharma ELISA reader tool, in units of ng/ml. Total cholesterol uses the enzymatic colorimetry method, Diasys® reagent, Biolyzer100 spectrophotometry, in mg/dl units. Data analysis was carried out with the Wilcoxon Signed Ranks Test with a significance level of 5% ($\alpha = 0.05$), the pre-data was compared with the post data. The study was conducted at the Biochemistry Laboratory of the Faculty of Medicine, University of Jember. The research ethics test was obtained from the University of Jember Ethics Committee in December 2017

3. Results And Discussion

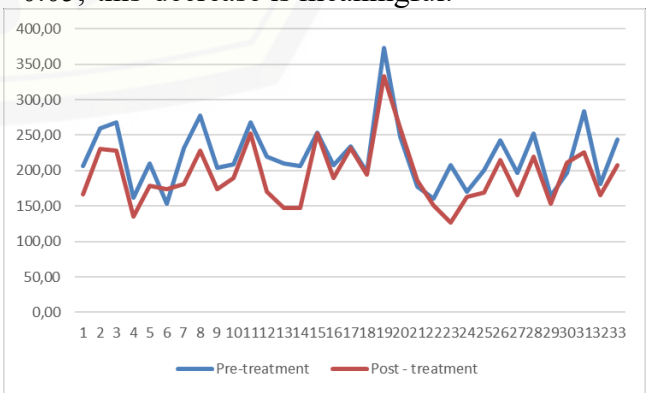
Results: measurement of pre-Apo-B data in 12 study subjects obtained mean 2.49 SD 0.117. The post data obtained mean 2.24 SD 0.177. The Wilcoxon Signed Ranks Test was obtained p-value 0.005, because p-value <0.05, this decrease was significant.



Source: 2018 primary data

Figure 1: Apo-B measurement results pre and post, n = 12 people

Results: measurement of total pre-cholesterol data in 33 study subjects obtained mean 226.6 min 153.3 max 373.33. The post data obtained mean 199.3 min 126.6 max 333.3. Wilcoxon Signed Rank Tests obtained p-value 0.000, Cor 0.772. Because p-value <0.05, this decrease is meaningful.



Source: 2018 primary data

Figure 2: Results of the measurement of total cholesterol, pre, and post, n = 33 people

This measurement is by research conducted by Saryono [8], Mustafa et al. [9], Niasari, et al. [7] that cupping can reduce cholesterol levels. As a result of keratinocyte clotting in the skin will experience hypoxia and induce hypoxia-inducible factor (HIF-1α) as an effort to self-defense (Ontoseno, 2004). HIF-1α activates macrophages in the skin which then produces proinflammatory genes such as IL-1. IL-4, IL-6, and TNF-α [2]. Interleukin-6 secreted by macrophages acts to stimulate the immune response, for example after trauma or tissue damage that leads to inflammation. The release of IL-6 stimulates young macrophage cells to mature and be able to do phagocytosis more efficiently. IL-6 also stimulates monocytes to produce inflammatory cytokines that play a role in local and systemic inflammation, resulting in accelerated proliferation and differentiation of macrophages [10].

LDL (low-density lipoprotein) is a source of cholesterol for an extrahepatic tissue. If LDL is very excessive, the LDL uptake system will be saturated so macrophages can take that excess LDL. Macrophages capture some LDL cholesterol before it is oxidized. The more LDL cholesterol levels in the plasma, the more macrophage cells will be achieved. Furthermore, macrophages will experience efflux, and nascent HDL will approach the macrophage to take LDL cholesterol.

Furthermore, the nascent HDL becomes adult HDL. After taking free cholesterol from macrophage cells, free cholesterol will be esterified to cholesterol ester by the enzyme Lecithin Cholesterol Acyl Transferase (LCAT). So HDL here functions as an absorbent of LDL cholesterol from macrophages and as a carrier of LDL cholesterol back to the liver so that cholesterol levels in the plasma decrease [11].

According to El-Sayed, et al., [3], cupping is a minor excretory surgical procedure that has a medical and scientific basis in cleansing the blood and interstitial spaces of causative pathological substances (CPS) cholesterol as the production of metabolic waste. Many research results report that cupping can reduce LDL cholesterol. HDL cholesterol functions as an absorbent of LDL cholesterol from macrophages and as a carrier of LDL cholesterol back to the liver with the help of pre-HDL [12]. Pre β-HDL has a role in the process of transporting back cholesterol (reverse cholesterol transport) which can increase the excess cholesterol efflux from the peripheral tissue back to the liver to be excreted through bile. The acceleration of macrophage migration also increases due to IL-6 stimulation [6].

Wet cupping treatment is a non-infectious inflammatory reaction that stimulates the release of chemical mediators including IL-1, IFN-γ, IL-6, IL-8, IL-18 which will activate macrophages so that cholesterol efflux occurs. Wet cupping treatment will enable LCAT (Lecithin Cholesterol Asil Transferase) which converts HDL to HDL3. Cholesterol binds to HDL3 to be carried to the liver and is formed as bile acids which will then be excreted through the intestine [13]. Through this process, the cholesterol in the circulation will decrease to be expelled through the gut [11].

4. Conclusion

The intervention of wet cupping therapy has the potential to reduce Apo-B levels and total cholesterol in the blood. Wet cupping therapy can be considered as an intervention that can lower cholesterol, in addition to the use of anti-cholesterol drugs. Further research needs to be done to measure the potential for prevention of atherosclerosis.

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PRODUCTION POTENTIAL PHARMACEUTICAL VARIETY OF SUGARCANE WITH MICROSPORE CULTURE

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Abstract

Sugarcane is popular crops that have a high economic value to product sugar and bioethanol. In addition to these products, it turns out that sugar cane plants can also be used as herbal remedies in the healing of the urinary system and some liver diseases. Based on the increasing need for sugar cane and the benefits contained in sugarcane, it is necessary to develop new varieties that have potential pharmaceutical variety. Microspore culture techniques can produce pure homozygous lines in one generation so that selection of superior varieties is easier. This study used a completely randomized design with microspore culture donor plants of sugarcane varieties isolated at different storage temperatures. The results of Kruskal-Wallis data analysis showed that the variation of microspore culture storage temperature had a significant effect of 0.000 on the number of microspore sugarcane embryogenesis. The low-temperature storage 4^0C produces 10 Callus-like, while the room temperature storage 25^0C can produce as many as 300 Embryos-like.

***Keyword**: embryogenesis, microspore culture, sugarcane*

1. Introduction

Sugarcane species (*Saccharum officinarum* Linn.) is the poace family that has potential crops value. This plant is widely found in tropical and sub tropical regions [3] .This plant is one of important crops around the world. Statistic show that 80 % of the world's sugar commodities come from sugarcane [9]. Sugarcane plants are widely cultivated in Indonesia and its reached 445.520 ha in 2016 with a productivity approximately 360713 tons/year [7]. Indonesia is a tropical country that is suitable in developing sugarcane. An increase in Indonesian sugar production is expected to improve the country's economy.

In general, sugarcane plants are used for sugar production dan bioenergy/bioethanol [17]. Sugarcane also has other potential in the health pharmaceutical aspect. Sugarcane is widely used as sugarcane juice. Sugarcane juice is actually known as a raw material for the production of refined sugar. And the other is in the processing sugarcane juice, secondary products are also produced in the form of brown sugar, molasses, and jaggery [12]. Sugar cane juice is very popular in India. This drink is used in traditional medicine systems for the healthy treatment such as urinary deseases (hemorrhage, dysuria, anuria etc.) [8]. Based on the research [10], Sugar juice can cure jaundice and liver disorders. Several sugarcane varieties tested have antioxidant aspects and also protect DNA damage so that it can counteract free radicals, reduce iron complexes and inhibit lipid peroxidation.

The use of sugar cane as a medicinal beverage has not been developed. currently, the development of sugarcane varieties only leads to the amount of sucrose content to be processed into sugar. In addition, conventional sugarcane breeding takes a long time. Plant breeding programs for the selection of genotypic varieties that have potential as medicines have been carried out [1] . The conventional selection of genotypes takes a long time. The selection was done in the form of leaf blade length, leaf width, fresh leaf weight, dry leaf weight, number of tillers, millable sugar cane, shoot size, sugarcane diameter, number of segments, segment length, plant height, long stem, brix% and sugarcane weight individuals. The results of this study indicate that sugarcane genotypes have high variability and need further research to obtain higher sugarcane yields due to juice and brix content.

Chance the selection of sugarcane varieties with microspore culture gives great hope for the development of sugarcane varieties which have the potential pharmaceutical. Based on the data above, it is necessary to develop sugarcane varieties that can be used as pharmaceutical product.

Biotechnology Sugarcane breeding has been developed in Indonesia. Biotechnology offers plant breeding techniques quickly [14] . One of the modern plant breeding techniques that have been widely applied to plantation crops is microspore culture. Microspore culture techniques are able to produce haploid and even double-haploids. With pure line varieties, selection of varieties will be easier because that are completely homogenous and homozygous in one generation [2] . So that in this study microspore culture techniques were carried out production potential pharmaceutical variety of sugarcane with microspore culture.

2. Material and Method

2.1 Plant material preparation

This research used Bululawang variety as donor plants of sugarcane microspore culture. The anther was collect after sugarcane flowering at 8-10 months. *panicle selection based on shapes that* still covered with flags leafs. Panicle harvested wrapped in newspaper and then stored in growth camber for 24 hours.

2.2 Microspore isolation

The first technique is pre pre treatment stress for anther. panicle is opened inside the laminar airflow to remove anther. Anther selection based on anther color, specifically yellow anther and then stored in a period of time 7 days before isolation of microspore culture. The isolation microspore culture began with 200 pounding anther which were slowly mixed with a mortar and stamfer in the mannitol 0.3M medium. if the microspores have appeared out then filtered using a 100 µm filter. suspended filtration was centrifuged using 10 ml mannitol medium as much as 10 ml by 4^0C cold temperature centrifugation at 750 rpm for 5 minutes. After that the pellet produced was transferred in a 4 ml petri dish containing MS medium at a density of 3×10^4 microspores per petridish. each petridish is coated with parafilm to prevent contamination. Isolation results are stored at different storage temperature variations 25^0C and 4^0C in dark conditions. data observed after 4 weeks. And data was analized by Kruskal-Wallis Test in SPPS aplications.

3. Result and Discussion

The application of microspore culture techniques to sugarcane plants has a very big challenge. this is because there is no literature on the success of microspore culture sugarcane techniques into perfect plants. The data reported in this study is the initial data on optimization of micropore culture techniques. Embryonic development will then be reported in the next article.

Based on observations made for 30 day culture, it was found that different storage temperatures could produce variations in embryo shape. at low temperatures 4^0C produced the form of the callus likes and at room temperature storage 25^0C produced *embryos-like* (Table 1).

Table1. Average Number of embryonic microspores based on storage temperature

	S1	S2	Sign
Number of embryos	10	598	0.000
Type of embryo	*Callus-like*	*Embryos-like*	0.000

** S(storage temperature): S1= 4^0C, S2 = 25^0C)*

The number of embryos produced is also very different. room temperature storage 25^0C can produce as many as 300 *Embryos-like*, while low temperature storage produces *Callus-like* (Figure 1).

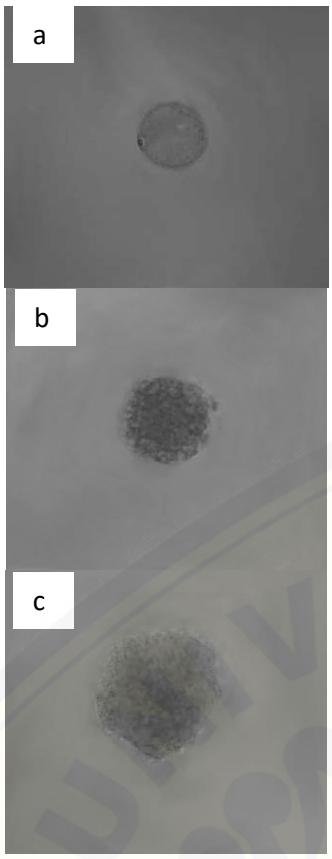

Figure 1. Microspore embryogenesis a. microspore b. *embrio-like* c. *callus-like*

There are several factors that can influence microspore culture such as genotype, stress treatments, culture media, microspore density and developmental stage in microspores [5]. Embryogenesis process in microspore culture is a unique system in which microspore cells are programmed specifically with stress treatment towards the embryogenesis pathway. stress treatment can be in the form of variations in incubation temperature to trigger embryogenesis [11]. The statement is in line with research Suaib *et al* [13] which states that the stress of temperature treatment can trigger embryogenesis. Microspore culture techniques in this study used stress pre-treatment on anther before isolation of microspore culture. Selected anther was stored in mannitol 0.3M solution for 7 days at 4^0C. At the other family poace (wheat) isolation, mannitol is effective in triggering microspore embryogenesis. Isolation of microspore culture of sugarcane in this study uses two different storage temperature variations to determine the temperature that is suitable to produce high embryogenesis [4].

Low temperature for storage of isolation results of microspore culture has not been developed much. Low temperature between 1-5^0C is developed as a stress treatment at anther culture in wheat (as in some other species) [18]. In this study the low temperature of 4^0C which was applied as the storage temperature of culture results had an effect on embryogenesis. but the number of embryos produced just 10 embryos average and callus-like shaped. Storage of isolation of microspore culture at 25^0C room temperature is more effective in increasing the number of embryos-like. Storage at room temperature 25^0C in isolation of sugarcane microspore culture produces 589 average embryos. This technique is widely applied to other plants such as brassica [15] and has been shown to successfully increase embryogenesis. Microspores have the ability to develop into haploid plants using in vitro culture by changing pathways to sporophytes by preventing the development of pollen (gametophytic pathways). This sporophytic process is carried out with stress treatment in microspores so that embryogenesis. The resulting embryo is truly homozygous making it easier to study plant breeding and variety selection [16]. So that in this study the treatment of microspore isolation was effective in producing sugarcane embryos.

4. Conclusion

Application of microspore culture techniques on sugar cane effectively produces embryos. the next stage is the regeneration of plants to become green plants so that the selection of potential sugarcane varieties in pharmacy can be done in a fast time.

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PRETREATMENT OF RICE HUSK FOR BUTANOL PRODUCTION AS THE INTERMEDIATE OF DRUG BIOPLASTICIZER

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Abstract

The growing population with higher life quality concern, disease prevention and treatment increased the needs for drugs and supplements. The digestible plasticizer, dibutyl sebacate, as the storage and delivery for the functional compounds are currently derived from petroleum. However, the depletion of fossil resources and environmental awareness has triggered the development of green chemistry. Research related to biomass waste valorization has gained the attention of researchers to get higher economic value. Rice is the largest crop in the world and generated large quantities of waste. Rice husk is a by-product of the rice milling process, and the main chemical constituents of rice husks are cellulose, lignin, and inorganic residues. Cellulose as the main constituents of rice husks (RH) could be utilized for biobutanol production, as the intermediate for dibutyl sebacate manufacture, employing *Clostridium* bacteria. High lignin content extended the challenges for the utilization of rice husk, which inhibits both enzyme activity to hydrolyze the cellulose and strain activity to produce butanol. Specific pretreatment process is necessary to prevent the formation of inhibitor from the lignin degradation. This article studied the pretreatment to dilute lignin and hemicellulose and its effects to the hydrolysis of cellulose into sugars and fermentation of sugars to butanol. Pretreatment with steam explosion provided highest sugar yield based on raw material and enzyme activity. As a result of low inhibitors concentration, a high concentration of biobutanol was produced from simulated glucose mixture.

Keywords: rice husk, biobutanol fermentation, pretreatment, hydrolysis

1. Introduction

Dibutyl sebacate is plasticizer and mainly used for pharmaceutical applications, plastics in medical devices, and food packaging industry. Dibutyl sebacate can be synthesized by reacting sebacic acid or sebacoyl chloride and butanol. Most of the hydrocarbon compounds including butanol are derived from petroleum cracking. However, depletion of fossil resources, the rising cost of oil, environmental awareness diverts the attention of researchers to utilize biomass instead. Research related to biomass waste valorization has gained the attention of researchers. The term "waste" comprises all organic material besides the primary material for which the plants were initially planted (e.g., rice husk from rice, a cocoa shell from cocoa, coffee grounds from coffee, etc.) Industrial food and agricultural processing have produced many wastes, four functional constituents derived from waste are polysaccharides, lignocellulosic, triglycerides, and proteins [1].

Lignocellulosic resources can be processed further to produce bioethanol, biobutanol, biodiesel, etc. Rice milling process produces two waste products, rice straw and husk, which always disposed of by burning. This has negative impacts on air quality and contributes to global warming and air pollution. Hence, two waste products could be utilized to gain more value, for example, conversion to biobutanol [2].

Rice husk was removed in the rice milling process and generated large quantities of waste. Conversion of lignocellulosic materials to biobutanol involve three processes: pre-treatment to solubilize lignin and hemicellulose, enzymatic hydrolysis of cellulose into fermentable sugars, and fermentation of sugars into biobutanol employing

Clostridium bacteria. Pre-treatment aims to provide enzymes gaining easy access to cellulose for the next step and optimizing enzymatic hydrolysis and fermentation process [3].

Different methods of pre-treatment will be discussed in this paper. Calculation about sugar yield per raw material and enzyme activity were evaluated in each pretreatment process. Biobutanol fermentation using simulated concentrated hydrolysate was also studied.

2.Methods

Acid or alkaline Pretreatment

Rice husk was collected from the local rice milling process. Moisture content must be reduced before the further process; the rice husk was oven dried at 50^{O}C For 24 h. The rice husk was screened to obtain uniform particle size ranged 2 mm with a sieve shaker. The solid was stored in tightly sealed plastic before continuing next process. A 5 g of rice husk was mixed with 95 ml of 0,5-1,0% (v/v) sulfuric acid or alkaline solution in a 250ml flask with a stopple and then autoclaved at 121^{O}C, 15 psi for 15-45 min. The solid residue was separated by Whatman filter paper and washed with distilled water until neutral pH. The sample was air dried and stored in tightly sealed plastic at the refrigerator. The difference between acid and alkaline pretreatment process is the soaking solution. The soaking solution of alkaline pretreatment is natrium hydroxide 1-3% (w/v) [4].

Steam explosion pretreatment

Moisture content was determined to be 9,98%; biomass was fumigated and air-dried at a temperature 34^{O}C, 84% RH. Rice husk was steam exploded into hot water (70-80^{O}C) using a cambium Steam Explosion with 35 mL reactor. The reactor was charged with 500 g of feedstock, sealed and heated with steam to the desired temperature (180-230^{O}C) for 10 min. The content of the heating chamber was exploded into 3.5 L of hot water. The slurry was collected and separated into solid and liquid phases by centrifuging through 100 m nylon mesh. The solid residue was washed to remove any water-soluble material. The samples were stored at -40^{O}C and freeze-dried before the further process [5].

Fungal pretreatment and hydrolysis

Substrates were hydrolyzed with *P. ostreatus* And the hydrolysis process terminated weekly (for the eight weeks) by autoclaving. The hydrolysate was used as substrates for the second stage hydrolysis using the *A. niger*. 10 mL of the *A. niger* spore suspension was poured onto the substrates in each flask and corked. These were subsequently incubated at 28 °C for five days. The incubation period was kept as a constant since it would be used for comparative analysis [6].

Lime pretreatment

Rice husk was milled, then mixed with lime in the composition of 15.0% w/v and 1.5% w/v respectively to form a slurry with water. The slurry was autoclaved at 121 °C for one h. The acidity of lime-pretreated rice husk was adjusted to the pH of 5.0 with concentrated HCl before enzymatic hydrolysis [7].

Microwave

Rice husk was pre-treated by using a BIOTAGE R Initiator + reactor (Biotage AB, Box 8, 751 03, Uppsala, Sweden). Milled husks (0.75 mg for each tube) had been added separately into 20 of 25 mL microwave pressure tubes (containing 14.25mL distilled water individually) to give a 5% (w/w) suspension. The tubes were then capped and treated at pre-designed pretreatment severities [8].

Enzymatic hydrolysis

The saccharification of the pretreated rice hulls with enzymes (commercial cellulase and *â*-glucosidase preparations, unless otherwise stated) was performed by shaking slowly at 100 rpm at 45 °C

and pH 5.0 for 72 h. All enzymes used in this study were used at a dose of 2 mL/100 g of rice hull unless otherwise stated. Samples (0.5 mL) were withdrawn and kept at -20 °C for analysis by HPLC [7].

Calculation method

The yield of sugar based on the enzyme activity. The amount of sugar produced in gram was divided with the enzyme activity used in the saccharification in FPU to calculate the sugar product per enzyme activity (g/FPU).

$$Sugar\ yield\ per\ enzyme\ activity = \frac{Mass\ of\ sugar\ produced}{Enzyme\ activity}$$

Biobutanol Fermentation

One milliliter of a spore suspension of *C. saccharoperbutylacetonicum* N1-4 was transferred to 9 mL of fresh PG medium (10%(v/v) inoculation) in a test tube. The broth was heat-shocked in a 100 ^{o}C water bath for 1 min, and then refreshed at 30^{o}C for 24 h anaerobically using Anaeropack. The refreshed culture broth was then inoculated into tryptone-yeast extract-acetate (TYA) medium containing 3 g/L CH3COONH4, 0.3 g/L MgSO4.7H2O, 2 g/L Bacto yeast extract, 6 g/L Bacto tryptone, 0.01 g/L FeSO4.7H2O, 0.5 g/L KH2PO4, and 20 g/L glucose at 10% (v/v) inoculum. After inoculation, the culture broth was incubated anaerobically for 15 h at 30^{o}C using an Anaeropack and then used as a seed culture. Tryptone-yeast extract (TY) medium containing 2.57 g/L (NH4)2SO4 instead of CH3COONH4 was used in main cultures. Three grams per liter of CaCO3 was added to TY medium to avoid drastic pH reduction. Mixed sugars containing 30 g/L glucose and 30 g/L xylose for the batch culture after preculture with glucose (G- G30X30) was used in the main culture and fermented for 72 h in 30^{o}C.

3. Results

Characterization of rice husk

The structure of raw rice husk is globular, and the surface is relatively nonporous (Ganieva, 2008). The chemical constituents of rice husks are cellulose, hemicellulose, and lignin. Table 1 showed the chemical analysis of rice husks which mostly contain silica and carbon. Compounds containing carbon can be converted into bio-based chemicals such as biofuels, biodiesel, biobutanol, etc.

Table 1. Chemical analysis of rice husks

Constituents	Percent
Carbon	66.7
SiO_2	22.3
H_2O	7.1
Al_2O_3	0.82
Fe_2O_3	0.78
K_2O	1.10
Na_2O	0.78
CaO	0.24
MgO	0.21

Pretreatment with acid and alkaline

Different pretreatment methods have been discussed in this paper, acid or alkaline method, steam explosion, and fungal method. Acid pretreatment using sulfuric acid is known to bioethanol production [3], lignin can be separated from lignocellulosic biomass by alkaline pretreatment. Thermal pretreatment is known to dilute most of the hemicellulose. Ref.[3] investigates the effect of residence time and acid or alkaline concentration on glucose content and lignin removal. From the conducted acids pretreatment experiment using sulfuric acid, there was no significant impact between various pretreatment time and glucose concentration. The husk pretreated with the highest concentration (1%) for 30 min produces higher glucose concentration.

The alkaline pretreatment experiment resulted, In the same way, the higher concentration of NaOH will produce a high glucose concentration. The residence time of 30 and 45 min also yielded in high glucose concentration. It may contribute to

high lignin removal in the highest concentration of acid and alkaline. Table 2 shows alkaline pretreatment reduce lignin content and yield in higher cellulose content comparing to acid pretreatment.

Different pretreatment methods resulted in different composition changes of rice husk, and the major component in pretreated solid is cellulose. Comparing the pretreatment methods, NaOH pretreatment is more effective than acid methods to retain cellulose and remove lignin.

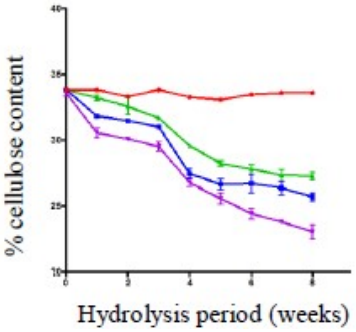
Table 2. Acid and alkaline pretreatment of RH

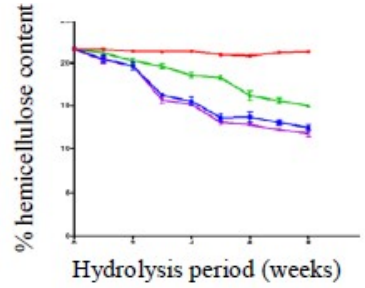
Component	Raw Rice Husk	Acid	Alkaline
Cellulose	37.6	51.9	54.2
Hemicellulose	15.2	6.68	14.2
Lignin	19.2	25.3	9.61
Hemicellulose dissolution (%)	-	73.0	43.8
Lignin removal (%)	-	19.1	69.8
Dry matter loss (%)	-	38.5	4.10

Pretreatment with steam explosion

Rice husk was pretreated by a steam explosion at a different temperature range, the yield of insoluble dry matter was calculated. Due to lignin and hemicellulose solubility, the cellulose content will increase from the initial rice husk content. Cellulose composition achieved peak at temperature 210 °C for 10 min. This operation condition yields maximum cellulose content. If temperature increased further, cellulose will break down and decrease the yield of cellulose content [5].

This pretreatment method affects on saccharification process. The glucose released from rice husk were achieved maximum yield at pretreatment temperature 210 C for 10 min.





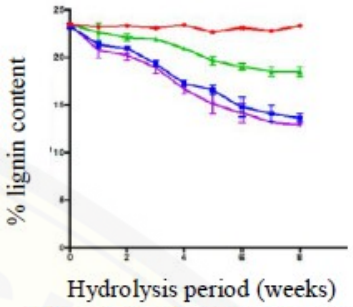


Figure 1. Simultaneous pretreatment and saccharification of RH using *P. ostreatus* and *A. niger* co-culture. Symbols ■ Control, ■ *P. ostreatus*, ■ *A. niger*, ■ *P. ostreatus and A. niger.*

Figure 1 shows the time course of pretreatment and saccharification using fungal pretreatment method. *P. ostreatus* and *A. niger* actively consumed cellulose, hemicellulose, and lignin. 30%, 50%, and 26% of cellulose, hemicellulose, and lignin relative to the control group were degraded, respectively. Fungal pretreatment and saccharification take a relatively longer time of 8 weeks compared with other pretreatment methods. The combination of this two fungus secreted cellulase, hemicellulase, and degrading lignin enzyme. Furfural and HMF as the main inhibitory product of saccharification were not detected [6].

Table 3. Data summary of pretreatment and saccharification performance

Pretreatment method	Hydrolysis	Sugar yield (g/L)	Inhibitor		Sugar production per enzyme activity (g/FPU)
			Furfural	HMF	
Acid	Enzymatic	21.9%	0.02%	ND	1.99
Alkaline	Enzymatic	25.7%	ND	ND	2.33
Steam explosion	Enzymatic	62.0%	0.10%	0.30%	31.0
Fungal	Fungal	0.6%	ND	ND	ND
Lime	Enzymatic	14.5%	ND	ND	0.18
Microwave	Enzymatic	35.0%	ND	ND	0.0008

Pretreatment using microwave was considerably attractive to reduce the use of chemicals for decreasing the recalcitrance of rice husk. With microwave severity level of 3.65, lowest (not detected) furfural and HMF was formed [8]. Sugar yield based on the raw material was optimized to 35.0% (Table

3). However, a large amount of enzyme was required during saccharification which caused lower sugar yield per enzyme activity.

Table 3 showed that the steam explosion resulted in the highest sugar yield per raw material of 62.0%. Lower enzyme activity was needed to obtain the hydrolyzed sugar, increased sugar yield per enzyme activity up to 31.0 g/FPU could be reached using this method. The result using steam explosion pretreatment and enzymatic hydrolysis was selected for the biobutanol fermentation with simulated sugar mixture.

Sugar mixture of 30 g/L glucose and 30 g/L Xylose was almost totally consumed (Figure 2). Total butanol concentration was highly produced from 48 h of fermentation as a result of rapid sugar consumption [9]. This remarkable performance was the advantages of the low inhibitors concentration in the mixture.

This study reviewed the pretreatment process for rice husk as lignocellulosic material. Steam explosion pretreatment method successfully produced the highest sugar yield based on raw material and sugar yield based on the enzyme activity. High butanol concentration was obtained using the simulated sugar mixture of the glucose and xylose contained in the hydrolysate.

4. Conclusion

Several pretreatment methods for rice husk were discussed in this paper. Among the studied methods, steam explosion demonstrated the highest sugar yield based on the raw material and based on the enzyme activity. Steam explosion is a prospective technique to reduce the usage of chemicals, both for pretreatment and neutralization process, using severe condition. As the effect of low concentration of the formed inhibitors, high butanol concentration was produced using the simulated glucose and xylose contained in the hydrolysate. This method is useful for the development of biomass utilization for butanol production as the intermediate in chemical manufacture and as biofuel.

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POSTER PRESENTATION

2nd INTERNATIONAL CONFERENCE ON AGROMEDICINE AND TROPICAL DISEASE

"Current Trends, Challenges, and Issues in Agricultural Health Medicine: From Rural to Urban, Ocean to Island and Molecular to Clinical"

Aston Jember Hotel and Conference Center, Jember, East Java
October, 20th – 21, 2018

HEPATOPROTECTOR EFFECT OF SPINACH LEAF ETHANOL (*Amaranthus tricolor* L.) ON HISTOPATHOLOGICAL OVERVIEW OF ISONIAZID-INDICATED MENCIT

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Abstract

Isoniazid (INH) is a tuberculosis first-line drug that has a risk of DILI (*Drug Induced Liver Injury)* due to the resulting reactive metabolites namely acetylhydrazine and hydrazine. Both of these metabolites are oxidized by CYP2E1 to produce molecules that can cause lipid peroxidation. Spinach leaves are one of the plants that contain antioxidants such as flavonoids, vitamin C, beta carotene, and chlorophyll. The results of the study by Al-Dosari showed that the administration of spinach leaf ethanol extract could reduce the ALP levels of mice with hepatotoxic effects due to CCl_4. The purpose of this study was to prove that the administration of spinach leaves ethanol extract could improve the histopathological picture of isoniazid-induced liver mice. This study is *true experimental* with a sample of 28 mice divided into 7 groups randomly, namely the normal group, the negative control group, 5 treatment groups P1, P2, P3, P4, P5 by giving spinach leaf ethanol extract 1.05mg / 20gBW, 2.1mg / 20gBW, 4.2mg / 20gBW, 8.4mg / 20gBW, and 16.8mg / 20gBW orally after 2 hours of administration of INH 100mg / kgBW / day. The treatment was carried out every day for 10 days and then assessed the liver histopathological damage score. Statistical analysis ($p < 0.05$) showed significant differences in liver histopathological scores between treatment groups, so it can be concluded that administration of spinach leaves ethanol extract can improve the histopathological picture of isoniazid-induced liver mice.

Keywords: spinach leaf ethanol extract, INH, liver histopathology

1. Introduction

Isoniazid (INH) is a tuberculosis first-line drug that has a risk of DILI (*Drug Induced Liver Injury)*. The hepatotoxic risk incidence because INH increases with age, rarely occurs below 20 years, 0.3% occurs at ages 21–35 years, 1.2% in ages 36–50, and 2.3% occur over 50 years of age [1]. INH produces reactive metabolites, namely acetylhydrazine and hydrazine which can be oxidized by cytochrome P450 2E1 (CYP2E1) into radical acetyl toxic molecules. The radical acetyl binds to cellular macromolecules causing liver damage. Hydrazine can reduce and even eliminate GSH activity which is an endogenous antioxidant so that free radicals accumulate and oxidative stress occurs. Histopathological picture of liver damage from rifampicin appears as dose-dependent necrosis, vacuolar degeneration, and inflammatory cell infiltration [2]

The hepatotoxic effect of INH requires antioxidants to protect against its reactive metabolites. Exogenous antioxidants can be obtained either synthetically or naturally. Synthetic antioxidants are used limitedly due to long-term use causing poisoning and carcinogenesis. Therefore, natural and safer antioxidants are needed. Natural antioxidants generally come from plants. Spinach leaves are one of the plants that contain antioxidants such as flavonoids, vitamin C, beta carotene, and chlorophyll. The antioxidant content in *Amaranthus* consists of polyphenols, flavonoids, betalains, phenolics and anthocyanins [3]. Flavonoids as antioxidants work through *radical scavenging*, *metal ion chelation* and protection on the membrane. The study by Al-Dosari (2010) showed that the ethanol extract of leaves of amaranth *(Amaranthustricolor L.)* at a dose of 250 mg / kg and 500 mg / kg yielded

significant results to decreased levels of ALP mice that experienced hepatotoxicity due to CCl_4 [4].This study aims to determine the effect of spinach leaf ethanol extract on the improvement of hepatic histopathology of mice induced by isoniazid.

2. Research methods

The type of research used was *true experimental* with the study *post-test only control group design.* The research was conducted at the Pharmacology Laboratory and Biochemistry Laboratory of the Faculty of Medicine, as well as the Biology Laboratory of the Faculty of Pharmacy, University of Jember. Samples were randomized with a total of 28 male mice (*Mus musculus*), healthy (active), 2-3 months old, and 20-30 grams of weight. The number of study groups was 7, namely the normal group (Kn) with normal saline, negative control group (K (-)) with INH 100 mg / kg BW / day orally, and 5 treatment groups P1, P2, P3, P4, P5 by giving spinach extract ethanol leaves 1.05mg / 20gBW, 2.1mg / 20gBW, 4.2mg / 20gBW, 8.4mg / 20gBW, and 16.8mg / 20gBW orally after 2 hours of administration of INH 100mg / kgBW / day. The independent variable in this study was the dose of spinach leaf ethanol extract and the dependent variable in this study was the histopathology of liver mice. The determination of the degree of liver damage was assessed using a score of 1 = normal, 2 = parenchymatic degeneration, 3 = hydrophic degeneration and 4 = necrosis. Analysis of the data used the *Kruskal wallis test*.

3. Results and discussion

Each treatment group liver preparations were observed and given a score with the results as shown in Table 1.

Table 1.

Group	The averagescore ± SD
K (n)	1.088 ± 0.012
K (-)	2.518 ± 0.155
P1	2.375 ± 0.032
P2	2.126 ± 0.195
P3	1,949 ± 0,158
P4	1,852 ±
P5	0,1201,563 ± 0,158

From these data it is known that in the normal control group has a minimum level of damage compared to other groups. The negative control group has the greatest damage level score compared to the others. The scoring data can be seen in Figure 1.

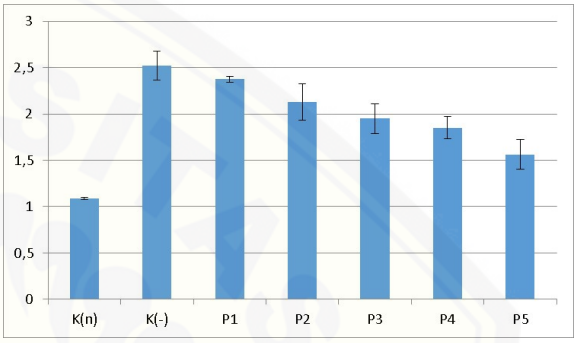
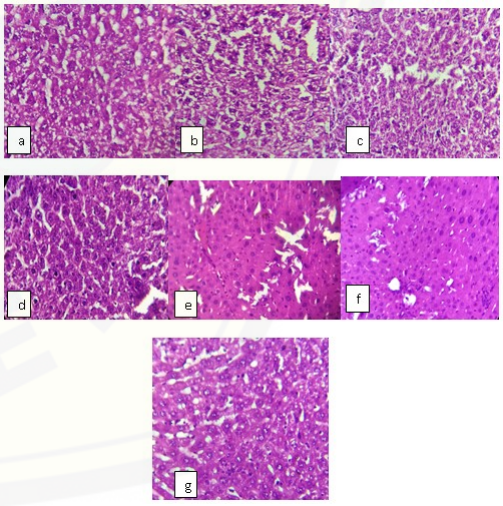


Figure 1. Average score of hepatocyte damage in the treatment group

The results of liver histopathology observation in each group are shown in Figure 2.



Note: a.K(N) b.K(-) c.P1 d. P2 e.P3 f.P4 g. P5

Figure 2.

The data is then analyzed using the Kruskall-wallis test. The statistical test results obtained p = 0.001. This means that there are significant differences in liver histopathology in the treatment group.

The result in this study were p=0.001 (p <0.05), because p <0.05, it can be concluded that at least two groups had a significantly different mean score of hepatocyte damage. Based on the results of the study, it was found that the average hepatocyte score in the K (N) group was significantly different from the K(-) group. This shows that administration of INH dose of 100 mg / kg for 10 days can cause significant hepatocyte damage.

On the results of histopathological observations in the negative control group, hydrophic degeneration and parenchymatic degeneration were found and some were necrotic. INH causes hepatitis due to the resulting reactive metabolites namely acetylhydrazine and hydrazine which are oxidized by CYP2E1 to produce toxic molecules such as CH3CO · and ROS free radicals (OH · and O2 ·). These oxidation results will bind to cellular macromolecules such as membrane lipids, nucleic acids, and hepatocyte proteins because they consist of unpaired electrons and act as oxidants [5]. Hydrazine can also reduce and even eliminate endogenous antioxidant activities such as GSH so that free radicals accumulate and oxidative stress occurs. This results in higher oxidation compounds than antioxidant compounds so that liver damage occurs [6]. Free radicals derived from the INH metabolite results in changes in the nature of the liver cell membrane due to lipid peroxide. This damaged liver cell membrane will cause the cell structure to be disrupted and will cause a degeneration process which causes extracellular fluid to go into the cell. Lipid peroxidation causes damage to cell membranes which will then reach the nucleus and damage cell function. Liver cell damage begins with the degeneration process before it will eventually become necrosis. Hydrophic degeneration and parenchymatic degeneration are reversible lesions that are sublethal and can be corrected if the oxidation exposure is stopped, but if the exposure is greater than the body's antioxidant ability to neutralize necrosis will occur.

According to Hariyatmi (2004), one of the changes induced by free radicals is the change in the nature of cell membranes and cytoplasmic membranes in cell elements such as mitochondria and lysosomes caused by peroxide fat [7]. After damaging the cell membrane, toxic effects can also reach the nucleus and damage it, which results in abnormal cell structure and eventually empties into necrosis [8].

The results of this study are also in accordance with other studies which state that INH can cause liver damage. Dong *et al.* (2014) showed that INH dosage of 100 mg / kgBW / day given for 10 days was able to cause SGOT to increase by ± 29 U / L from the normal group ± 17 U / L while SGPT increased by ± 33 U / L from the normal group ± 18 U / L [9]. The use of INH in the treatment of tuberculosis can be used singly as prophylaxis and in combination with other OATs as therapy. In this study it was shown that a single use of INH can cause hepatotoxic, so further research can be conducted with the use of other OAT combinations. The use of INH with other OATs such as RMP and PYR can increase liver damage because it can induce CYP2E1 excessively [10].

This study showed that there was a difference in liver histopathology in the treatment group given the ethanol extract of spinach leaves with a negative control group. This shows that the dose of spinach ethanol extract leaves was 4.02 mg / 20 gBW, 8.04 mg / 20 gBW, and 16.8 mg / 20 gBW was able to significantly repair the liver cell damage induced by INH. This significant difference shows that the ethanol extract of spinach leaves has the ability of hepatoprotector to prevent liver damage from free radicals.

The results of the study of Rajalakhsmi *et al.* (2011) showed that the ethanol extract of spinach leaves has a lot of antioxidants including flavonoids, vitamin C, beta carotene, and chlorophyll [11]. These antioxidants are able to donate electrons to free radicals to become stable. The type of flavonoids found inspinach leaves *Amaranthus tricolor* L.is routine

[12]. According to Ghasemzadeh *et al.* (2012), other types of flavonoids found inspinach leaves *Amaranthus tricolor* L.are catechins [13]. Catechins and routine have properties *radical scavenger* very strong because ring B flavonoids have catechol groups with stable semiquinone radicals to bind free radicals [14]. Flavonoids as antioxidants have the most levels found in spinach leaves, so in the next study can be carried out to take isolates of flavonoid active substances namely routine and catechins as hepatoprotectors with inductors other than isoniazid.

4. Conclusion

The administration of ethanol extract of spinach leaves (*Amaranthus Tricolor L.*) can improve the histopathological picture of isoniazid-induced liver mice.

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THE INFLUENCE OF EDUCATIONAL STAGE ON STUDENTS' SKILL IN PERFORMING PHYSICAL EXAMINATION (A STUDY IN FACULTY OF HEALTH SCIENCE UNIVERSITAS MUHAMMADIYAH PONOROGO)

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Abstract

Generally, the goal of education is to develop students' ability in three domains of learning; cognitive, affective, and psychomotor skills. Physical assessment is a mandatory subject that requires not only students' cognitive skill, but also their psychomotor skill. Physical assessment subject is a course focusing on understanding the correct examination techniques for each patient. This is a descriptive correlational study, conducted with students of Diploma Degree (n=97) and Bachelor Degree (n=103) of Nursing study program. The students are those who have been taught the lesson about physical assessment through lecturing and demonstration method. Independent T-test with significant level 0.05 is used. Results showed that the average score of students' skill in physical assessment for Diploma Degree students is 48.53 with standard deviation 7.148 while the average of students' skill in physical assessment for Bachelor Degree students is 6.69 with standard deviation 7.511. The p value is 0.000 and can be inferred that there is significant differences in term of students' skills in performing physical assessment between students of Diploma degree and Bachelor degree. Some experts claimed that the higher one's educational stage, the more comprehensive their psychomotor skills are. However, the result of this study showed that students of Diploma degree have a higher average score of skills in performing physical assessment. Therefore, this result is somehow in line with the objective of diploma degree as a vocational education that requires more skills.

Keywords: Educational Stages, Skills, Physical Assessment

1. Introduction

Nursing education is a type of vocational education that requires different body of knowledge than that of other vocational educations. Nurses have the characteristics of giving importance to others, having a profession, there are standards and professional ethics, accountability, autonomy [1]. In Indonesia, there are various levels of nursing education where all require good skills from students. One of the competencies that require skills is the physical examination. Physical examination is one subject that not only requires cognitive abilities but also requires psychomotor abilities. Nurses have the characteristics of giving importance to others, having a profession, there are standards and professional ethics, accountability, autonomy[1]. In Indonesia, there are various levels of nursing education where all require good skills from students. One of the competencies that require skills is the physical examination. Physical examination is one subject that not only requires cognitive abilities but also requires psychomotor abilities.

Physical examination is part of the assessment phase in the process of nursing care. The assessment phase consists of anamnesis and physical examination that serves to establish nursing diagnoses. Physical examination is an action to find something abnormal in the patient. A good ability in students to carry out physical examinations will determine the success of nursing care. Therefore, quality skills are needed from students as prospective nurses so that nursing services are provided according to competence, meet standards and prioritize ethical and moral principles.

In Nursing Diploma education more towards skill learning because Diploma Degree of Nursing is vocational education,

but still aims to produce nurses who master nursing and work professionally. During the teaching learning process, physical assessment skill that is included in the subject of Basic Nursing is a skill that should be mastered by students in Diploma and Bachelor degree. This Basic Nursing Material aims to develop student behavior which consists of three domains, namely cognitive, affective and psychomotor domains so that it will produce competent nurses.

Although the physical examination material is equally given to Bachelor Degree or Diploma degree of Nursing, the mastery of the material differs depending on the ability of each absorptive person. Wawan's theory (2010) says that the higher one's education, the easier it is to receive information so as to be able to make decisions in action [2] According to Wawan (2010) and Siagian in Saselah (2010) that with high education will increase motivation, desire, and intellectual maturity[3].

The results of Sujiono and Munawaroh (2017) study of Nursing students found that the average physical examination ability students of 6th semester was 24.11 with a standard deviation of 6,664, while the average physical examination ability students of 8th semester was 25,18 with a standard deviation of 8,173 [4]. This illustrates that with the increase in the study period will increase experience and maturity so that the ability to do a skill will also increase. The results of the study also found that there were still many respondents who carried out physical inspection actions not in accordance with the Standard Operating Procedure. According to Rutami Research Results (2012) that the inhibiting factor in the implementation of the nursing assessment process is the lack of the ability of nurses to collect comprehensive assessment data, lazy to do studies, high nurses' workload, and nurses perceive that studying is time-consuming[5].

The phenomenon that occurs in students of Diploma Degree, Faculty of Health Sciences University of Muhammadiyah Ponorogo that physical examination is a material that is difficult to learn compared to other clinical skills. The result of pilot survey for seven students revealed that six out of seven students do not recall this skill anymore. This result is in line with the prior study conducted by Sujiono and Siti Munawaroh (2017). In this study, six students of 8th semester and three students of 6th semester of Bachelor Degree of Nursing study program were involved as participants. The result showed that all students have already forgotten the skill as they no longer practice it. Moreover, six respondents claimed that they were once confused because they only read the manual procedures of physical assessment without practicing it[4].

2. Material and Method

The subjects of this study are 103 Bachelor degree students who are in 6th and 8th semester of academic year 2016/2017 (the result of study conducted by Sujiono and Munawaroh, 2017) and 97 Diploma degree students who are in 4th and 6th semester of academic year 2017/2018. Data are collected by using observation checklist of physical assessment's standard operational procedure. The checklist is fulfilled by observing how well the students conduct the assessment based on the procedure. The score is ranged from 0 to 2 with detail: 0 means the procedure is not performed, 1 means that the procedure is performed but less correct, and 2 means that the procedure is performed correctly. In addition, the data are analyzed by using independent T-test with significant level 0.05.

3. Results and Discussion

The distribution of students' performance in performing physical assessment based on gender can be seen in Table 1 and 2.

Table 1. Average distribution of Students' performance in performing physical assessment based on gender in Diploma degree of Nursing study program

Gender	Mean	SD	SE	N
Male	46,16	7,418	8,207	19
Female	49,10	7,511	7,151	78

Table 2. Average distribution of Students' performance in performing physical assessment based on gender in Bachelor degree of Nursing study program

Gender	Mean	SD	SE	N
Male	23,27	6,857	1,194	33
Female	25,27	7,769	0,929	70

The results showed that the ability to perform physical examinations on female students of Diploma Degree was higher than that of men students. This also happens to undergraduate nursing students. This result is in line with Cahyani's (2010) study that women have a higher score than men in hand washing [6]. Hand washing is also a clinical competence that must be mastered by students. According to Kurt Lewin's model in terms of background (background factors), such as age, gender, ethnicity, socioeconomic status, mood, personality traits, and knowledge affect individual attitudes and behavior towards something. According to researchers, women are more diligent and diligent in undergoing or working on complicated things so as to get better results.

The comparison of students' performance in performing physical assessment based on Education level in Diploma degree and Bachelor degree can be seen in Table 3. The research result showed that the average score of students' skill in physical assessment for Diploma Degree students is 48.53 with standard deviation 7.148 while the average of students' skill in physical assessment for Bachelor Degree students is 6.69 with standard deviation 7.511. From this test, the p value is 0.000. From this result, it can be inferred that there is significant differences in term of students' skills in performing physical assessment between students of Diploma degree and Bachelor degree.

Table 3 Average distribution of Students' performance in performing physical assessment based on educational level

Educational Level	Mean	SD	Min-Max	SE	P *Value*	N
Diploma Degree	48,53	7,418	29-61	0,753	0.000	97
Bachelor Degree	26,69	7,511	11-45	0,74		103

The result showed that students of diploma degree have higher average distribution in performing physical assessment than that of bachelor degree students. This result is not in line with theory of Notoatmodjo (2010) who claimed that someone with higher educational stage may receive information easier [7]. Atitude and behavior can be seen from one's education. Referring to aforementioned theory, the ability of Bachelor degree students are supposed to better than their counterpart, the Bachelor degree students. In addition, both students have been taught about physical assessment and have practiced in laboratory for self-study.

Curriculum for diploma degree of Nursing study program is arranged to provide vocational outcomes. A vocational nursing education requires the ability to perform good clinical skills as its outcomes are supposed to be nurse practitioners. Although students of Diploma degree seem to be more prospective, it does not mean that students of Bachelor degree do not have such clinical competences in nursing education. In addition, nursing students in Bachelor degree also have been taught the physical assessment material through demonstration method and independent laboratory practices. Laboratory learning by having individual practices is expected to improve students' ability in performing physical assessment[4]. Thus, the aforementioned technique is considered as an effective way to help students master nursing technical ability.

The results of the study of the range of scores on Diploma Degree of Nursing are the minimum-maximum value of 29-61 which is higher than the minimum-maximum value of S1 Nursing, namely 11-45. In general, these results indicate that

the value of the ability to perform physical examinations is still lacking. Physical examination is a process to get objective data from patients by obtaining additional data about the patient's disease history. If a student as a prospective nurse is not right in taking a physical examination, it will have an impact on professionalism at work. Nurses are one of the healthcare providers in the hospital who is required to be able to work professionally and competently. Nursing education must be able to produce students' ability to combine cognitive, psychomotor and affective skills. These three skills will help students develop their competence in the field [8].

4. Conclusion

There is significant differences in term of students' skills in performing physical assessment between students of Diploma degree and Bachelor degree. students of diploma degree have higher average distribution in performing physical assessment than that of bachelor degree students. Lecturers should use a variety of learning methods, especially those that require skills. Learning media using video is one way to increase student learning interest. Development of the analytical power of undergraduate nursing students must be developed to be able to think critically so as to produce professional nurses.

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IDENTIFICATION OF SPECIES COMPOSITION AND DOMINATION OF ANOPHELES MOSQUITOES IN THE BANGSRING BEACH BANYUWANGI

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Abstract

The Bangsring village of Wongsorejo sub-district, Banyuwangi district is one of the receptive areas of malaria in the province of East Java. Indigenous cases in this region can occur due to high population mobility to endemic malaria areas and the existence of *Anopheles* mosquito habitat in the lagoon along the coast. This study aimed to determine the composition of *Anopheles* species and the dominant species that have potential as malaria vectors. Mosquito collection was conducted by catching mosquitoes at night inside and outside the house and in the cages of livestock. Collection mosquitoes in the morning were also done to catch mosquitoes that rest on the inside and outside the house. The results of the collecting mosquitoes showed that there were five *Anopheles* species, e.g. *An.sundaicus, An. subpictus, An. vagus, An. barbirostris*, and *An. indefinitus*. *Anopheles sundaicus* was the dominant species with the highest density compared to other species. The highest density of *An.sundaicus* was in January, and the lowest density was in June. The *Anopheles* mosquitoes in that area tend to be zoophilic and exophagic. There were four lagoons which become the breeding place for *An. sundaicus*. *Anopheles sundaicus* is one of the main vectors of malaria in Indonesia, so this mosquito has the potential to transmit malaria.

Keyword: Anopheles, composition, dominant, vector, malaria

1. Introduction

Malaria remains an important infectious disease that caused the morbidity and mortality in 104 countries and territories, including Indonesia. Malaria is one of vector-borne disease that naturally spreads from one person to another by female *Anopheles* mosquitoes. There are 30 species among 400 different species of *Anopheles* mosquitoes that could transmit malaria as important vectors [1]. In Indonesia, there are 24 species of Anopheles mosquitoes can be vectors of malaria and ten of these are vectors of major importance. The main vector of malaria in Indonesia includes *Anopheles aconitus, An.balabacensis, An.barbirostris, An.farauti, An.koliensis, An.letifer, An.maculatus, An.punctulatus, An.subpictus,* and *An.sundaicus*. Four important species for malaria transmission in Java and East Nusa Tenggara (NTT) are *An.aconitus, An.barbirostris, An.subpictus* and *An. sundaicus* [2,3].

Anopheles sundaicus is an important malaria vector for coastal areas On the islands of Java, Bali, Sumatra, Kalimantan and the islands of West Nusa Tenggara [3]. This species has a wide distribution area in Indonesia and act as the main vector [4]. Its distribution includes coastal areas in East Java (Banyuwangi, Trenggalek) [5], West Java (Pelabuhan Ratu, Tasikmalaya) [6], East Nusa Tenggara (West Sumba) [7], West Nusa Tenggara (East Lombok and West

Sumbawa Island) [8,9,10], North Sumatra (Teluk Dalam, Nias, South Tapanuli) [11] and South Lampung [12].

Banyuwangi Regency is one of the districts in East Java that is prone to malaria. In the period 1998-2002 Banyuwangi was one of the districts that reported the existence of villages with high case incidence (HCI). In 2008, 2010 and early 2011 there was an outbreak in the Bangsring Village which is a coastal area [11]. The previous study has found seven species, e.g., *An.sundaicus, An.vagus, An.subpictus, An.flavirostris, An.annularis, An.barbirostris dan An.indefinitus* in the Bangsring village, sub-district Wongsorejo [4]. The study also showed that *An.sundaicus* was the dominant vector in Bangsring Village and had biting activities especially outside the home and around the cage [4, 5]. Therefore, *An.sundaicus* had a potency to transmit malaria in this region.

The study aimed to identify the composition of *Anopheles* species in the Bangsring village so that the dominant vector would be known.

2.Method

Landing collection of *An.sundaicus* mosquitoes were conducted in Bangsring Village, Wongsorejo sub-district, Banyuwangi district, East Java. *Anopheles sundaicus* adult female was captured using an aspirator during landing around the cages of livestock, inside the house (indoor) and outdoors, from 18:00 pm to 06:00 am every 40 minutes in one hour. The mosquitoes were also collected in the morning from 06:00 am to 08:00 am when resting in the house (indoor). The collected mosquitoes were put in the paper cup. Mosquitoes were morphologically identified using identification key of Reid [13] in anesthetized conditions and under stereomicroscope.

3. Results and Discussion

The collection of *Anopheles* mosquitoes in Bangsring Village found five species, e.g., *An. sundaicus, An. subpictus, An. vagus, An. barbirostris,* and *An. Indefinitus*, with *An.sundaicus* as the dominant species. The results of the study conducted by Mardiana et al., [5] also shown *An.sundaicus* as the dominant vector in Bangsring Village and has biting activity, especially outside the house and around the cage. The composition of the species was the same as previous studies which found seven *Anopheles* species in the same region, e.g. *An.sundaicus, An.vagus, An.subpictus, An.flavirostris, An.annularis, An.barbirostris* and *An.indefinitus. Anopheles sundaicus* mosquitoes were always found indoor, outdoor and around the cage at night. Therefore, *An.sundaicus* was most likely a malaria transmission vector in the region [4].

The *Anopheles* species were identified using Reid [14] based on morphological characteristics. *Anopheles sundaicus* has morphological features as follows: on the head, the palpus was blackish brown with 3 bracelets with white edges that were the same length in black, there are 4 or more black spots on costa and first wing, in the hind limbs, the femur, tibia, and tarsus have pale spots and a dark fifth tarsus section. The difference between male and female *Anopheles sundaicus* can be seen in the proboscis section. The male *An.sundaicus* has a thick hairy antenna while the female's antenna is a little hairy (Figure.3).

The collection of *An.sundaicus* mosquitoes in different months (seven months) shown a different density of mosquitoes. The highest density occurred in January (451 mosquitoes), while the lowest in June (83 mosquitoes). This result

is different from research conducted by Shinta *et al.,* [13] which measured mosquito density from May to October which coincides with summer. The results showed the highest density in July and the lowest in May and October. Other study conducted from May to November showed similar results, the lowest density in June, but the highest occurred in May and September [15]. These varied results occurred because the density of *Anopheles* mosquitoes was affected by climate. These climate factors include temperature, humidity, and rainfall [13]. The increased humidity was directly proportional to the increase in mosquito density. High rainfall may cause the density of mosquitoes to decrease. The temperature has an optimum limit for mosquito breeding between 26-28ºC [15]. The global warming that occurs at this time affects the climate conditions in Indonesia, resulting in a change in the time of the rainy and dry season. It will also result in changes in the pattern of *An.sundaicus* mosquito density fluctuations.

The breeding place of *An.sundaicus* mosquitoes in the Bangsring Village was a lagoon located near the beach, and there were four lagoons at a different location. The lagoons include Laguna Kandangan (± 4000 m2), Laguna Loji Utara (± 2000 m2), Laguna Loji Selatan (± 2000 m2), and Laguna Kluwih (± 1000 m2). Laguna and stagnant river lade are preferred breeding place for *An.sundaicus* larvae. The breeding place had the characteristics of being open, exposed to direct sunlight, lots of plants and algae / floating moss. Many larvae were found under floating water plants such as water grass, algae, twigs, leaves, and litter. *An.sundaicus* larvae mainly develop in habitats with brackish water in coastal areas, although in some places they are also found in freshwaters such as in India, Nicobar Island, Malaysia, Sarawak and Indonesia [16].

Figure 1. Distribution of malaria and import patients in East Java Province in 2011 [11]

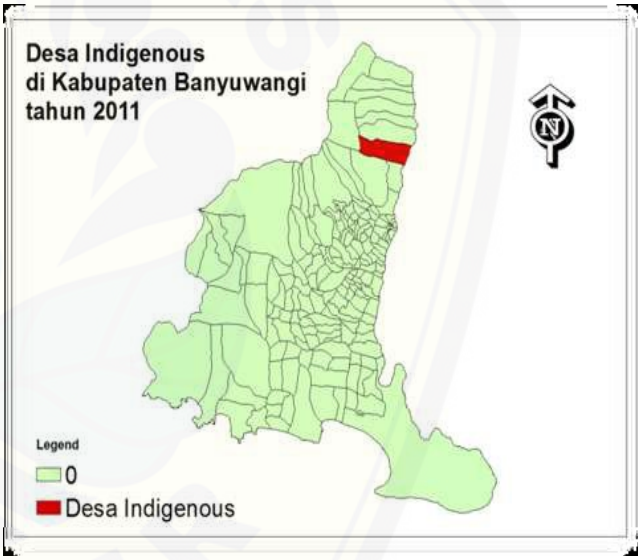

Figure 2. The map of Bangsring village in Banyuwangi district as an indigenous area of malaria [11]

Desa Bangsring	Density
Anopheles sundaicus	671
Anopheles vagus	5
Anopheles subpictus	1
Anopheles barbirostris	4
Anopheles indefinitus	1

Table 1. The Composition of *Anopheles* species in Bangsring village

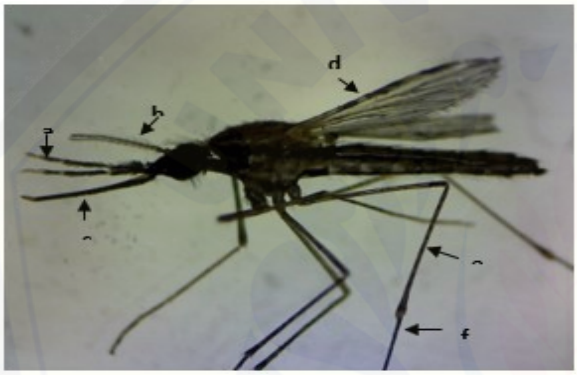

Figure 3. The Morphological feature of female *Anopheles sundaicus* mosquito (a) the palpus was blackish brown with 3 bracelets with white edges that were the same length in black,(b) antenna, (c) proboscis, (d) 4 or more black spots on costa and first wing, (e,f)in the hind limbs, the femur, tibia, and tarsus have pale spots and a dark fifth tarsus section.

Figure 4. Kandangan Lagoon as breeding place of *Anopheles sundaicus* in Bangsring village

The composition of the *Anopheles* species in Bangsring Village included *An. sundaicus, An. subpictus, An. vagus, An. barbirostris,* and *An. Indefinitus.* The dominant species was *An.sundaicus* that had the highest density in January and lowest density in June. *Anopheles sundaicus* could be a potential vector for spreading malaria in the coastal area of the Bangsring village.

ACKNOWLEDGMENT

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THE EFFECT OF HARMONICA EXERCISE ON INSPIRATORY CAPACITY AND QUALITY OF LIFE OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS IN AGROINDUSTRIAL COMMUNITY

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Abstract

Chronic inflammation in chronic obstructive pulmonary disease (COPD) causes respiratory muscle dysfunction and decreased respiratory muscle capacity. Incongruity of the capacity and the burden of the respiratory muscle results in increased symptoms of breathlessness, decreased inspiratory capacity, exercise capacity, and quality of life. The objectives of the study were to analyze the effect of harmonica exercise as a pulmonary rehabilitation modality on inspiratory capacity, shortness of breath symptoms, exercise capacity, and quality of life on stable COPD patient.Clinical trials with pre and post test group design were performed on 30 stable COPD patients at the Respiratoy clinic in August - September 2017 taken by purposive sampling. Inspiratory capacity (IC) (spirometry) and quality of life (SGRQ) were measured at baseline and after 6 weeks in the harmonic and control exercises group. A total 30 stable COPD subjects met criteria and divided into two groups. The harmonica training group increased IC(1.78 ± 0.30) litre and SGRQ score (33.87 ± 6.05) after exercise were had significant differences ($p<0.005$). Harmonica exercises increase IC and improve the quality of life of people with stable COPD. The harmonica exercise had benefit and could be applied as a pulmonary rehabilitation program in stable COPD patients of agroindustrial community.

Keywords: COPD, inspiratory capacity, quality of life, SGRQ

1. Introduction

Chronic inflammation in chronic obstructive pulmonary disease (COPD) causes respiratory muscle dysfunction and decreased respiratory muscle capacity. Incongruity of the capacity and the burden of the respiratory muscle results in increased symptoms of breathlessness, decreased inspiratory capacity, exercise capacity, and quality of life. The objectives of the study were to analyze the effect of harmonica exercise as a pulmonary rehabilitation modality on inspiratory capacity, shortness of breath symptoms, exercise capacity, and quality of life on stable COPD patient.

2. Methods

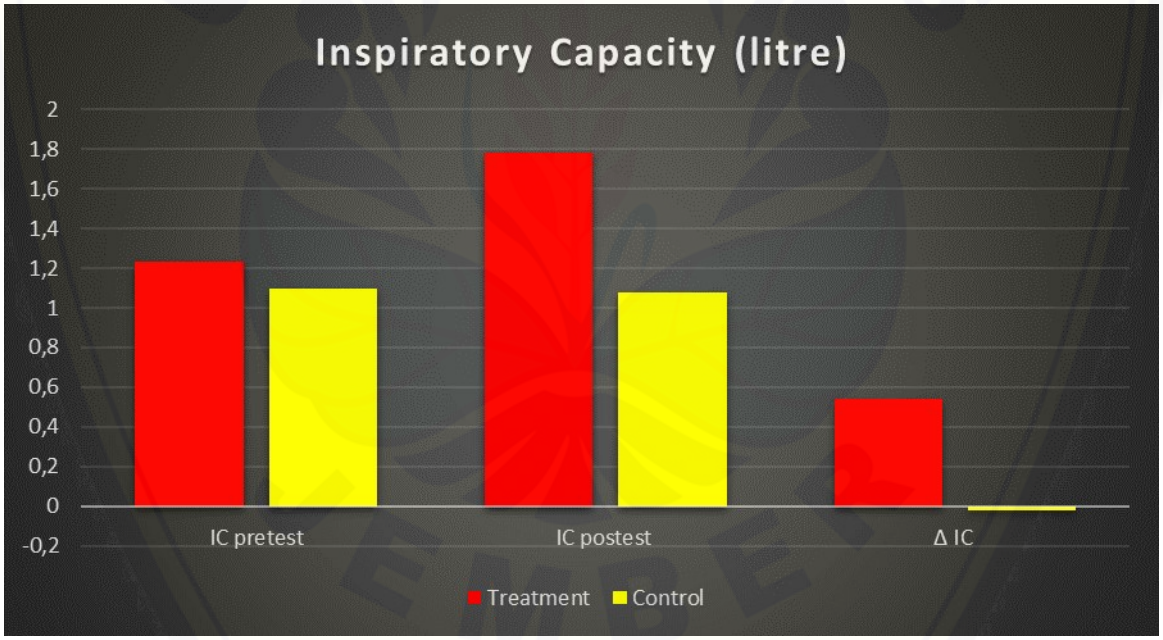
Clinical trials with pre and post test group design were performed on 30 stable COPD patients at the Respiratoy clinic in August - September 2017 taken by purposive sampling. Inspiratory capacity (IC) (spirometry) and quality of life (SGRQ) were measured at baseline and after 6 weeks in the harmonic and control exercises group.

3. Results and Discussion

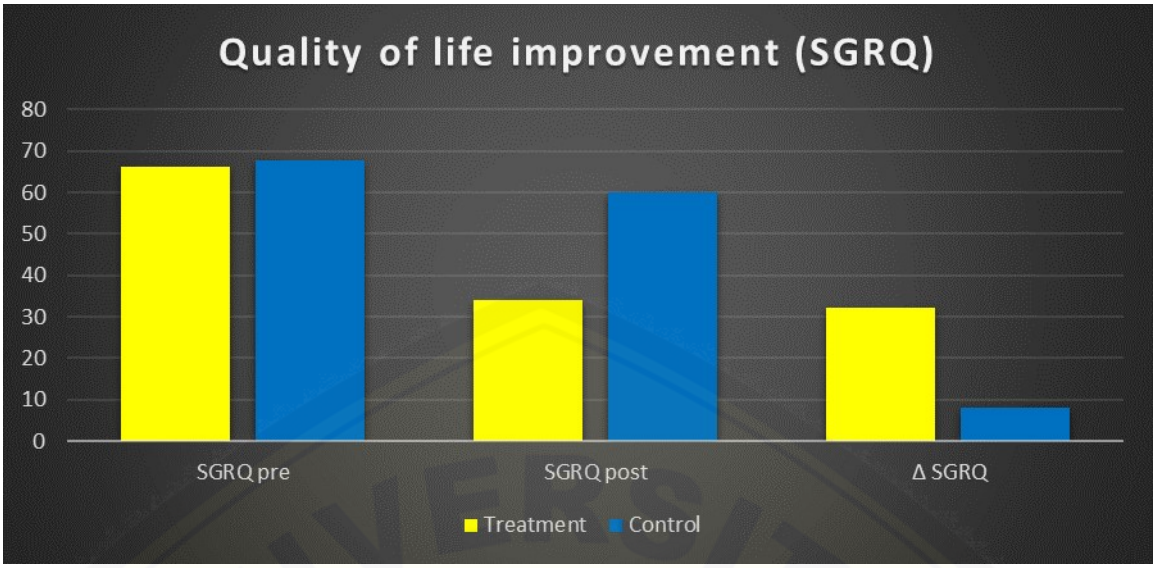
A total 30 stable COPD subjects met criteria and divided into two groups. The harmonica training group increased IC(1.78 ± 0.30) litre and SGRQ score (33.87 ± 6.05) after exercise were had significant differences ($p<0.005$).

Harmonica rehabilitation activity

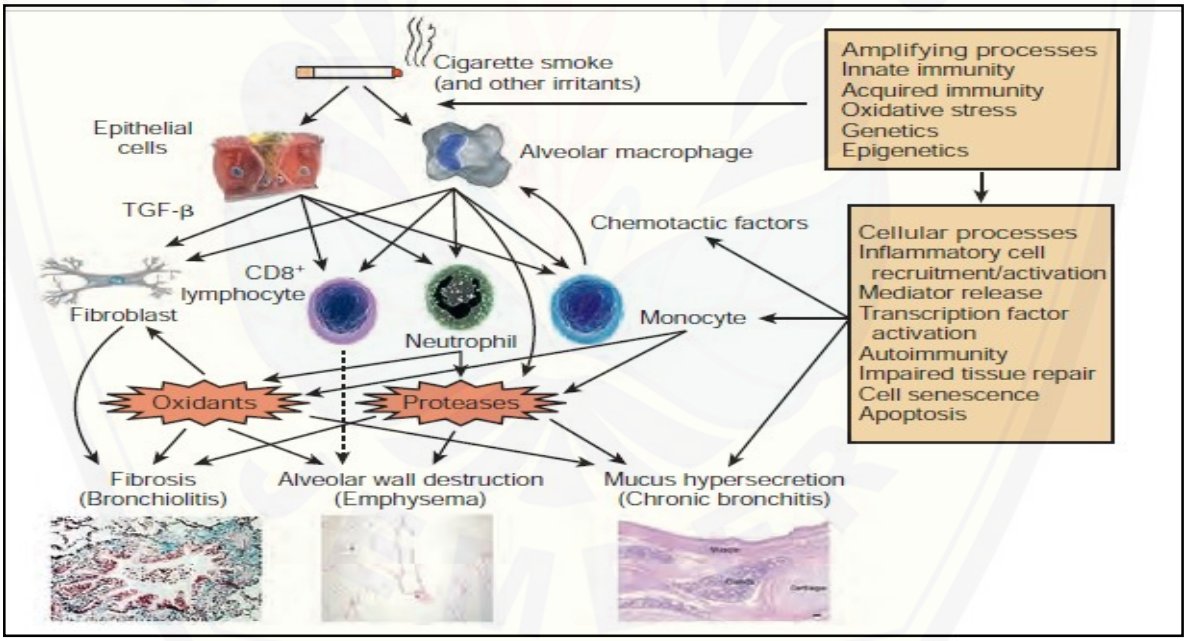
Inspiratory capacity differrence post and pre test between groups



Quality of life (SGRQ) improvement between groups



Pathogenesis of COPD



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4. Conclusion

Harmonica exercises increase IC and improve the quality of life of people with stable COPD. The harmonica exercise had benefit and could be applied as a pulmonary rehabilitation program in stable COPD patients of agroindustrial community.

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THE EFFECTS OF PESTICIDES ADMINISTRATION ON PREGNANCY AND THE INCIDENCE OF CONGENITAL DEFECTS IN FEMALE WHISTAR RAT

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Abstract

Infant and child mortality rates in Indonesia are high (43%-48%) some of them were caused by congenital abnormalities. Congenital abnormalities in Jember is 8.8%. This problem must be investigated because Jember Regency is an agricultural and plantation area that is very likely to use excessive pesticides, causing congenital abnormalities. This study was to determine the effect of pesticides on congenital abnormalities in wistar rats, specifically determine what type of pesticide is most influential on the occurrence of congenital disorders. This study was conducted on female wistar white rats exposed to pesticides from seven days before mating until 21 days after mating. The mating process took three days periode. In the end of study both group were observed for pregnancy and birth. The new born were orbserved for the occurrence of congenital disorders. Pesticides used in this study are the most widely used types in Jember based on sources of information from distributors whic is herbicides (glyphosate) and insecticides (carbamat). All the rat in negative control group's were pregnant and having normal offspring. The herbicide group's having one birth with one infant born without head (anencephaly), the other rats are not pregnant (infertile). The insecticide group's having one pregnancy with miscarriage, the other rats are not pregnant (infertile). Pesticides administration before matting can cause the possibility of infertility, misscariage and congenital abnormalities.
Keyword: pesticides, infertility, misscariage, congenital abnormalities.

1. Introduction

In the last 10 years, the Neonatal Mortality Rate in Indonesia has tended to be stagnant at 20/1000 live births to 19/1000 live births. However, the proportion of neonatal deaths to children under five years tends to increase from 43% to 48%. Based on the 2010 Riskesdas report, in Indonesia congenital abnormalities contribute 1.4% of 0-6 days of infant mortality and 18.1% of 7-28 days infant mortality. In addition, abnormalities also contributed 5.7% in under-five years mortality. According to the Ministry of Health data, the environmental impact and nutrition greatly affect the occurrence of congenital abnormalities. In 2013 the infant mortality in Bondowoso was almost 12 percent and the cause of this study was pesticides. The results of the study in Kedunguter Village, Brebes District, Brebes Regency, from the results of the cholinesterase test showed that there were 29 respondents who experienced poisoning (78.4%). Based on the Republic of Indonesia Ministry of Health, the normal limit allowed for blood cholinesterase levels is <75%. The results of examination of the levels of cholinesterase are known to have an average value of 73,946 with the lowest measurement of 50,0 and the highest of 87,5 (Muamilatul et al., 2012)

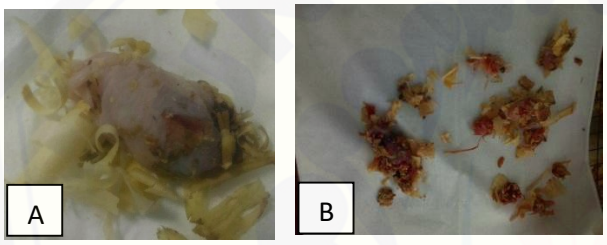
This problem must be investigated because Jember Regency is an agricultural and plantation area that is very likely to use excessive pesticides, causing congenital abnormalities. This study was to determine the effect of pesticides on congenital abnormalities in wistar rats. The purpose is specifically to find out what type of pesticide has the most influence on the incidence of teratogenic abnormalities, and when or at gestational age how many pesticides affect congenital abnormalities in wistar rats. The importance of the research proposed in supporting the achievement of the UNEJ research strategic plan characterized by agro-industry is the decline in disability rates as

a result of agricultural intensification programs due to the use of pesticides.

2.Methods

This study was conducted on female wistar white rats exposed to pesticides from seven days before mating until 21 days after mating. The mating process took three days periode. In the end of study both group were observed for pregnancy and birth. The new born were orbserved for the occurrence of congenital disorders. Pesticides used in this study are the most widely used types in Jember based on sources of information from distributors whic is herbicide (glyphosate) and insecticide (carbamat).

3.Result



A. The offspring of the herbicide groups born whitout head (anencephaly); B. The offspring of the insectiside groups was miscarriage

All the rat in negative control group's were pregnant and having normal offspring. The herbicide group's having one birth with one infant born without head (anencephaly) and the other rats are not pregnant (infertile). The insecticide group's having one pregnancy with miscarriage and the other rats are not pregnant (infertile).

4.Conclusion

Both herbicide and insecricide administration before matting can cause the possibility of infertility, misscariage and congenital abnormalities.

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