

Conference Paper

The Anti-tubercular Activity of Noni Fruitsto Inhibition Growth of Multi Drug Resistant-Tuberculosis Bacteria

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Abstract

Multidrug-resistant tuberculosis (MDR-TB) is a tuberculosis infection that is resistant to the treatment at least two of the most powerful anti-tuberculosis drugs, such as Isoniazid and Rifampisin. Increased cases of MDR-TB in morbidity and mortality become obstacles in the control of tuberculosis (TB), thus requiring supportive treatment of natural ingredients that can contribute in the treatment of TB, such a noni fruit. The main objective of this study was extract of noni fruits to inhibition the growth of strain MDR-TB bacteria, and compered it with the anti-TB drugs. The *Morinda c. Linn* (Noni) fruits was extraced by ethanol (96%). The extract was filtered through whatman No.1 filter paper, evaporated to dryness on a water bath until the solvent evaporated completely and yield of the crude extract. The experiment were divided into 3 groups, i.e.: negative control: group I; positive control: group II; crude extracts noni fruit: group III: combinations of crude extracts noni fruit and anti-TB drugs (K, AK, and OF). Each group was divided into three groups' doses of 30 mg/ml, 40 mg/ml and 50 mg/ml. The Anti-tuberculosis activities of extracts noni fruit and K, AK, and OF against TB-MDR bacteria were tested by susceptibility test using proportion method in Lowenstein-Jensen (LJ) media. The anti-tubercular activity of noni fruits was determined by the minimum inhibitory concentration (MIC) of the bacterial growth at various doses 30 mg/ml, 40 mg/ml, and 50 mg/ml. The research design used post-test only contol group, and analyzed using analysis of variance and post hoc test. The extracted of *Morinda c.Linn* (noni) fruits have antitubercular activity to inhibiton of growth MDR-TB bacteria at various doses (p value=0,000). At a dose 30 mg/ml the mean rate of the growth colonies of MDR TB-bacteria whit the mean rate $59,00 \pm 27,81$, and at a dose 40 mg/ml was $1,50 \pm 2,81$. While at a dose of 50 mg/ml the bacterial colonies of MDR-TB did not grow in media. The combination of *Morinda c.Linn* (noni) fruit with anti-tuberculosis drugs, was the smallest groups to inhibit and eliminate MDR-TB bacteria at a dose 30 mg/ml ($0,00 \pm 00,00$). The experimental results confirmed the extracted of *Morinda c.Linn* (noni) fruits have antitubercular activity as well as anti-TB drugs, and the combination of the extracted of *Morinda c.Linn* (noni) fruits and anti-TB drugs was the best groups to inhibiton of growth MDR-TB bacteria.

Keywords: TB-MDR Bacteria, *Morinda c. Linn* (Noni), Anti-TB drugs, Anti-tubercular activity, MIC.

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Published: 15 March 2021

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Selection and Peer-review under
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1. Introduction

Tuberculosis (TB) is a contagious infectious disease that became the public health problems in the world [1]. Handling and controlling of TB is more complicated since the front line anti-tuberculosis drugs have gradually become ineffective for TB therapy [2]. The drug resistance could occurred due to the inadequate and unregularly drug utilization that cause gene mutation of the drug target such as Kat-G gene for isoniazid and rpo-B gene for rifampicin [3, 4]. Increased cases of Multidrug-resistant tuberculosis (MDR-TB) become obstacles in TB control. According WHO Global Tuberculosis Control, MDR-TB patients receiving treatment have a cure rate of less than 60% because TB treatment takes a long time, so that patients with treatment dropouts and bacteriological numbers remain positive causing resistant bacteria to develop into extensively drug-resistant tuberculosis (XDR-TB), abbreviation for strains resistant to first and second line anti-tuberculosis drugs [5, 6]. Due to the facts mentioned above and to TB latency, new anti-TB drugs and better therapeutic strategies against TB are urgently required. New drug candidates should shorten standard treatments and effective against MDR-TB bacteria. In spite of the efforts require supportive treatment from a natural product that has therapeutic effects for health such as noni (*Morindacitrifolia Linn*) [7, 8].

Noni is one of the medical plants that have been reported to have a therapeutic and nutritional value [7, 9, 10]. The leaves was largely used in traditional medicine and has been uses for arthritis, atherosclerosis, boils, burns, cancer, chronic fatigue syndrome, circulatory weakness, cold sores, congestion, constipation, diabetes, gastric ulcers, gingivitis, heart disease, hypertension and infections [11, 12]. Root of noni has been reported significant inhibitory effects on the proliferation of human lung and colon cancer cells [13, 14]. The fruit juice of noni contains a polysaccharide-rich substance with antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory activity and enhance the immune effects [15, 16]. Previous pharmaceutical study showed that, extract of noni fruits effectively inhibit gram-positive and gram-negative bacteria such as *Staphylococcus aureus*, *P.aureginosa* strains *Streptococcus mutans* [17] *Bacillus subtilis*, *Proteus morgaii*, *Pseudomonas*, *Escherichia coli* [7, 18] moreover, the plants also utilizes to control the groups of pathogen bacteria, such *Salmonella* and *Shigella* [19].

Another anti-microbial in vitro assay was conducted on ethanol extract and hexane fractions noni leaves at 100 mg/ml reported to have an anti-tubercular activity in *Mycobacterium tuberculosis* cultures, with inhibition rates of 89% and 95% respectively [20]. [21] revealed that the ethanol extract of noni fruits showed that noni possessed in vitro anti-mycobacterial effect against *Mycobacterium tuberculosis* bacteria at minimum

inhibitory concentrations (MIC) 40 mg/ml [21]. The anti-mycobacterial activities of noni lead by the presence of active constituents such as secondary metabolites and *lectins* [22]. According phytochemical investigations of noni, the fruit contains of approximately 200 compounds from different parts of noni [23]. A number of major components in the noni plant was a *scopoletin*, *flavonoids*, *octoanic acid*, *potassium*, *vitamin C*, *terpenoids*, *alkaloids*, *antraquinones*, which the ranged of the compounds antibacterial was 5.94g to 36.52g/100g of dry material [24–26]. The antimicrobial activity is generally attributed to various phytochemicals in noni extract that target bacterial cell membranes and cellular biochemical pathways [23, 27]. The noni molecules react with lipids in bacterial cell membranes, resulting in increasing the cell-membrane permeability, disrupting the membranes, and breaking cell homeostasis, inactivate enzymes and denature proteins in bacteria [15, 19]. Therefore the bacterial cell wall will be leakage of intracellular components, but also facilitate the movement of antimicrobial compounds into the cytoplasm, inducing cell death [28].

This study is to investigated the in-vitro anti-tubercular activity of extract noni fruit when is used as individual or combination with second-line anti-TB drugs against TB-MDR strain bacteria at doses of 30mg/ml, 40mg/ml, and 50mg/ml. Minimum inhibitory concentration (MIC) values were used in order to assess the synergistic activity of extract noni fruit.

2. Material And Methods

2.1. Study design

The research design used post-test only control group design [29].

2.2. Samples

The samples of this research are strain bacteria of *Mycobacterium tuberculosis* resistant to the first-line anti-tubercular drugs (rifampicin and isoniazid) based on drug-susceptibility sensitive test at the Health Laboratory of West Java Province was derived from sputum of patients with active TB as much as 54 strain. The sample was added aseptically to the Lowenstein Jenson (LJ) medium and incubated at 37°C for 2 weeks with an optimum pH of 7.0.

2.3. Reagents and antibiotics

Reagent utilized in this study were, ethanol, etilasetat, n heksana, aquabidestilata, iron (II) klorida, natriumhidroksida 1 %, (CV. GriyaSaranaMeditama, Bandung, Indonesia), *dimethyl sulfoxide* (DMSO), Ammonium sulphate, L. glutamic acid, sodium citrate (PT. Biogen Scientific Indonesia), Lowenstein Jensen (LJ Merck no. 105400), and glyserol (Merck cat. no. 104094). The antibiotics kanamycins (K), amikacin (AK), danofloxacin (OF) were purchased from Sigma-Aldrich.

2.4. Plant material and Extraction

The plant material of the present study is fresh mature of noni fruit that was collected during the rainy season in Cibeber, South Cimahi (2.8 Km from Cimahi city), West Java province, Indonesia. The plant voucher specimens of noni fruit (EN-no. 241571) were identified and authenticated at the Biomedical Sciences Laboratory of the School of Health Sciences Jenderal Achmad Yani Cimahi. The fresh mature noni fruit was cleaned and sliced into small pieces, shade-dried at 50°C and ground to powder. The powdered noni fruit material (500 gr) was macerated with 500 ml ethanol (96%) in the increasing order of polarity from non-polar to high polar at 50°C for 2 hours. The extract was filtered through whatman No.1 filter paper, evaporated to dryness on a water bath until the solvent evaporated completely and yield of the crude extract.

2.5. Experiment protocol

The experiment were in-vitro experiment which divided into 3 groups, i.e.: negative control: group I; positive control: group II; crude extracts noni fruit: group III; combinations of crude extracts noni fruit and anti-TB drugs (K, AK, and OF). Each group was divided into three groups' doses of 30 mg/ml, 40 mg/ml and 50 mg/ml. The Anti-tuberculosis activities of extracts noni fruit and K, AK, and OF against TB-MDR bacteria were tested by susceptibility test using proportion method in Lowenstein-Jensen (LJ) media. The crude extract of noni fruit was dissolved in 1 ml of 20% DMSO (*dimethylsulfoxide*), and anti-TB drugs was prepared in deionized water. The inoculum was adjusted 100 ml dilution 10^3 of TB-MDR bacteria by comparison with Mc. Farland standard to 10^8 CFU/ml and spotted onto LJ media. All assays were run in duplicate, and kanamycin (30 mg/ml), amikacin (40 mg/ml) and ofloxacin (2 mg/ml) were utilized as positive controls. Cultivation was done at 37°C for 8 weeks.

2.6. Anti-tuberculosis activity

The anti-tuberculosis activity of extract noni fruit and anti-TB drugs (K, AK, or OF) were measure based on minimum inhibitor concentration (MIC) values expressed as the lowest concentration inhibition growth of colonies TB-MDR bacteria, while the determination of minimum bactericidal concentration (MBC) was indicated by clear zones around the medium using susceptibility test [28, 30].

2.7. Statistical analysis

All data were analysis using univariate analysis of variance, and the differences means \pm standard deviation between each group were evaluated by least significant difference (LSD) and Duncan's. Statistical analysis in this study using confidence interval 95% ($p \leq 0.05$). The experimental protocol of the current study has been approved by the Commission of Health Research Ethics Faculty of Medical, Diponegoro University and General Hospital dr. Kariadi Semarang with the issuance of Ethical Clearance letter.

3. Result

The qualitative observation from the crude extract of noni fruit and the combination of extract noni fruits with anti-TB drugs showed a significant activity of anti-tuberculosis against MDR-TB bacteria ($p < 0.05$). The anti-tuberculosis of crude extract noni fruit have different mean number of growth colonies TB-MDR bacteria at various doses 30 mg/ml, 40 mg/ml, and 50 mg/ml (Fig. 1).

The mean rate growth of colonies TB-MDR bacteria on the crude extract noni fruit at 30 mg/ml dose showed smaller mean compared to negative control (174.67 ± 44.58) but greater than the positive control (49.83 ± 77.57). At the 40 mg/ml dose the growth colonies of TB-MDR bacteria had inhibited the growth colonies of TB-MDR bacteria better than the positive controls, and a dose of 50 mg/ml the bacterial colonies of TB-MDR did not growth in LJ media (Table 1). According to that data, it can be shown that extract noni fruit can inhibit the growth of TB-MDR bacteria at doses 30 mg/ml and as bactericidal at 50 mg/ml.

CN=control negative, CP=control positif, EN= extract noni fruit, CEA= combination of extract noni fruit and anti-TB drugs. Inhibitory zones in mm, represented as mean \pm SD values (n=72), 0= no zone. The values followed by different superscript differ significantly at $p < 0.05$

The combinations of extract noni fruit with anti-TB drugs (K, AK, or OF) showed that at a dose of 30 mg/ml, TB-MDR bacteria colonies didn't grow on the LJ media. The combination extract noni fruit and second-line anti-TB drugs was the best inhibitor compared with the single treatment of extract noni fruit, positive control, and negative control ($p < 0.05$). The combination of extract noni fruit and second-line anti-TB drugs showed synergistic activity against TB-MDR bacteria (Table 2).

CN=control negative, CP=control positif, EN= extract noni fruit, CEA= combination of extract noni fruit and anti-TB drugs Inhibitory zones in mm, represented as mean \pm SD values (n=72). The values followed by different superscript differ significantly at $p < 0.05$. the mark indicate significant differences compared with the crude extract (LSD post hoc test; * = $p < 0.05$; ** = $p < 0.01$)

The inhibitor of single noni fruit showed that extract noni is the best inhibiting the growth of *Mycobacterium tuberculosis* resistant of $\geq 80\%$ compared to a negative control and 24% when compared with the positive control (table 3).

4. Discussion

The anti-TB drugs were formulations or as fixed-doses combination were the major rule in killing of MTB or TB-MDR bacteria [2]. Anti-TB drugs are strong bactericidal and bacteriostatic in inhibiting the growth of *Mycobacterium* bacteria. The target of Treatments of infectious diseases such as MDR TB facing serious problem worldwide, as microorganisms become resistant to multiple antimicrobial agents, which lead to increase TB cases incidence [31]. Based on that phenomena, development of new therapeutic from local plants traditionally such as noni fruit that may can efforts the affectivity of anti-TB for the treatment of TB [26].

Noni fruit is one of the medical plant as well as some other compounds in noni root and leaves, are all proved as antibacterial agents [24, 25]. The results of the present study showed the crude extract noni fruit have a significant difference of the growth of TB-MDR bacteria. The antibacterial activity of noni against certain infectious bacterial strains was reported [7, 11, 27, 32, 33]. [17] reported that noni fruit extract can inhibit zone diameter *Streptococcus* mutant growth in 330.66 mm^2 [17]. Another study of affectivity noni against *E. coli*, *Salmonella typhi*, and *Bacillus cereus*, *C. albicans*, and *S. aureus* reported by Usha (2010). Anti-tubercular effects of Saludes research, reported that leaves from noni has been found killed 89 percent of the bacteria *M. tuberculosis* [20], and noni fruits killed *M. tuberculosis* bacteria at 40 mg/ml [21].

The anti-tubercular activity extract noni might be influenced by the presence of secondary metabolites as phenolic compounds [34]. Phenolic compounds contained in the noni fruit ranged from 5.94 to 36.52 g /100 g of dry material [26, 35]. Phytochemicals in noni molecules are associated with the defense mechanisms of plants by their repellent or attractive properties, protection against biotic and abiotic stresses, and maintenance of structural integrity of plants [30, 36]. In addition the molecules of noni react with lipids in bacterial cell membranes, resulting in increasing the cell-membrane permeability, disrupting the membranes, and breaking cell homeostasis, inactivate enzymes and denature proteins in bacteria [18, 19]. Therefore the bacterial cell wall will be leakage of intracellular components. It further disrupts the transport of important organic ions into the cells resulting in inhibition of growth even in cell lysis [36–38].

The decrease of MIC value showed that the combination of extract noni fruit and anti-TB drugs found to have synergistic activity that can inhibit the growth of TB-MDR at low doses. The combination of extract noni fruit and anti-TB drugs possible, makes synergism and complementary effects of active substances that can enhance the activity were more effective than the individual agents [39, 40]. The combinations will be improved bacterial potential of extract noni fruit and anti-TB drugs as combined antimycobacterial cell wall [41]. Based on several studies the extract noni effectively have an antibacterial activity that can inhibit and eliminate the bacteria. Another study showed that noni fruit was found relatively non-toxic [10, 35].

5. Conclusion

The present study brought out the fact that extract have anti-tuberculosis activity against TB-MDR bacteria. The combination of extract noni fruit and anti-TB drugs showed significant most active compound compared the individual drugs. Based on the results obtained, noni fruits can be a potential source of drugs as an adjuvant therapy for anti-tuberculosis drugs. However, further studies required to carry out in vivo, ex vivo and clinical trial investigations of noni for alternative treatment of tuberculosis problems.

Conflict of interest

This study is important because lung Tuberculosis remains a global health problem, especially MDR TB disease increased even XDR TB due to drug resistance. Treatment strategy is required for supportive treatment of natural ingredients that *Morinda citrifolia* (noni), which may contribute to the treatment of TB. Noni is a plant that is easy to obtain

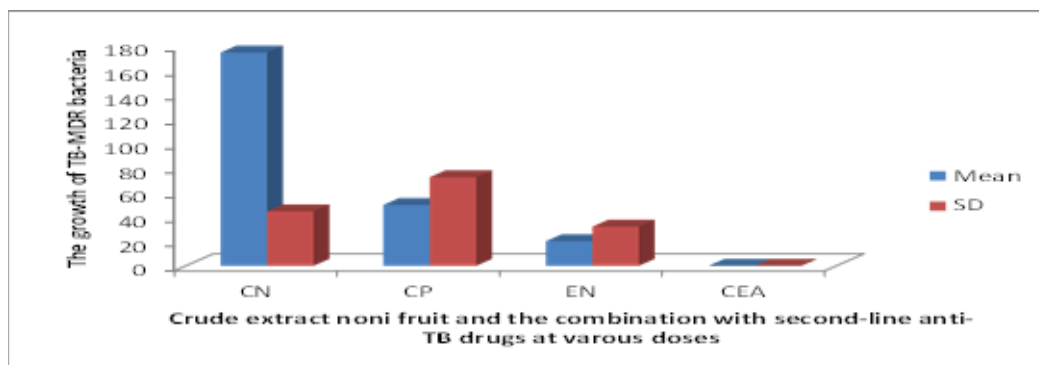


Figure 1

TABLE 1: Antimicrobial activity of crude extracts noni fruit and the combination with second-line anti-TB drugs

Sample	Doses		
	30 mg/ml	40 mg/ml	50 mg/ml
CN	174.67±44.58	174.67±44.58	174.67±44.58
CP			
K	0±0	0±0	0±0
AK	0±0	0±0	0±0
OF	49.83±72.57	49.83±72.57	49.83±72.57
EN	59.00±27.81	1.50±2.81	0±0
CEA	0±0	0±0	0±0

TABLE 2: Combination testing of extract noni fruit and anti-TB drugs against TB-MDR bacteria

Sample		Mean	SD	p value
CE (20.17± 32.072)	CN	174.67	± 44.5751	0.000*
	CP	49.83	± 72.567	
	CEA	0.00	0.00	

TABLE 3: The effectiveness noni fruit compared the control of Mycobacterium tuberculosis

Compounds	% Effectivity	
	control +	control -
control +		35
Extract Noni	24	87

and have side effects were relatively minor and easily accessible by the public. This study aims to explain the influence the anti-tubercular activity of extract and compounds noni fruit to inhibition the growth of MDR TB bacteria. This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.

Acknowledgments

We thanks to Prof. dr. Edi Dharmana, Sp.Par(K), M.sc, Ph.D; Prof dr.HardhonoSusanto, PAK(K); Dr. Muchlis AUS., SpPD-KPTI;Dr. dr Sri Andarini I., M.Kes, Dr. Dyan K. Nugrahaeni, SKM., MKM and to their contribute for this research.

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