

## POTENTIAL ANTIBACTERIAL EXTRACTS OF MANGROVE GENUS AVICENIA ROOT AND LEAVES FROM KOMBI BEACH MINAHASA

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### Abstract

This study aimed to obtain the phytochemical content of the crude extract of leaves and roots of *Avicenia* spp from Kombi Beach Minahasa. Obtaining data on the activity of inhibiting the growth of infective microbes on external wounds of crude extract of leaves and roots of *Avicenia* spp from Kombi Beach, Minahasa. To determine the concentration of minimum growth inhibition of crude extract of leaves and roots of *Avicenia* spp from Kombi Beach, Minahasa. Knowing the crude extract (crude extract) leaves and roots of *Avicenia* spp originating from the Minahasa Kombi Beach is bactericidal or bacteriostatic. This research consisted of extraction, phytochemical analysis and antibacterial activity test. The results showed that the dominant secondary metabolites in the root and leaf extracts were alkaloids, flavonoids, saponins and tannins. The chloroform extract inhibited the growth of bacteria in infected wounds more than the ethanol and n-hexane extracts, at concentrations of 15 and 30 ppm for the test solution of ethanol extract with an inhibitory diameter of < 7 mm and a concentration of 50 ppm with an inhibitory diameter of > 10 mm indicating that the high bioactive activity antibacterial.

**Keywords:** *antibacterial, crude extract, leaf, avicenia*

### INTRODUCTION

Infective diseases caused by pathogenic microbial infections are still a major problem in developing countries. Many factors influence the high prevalence of infective diseases in developing countries, but the main factor is poor sanitation. Infectious diseases and cancer are one of the problems in the health sector that from time to time continue to grow. Infection is a disease that can be transmitted from one person to another or from animals to humans. Infections are caused by various microorganisms such as bacteria, viruses, rickettsiae, fungi, and protozoa. These organisms can attack the whole body or part of it (Gibson, 1996).

To inhibit infection in external wounds, antiseptic is used on new wounds by means of smears while on old wounds, antibiotics are used. Antibiotics are always given as a drug companion in various types of infective diseases by microbes even in inflammation (inflammation). In general, patients do not

finish the antibiotics given by doctors when the disease has improved. Therefore, a report from the Ministry of Health of the Republic of Indonesia in 2010 stated that cases of bacterial resistance to antibiotics in Indonesia had reached a level above 45%.

Indonesia is a country rich in flora and fauna with pharmacological potential. More than 65% of drugs are derived from active plant ingredients. As a tropical country, infective diseases caused by microbes in Indonesia are relatively high, especially with climate change which has changed the pattern of the rainy season. Plants produce secondary metabolites as a form of self-defense against pathogenic microbial infections. These secondary metabolites have medicinal potential, among others, as antibacterial.

Mangroves are plants that live in the transition area between fresh water and sea water (brackish water). This ecological condition causes mangroves to have specific physiological and biochemical mechanisms. Held and heldt (2005) state that plants tend to produce special secondary metabolites when living in extreme environmental stresses. Thus, it is suspected that mangrove plants produce secondary metabolites that have pharmacological potential. This study aims to: Obtain the phytochemical content of the crude extract of leaves and roots of *Avicenia* spp from Kombi Beach, Minahasa. Obtaining data on the activity of inhibiting the growth of infective microbes on external wounds of crude extract of leaves and roots of *Avicenia* spp from Kombi Beach, Minahasa. To determine the concentration of minimum growth inhibition of crude extract of leaves and roots of *Avicenia* spp from Kombi Beach, Minahasa. Knowing the crude extract (crude extract) leaves and roots of *Avicenia* spp originating from the Minahasa Kombi Beach is bactericidal or bacteriostatic.

This research is very useful in exploring the wealth of natural resources, especially plants with pharmacological potential. This study is an initial study using an antibacterial bioassay where cases of infective disease are very high while antibiotic resistance is also very high. The results of this study can provide an alternative to antibiotics for infective diseases of plant origin.

## MATERIALS AND METHODS

### Place of Research

This research was carried out at the Biology Laboratory of FMIPA Manado State University and the Microbiology Laboratory of Pests and Plant Diseases, Faculty of Agriculture, Sam Ratulangi University Manado.

### Materials and Tools

The equipment used in this research are rotary evaporator (Heidolp), artificial incubator, oven, laminar airflow, petri dish, beaker, measuring cup, tweezers, incubator, stirring rod, pH indicator, funnel, filter paper, hot plate, magnetic stirrer, autoclave, label, test tube, inoculation needle, Bunsen lamp, sterile cotton buds, ruler/ micrometer, micro pipette.

The materials used in this study were flowers and leaves of *Avicenia* spp, agar media (NA), 70% and 95% ethanol, sterile distilled water, bacteria from infectious wounds, antibiotics: streptomycin and ampicillin (pharmaceutical chemicals), cotton, tissue rollers, sucrose, disc paper, Ammonia, chloroform, H<sub>2</sub>SO<sub>4</sub>, HCl, Mg, FeCl<sub>3</sub>, Meyer and Wagner reagent, ether, acetic acid.

### **Preparation of Crude Extract of Leaves and Roots of *Avicenia* spp**

Samples of leaves and roots of *Avicenia* spp were washed with running water and washed with detergent then rinsed thoroughly and drained. Then the extract was made into wet extract simplicia and dry extract simplicia using ethanol and n-hexane. For wet simplicia mashed using a mortar, and for dry simplicia dried and mashed using a blender.

In the extraction process, a ratio of 1: 5 (simplicia: solvent) is used, which is 50 grams of simplicia extracted with 250 milliliters of 70% ethanol for the ethanol extract and for the distilled water extract 50 grams of simplicia dissolved in 250 milliliters of distilled water and macerated in a closed vessel for 2x24 hours and shaken occasionally. Then it is filtered using filter paper to produce filtrate, which is then evaporated in a rotary evaporator at a temperature of 35-37°C (48-50 rpm). So that a crude extract will be produced from the leaves of the Mangrove Plant which will be used to be tested in the next stage.

### **Phytochemical Analysis**

Phytochemical Analysis Methods (Harborne 1996) in Mokosuli YS, (2008). Alkaloid Test: A total of 0.1 grams of extract was added with 3 mL of chloroform and 3 drops of ammonia. The chloroform fraction was separated and acidified with 10 drops of 2 M H<sub>2</sub>SO<sub>4</sub>. The acid fraction was taken, then Meyer and Wagner's reagent was added. The presence of alkaloids was indicated by the formation of a white precipitate by the Meyer reaction and a brown precipitate by the Wegner reaction. Saponin and Flavonoid Test, 1 gram of extract was put in a beaker then added 100 ml of hot water and boiled for 5 minutes, after that it was filtered and the filtrate was used for testing. Saponin test: carried out by shaking 10 ml of the filtrate in a closed test tube for 10 seconds and then left for 10 minutes. The presence of saponins is indicated by the formation of stable foam. Another 10 ml of the filtrate was added with 0.5 grams of magnesium powder, 2 ml of carbohydrate alcohol (a mixture of 37% HCl and 95% ethanol in a ratio of 1:1) and 20 ml of amyl alcohol and then shaken vigorously. The formation of red, yellow and orange colors on the amyl alcohol layer indicates the presence of flavonoids. Tannin test: 0.1 grams of extract added 2 mL of water and then boiled for several minutes. Then filtered and the filtrate was added with 1 drop of 1% FeCl<sub>3</sub> (w/v). Dark blue or greenish black color indicates the presence of tannins. Triterpenoid and Steroid Test: 0.1 grams of extract added 2 mL of 30% ethanol then heated and filtered. The filtrate was evaporated and then 1:1 ether was added. The ether layer was added with Lieberman Burchard's reagent (3 drops of acetic anhydride and 1 drop of concentrated H<sub>2</sub>SO<sub>4</sub>). Red and green colors indicate the presence of triterpenoids and green colors indicate the

presence of steroids.

### Antibacterial Activity Test

#### - Preparation of Infective Bacterial Inoculants

Bacterial samples were taken at the Bethesda Tomohon Hospital for infected or festering wounds. Bacterial samples were taken from a wound that was actively infected or had pus, then took the pus fluid using a sterile cotton swab and immediately cultured or scratched on the agar (NA) media that had previously been prepared aseptically.

#### - Bacterial Breeding

Prepare near the fire all equipment and materials to be prepared. Burn the inoculation needle over the fire until the entire wire glows, allow the needle to cool for about 30 seconds before use. Then take a few bacterial colonies and separate them, and with your left hand take it at an angle and open the plug using the right little finger, heat the mouth of the tube that is not clogged by doing it over a fire, insert a needle that already contains bacteria into the tube so that it tilts then scratch it on the surface so that it is tilted in a zigzag manner, reheat the mouth of the tube and then close it with a stopper. Re-illuminate the inoculation needle before storing and using it again. Label the newly inoculated tubes according to the type of colony and origin. Incubate at room temperature and put in an incubator at a temperature of 22-30°C for 2x24 hours (Irobi et al. 1996; Russel and Fur, 1977; Akarele et al. 2008).

#### - Antibacterial Activity Test

Preparation of mother liquor for antibacterial activity test

The mother liquor was made in 500 ppm (mg/l) i.e. 50 mg of the extract was dissolved in 1000 ml of distilled water. Then the mother liquor was made in several concentrations of the test solution, namely 50, 30, 15 ppm (mg/l) and streptomycin and ampicillin at a concentration of 15 ppm and aseptically.

Determination of the concentration of the solution using the formula:

$$\text{ppm1} \cdot v1 = \text{ppm2} \cdot v2$$

note:      v1        = volume 1,  
              v2        = volume 2  
              ppm1    = main solution concentration  
              ppm2    = concentration of the solution to be made

### Agar Diffusion Method

The implementation of this stage is carried out in an aseptic laminar airflow. The NA media was well made and then the test solution was given using a micropipette. After that, take the bacteria that have been inoculated and incubated for 18 hours using a sterile cotton bud and circled in the media that has been dissolved by the test solution (50, 30, 15 ppm) and the test solution using streptomycin and ampicillin at a concentration of 15 ppm. Then it was incubated for 72 hours at 320C after 30 minutes

of inoculation. Then the diameter of the inhibition of bacterial growth was measured with a micrometer and the lowest concentration that prevented the growth of bacteria. Each was replicated 3 times.

### Research Variables

The variables measured in this study are:

1. The growth inhibition of bacteria from the crude extract of leaves and roots of *Avicenia* spp at various concentration levels, was measured by micrometer clear zone formed on KNA media.
2. The minimum concentration of bacterial growth inhibition is the smallest concentration of extract that can inhibit the growth of the test bacteria.

### Data Analysis

The research data were analyzed qualitatively and quantitatively.

## RESULTS AND DISCUSSION

The results of phytochemical testing using the Harborne method (1996) showed that ethanol and aquades extracts from the roots and leaves of *Avicenia* spp contained alkaloids, flavonoids, saponins, tannins, triterpenoids and steroids (Table 1).

Table 1. Phytochemical analysis data of flowers and leaves of *Avicenia* spp (*Impatiens balsamina* Linn)

No.	Compound	Root	Leaf
1.	Alkaloids		
	- Meyer	+++	+++
	- Wagner	+++	++
2.	Saponins	+++	++
3.	Flavonoids	+++	+++
4.	Tannin	+	++
5.	Triterpenoids	++	+
6.	Steroids	+	+

Where (+++ ), (++) , (+) , (-) indicate the high and low content of compounds qualitatively which is characterized by the presence of deposits, and the intensity of color (Harborne, 1996). The parameters measured were the amount of white precipitate through the separation by Meyer's reagent and the presence of brown precipitate through the separation by Wagner's reagent in the Alkaloid test, the amount of stable foam/foam in the saponin test, the formation of red yellow, orange in the amyl alcohol layer in the flavonoid test, the formation of dark blue or blackish green in the tannin test, the formation of red, green in the triterpenoid test and green in the steroid test.

The antibacterial activity of crude extract of roots and leaves of *Avecenia* spp obtained from Kombi Beach against bacteria in infected wounds can be calculated by measuring the diameter of the inhibition (DDH) of bacterial growth (Figure 5). Each tested ethanol extract (A1), chloroform extract (A2) and n-hexane extract (A3). The results of the measurement of the diameter of the inhibition zone for bacterial growth at several concentrations of the test solution (Figures 3 and 4).

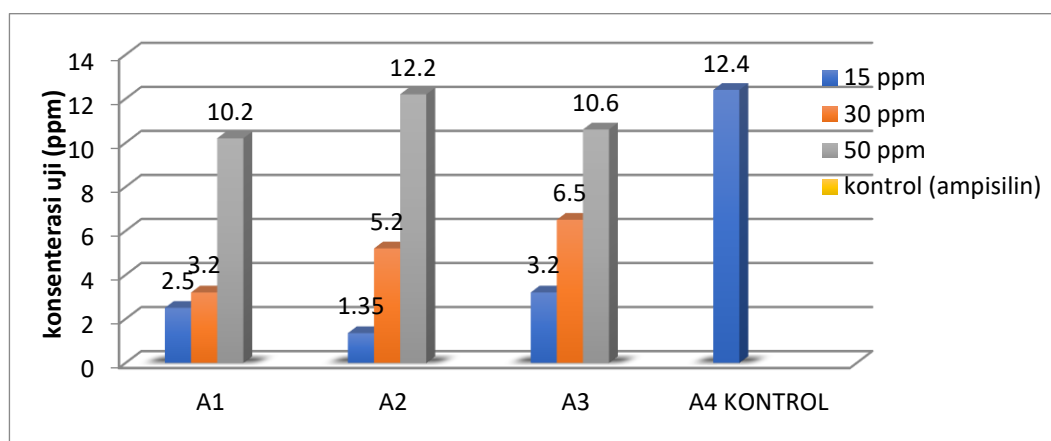


Figure 3. Graph of Bacterial Growth Inhibitory Zone Diameter Measurement

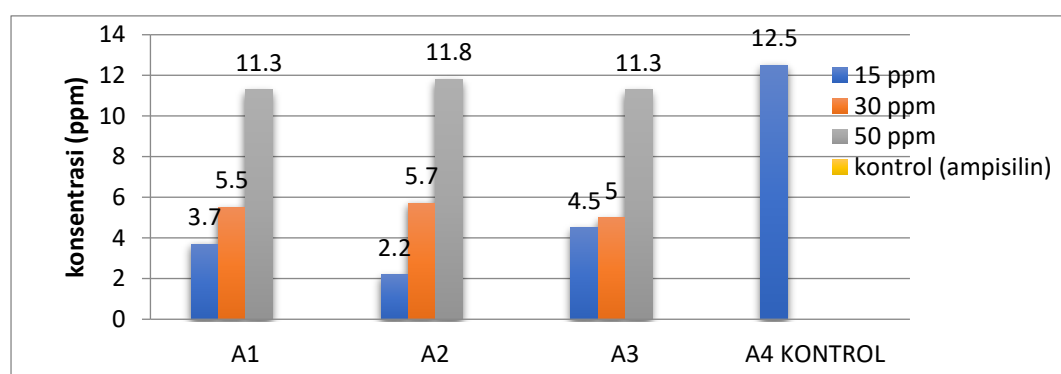


Figure 4. Graph of the Diameter Measurement of the Inhibitory Zone for Bacterial Growth D

From the results of the measurement of the diameter of the growth inhibition of bacteria, it can be seen that the minimum concentration of growth inhibition was obtained from the leaf and root extract of *Avecinia* spp.

Table 2. Minimum Growth Inhibitory Concentration of Crude Extract of Leaves and Roots of *Avicenia* spp

Simplicia	Ethanol extract		Chloroform extract		N-hexane extract		Ampicillin (control)	
	KHTM (ppm)	DH (mm)	KHTM (ppm)	DH (mm)	KHTM (ppm)	DH (mm)	KHTM (ppm)	DH (mm)
A1	15	3	15	1				
A2	15	1,67	15	0,83				
A3	15	2,67	15	1				
A4	15	1.67	15	0,83				
					15	11,33	15	15,33

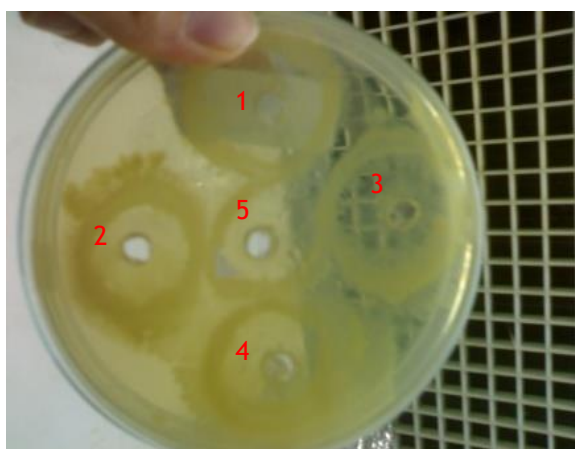


Figure 5. Minimum Growth Inhibitory Concentration

In the process of maceration of the extract with ethanol solvent, it has a sharp smell typical of *Avicenia* spp. The 70% ethanol extract took longer to evaporate with rotapavor because it contained a fairly high water content. The evaporation process is carried out in 2 ways, namely using a rotary evaporator with a temperature of 35-40°C and 60rpm, and the second is carried out with an artificial incubator with a temperature of 32°C. Using a rotary evaporator produces extracts that are faster than those using an artificial incubator. This is because the temperature in the rotary evaporator is higher and is equipped with a rotation that accelerates the evaporation of water. The use of a rotary evaporator with a relatively high temperature can cause damage to the compounds in the extract which have antibacterial properties. Then those using artificial cash, the filtrate is only put in small bottles and petridish in the cash and the temperature is only 32°C so that it will produce a better extract. Using a rotary evaporator within  $\pm 8$  hours will produce an extract, and in an artificial incubator it reaches  $\pm 5$  days for ethanol solvent, and  $\pm 7$  days for aquades solvent.

The results of the evaporation process using a rotary evaporator and an artificial incubator have the same shape, color, and smell. The form is like dry paste (dry extract), thickened paste (wet extract). The leaf extract is blackish green and the root extract is blackish brown. The resulting extract was analyzed for its photochemical content using the Harborne method, where it would be seen what class of



compounds were contained in the leaves and roots of *Avecenia* spp. It turns out that leaf and root extracts contain compounds of Alkaloids, Saponins, Triterpenoids, Tannins, Steroids, and Flavonoids. However, the concentration of alkaloids and flavonoids is relatively higher.

In a study conducted by Pholoengan, et al. (2006) on Antimicrobial and Phytochemical Activities of Some Medicinal Plants, some plants have high alkaloid content, namely *Baccaurea lanceolata* Mig. (++++), *Cinnamomum jaanicum* BL. (+++), *Cinnamomum porrectum* BL. (+++), *Ficus annulata* BL. (+++), *Garcinia Bacana* Mig. (+++), *Piper miniatum* BL. (+++), and *Pittosporum ferrugineum* W. Ait. (++++). And contains medium triterpenoids (++) namely *Cinnamomum jaanicum* BL., *Ficus annulata* BL., *Garcinia bancana* Mig., *Pittosporum ferrugineum* W.Ait., *Syzygium racemosum* BL., and *Uncaria Glabrata* BL. Saponin compounds in moderate amounts (++) were found in the plant species *Aglaia argentea* BL., *Baccaurea lanceolata* Mig., *Ficus annulata* BL., *Garcinia bancana* Mig., *Piper miniatum* BL., *Pittosporum ferrugineum* W.Ait., *Syzygium racemosum* BL. and *Uncaria Glabrata* BL.

From the results of phytochemical analysis of crude extracts of leaves and roots there are several compounds that are high in content, namely Alkaloid compounds (+++) which are characterized by the number of white deposits resulting from the separation of the Meyer reaction and brown deposits produced by Wagner's reagent. Saponins (++) are characterized by the amount of foam/foam produced, Tannins (+++) form a blackish green color. From the results of photochemical analysis, it turns out that *Avicenia* spp plants have compounds that can be antibacterial. The compounds contained function: (1) damage the cell wall resulting in lysis or inhibit the process of cell wall formation in growing cells; (2) changing the permeability of the cytoplasmic membrane which causes the leakage of nutrients from within the cell; (3) denature cell proteins; (4) damage the metabolic system in the cell by inhibiting the work of intracellular enzymes.

Based on the results from table 2, the leaf extract has a higher antibacterial activity, so that in the test the antibacterial activity of the leaf shows a greater inhibition zone than the root extract. This is because the leaf extract is thought to have a high concentration of bioactive metabolites compared to the root extract. Plant leaves are the center of carbohydrate biosynthesis so that the raw materials for making secondary metabolites are quite available compared to roots.

From the results of the test the minimum growth inhibitory concentration (KHTM) with a concentration of 50, 30, 15 ppm (mg/ml), and antibiotics streptomycin and ampicillin as a positive control with a concentration of 15 ppm. Shows the difference in the diameter of the inhibition ranging from concentrations of 50, 30, 15 ppm. According to Salvador et al. 2007, and Tuney et al. 2006, the inhibition of bacterial growth refers to the value: < 7 mm where the diameter of the inhibition zone is little activity, 7-10 mm is moderate activity (active), the inhibition zone diameter >10-15mm indicates activity (a level suitable for use as an antibiotic).

At concentrations of 15 and 30 ppm for the test solution of ethanol extract with an inhibitory diameter of < 7, it shows that there is still a lack of bioactive activity, while at a concentration of 50 ppm with an inhibitory diameter of > 10, it shows that high antibacterial bioactive activity can be seen, and can



be seen in Figure 5. And for the test solution Aquades extract at concentrations of 15 and 30 ppm with an inhibitory diameter of  $< 7$  indicates that the bioactive activity is still low and for 50 ppm with an inhibitory diameter of 7-10 it shows that it actively inhibits bacterial growth in Figure 6.

Research conducted by Parwata (2008) on "Antibacterial Test of Essential Oils from the Rhizome of Galangal (*Alpinia galanja* L.)". The test results of essential oil activity against *Escherichia coli* bacteria at concentrations of 100 ppm and 1000 ppm showed the diameter of the inhibition area was 7 mm and 9 mm, and was only able to inhibit *Staphylococcus aureus* at a concentration of 1000 ppm at 7 mm. Another research on "Activity Test of *Gracilaria* sp. Extract. Based on Source of Extraction". The results of the activity test of the dry extract of *Gracilaria* sp. against *Staphylococcus aureus* bacteria at a concentration of 500 ppm, showing an inhibitory diameter of 10.42 mm, and inhibiting *Escherichia coli* bacteria at a concentration of 500 ppm at 9.31 mm.

Based on the above studies, it turns out that the leaf extract has a greater inhibitory power of 10-12 mm at a concentration of 50 ppm on bacteria from infected wounds. So that the dried leaf extract of the *Avicenia* spp (*Impatiens balsamina* Linn) plant with ethanol as a solvent at a concentration of 50 ppm of 12.0 mm has fulfilled the level requirements as an antibiotic.

## CONCLUSION

1. The predominant secondary metabolites in the root and leaf extracts are alkaloids, flavonoids, saponins and tannins.
2. The chloroform extract inhibited the growth of bacteria in infected wounds more than the ethanol and n-hexane extracts, at concentrations of 15 and 30 ppm for the test solution of ethanol extract with an inhibitory diameter of  $< 7$  mm and a concentration of 50 ppm with an inhibitory diameter of  $> 10$  mm, indicating that the high antibacterial bioactive activity.

## REFERENCES

- Akarele JA. 2008. Antimicrobial Activity Of The Ethanol Extract And Fractions Of The seeds Of *Garcinia kola* Heckel (Guttiferae). *Afr. J. Biotechnol.* Vol. 7 (2):169-172.
- Bidiarso R. 1996. Survey Kesehatan Rumah Tangga. Jakarta : Badan penelitian dan Pengembangan Kesehatan DepKes RI.
- Ajijah A. 2004. Sensitivitas *Salmonella typhimurium* Terhadap Ekstrak Daun *Psidium guajava*. *Bioscientiae* Volume 1(1):31-38.
- Cutisurb surbact . 2007. Pencegahan Pada Luka Trauma Superfisial. PT BSN Medical Indonesia
- Djulkarnain,dkk. 2009. Tanaman Obat Bersifat Antibakteri.
- Hartini, dkk. 2006. Daya Antibakteri Campuran Ekstrak Etanol Buah Adas (*Foeniculum Vulgare* Mill) Dan Kulit Batang Pulasari (*Alyxia Reinwardtii* Bl). *Jurnal Farmasi.* hal 191-196
- Irobi ON Mon-Young, Anderson WA (1996). Antimicrobial activity of Annatto (*Bixa orellana* extract). *Int. J. Pharm* Vol.34:87-90
- Mokosuli YM. 2008. Aktivitas Antioksidasi Dan Antikanker Ekstrak Kulit Batang Langsung (*Lansium domesticum* L.) [Tesis]. SPS Institut Pertanian Bogor.
- Noor, dkk. 2006. Analysis of Secondary Compounds and Testing of Antibacterial Activity of *Minusops elengi* L Extract on *Salmonella typhi* and *Shigella boydii*. Seminar Nasional Teknologi Peternakan dan Veteriner pp.986-992.

- Parwata, dkk. 2008. Isolasi Dan Uji Aktivitas Antibakteri Minyak Atsiri Dari rimpang Lengkuas (*Alpinia galanga* L.). Jurnal Kimia 2(2):100-104
- Pholoengan, dkk. 2006. Aktivitas Antimikroba Dan Fitokimia Dari Beberapa Tanaman Obat. Seminar Nasional Teknologi Peternakan dan Veteriner 2006 (pp. 974-978).
- Rasional. 2001. Antibakteri. Media Informasi Peresepan Rasional Bagi Tenaga Kesehatan Indonesia. Vol.2(1).
- Russel AD, Fur JR (1997). Antibacterial activity of a new chloroxyleneol preparation containing ethylenediamine tetra acetic acid. *J. Appl. Bacteriol* 43: 253-260.
- Salvador, N. A. G. Garreta, L. Lavelli and M. A. Ribera. 2007. Antimicrobial Activity of Iberian Macroalgae. *Scientia Marina*, vol.1:101-113
- Tuney, Inci., B. H. Cadirci, D. Unal and A. Sukatar. 2006. Antimicrobial Activities of the Extracts of Marine Algae from the Coast of Uria (Izmir, Turkey). *Turk J Biol* vol.30:171-175