mutiara medika
Jurnal Kedokteran dan Kesehatan
Vol 21 No 1 Page 45-50, January 2021

Mutiara Medika: Jurnal Kedokteran dan Kesehatan
http://journal.umy.ac.id/index.php/mm

Effects of Anti *Mycobacterium tuberculosis* Nanoherbal Legundy Leaf Extract (*Vitex trifolia*)

Atika Dwiyanti,[1] Siti Mulyani,[1] Oche Axena Yulhan,[1] Humairah Medina Liza Lubis,[2] Ance Roslina [3]

[1] Medical Student of Faculty Medicine Muhammadiyah University of North Sumatra.
[2] Section of Anatomical Pathology, Faculty of Medicine Muhammadiyah University of North Sumatra.
[3] Department of Microbiology, Faculty of Medicine, Muhammadiyah University of North Sumatra

DATA OF ARTICLE:
Received: 21 Oct 2020
Reviewed: 23 Oct 2020
Revised: 28 Dec 2020
Accepted: 16 Jan 2021

*CORRESPONDENCE:
atika.dwiyanti98@gmail.com

DOI:
10.18196/mmjkk.v21i1.6497

TYPE OF ARTICLE:
Research

Abstract: Tuberculosis is an infectious disease caused by Mycobacterium tuberculosis. Indonesia ranks 2nd largest in the world after India. Resistance to anti-tuberculosis drugs is one of the difficulties in treating tuberculosis. This research aim to determine the effectiveness of legundi (*Vitex trifolia*) leaf extract nanoherbal in killing *Mycobacterium tuberculosis*. This study was conducted with an experimental design using *M. tuberculosis* colonies taken from 25 samples of aspirate of tuberculosis patients with lymph nodes and then given the ethanol extract of legundi leaves (70% and 90%) with the process of making nanoparticle extracts. The evaluation of the observations was in the form of measuring the inhibition zone for bacterial growth which were categorized as weak (<5mm), moderate (6-10mm), strong (11-20 mm) and very strong (> 21mm). The results of the study from 6 repetitions found that the inhibition zone category was 0% weak, 39% moderate, strong 52.3%, and very strong 8.7%. Comparison of the concentration of legundi leaf extract to the diameter of the bacterial growth inhibitory zone was analyzed using the Kruskal Wallis test, which found a significant relationship with p value = 0.000 (<0.05). Legundi's leaf extract can be used as an antibacterial agent for *M. tuberculosis*.

Keywords: *Vitex trifolia* leaf extract; antibacterial; *Mycobacterium tuberculosis*

Abstrak: Tuberkulosis (TB) adalah penyakit infeksi menular yang disebabkan oleh *Mycobacterium tuberculosis*. Indonesia menempati urutan ke-2 terbesar didunia setelah India. Resistensi terhadap Obat Anti Tuberkulosis menajdi salah satu kesulitan pengobatan Tuberkulosis. Penelitian ini bertujuan untuk mengetahui efektivitas nanoherbal ekstrak daun legundi (*Vitex trifolia*) dalam membunuh Mycobacterium tuberculosis**.** Penelitian ini dilakukan dengan rancangan eksperimental menggunakan koloni *Mycobacterium tuberculosis* yang diambil dari aspirat pasien tuberkulosis kelenjar getah bening sebanyak 25 sampel kemudian diberikan ekstrak etanol daun legundi (konsentrasi 70% dan 90 %) dengan proses pembuatan ekstrak nanopartikel. Evaluasi hasil pengamatan berupa pengukuran zona hambat pertumbuhan bakteri yang dikategorikan lemah (<5mm), sedang (6-10mm), kuat (11-20 mm) dan sangat kuat (>21mm). Hasil penelitian dari 6 kali pengulangan didapatkan kategori zona hambat lemah 0%, sedang 39%, kuat 52,3%, dan sangat kuat 8,7%. Perbandingan konsentrasi ekstrak daun legundi terhadap diameter zona hambat pertumbuhan bakteri dianalisis menggunakan uji Kruskal Wallis ditemukan hubungan yang bermakna dengan nilai p = 0,000 (<0,05). Ekstrak daun legundi dapat digunakan sebagai antibakteri *Mycobacterium tuberculosis*.

Kata Kunci: ekstrak daun *Vitex trifolia;* antibakteri; *Mycobacterium tuberculosis*

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. According to the Basic Health Research (Riskesdas) in 2017 on each 100 thousand populations of Indonesia found 138 people diagnosed TB cases by health workers and 64 people with smear-positive. In Medan, based on the 2016 Medan City Health Profile, total number of TB cases was 6418 cases, the number of new TB cases with positive of Acid Resistant Bacteria (ARB) examinations was found 2829 cases and TB cases in children 0-14 years 231 cases. Although pulmonary TB is the most prevalent, extrapulmonary TB is also an important clinical problem. Based on epidemiology extrapulmonary TB constitutes 15-20% of all TB cases, of which TB lymphadenitis is the most prevalent form (35% of all extrapulmonary TB). Primary infection occurs after a person inhales *M. tuberculosis*, after passing through the mucociliary barrier of the airway, the TB bacilli will reach the alveoli. The germs spread through the lymph flow to the hilar lymph nodes causing a local lymphangitis process.[1 2 3 4]

The relatively long time for TB treatment (6 - 8 months) is the reason TB patients find it difficult to recover because TB patients drop out of drugs after feeling healthy even though the treatment process has not been completed. Antituberculosis drugs (OAT) which is used consisting of *isoniazid* (H), *Ethambutol* (E), *Pyrazinamide* (Z), *Rifampicin* (R) and *streptomycin* (S). The use of OAT itself has side effects. Isoniazid has the effect of peripheral neuropathy and hepatitis, *Ethambutol* additions have the effect of loss of vision which progressively because of retrobulbar neuritis, Pirazinamid has the effect of liver damage (hepatotoxicity) and arthralgia, Rifampicin effect on gastro-intestinal tract, such as nausea, loss of appetite and mild abdominal pain, diarrhea sometimes arise, and streptomisin has become hypersensitive and skin effect occurs hearing loss.[2 5 6]

Because of the many side effects, multiple resistance and frequent failure of tuberculosis treatment, several studies have examined alternative tuberculosis treatment options. Herbal medicine is more easily accepted by the community because besides being familiar with the community, it is cheaper and easier to obtain. One of the plants in Indonesia that is accepted by the community is legundi. So far, there have been many studies that reveal the benefits and properties of this legundian plant both from the leaves, stems, fruits and seeds, especially their effects as antibacterial, antifungal, insecticide, anticancer, analgesic, tracheospasmolytic, allergy and antipyretic.[6 7]

Legundi leaves contain essential oils which are composed of sesquiterpenes, terpenoids, ester compounds; alkaloids (vitrisin, flavonoid glycosides (artemetin and 7 desmethyl artemetin) and non-flavonoid components friedelin, ß-sitosterol, glucosides and hydro-carbon compounds (Listiawati, 2010). Flavonoids and essential oils are phenolic class compounds that will kill bacteria by coagulating or denaturing the protoplasm of the cell protoplasm, or causing cell lysis by changing the structure of the cell membrane resulting in leakage of cell contents. While the saponin mechanism is thought to damage the lipid membrane so that it can penetrate the cell wall and kill bacteria.[8]

Legundi leaves are proven to be antibacterial for *Staphylococcus aureus* and *Eschericia coli* with a minimum kill rate of 20%. Research conducted by Lubis HML in 2016 proved that giving legundian fruit at a dose of 0.5 g/kg / day and 1 g/kg/day for 2 weeks can reduce the size of skin tumors due to the flavonoid content of these plants. Research *in vitro* have been developed which are often taken from sputum specimens but were taken from lymph node aspirates cervical lymph undiscovered related literature. For this reason, the effect of legundi leaf extract will be assessed in the treatment of tuberculous lymphadenitis so that the use of synthetic drugs can be avoided.[9 10]

MATERIALS AND METHOD

Legundi leaves were from Yogyakarta city and identified in the Laboratory of Herbarium Medanense (Meda), University of North Sumatra. Prepare legundi nano leaf extract by washing legundi leaves with water, draining then drying in an oven at 40° C. Dry materials are pollinated using nano technology using a *High Energy Ballmill tool* and making legundy leaf ethanol extract consisting of a concentration of 70 % and 90%.[11]

Mycobacterium tuberculosis colonies were taken from aspirates of tuberculosis lymphadenitis patients from the Laboratory of Anatomical Pathology, Faculty of Medicine, Muhammadiyah University of North Sumatra. The result shows that 5 from 25 samples were positive level 1 (1+), 8 samples were positive level 2 (2+), and 12 samples were positive level 3 (3+) (Figure 1, 2 and 3).[12]

Fig. 1 Microscopic Acid Fast Bacilli 1+ (Ziehl Neelsen, 1000x)

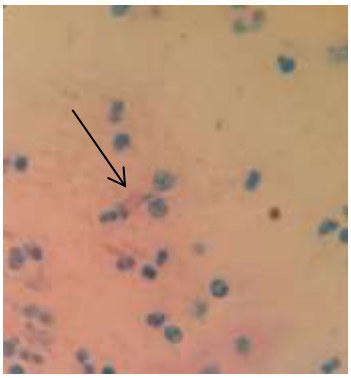

Fig. 2 Microscopic Acid Fast Bacilli 2+ (Ziehl Neelsen, 1000x)

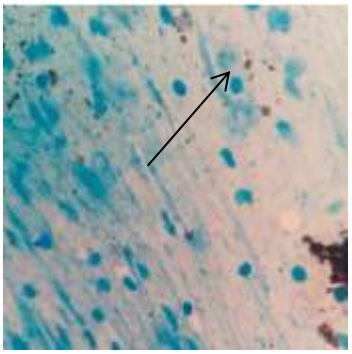

Fig. 3 Microscopic Acid Fast Bacilli 3+ (Ziehl Neelsen, 1000x)

The culture process of *M. tuberculosis* colonies using *Lowenstein-Jensen* medium as much as 150 petri dishes for 6 repetitions of the treatment. Evaluation of the observations in the form of bacterial growth inhibition zone categorized as weak if inhibition zone diameter ≤ 5mm, 6-10mm in diameter when the medium category, a category stronger as the diameter of 11-20 mm, the category of very strong when diameter ≥ 21 mm. Statistical analysis of the results inhibition zone diameter with statistical Kruskal Wallis analysis.[13]

RESULT

The results of anti-*Mycobacterium tuberculosis* examination for nanoherbal leaves of legundi (*V. trifolia*) with a concentration of 70% and 90% , are shown in Table 1 and Table 2.

Table 1.Percentage of Inhibition Zone Diameter Category at 70% Concentration

Inhibition Zone Diameter	Diameter	Total	Percentage
Weak	≤ 5 mm	0	0
Moderate	6-10	44	29.3%
Strong	11-20	101	67.3%
Very strong	≥ 21 mm	5	3,4%
Total		150	100%

Table 2.Percentage of Inhibition Zone Diameter Category at 90% Concentration

Inhibition Zone Diameter	Diameter	Total	Percentage
Weak	≤ 5 mm	0	0
Moderate	6-10	5	3.3%
Strong	11-20	98	65.4%
Very strong	≥ 21 mm	47	31.3%
Total		150	100%

Table 3. Percentage of Total Inhibition Zone Categories

Inhibition Zone Diameter	Percentage
Weak	0%
Moderate	39%
Strong	52.3%
Very strong	8.7%
Total	100%

In the examination of the inhibition zone measurement for the growth of *M. tuberculosis*, it was found that 39% had a moderate inhibition zone, 52.3% had a strong inhibition zone, 8.7% had a very strong inhibition zone (Figure 4, 5 and 6). The results of the inhibition zone diameter had analyzed using the Kruskal Wallis test found a significant difference with a p value = 0.00 (<0.05). Furthemore the Mann Whitney test shows that there is a significant difference

Table 4. Comparison of Analisis each Leaf Extract Concentration Legundi

Concentration	Concentration	P value
70%	90%	0.00 (≤0.05)

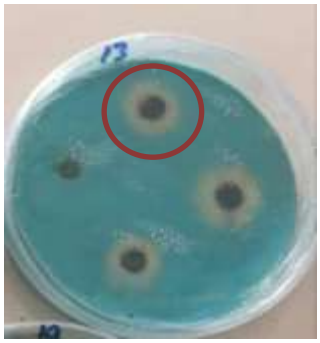

Figure 4 . The result of a moderate inhibition zone in diameter of 6-10 mm

Figure 5 . The result of a strong inhibition zone in diameter of 11-20 mm

Figure 6. The result of a very strong inhibition zone in diameter of ≥21 mm

DISCUSSION

Of the many traditional plants that are useful for the treatment, Legundi (*V. trifolia*) is a plant that is not so popularly used in Asia, especially in Indonesia un tuk treatment of tuberculosis. *Vitex trifolia* (Family-Verbenaceae) is an aromatic tree, which can grow up to 4m and is found from the foothills of the Himalayas to the south throughout most of India. Legundi's leaf extract is used as an anti-cancer, while the fruit is good for amenorrhea. *Vitex trifolia* flowers mixed with honey are used in cases of fever accompanied by vomiting and dehydration. The methanol extract of this plant has been reported to have strong antioxidant activity. Legundi leaves are proven to be antibacterial for *Staphylococcus aureus* and *Eschericia coli* with a minimum blood content of 20%.[3 9 14]

The content of flavonoids and essential oils from legundi leaves works to kill bacteria by coagulating or denaturing cell protoplasmic proteins, or causing cell lysis by changing the structure of the cell membrane resulting in leakage of cell contents. From the experiments conducted, it was proven that legundian leaves can inhibit the development of *M. tuberculosis* bacteria by observing the improvement of the inhibition zone at all the concentrations of legundian leaf extract examined. In the early stages of this bacterial infection the macrophages will engulf the bacteria and form granulomas. *Mycobacterium tuberculosis* is an inactive state, actively replicates but is killed by the immune response, or is metabolically altered by a limited replicative cycle, making OAT treatment more difficult. The action of flavonoids and essential oils that remove cell contents including the *M. tuberculosis* bacillus makes it easier and more adequate for the action of legundian leaves.[5 8 15]

Immunopathology regarding the development of tuberculosis is very intensively carried out and requires deeper research, especially in terms of the development of very potential traditional medicines. From the results of this research, it is proven that legundian leaf extract can be used as an antimicrobial against *M. tuberculosis* and can be continued for further research on tuberculosis immunotherapy.

CONCLUSION

The results of the study from 6 repetitions found that the inhibition zone category of legundy leaf extract was 0% weak, 39% moderate, strong 52.3%, and very strong 8.7%. Comparison of the concentration of legundi leaf extract to the diameter of the bacterial growth inhibitory zone was analyzed using the Kruskal Wallis test, which found a significant relationship with p value = 0.000 (<0.05). Legundi's leaf extract can be used as an antibacterial agent for *Mycobacterium tuberculosis*.

ACKNOWLEDGEMENT

Thanks to Directorate General of Learning and Student Affairs, Ministry of Research, Technology and Education through Student Creativity Program (PKM) 2019 which has support the research.

REFERENCES

1. Banuls AL, Sonou A, Van Anh NT, Godreuil S. Mycobacterium tuberculosis : ecology and evolution of a human bacterium. *J Med Microbiol.* 2015 Nov; 64 (11): p. 1261-1269.
2. Dinas Kesehatan Provinsi Sumatera Utara. Profil Kesehatan Kota Medan. Medan: 2016 [cited 2020 Mar 10]. Available from: Profil Kes Prov Sumut 2016 eBooks.
3. Yunita SI, Kusmiati T. TB MDR Primer dengan Limfadenitis TB pada Wanita SLE. *J Respirasi:* 2015 Sep; 1 (3): p. 81-87.
4. Kemenkes RI. Profil Kesehatan Indonesia 2017. Indonesia : 2018. [cited 2020 Mar 10]
5. Flynn JL, Chan J. Immunology of Tuberculosis. *Annual Review of Immunology.* 2001 Apr;19 (1); p. 93–129.
6. Perhimpunan Dokter Paru Indonesia. Tuberkulosis Pedoman Diagnosis dan Penatalaksanaan di Indonesia. Jakarta: [cited 2020 Mar 20] PDPI; 2006.
7. Mustanir dan Rosnani. Isolasi Senyawa Bioaktif Penolak (Repellent) Nyamuk dari Ekstrak Aseton Batang Tumbuhan Legundi (*Vitex trifolia*). *Buletin Littro*. 2008 ;19 (2): p. 174-180
8. Listiawati Y. Uji Efek Antipiretik Ekstrak Etanol Daun Legundi (*Vitex trifolia* Linn) pada Kelinci yang Diinduksi Vaksin Dpt-Hb. Universitas Muhammadiyah Surakarta. 2010 [cited 2011 Jan 31]. Available from : http://eprints.ums.ac.id/10093
9. Tiwari N, Thakur J, Saikia D, Gupta MM. Antitubercular diterpenoids from *Vitex trifolia*. *Phytomedicine*. 2013 May 15;20(7):605-10.
10. Lubis HML, Hariaji I. Ekstrak Buah Legundi (*Vitex trifolia*) Mampu Menghambat Pembelahan dan Pertumbuhan Sel Tumor Kulit Tikus. *Mutiara Medika Jurnal Kedokteran dan Kesehatan*. 2017 Jan 17 (1): p. 1-6.
11. Jawetz, Melnick, Adelberg. *Mikrobiologi Kedokteran*. 27th ed. Jakarta: EGC; 2016
12. Anggraini A, Yuniningsih S, Sota MM. Pengaruh Ph Terhadap Kualitas Produk Etanol dari Molasses melalui Proses Fermentasi. *Jurnal Reka Buana*. 2017; 2 (2): p. 99-105.
13. Mahardika HA, Sarwiyono, Surjowardojo P. Ekstrak Metanol Daun Kersen (*Muntingia calabura* L) sebagai Antimikroba Alami terhadap Bakteri *Staphylococcus aureus* Penyebab Mastitis Subklinis pada Sapi Perah. *Jurnal Ternak Tropika*. 2014;15 (2): p. 15-22.
14. Geetha V, Doss A, Doss AP. Antimicrobial Potential of *Vitex trifolia* Linn. *Anc Sci Life*. 2004 Apr; 23 (4): 30-2
15. Amanda EA, Oktiani BW, Panjaitan FUA. Efektivitas Antibakteri Ekstrak *Flavonoid Propolis Trigona Sp (Trigona thorasica)* terhadap Pertumbuhan Bakteri *Porphyromonas gingivalis*. *Jurnal Kedokteran Gigi*. 2019; 3 (1): p. 23-28.