

## ANTIMALARIAL AND ANTIBACTERIAL BIOACTIVITY OF LANGSAT (*Lansium minahasae* L.) BARK EXTRACT

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### Abstract

This research is focused to obtain the data of the phytochemical compounds class that is contained in langsat bark, obtain the data of malaria antiparasite activity of langsat bark extract *in vitro*, and obtain the data of langsat bark extract antibacterial activity. The plant sample is obtained from Minahasa and Southeast Minahasa. Both wet and dry langsat bark extraction is conducted using maseration method. Phytochemical analysis uses Harborne and UV-Vis spectrophotometer method. The antimalarial activity analysis is conducted *in vitro* on *P. falciparum* culture, whereas the antibacterial activity uses diffusion method. The research result shows that dry Langsat bark extracts (KBLK) that are from Minahasa (KBLKMI) and Southeast Minahasa (KBLKMT) have % highest extract yield, compared with the wet Langsat bark extract (KBLB). The highest extract yield KLBKMT percentage is 5.78% and the lowest is n-butanol:water fraction, that is 0,663%. The thoroughly detected contents of phytochemical classes (alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids, anthocyanin and quinone) is found on the KLBK extract. The highest intensity is found on KLBKMT. The strongest antimalarial activity in the 1000 erythrocyte that is infected by plasmodium, stated with LC<sub>50</sub>, is found on KLBKMT extract, i.e. 57056,1 µg/ml and the weakest is KLBBMT extract, i.e. 80813,8 µg/ml. The strongest antibacterial activity is stated with the diameter of formed inhibition zone is KLBKMT extract, that its average is 12.5 mm in all experiment isolates.

**Keywords:** bark, *Lansium minahasae* L., antimalarial, antibacterial

### 1. Introduction

Malaria is an infectious disease which its morbidity and mortality rates tend to increase in tropical countries. Malaria is caused by protozoa of genus *Plasmodium* infection that is transmitted through the bite of female *Anopheles* mosquito. Malaria may also be transmitted directly through blood transfusion, hypodermic needle, and from pregnant mothers to their babies. In human, there are 4 kinds of *Plasmodium*, i.e. *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*.

Malaria infection cases approximately amounts to 300-500 million people and cause death of about 1 million people per year especially to children. Malaria causes about 273 millions clinic cases and 1.12 millions deaths per year, more than 40% of world population (2.1 billion people) is predicted at risk of contracting the disease (WHO, 2003). More than 1.2 billion inhabitants or 85% of the population total in Southeast Asia are at risk for malaria. Approximately, 30% of inhabitants, which are at this risk, live in moderate to high risk areas of malaria. India, Indonesia, Myanmar and Thailand are the risk area of malaria.

As a tropical country, Indonesia has a lot of tropical forest, terrestrial waters regional such as lake, river, and marshy area. In addition, poor environmental sanitation in residential areas gives some contribution to the growth of *Anopheles* mosquito as *Plasmodium* agent which causes Malaria.

From 1998-2004, there were exceptional Malaria cases in various region of Indonesia with huge patient number per year that is about 37-10.678 people and various death victims. The highest death case number is in North Maluku that is 162 people of 1174 patients. In 2005, the malaria cases reduced in Jawa and Bali, but do not in East Indonesia.

Malaria clinical case is still relatively high in East Indonesia. The most often found *Plasmodium* is *P. falciparum* and *P. vivax* or a mixture of both, whereas *P. malariae* is only found in Nusa Tenggara Timur and *P. ovale* is found in Papua (Kristanto and Harijanto, 2000). About 14 kinds of malaria vector *Anopheles* has been known in

Indonesia causing the high rates of malaria infection.

Malaria is not only contracted by adults but ranging from babies, pregnant mothers, to adults. At the *P. falciparum* infection case, it can cause the impaired brain function to death. The death number at falciparum malaria patients is 39% (18/46) and the death of cerebral malaria patients are 50% (16/32) (Tjitra, 1994).

The indicator of malaria examination is based on the sightings of parasite in the blood (parasitemia/P) and the enlargement of the lymph glands (splenomegali/S). The enlargement of the lymph glands shows that the person has long been exposed to malaria, because the new lymph glands enlarge after the repeated malaria infection/exposure.

The clinical treatment of malaria in Indonesia that is suggested by doctors (in Puskesmas and Hospital) is antimalarial drug such as chloroquine, amodiaquine, sulfadoxine-pyrimethamine, mefloquine, kinin and artemether. Standard malaria drug that is used in Indonesia for all parasites is Chloroquine (Kusumawardhani, 2006). One of the new developed drugs is artemisinin. However, artemisinin monotherapy treatment only weakens Plasmodium, not kill. In Thailand, sulfadoxine-pyrimethamine (SP) is almost 100% effective in curing malaria when introduced in 1977. Yet, over the last 5 years only 10% of cases can be cured, because of the resistance. Resistance also occurred to atovaquone a year after it was introduced in 1997. Chloroquine which is known to have lost its effectiveness in almost all part of the world was used by 95% of African children from 1999 to 2004 (Depkes, 2006).

Experienced serious problem in the context of Malaria treatment is *Plasmodium* resistance on MOH (Ministry of Health) standard drugs that have been used since 1984. Anti-malarial drug counterfeiting, incorrect dose, delayed treatment, and different behavior of malaria in many regions in Indonesia exacerbate the problem of Malaria treatment. The research result of Eijkman and Australia institute in 1997-2004 about Malaria from the side of drug pathogenesis and resistance show that in several provinces in Indonesia almost all parasites are carrying the chloroquine mutated gene (Kompas, 2007). One way to overcome it is to search a new ingredient for the drug that is derived from nature, especially from plants. From the previous researches, it is known that a number of compounds that have chemical structure such as alkaloids, terpenoids, quinolones, and phenolic contain antiprotozoa active substance that can be isolated from high level plants. It is reported that the compounds effectiveness mentioned above to protozoa had been tested *in vitro* and *in vivo* on experimental animals (Simanjuntak *et al.*, 1995).

Malaria treatments by society in ethnobotany way are often found in malaria endemic areas. Essential oils *Virola surinamensis* (Rol) had been used since the ancient time by the Amazon Indians Waiapi as an antimalarial drug (Lopez *et al.* 1999). *Lansium domesticum* L. seed, fruit, and leaves had been used in Sabah Malaysia as antimalarial drug. Fruit peel extract of *Lansium domesticum* is proven significantly prevent the life cycle of *Plasmodium falciparum* which is chloroquin-resistant (Yapp, DT and Yap, S.Y, 2003). Kusumawardhani, D. (2006) reports that the content of andrographolide on Sambiloto (*Andrographis paniculata* Ness) in the ethanol extract form is 96% more effective than isolate or singgel compound form on growth inhibition of *Plasmodium*.

As endemic disease in Indonesia, the rural communities have had malaria treatment wisdom using traditional drug. Several types of traditional drug which are empirically used as malaria drug in Sulawesi Utara, e.g. papaya, seed and bark of langsung, bitter melon fruit, pangi seeds, and ylang flowers. Most of the plant parts are boiled and drunk.

In addition to be a part of traditional treatment, available in nature, cheap, and their negative effect are very less, the utilization of traditional drug that is taken from plant such as leaves, seed, root, etc is also believed providing other benefits for health. However, the scientific proof of antimalarial activity is still rarely reported and phytochemical analysis of antimalarial active components that is actually potentially exploitable is not widely practiced. Until now, there is no research report about antimalarial and antibacterial from bark extract of langsung [*L. minahasae* L.]. The research in this first year aims to: Obtain the data of phytochemical compounds class which is contained in plants, that have Minahasa ethnomedical antimalaria activity, Obtain the data of plant crude extract antimalarial activity *in vitro*, and Obtain the data of plant crude extract antibacterial activity *in vitro*.

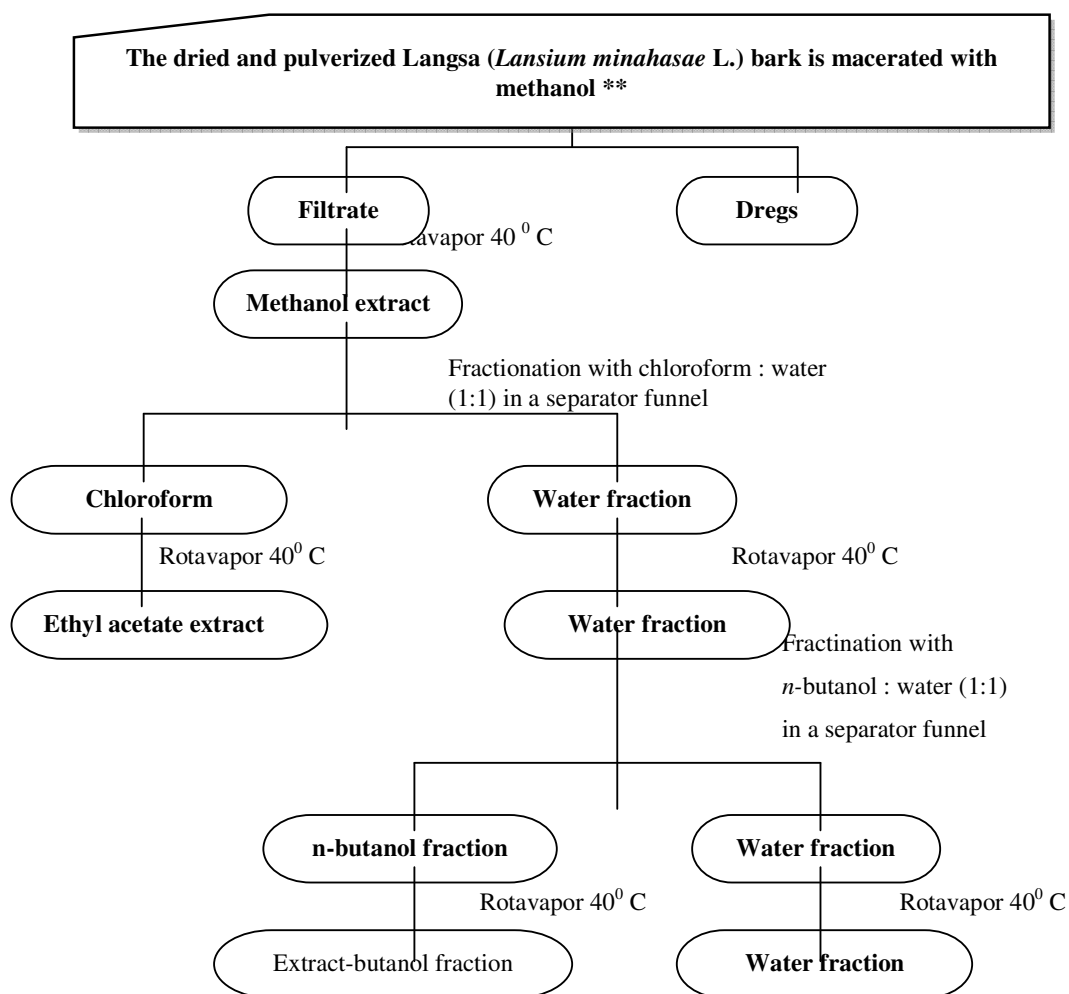
## 2. Ingredients And Methods

The ingredients that are used in this research are methanol, ethyl acetate, n-butanol, dimethylsulfoxide, HCl, H<sub>2</sub>SO<sub>4</sub>, acetic anhydride, FeCl<sub>3</sub>, NA media, NB media, methanol, 96% ethanol, ethyl acetate, chloroform, distilled water, RPMI 1640 medium, HEPES buffer, natrium bicarbonate, chloroquine diphosphate, gentamicin,

human serum, human erythrocytes, CPD anticoagulant, Giemsa dye and emersi oil. The tools that are used in this research are Heidolp rotary evaporator, UV-Vis spectrophotometer Parkin Elmer, pH meter, analytical balance, Eppendorf micropipette, water heater, mammert oven, Eppendorf centrifuge tools, UV light, laminar flow, incubators, refrigerators , autoclave, desiccator with candle holder (candle jar), microscope, object glass, 0.22 mm Millipore membrane filter, sterile tube, sterile beaker, Erlenmeyer flask, microplate (micro well plates) with 24 wells, bottles or sterile flasks medium, centrifuge tubes tools, sterile syringes, measuring pipette, sterile Pasteur pipettes and pH meters.

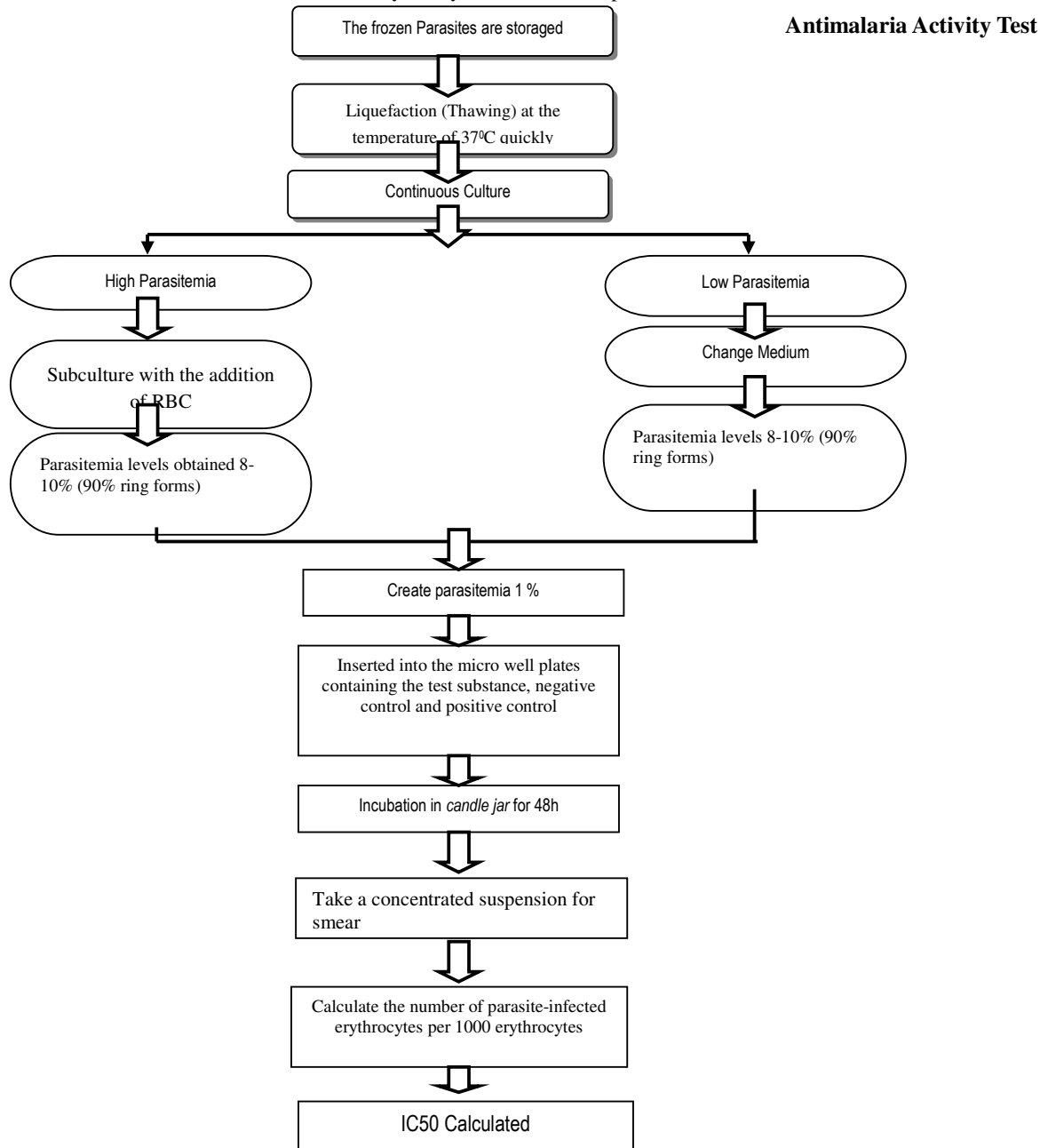
### 2.1 Plant Sample

Langsat (*L. minahase L.*) bark is obtained from the Kawangkoan District, Tareran District Minahasa and Toluuan District Southeast Minahasa.



Picture 1. Fractination and extraction steps

After the experiment is conducted *in vitro*, the inhibition of each extracts on *P. falciparum* (Picture 2), extract that has the best inhibition is further analyzed by TLC and UV Sepktrofotometri is.



Picture 2. Antimalaria activity test *in vitro* (Harijanto, 2000)

## 2.2 Experiment Result Observation

### 2.2.1 Parasite Growth (% Growth) (Harijanto, 2000)

The observation is conducted on parasite culture at 0 hour and 48<sup>th</sup> hour (0 hour before and 48 hours after incubation).

$$\% \text{ growth} = \frac{\text{Amount of infected erythrocytes}}{1000 \text{ erythrocytes}} \times 100 \%$$

### 2.2.2 Inhibition of Parasite Growth (Harijanto, 2000)

The prepared blood smear is examined using microscope with 10x100 magnification. The observation is conducted on the amount of parasite infected erythrocytes in 1000 erythrocytes, and then calculated its % inhibition.

$$\% \text{ growth} = 100 - \frac{X_e}{X_k} \times 100 \%$$

$X_e$  = amount of malaria parasite infected erythrocytes in test substance per 1000 erythrocytes

$X_k$  = amount of parasite in negative control per 1000 erythrocytes

### Analysis Method

The obtained data is analyzed using SPSS 13 program with probit analysis.

### Antibacterial activity test

Antibacterial activity test use diffusion method.

### Data Analysis

The data of research result is analyzed using SPSS IBM 20.

## 3. Result And Discussion

### 3.1 Extraction and Phytochemicals Class Analysis

Langsat (*L. Minahasae* L.) bark is obtained from Kali Village Tombatu District Minahasa Tenggara and Kawangkoan Minahasa. The obtained langsung bark then is divided into two parts, i.e. wet sample and dry sample. The extraction is conducted using maceration methods or plant botanical soaking, i.e. the langsung bark is mashed so that the dry sample become powder, and the wet sample become pasta.

The extraction is conducted gradually, i.e. using p.a. methanol for 3 x 24 hours and then filtrated in rotavapor to separate the solvent and extract, whereas the fractination dregs with ethyl acetate : water (1:1 b/v). Each solvent fraction is evaporated using Heidolp rotavapor. Water fraction is fractinated further with n-butanol : water (1;1 b/v). Each fraction in rotavapor so that the crude extract obtained (Table 1).

Job-shop production refers to a manufacturing environment that produces goods in small batches according to customer specifications. Usually, one or several types of products are deliverable, while the incoming orders may differ in the design, quantity, process flow, or urgency. Flexibility is allowed in terms of switching between machines, methods, and resolving problems in production. Depending on the nature of business, each of the workers hired may need to possess a certain range of skills to handle different tasks or machines, whereas the total number of workers may be adjusted in response to the varying demand. In practice, transferability of permanent workers and recruitment of temporary or contract workers will help make such adjustment feasible, thus admitting of the idea of WOZIP.

**Table 1** Wet Langsat Bark (KBLB) and Dry Langsat Bark (KBLK) Extract Yield

Sample	Solvent	% Yield
KBLK_MI	p.a methanol	5,341
KBLB-MI	p.a methanol	4,361
KBLK_MT	p.a methanol	5,781
KBLB-MT	p.a methanol	4,589
chloroform KBLK_MI fraction	chloroform : water	1,423
n-butanol KBLK_MI fraction	n-butanol : water	0,563
chloroform KBLK_MT fraction	chloroform : water	1,323
n-butanol KBLK_MT fraction	n-butanol : water	0,663

Plant substance extraction is the very important step in obtaining plant secondary metabolite to be utilized as drug. The extraction is conducted in maceration way and lain on shaker (45-50 rpm). Maceration method where the simplicia immersion is conducted at room temperature so that the volatile compounds do not disappears. It is conducted with shaker so that the collision is always occurred between the langsat bark powders so that the secondary metabolite extraction process in plant cell will be more maximal. The very important factor that affects the extraction result is solvent, time and temperature in conducting the extraction (Mokosuli, 2008). There are many methods in extracting plant substances, e.g. percolation, socolation and steam distillation methods. Percolation method is just good to use on soluble organic compounds whereas the socolation and steam distillation are just good on heat-resistant compounds.

The evaporation, using rotavapor, result is blackish brown methanol extract and light green chloroform extract:water (1:1). The extract is typically langsat bark smell and bitter. Yield is the percentage between obtained extract on the (weight) number of extracted simplicia (Depkes, 1987). KBLK\_MI is maserated with p.a. methanol (1:5) for 3 x 24 hours and produce 5.34% yields. The KBLK\_MI MeOH dregs is fractionated with chloroform:water solvent, produce 1.43% yields. Then, the same treatment is applied to KBLB\_MI MeOH. KBLB\_MI MeOH produce 4.36%. The wet langsat bark dregs are not fractinated. KBLK\_MT is macerated with p.a. methanol (1:5) for 3 x 24 hours, and produces 5.78% yields. KBLK\_MT MeOH dregs is fractionated with chloroform:water solvent, and produce 1.32% yields. The same treatment is applied to KBLB\_MT MeOH. KBLB\_MT MeOH produce 4.58% yields. The wet langsat bark dregs are not fractinated. The yields of each extract and fraction are shown at table 3.

Maseration method produces greater total and more stable yield than the other extraction methods. Extraction on propolis uses ethanol solvent with comparing some extract methods, i.e. UE (*Ultrasound Extraction*) and MAE (*Microwave Assisted Extraction*), and evidently the maceration method produce greater percent of total yields 55.58% than UE and MAE methods with its each produced yields 41% and 53%. This confirmed that extraction with methanol solvent using the maceration methods produce greater yield percent than the other methods. KBLK\_MI and KBLB\_MI ethanol extract have the highest yield percentage. The high yield of dry simplicia is due to the less water content in simplicia then the extraction or the methabolit withdrawal from the plant cell by the used solvent are more effective. The plant simplicia extraction for the purpose of the best herbal medicine uses alcohol solvent. The residues of other solvents have toxic risks for experiment animal. Furthermore, the dregs of methanol extract is extracted and macerated with chloroform:water which are semi-polar. It is expected that the secondary metabolites that have not been withdrawn by the ethanol solvent can be withdrawn by this solvent. Emphirically, the wet langsat bark which is used by Minahasan people as drug substance is boiled with water and its juice is taken. Water is polar solvent but the boild with high temperature can damage some secondary metabolite at langsat bark which have antimalarial, antibacterial and others bioactivity.

### 3.2 Phytochemistry Analysis

Phytochemistry analysis is a way to know the secondary metabolite contents in a plant sample. In this research, the phytochemistry analysis use Harborne Method (1996). The compounds that are analyzed are alkaloids,

saponins, flavonoids, tannins, steroids and triterpenoids (Table 2).

**Table 2.** The phytochemistry analysis result of wet langsat bark (KBLB) and dry langsat bark (KBLK)

Kind of Extract	Compounds group							
	A	F	S	T	Tri	Str	Ant	Kui
KBLK_MI	++	++	+	++	+	+	-	++
KBLB_MI	+/-	+++	++	++	-	-	+	+
KBLK_MT	+++	++	+	++	+	+	-	+++
KBLB_MT	+/-	+	+	++	+/-	+	-	++
chloroform KBLK-MI fraction	+	+	+	+	++	+	+	+
n-butanol KBLK_MI fraction	+	-	-	+	-	+	+	+
chloroform KBLK-MT fraction	++	+	-	+	+	+	+/-	++
n-butanol KBLK_MT fraction	+/-	+	-	+	-	+	-	+

Caption:

(+) sign shows the level of color intensity which also show the content in experiment sample

A = alkaloids, F = flavonoids, S = saponins, Tri = triterpenoids, Str = steroids, Ant = anthocyanin, Kui = quinone

KBLK MeOH extract contains almost almost the entire group of phytochemical compounds that are identified, except Anthocyanin. KBLB does not contain triterpenoids and steroids compound but contains saponin with higher intensity. Chloroform:water KBLK is identified containing all groups in low intensity. KBLB does not contain alkaloid compounds group but contains phenolic compounds, i.e. flavonoids, saponins and tannins with high intensity. It is due to KBLB when is extracted with methanols, it still has high water content, and when its dregs are extracted with chloroform:water that is semi-polar, the compound classes that have not been withdrawn well at ethanol solvent, are withdrawn well at chloroform:water solvent. Triterpenoid and steroid are only formed slight sediment when it is given Wagner reagent. Triterpenoid and steroid are non-polar lipid-derived secondary metabolites so that it needs non-polar solvent to be able to extract it well.

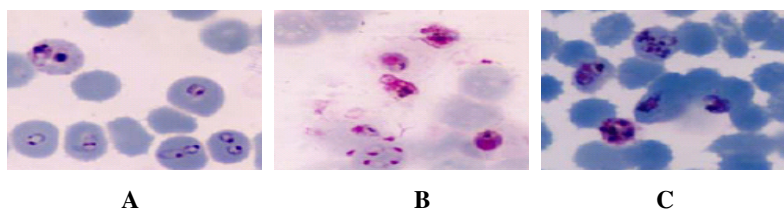
Dry and wet (10% water content) langsat bark extract with ethanol withdraw almost all group of secondary metabolites, i.e. alkaloids, flavonoids, tannins, saponins and triterpenoids, anthocyanin and quinones. It is due to methanol is a solvent that has two groups of different polarities, i.e. hydroxyl group which is polar and alkyl groups which are non-polar. By the existence of this group, the compounds with different polarity levels will be extracted in ethanol. From this phytochemistry analysis result, the best KBLK MeOH\_MI and KBLK MeOH\_MT extract contain compound groups that is expected have antiplasmodium (malaria) and antibacterial activity.

### 3.2 Langsat Bark Extract Antimalaria Activity *in vitro*

*P. falciparum* culture, which is stored frozen, then is thawed at 37<sup>0</sup>-38<sup>0</sup> C rapidly. It is conducted continuous culture to antimalarial activity test. The *P. falciparum* continuous culture is classified into two groups, i.e. high and low parasitemia group. High parasitemia groups, then is sub-cultured by adding the erythrocyte. It conducts the analysis using microscope to know the parasitemia level, that is 8-10% or 90 percent identified a ring-shaped. The obtained culture condition is made into a 1% parasitemia, and then the culture is filled in the test wells plate. Micro wells are filled with test solution (100 ppm, 200 ppm and 250 ppm were made triplo), negative control and positive control. After it is incubated in candle jar for approximately 48 hours, concentrated suspension was taken to make smear on object glass. The smear is observed using microscope to know the parasite infection level in 1000 erythrocytes. The observation is conducted in ring, trophozoite and schizont phase (Picture 3). The observation result shows that the increasement of test solution concentration is directly proportional to the number of infected erythrocyte but not directly proportional to infection stage (Table 3).

**Table 3** Parasite Growth and Retardation Based on Infected Erythrocyte Calculation on Each Stage in 1000 Erythrocytes at Thin Blood Smear that were Stained with Giemsa Dye.

Extract	Concentration (ppm)	Amount of infected erythrocyte			Total	Growth Percent
		Ring	Trophozoite	Schizont		
Negative control	0	20	1	16	37	3.7
Positive control (chloroquine diphosphate)	20	13	2	1	16	1.6
KBLK_MI	100	12	10	13	35	3.5
	200	15	8	11	34	3.4
	250	18	5	14	37	3.7
KLBK_MT	100	14	10	14	38	3.8
	200	13	10	12	35	3.5
	250	17	7	16	40	4
KLBB_MI	100	10	6	10	26	2.6
	200	7	4	8	19	1.9
	250	16	6	9	31	3.1
KLBB_MT	100	9	8	12	29	2.9
	200	8	7	10	25	2.5
	250	12	8	6	26	2.6



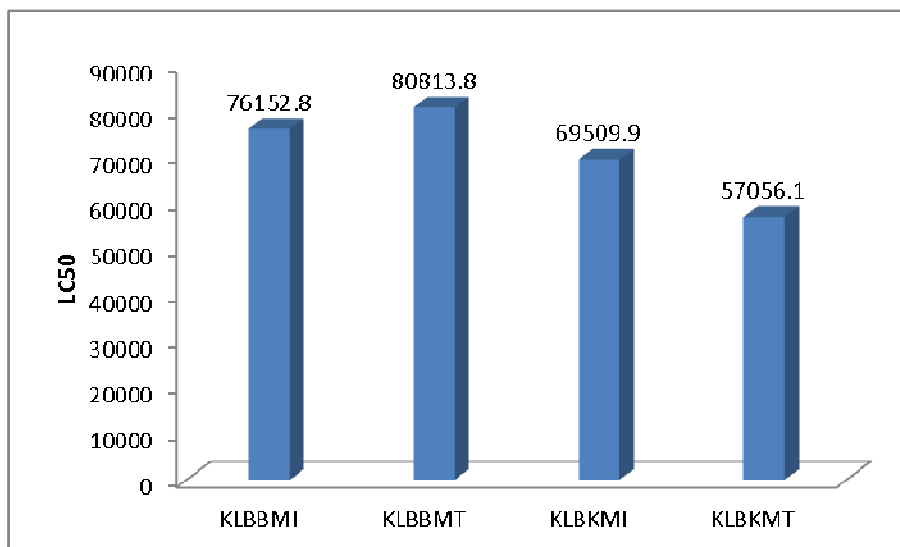
Picture 3. *P. falciparum* parasite stage in the thin blood smear with Giemsa dye (A : ring, B : trophozoite dan C : schizont)

Highest growth inhibition was found in the treatment of dry olive bark extract both samples from Minahasa and Southeast Minahasa. This result, if it is projected with phytochemistry group analysis result, the most complete secondary metabolite contents is found in dry langsung bark extract. The quinones, alkaloids and steroids contents from variety research reports are toxic to *Plasmodium* spp by damaging the cell membrane and is cytotoxic.

### 3.3 Lethal Concentration 50 of Langsung Bark Extract

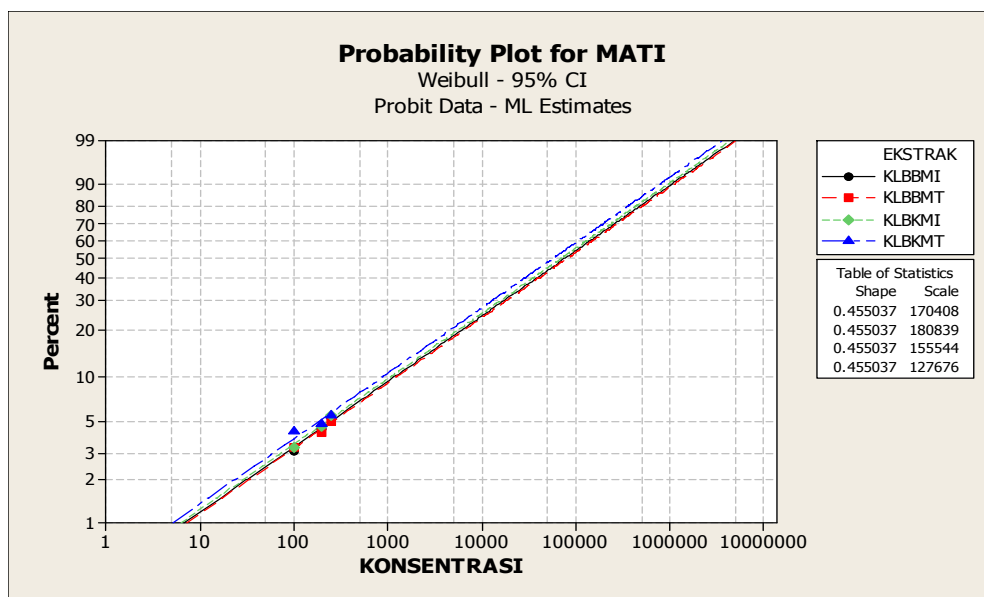
This data then is used to obtain IC50 value using probit analysis on SPSS IBM 20 program. The analysis result can be seen on appendix, while the probit graph vs % parasite growth inhibition at 48 hours incubation can be seen at Picture 3.2. From the probit analysis result, it is obtained the information about Inhibitor Concentration 50 (Inhibitor Concentration = IC50) from each kind of langsung bark extract on *P. falciparum* growth at 48 hours incubation. This IC50 value shows the extract concentration that can inhibit 50% of parasite growth. The smaller the IC50 value, the greater the inhibitory effectiveness of guava leaves against parasite growth (Picture 4 and Picture 5).





Picture 4. Langsung Bark Extract IC50 Comparison

The plant extract effectiveness on malaria parasite which is used traditionally needs to be scientifically tested. The stages of extract bioactivity test after its phytochemistry group contents are known, starts with *in vitro* test. Antimalaria activity *in vitro* using *P. falciparum* culture can be used as preliminary test to evaluate a prospective natural substance as antimalaria.



Picture 5. Extract probability plot on *P. falciparum* infected erythrocyte

The langsung bark has been empirically used by Minahasa societies as antimalarial drug since the ancient time. Minahasa is known as Malaria endemic areas. From the interview with the local community, it is know that langsung bark effectively treats malaria. In the event of an outbreak of malaria in the 1950s, where there was civil war known as Permesta, the people used langsung bark as malaria drug that can decrease the death risk at the time.

The antiparasite activity of Langsung (*L. minahasae* L.) bark extract from Minahasa is analized to know the optimum levels of antimalaria activity of various types of extract and sample origin. The antimalaria activity test

in this research use 3D7 Plasmodium falciparum isolates which were cultured continuously using the Trager and Jensen modification method (1976). *P. falciparum* isolate is cultured using RPMI 1640 medium, HEPES buffer, sodium bicarbonate solution and human serum, and after that it is put in a desiccator containing wax and incubated in incubator with 5% CO<sub>2</sub> and 37°C. An asexual cycle (skizogoni) of *P. falciparum* takes 48 hours. Therefore, in this research, the observation is conducted at 48 hours in accordance with the asexual cycle. After it is incubated for 48 hours, the thin blood smear is made and stained with Giemsa dye. The smear is observed using microscope at 1000 times magnification and calculated the amount of malaria parasite infected erythrocyte per 1000 erythrocytes.

### 3.4 Langsat Bark Extract Antibacterial Activity in vitro

The antimicrobial activity test is conducted in aseptically in jelly diffusion method. For the antibacterial activity test, the bacteria culture which has been aged between 18 and 24 hours in *nutrient broth* (NB) is poured into petri dish and added with 15 ml of jelly nutrient (NA) at 45°C. After the jelly is frozen, the disc paper (6mm diameter) which had been soaked in essential oils with concentrations of 2%, 4%, 6%, 8% and 10% is included into ethanol pa. As a control at each petri dish, the disc paper that has been moistened with ethanol pa is put. These petri dishes are incubated in reverse way for 24 hours at 35-37°C. The clear zone around the disc paper shows the positive test, diameter of obtained clear zone is measured using micrometer, and compared with ampicillin and tetracycline standard compounds.

The result of antibacterial activity test with 2, 4, 6, 8 and 10% concentration (% v/v) in ethanol pa is presented at Table 4 that shows that dry and wet langsat bark extract with 2% to 4% concentration is not able yet to inhibit the growth of tested bacteria. The 6% concentration can only inhibit the growth of *E. coli* and the diameter of its inhibition zone (DDH) is 9 mm. The 8% concentration can inhibit the growth of *E. coli* and *S. aureus*, and the diameters of their inhibition zone are 10 mm and 7 mm. From the result of this activity test with 10% concentration of langsat bark extract is not able yet to inhibit the growth of *E. coli*, assumed that galangal essential oil was not active against *E. coli*.

**Table 4.** Diameter (mm) zone of bacterial growth inhibition by KLBKMI

Concentration of Experiment Extract	Bacteria Isolate				
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	Infection wound isolate 1	Infection wound isolate 2	Infection wound isolate 3
<b>KLBKMI</b>					
2%	2	-	2.5	1.5	1
4%	2.5	-	2.5	2	2
6%	10	5.5	8	9	11
10%	14	12	13	14	14
<b>KLBKMT</b>					
2%	3	-	3.5	2	2
4%	3	1	3	4	3
6%	12	8	10	12	12
10%	15	12	12	14.5	15
<b>KLBBMI</b>					
2%	-	-	-	-	-
4%	1	-	-	-	1.5
6%	3	1	4	5	6
10%	10	3	6	8	7
<b>KLBBMT</b>					
2%	-	-	-	-	-

4%	-	-	-	-	-
6%	2.5	-	3	2	2
10%	9	2	5	6	8
	<b>Control</b>				
Ampisili	14	18	20	21	20
Tetracycline	14	17	22	21	22

- : not form a significant barrier zone

Concentration of each control is 30 µg

## 5. Conclusion

From these results, it can be concluded that:

1. Dry langsat bark extract (KBLK) both from Minahasa (KBLKMI) and Southeast Minahasa (KBLKMT) have % highest extract yield compared with wet bark extract (KBLB). The extract yield highest percentage is in KLBKMT that is 5.78% whereas the lowest one is n-butanol : water fraction's that is 0.663%.
2. The phytochemistry class content that is comprehensively detected (alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids, anthocyanin and quinone) is found in KLBK extract. The highest intensity is found in KLBKMT.
3. The strongest antimalarial activity in the 1000 erythrocyte which is infected by plasmodium, is expressed with found LC<sub>50</sub> value in KLBKMT extract that is 57056.1 µg/ml and the weakest one is in KLBBMT extract that is 80813.8 µg/ml.
4. The strongest antibacterial activity that is expressed with diameter of the formed inhibition zone is in KLBKMT extract that has average 12.5 mm in all tested isolate.

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