

Effectiveness of Antibacterial Leaf Extract *Jatropha curcas* Linn with *Klebsiella pneumoniae* Bacteria

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Abstract

Background: This study aims to determine the effectiveness of jatropha leaf extract (*Jatropha curcas* L) with a concentration of 30%, 50%, 70%, 90%, and 100% against bacteria *Klebsiella pneumoniae* with amoxicillin-clavulanic acid as a comparison.

Method: The type of research used is true experiment with design posttest only. Antibacterial effectiveness test of leaf extract (*Jatropha curcas* L) against *Klebsiella pneumoniae* by agar diffusion/Kirby-Bauer using disc paper in which there is jatropha leaf extract then put into culture media (MHA) this method is useful for observing the diameter of the inhibition zone extract then the results are batch-to-batch good.

Results: The data processing method used in the research trial was Shapiro-Wilk, namely one-way ANOVA to determine the antibacterial effect of jatropha leaf extract and the Least Significant Difference (LSD) test to determine the difference in antibacterial effects between the 2 treatment groups.

Conclusion: From this test, it shows that the research data is normally distributed because $p > 0.05$ in the statistical test oneway ANOVA shows a value of $p = 0.000$ ($p < 0.05$), that is, there is a significant difference in the antibacterial effect of jatropha leaf extract (*Jatropha curcas* L) on bacteria. *Klebsiella pneumoniae* in the analysis results posthoc LSD showed that there was a significant difference between the extract group and the other extract treatment groups.

Keywords: *Klebsiella pneumoniae*; *Jatropha curcas* L; Jatropha leaf extract

INTRODUCTION

Lung disease is a very dangerous disease because the rate of transmission occurs very quickly. Indonesian people have very little knowledge about the dangers of lung disease and the symptoms they experience so that if left without proper treatment there is a risk of disease transmission and even death), Chronic Obstructive Pulmonary Disease (COPD), Bronchial Asthma, Lung Cancer, and Pneumonia. [1]

Non-smoking tobacco products can increase the risk factors for disease transmission. From research conducted in Saudi Arabia, there are 55 types of bacteria in non-smoking tobacco products, consisting of 28 types of gram-positive bacteria and 27 types of gram-negative bacteria. There was found *Klebsiella pneumoniae* bacteria were the bacteria are bacteria that are resistant to antibiotics. [2]

Infections caused by pathogenic bacteria that are resistant to antibiotics have increased health care and community-acquired infections. Antibiotic resistance is a serious and growing threat to public health and health care providers worldwide. Factors for the emergence of the spread of antibiotic resistance worldwide include inappropriate use of antibiotics, health care settings in

prescribing, and widespread community use resulting in an increasing number of immunocompromised individuals, increased global travel, and migration from countries with high levels of the pathogen. Antibiotic resistance is higher with an insufficient number of new antibiotics in development. Based on the table Antibiotic-resistant bacteria in the United States of America, Europe, and the world in 2015 in Europe the bacteria *Klebsiella pneumoniae* (17%) experienced resistance to antibiotic drugs. [3]

In Indonesia, from the table of microbial surveillance data on type A/intensive care type hospital with laboratory testing of blood specimens of gram-negative bacteria *Klebsiella pneumoniae* resistance to antibiotics amoxicillin-clavulanic acid (55.5%), ampicillin-sulbactam (2.1%), azithromycin (55.5%), cefepime (8.5%), cefoxitin (50%), ceftazidime (10.6%), ceftriaxone (6.4%), ciprofloxacin (51.1%), ertapenem (87.2%), gentamicin (33.9%), imipenem (50%). [4]

Klebsiella pneumoniae is a gram-negative bacterium that is commonly found in the human digestive tract, a type of bacteria that is an opportunistic pathogen that usually infects the urinary tract, pneumonia, cystitis, surgical wound infections, and

life-threatening infections such as endocarditis and sepsis. The number of side effects caused by antibiotics makes many people use traditional medicinal plants as an alternative to treat infectious diseases.

Indonesian people have always used the surrounding plants to meet their needs such as food, beauty, and various health problems. This is easy to do, considering that plants can be planted in various places and do not require a large area of land. Natural ingredients have to be trusted by the community for a long time because they can treat diseases without side effects compared to drugs that use synthetic materials.

Jatropha (*Jatropha curcas* L) can become an antimicrobial plant because it has latex metabolite compounds as its main component. In addition, there are metabolites, tannins, polyphenols, and polysaccharides as enzyme inhibitors. It is known that *jatropha* leaves are antiparasitic, while *jatropha* leaf extract and seeds are reported as antibacterial. In the *jatropha* leaves and bark, it is known that the most abundant elements are tannins, flavonoids, cardiac glycosides, phlorotannins, terpenoids, and alkaloids. However, it depends on the concentration of *jatropha* leaves, microbes, and parts used. Tannins with proline-rich proteins form an

irreversible complex that inhibits protein synthesis [5].

Jatropha leaves, leaves, fruit, and stems can inhibit *Klebsiella pneumoniae* bacteria because they contain flavonoid compounds as inhibitors of protein synthesis which are anti-microbial, anti-allergic, anti-inflammatory, anti-oxidant, and analgesic. the research was developed on the effectiveness of *jatropha* leaf extract (*Jatropha curcas* L) as an antibacterial against *Klebsiella pneumoniae* bacteria.

METHODS

1. Study Design

The type of research used was a true experiment with a post-test-only design that aims to determine the antibacterial effectiveness of *Jatropha* leaf extract (*Jatropha curcas* L) at concentrations of 30%, 50%, 70%, 90%, and 100% against *Klebsiella pneumoniae* bacteria.

2. Population and Sample

The sample used in this study was *jatropha* leaves (*Jatropha curcas* L) derived from *jatropha* leaf trees obtained in Sidodadi Village, Aceh Tamiang Regency. *Klebsiella pneumoniae* bacteria were purchased from the USU FMIPA Microbiology Laboratory.

The research location for the manufacture of extracts was carried

out at the Traditional Medicine Laboratory, Faculty of Pharmacy, the University of North Sumatra in June. Meanwhile, to see the effectiveness of the extract as an antibacterial on *Klebsiella pneumoniae* bacteria, it was carried out at the Prima Indonesia University Laboratory in March.

3. Study Instruments

The tools used in this research are rotary evaporator, beaker glass, petri dish, incubator, water bath, laminar airflow, vortex mixer, paper disc.

The research materials that will be used are jatropha leaves (*Jatropha curcas* L), *Staphylococcus aureus* bacteria, amoxicillin-clavulanic acid antibiotics, DMSO (dimethyl

sulfoxide), MHA media (Muller Hinton Agar).

The method of making jatropha leaf extract using the maceration method. Weigh 500 grams of *Simplicia* powder then put it in a container.

4. Data Analysis

Data was analyzed by using one-way ANOVA and posthoc LSD parametric statistical tests.

RESULT

In *Jatropha* leaf extract (*Jatropha curcas* L) a phytochemical screening test was carried out so that the results were obtained as shown in the table.1 below:

Table 1. Resultsof Phytochemical Screening of *Jatropha* Leaf Extract (*Jatropha curcas* L)

No	Parameter	Result	Sign
1	Alkaloid	Positive	+
2	Steroid	Positive	+
3	Triterpenoid	Positive	+
4	Saponin	Negatif	-
5	Flavonoids	Positive	+
6	Tanin	Positive	+

The antibacterial effectiveness of *Jatropha* leaf extract (*Jatropha curcas* L) at concentrations of 30%, 50%, 70%, 90%, 100% against *Klebsiella pneumoniae* bacteria us-

ing the diffusion method. The results showed that all concentrations produced an inhibition zone.

Table 2. Results of Inhibition Zone Measurement of Jatropha Leaf Extract (*Jatropha curcas* L) against *Klebsiella pneumoniae* bacteria

Repetition	Inhibitory Zone Diameter (mm) at a concentration of Jatropha (<i>Jatropha curcas</i> L) Leaf Extract					Negative Control	Positive Control
	30%	50%	70%	90%	100%		
1	12,6	14,0	15,3	17,7	20,9	0	0
2	11,5	15,0	15,5	16,2	19,4	0	0
3	13,2	12,6	13,2	12,5	19,8	0	0
4	12,5	12,5	13,8	13,8	16,5	0	0
Median (mm)	12,450	13,525	14,450	15,050	19,150	0	0
Deviasi Standart	0,7047	1,1983	1,1269	2,3388	1,8771	0	0

Description: Positive Control (*Amoxicillin-clavulanic acid*), Negative Control (DMSO)

Data from the antibacterial effectiveness test of Jatropha leaf extract (*Jatropha curcas* L) against *Klebsiella pneumoniae* bacteria, then tested for normality with Shapiro-Wilk. The results of the normality test can be seen in Table 3 below.

Table 3. Normality Test Data for Antibacterial Effects of Jatropha Leaf Extract (*Jatropha Curcas* L) Concentration of 30%, 50%, 70%, 90%, 100%, Positive Control (Amoxicicilin-Clavulanic Acid) and Negative Control (DMSO) against *Klebsiella* Bacteria *Pneumoniae*

Concentration	<i>p value</i>	Description
30%	0,669	Normal
50%	0,355	Normal
70%	0,335	Normal
90%	0,791	Normal
100%	0,449	Normal
Positive control	0	-
Negative control	0	-

The results of the one-way ANOVA test were used to see the antibacterial effect of jatropha leaf extract (*Jatropha curcas* L) at concentrations of 30%, 50%, 70%, 90%, 100%, positive control (*Amoxicillin-clavulanic acid*), and negative control (DMSO) against bacteria. *Klebsiella pneumoniae* can be seen in Table 4 below.

Table 4. Oneway ANOVA Test Results Antibacterial Effect of Jatropha Leaf Extract (*Jatropha Curcas* L) Concentration of 30%, 50%, 70%, 90%, 100%, Positive Control (Amoxicicilin-Clavulanic Acid) and Negative Control (DMSO) against Bacteria *Klebsiella pneumoniae*

Group	$\bar{x} \pm SD$	<i>p-value</i>	Description
30%	12,450±0,7047 mm	0,000*	There is a significant difference
50%	13,525±1,1983 mm		
70%	14,450±1,1269 mm		

Group	$\bar{x} \pm SD$	<i>p-value</i>	Description
90%	15,050±2,3388 mm		
100%	19,150±1,8771 mm		
Positive control	0		
Negative control	0		

Description: there is a significant difference ($p < 0.05$)

The posthoc LSD test was used to determine the difference in antibacterial effects

between the 2 treatment groups, more details are seen in table 5 below

Table 5. Posthoc LSD Test Results Antibacterial Effects of *Jatropha Curcas* L Leaf Extract Concentrations of 30%, 50%, 70%, 90%, 100%, Positive Control (Amoxicillin-Clavunalic Acid) and Negative Control (DMSO) against Bacteria *Klebsiella pneumoniae*

Kelompok	<i>p value</i>	Keterangan
Extract 30% - extract 50%	0,262	No difference
Extract 30% - extract 70%	0,044	There is a difference
Extract 30% - extract 90%	0,011	There is a difference
Extract 30% - extract 100%	0,000	There is a difference
Extract 30% - positive control	0,000	There is a difference
Extract 30% - negative control	0,000	There is a difference
Extract 50% - extract 70%	0,333	No difference
Extract 50% - extract 90%	0,117	No difference
Extract 50% - extract 100%	0,000	There is a difference
Extract 50% - positive control	0,000	There is a difference
Extract 50% - negative control	0,000	There is a difference
Extract 70% - extract 90%	0,527	No difference
Extract 70% - extract 100%	0,000	There is a difference
Extract 70% - positive control	0,000	There is a difference
Extract 70% - negative control	0,000	There is a difference
Extract 90% - extract 100%	0,000	There is a difference
Extract 90% - positive control	0,000	There is a difference
Extract 90% negative control	0,000	There is a difference
Extract 100% - positive control	0,000	There is a difference
Extract 100% - negative control	0,000	There is a difference
Positive control - negative control	1,000	No difference

DISCUSSION

Based on table 1 above, the results of research on phytochemical screening tests indicate that *Jatropha* leaf extract (*Jatropha curcas* L) contains secondary me-

tabolites in the form of alkaloids, steroids, triterpenoids, flavonoids, tannins. The method of testing the compound content of *Jatropha* leaf extract (*Jatropha curcas* L) is qualitative which is only to detect the presence or absence of metabolites in this study. The test was carried out on *jatropha*

leaf extract (*Jatropha curcas* L) at concentrations of 30%, 50%, 70%, 90%, 100%. The formation of an inhibition zone or clear zone around the paper disc on MHA media indicated an inhibition zone for the growth of *Klebsiella pneumoniae* bacteria. Whereas in the positive control (Amoxicillin-clavulanic acid) and the negative control (DMSO) no inhibition zone or clear zone was found. Based on table 2 above, the results showed that the average inhibitory diameter of *Jatropha* leaf extract (*Jatropha curcas* L) at concentrations of 30%, 50%, 70%, 90%, 100% in inhibiting *Klebsiella pneumoniae* was 12.450 ± 0.7047 mm; 13.525 ± 1.1983 mm; 14.450 ± 1.1269 mm; 15.050 ± 2.3388 mm; 19.150 ± 1.8771 mm. while the positive control (Amoxicillin-clavulanic acid) and the negative control (DMSO) had no inhibition.

Based on table 3 above, the results of the Shapiro-Wilk normality test on the antibacterial effectiveness of *Jatropha* leaf extract (*Jatropha curcas* L) against *Klebsiella pneumoniae* bacteria indicate that the data in this study were normally distributed because the p value > 0.05 . Thus, data analysis can be continued with one-way ANOVA and posthoc LSD parametric statistical tests. The test was carried out on *Jatropha* leaf extract (*Jatropha curcas* L) at concentrations of 30%, 50%, 70%, 90%, 100%. The formation of an inhibition zone or clear zone around the paper disc on MHA media indicated an inhibition zone for the growth of *Klebsiella pneumoniae* bacteria. Whereas in the positive control (Amoxicillin-clavulanic acid) and the negative control (DMSO) no inhibition zone

or clear zone was found. Based on table 2 above, the results showed that the average inhibitory diameter of *Jatropha* leaf extract (*Jatropha curcas* L) at concentrations of 30%, 50%, 70%, 90%, 100% in inhibiting *Klebsiella pneumoniae* was 12.450 ± 0.7047 mm; 13.525 ± 1.1983 mm; 14.450 ± 1.1269 mm; 15.050 ± 2.3388 mm; 19.150 ± 1.8771 mm. while the positive control (Amoxicillin-clavulanic acid) and the negative control (DMSO) had no inhibition.

Based on table 4 above, the results of the Oneway ANOVA statistical test indicate that the value of $p = 0.000$ ($p < 0.05$), the research hypothesis is accepted, namely that there is a difference in the antibacterial effect of *Jatropha curcas* Linn (*Jatropha curcas* Linn) leaf extract against *Klebsiella pneumoniae* bacteria.

Based on table 5 above, the results of the Posthoc LSD test showed that there was a significant difference between the 30% extract group and the 70% extract group ($p=0.044$), the 30% extract group and the 90% extract group ($p=0.011$), the 30% extract group and the 30% extract group. 100% ($p=0.000$), extract group 30% - positive control ($p=0.000$), extract group 30% with negative control ($p=0.000$), extract group 50% and extract 100% ($p=0.000$), extract group 50% with positive control ($p=0.000$), 50% extract group with negative control ($p=0.000$), 70% extract group with 100% extract ($p=0.000$), 70% extract group with positive control ($p=0.000$), 70% extract group with negative control ($p=0.000$), 90% extract group with 100% extract ($p=0.000$), 90% extract

group - positive control ($p=0.000$), 90% extract group with negative control ($p = 0.000$), 100% group with positive control ($p = 0.000$), and 100% extract group with negative control ($p = 0.000$).

The group that showed the results of the Posthoc LSD test that there was no significant difference was obtained in the 30% extract group with 50% extract (0.262), 50% extract group and 70% extract (0.333), 50% extract group and 90% extract (0.117) , 70% extract group with 90% extract (0.527), and positive control with negative control ($p=1,000$).

The antibacterial effectiveness of jatropha leaf extract (*Jatropha curcas* L) at concentrations of 30%, 50%, 70%, 90%, and 100% against *Klebsiella pneumoniae* bacteria in this study was due to the presence of active compounds that have antibacterial properties. One of the active compounds contained in Jatropha leaf extract (*Jatropha curcas* L) is an alkaloid. According to Tiwa et al 9, alkaloids can act as an antibacterial by disrupting the composition of the peptidoglycan component of bacterial cells so that the bacterial cell wall is not fully formed and results in lysis.

The mechanism of action of tannin compounds in Jatropha leaf extract (*Jatropha curcas* L) as an antibacterial by inactivating adhesin and enzymes in microbial cells. In addition, tannins also interfere with the protein transport process in the inner layer cells. Imperfect cell wall formation is a target for tannins in poly-

peptides that result in cell lysis in bacteria by osmotic and physical pressure so that bacterial cells die.

Then, there are flavonoid compounds in jatropha leaf extract (*Jatropha curcas* L). The mechanism of action of flavonoid compounds in the form of extracellular protein complex compounds is polar so that it can damage bacterial cell membranes and release intracellular compounds. Flavonoid compounds can also inhibit DNA-RNA synthesis by an accumulation of nucleic bases so that energy metabolism is disrupted. [6]. Inhibition of membrane binding enzymes such as ATPase and Phospholipase 2 is what inhibits bacterial growth. In addition, Jatropha leaf extract (*Jatropha curcas* L) also contains steroid/triterpenoid components, its mechanism of action is to interfere with the transport of important ions into bacterial cells

CONCLUSION

There is an antibacterial effect of Jatropha leaf extract (*Jatropha curcas* L) against *Klebsiella pneumoniae* bacteria. There was an increase in the average diameter of the inhibition zone in each treatment with each increase in the concentration of jatropha leaf extract (*Jatropha curcas* Linn) tested. The mean diameter of *Klebsiella pneumoniae* bacteria with concentrations of 30%, 50%, 70%, 90% and 100% were 12.450 ± 0.7047 mm; 13.525 ± 1.1983 mm; $14,450\pm 1.1269$ mm; 15.050 ± 2.3388 mm; $19,150\pm 1.8771$ mm.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest in this study.

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