

Antibacterial and antioxidant activities of *Trema cannabina* and *Cratoxylum sumatranum* leaf extracts

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ABSTRACT

The Borneo Research Forest contains plant species with underexplored bioactive potential. This study evaluated the antibacterial and antioxidant activities of ethanolic leaf extracts of *Trema cannabina* Lour. and *Cratoxylum sumatranum* (Jack) Blume. Antibacterial activity was tested against *Ralstonia solanacearum* and *Propionibacterium acnes* by agar-well diffusion at 2,500, 5,000, and 10,000 ppm. Chloramphenicol (100 ppm) and 40% ethanol served as positive and negative controls. Antioxidant activity was measured by the DPPH radical-scavenging assay at 25, 50, and 100 ppm, with vitamin C as the positive control. *Cratoxylum sumatranum* produced the strongest antibacterial response. At 10,000 ppm, its relative inhibition was 60.0% against *R. solanacearum* and 90.7% against *P. acnes*. *Trema cannabina* reached 33.5% relative inhibition against *R. solanacearum* at the same concentration but showed no detectable inhibition of *P. acnes*. Both extracts scavenged DPPH radicals in a concentration-dependent pattern. At 100 ppm, inhibition reached 76.24% for *T. cannabina* and 78.31% for *C. sumatranum*, compared with 98.24% for vitamin C. These findings identify *C. sumatranum* as the more promising broad-spectrum antibacterial extract, while both species warrant further phytochemical characterization and dose-response testing as sources of natural antioxidants.

INTRODUCTION

Bacterial diseases affecting crops and humans continue to create demand for new bioactive materials from renewable sources. *Ralstonia solanacearum* is a destructive soil-borne pathogen with a wide host range and is especially important in tropical and subtropical agriculture. Its persistence in soil, water, alternate hosts, and infected planting material makes bacterial wilt difficult to manage. Recent work has therefore renewed interest in plant-derived molecules that suppress pathogen growth, biofilm formation, or virulence [1,2]. *Propionibacterium acnes*, previously known as *Propionibacterium acnes*, is an anaerobic Gram-positive member of the normal skin microbiota but contributes to acne-associated inflammation when follicular conditions favor overgrowth and lipase activity [3]. Repeated antimicrobial exposure can select less-susceptible populations, reinforcing the value of screening chemically diverse plant extracts as early-stage leads rather than as untested therapeutic replacements.

Tropical forests are reservoirs of secondary metabolites that mediate plant defense and may also show antibacterial or antioxidant activity. The Borneo Research Forest of Universitas Borneo Tarakan supports numerous species used locally or traditionally. Among them, *Trema cannabina* Lour. and *Cratoxylum sumatranum* (Jack) Blume are plausible sources of bioactive compounds. Ethanolic *T. cannabina* leaf extracts have been reported to contain alkaloids, steroids, and tannins [4], and later studies confirmed antioxidant and selective antimicrobial activities of the leaves [5]. Members of *Cratoxylum* are rich in xanthenes, benzophenones, flavonoids, terpenoids, and other phenolic constituents [6,7]. These chemical classes can interact with microbial membranes and proteins, interfere with enzymes and nucleic-acid processes, or donate electrons and hydrogen atoms to stabilize free radicals [8].

Crude-extract screening is an exploratory step between ethnobotanical observation and compound-level development. A measurable zone of inhibition can justify fractionation, but it does not identify the active constituent, prove bactericidal action, or

establish safety. Likewise, strong radical scavenging in a chemical assay does not directly demonstrate physiological antioxidant efficacy. For that reason, early comparative studies are most useful when they report the assay conditions, reference controls, concentration range, and analytical limitations transparently. Such information allows promising species to be prioritized while preventing preliminary in vitro findings from being interpreted as finished agricultural products or clinical treatments.

Antibacterial screening is particularly relevant when it spans organisms from different biological contexts. *R. solanacearum* is a Gram-negative phytopathogen with an outer membrane that can restrict the entry of many compounds, whereas *P. acnes* is a Gram-positive human-associated bacterium. A single extract that inhibits both would suggest a comparatively broad spectrum of activity; an organism-specific response would indicate selectivity or differences in extract penetration and cellular targets. Medicinal-plant screening against *P. acnes* has shown that activity varies substantially among species and cannot be predicted solely from total phenolic or flavonoid content [9]. Similar variability is expected for phytopathogens because extract composition, solvent polarity, concentration, and assay format all influence the observed inhibition.

Antioxidant screening complements antibacterial testing because oxidative stress and radical-scavenging capacity provide a second functional measure of extract bioactivity. The DPPH assay is widely used as a rapid spectrophotometric method: antioxidants reduce the purple DPPH radical, and the decline in absorbance can be expressed as percentage inhibition. Although the assay does not reproduce a living biological system, it permits standardized comparison across extract concentrations and against a reference antioxidant [10]. Evaluating antibacterial and DPPH-scavenging activity together can therefore identify extracts that deserve more detailed fractionation, phytochemical profiling, toxicity assessment, and mechanism-based testing.

Despite ethnobotanical use and published evidence for related pharmacological properties, comparative data for *T. cannabina* and

C. sumatranum collected from the Borneo Research Forest were limited, especially against both *R. solanacearum* and *P. acnes* under the same experimental framework. This study aimed to determine whether ethanolic leaf extracts of the two species inhibited these bacteria and scavenged DPPH radicals, and to identify the most effective tested concentrations. The working hypothesis was that both extracts would show measurable, concentration-related bioactivity, with differences between plant species and target bacteria reflecting their distinct secondary-metabolite profiles.

MATERIALS AND METHODS

Research site

The experiment was conducted in the Plant Protection Laboratory, Agrotechnology Study Program, Faculty of Agriculture, Universitas Borneo Tarakan, Tarakan, North Kalimantan, Indonesia. Fresh leaves of *Trema cannabina* and *Cratoxylum sumatranum* were collected from the Borneo Research Forest. The study used an in vitro laboratory design to compare two plant extracts, two bacterial targets, and multiple extract concentrations.

Materials and experimental design

The main materials were fresh leaves of both plant species, 96% ethanol for extraction, 40% ethanol as the antibacterial negative control, chloramphenicol as the antibacterial positive control, DPPH (1,1-diphenyl-2-picrylhydrazyl), dimethyl sulfoxide (DMSO), analytical-grade ethanol and methanol, nutrient agar, nutrient broth, glucose, sterile distilled water, cultures of *Ralstonia solanacearum* and *Propionibacterium acnes*, and vitamin C as the antioxidant reference. Equipment included an oven, desiccator, blender, shaker, rotary vacuum evaporator, autoclave, laminar-flow cabinet, spectrophotometer, micropipettes, Petri dishes, glassware, and a cork borer.

For antibacterial testing, each extract was evaluated at 2,500, 5,000, and 10,000 ppm against both bacteria. Chloramphenicol at 100 ppm was the positive control and 40% ethanol was the negative control. Each treatment was performed in triplicate. For antioxidant testing, each extract was assessed at 25, 50, and 100 ppm, and vitamin C was tested at the same concentrations.

Sample preparation and extraction

Leaves were sorted to remove soil, foreign material, and damaged tissue. To determine the moisture factor, 1 g of chopped fresh leaf material was placed in a pre-weighed Petri dish in three replicates, dried at 105 °C for 24 h, cooled in a desiccator for 15 min, and weighed. The remaining leaves were shade-dried at room temperature away from direct sunlight, dry-sorted, and milled into simplicia powder.

A known mass of powdered sample was submerged in 96% ethanol and macerated for 72 h at room temperature, with agitation twice daily. The suspension was filtered to separate filtrate and residue. The filtrate was concentrated with a rotary vacuum evaporator, and the concentrated extract was finished at approximately 40 °C until a stable crude extract was obtained. Extraction yield was calculated as the mass of crude extract divided by the initial dry plant-material mass, multiplied by 100%.

Antibacterial assay

Antibacterial activity was determined by agar-well diffusion. Growth medium was prepared from agar, nutrient broth, glucose, and distilled water, then sterilized with the required glassware. Approximately 20 mL of sterile medium was poured into each Petri dish and allowed to solidify in a laminar-flow cabinet. Bacterial suspensions were standardized spectrophotometrically at 600 nm to 70-75% absorbance. A 100 µL aliquot of suspension was spread over the agar surface and left to dry for approximately 10 min.

Wells were cut aseptically with a cork borer and loaded with 20 µL of test solution at the assigned concentration. Plates were sealed and incubated at 37 °C for 24 h. The diameter of the clear inhibition zone was measured in millimeters. Relative inhibition was calculated as (x/y) × 100, where x was the inhibition-zone diameter of the extract treatment and y was the inhibition-zone diameter of the chloramphenicol positive control. Zone-strength interpretation followed the conventional categories proposed by Davis and Stout

[11]: ≤5 mm, weak; 5-10 mm, moderate; 10-20 mm, strong; and ≥20 mm, very strong.

DPPH radical-scavenging assay

Antioxidant activity followed a DPPH spectrophotometric procedure adapted from Arung et al. [12]. Three milligrams of each crude extract were dissolved in 1,000 µL DMSO. For each measurement, 33 µL of extract solution and 467 µL ethanol were combined with 500 µL of 60 µM DPPH prepared in ethanol, giving a final volume of 1 mL. Samples were incubated for 20 min at room temperature under minimal light and measured at 514 nm. Extract concentrations were 25, 50, and 100 ppm. DPPH-scavenging activity was calculated as [(Acontrol - Asample)/Acontrol] × 100, where Acontrol was the absorbance of the DPPH control and Asample was the absorbance after addition of the extract.

Data analysis

Results were summarized descriptively in tables and compared across plant species, target bacteria, and concentrations. The study did not apply inferential statistical tests; consequently, the reported percentages describe the observed triplicate experiment and should not be interpreted as proof of statistical significance. The concentration producing the greatest observed relative inhibition was identified for each assay.

RESULTS

Drying 1 g fresh samples produced oven-dry masses of 0.45 g for *Trema cannabina* and 0.48 g for *Cratoxylum sumatranum*, corresponding to moisture factors of 0.45 and 0.48. Maceration of 133.20 g *T. cannabina* leaf material yielded 17.6110 g crude extract (13.2%), whereas 140.20 g *C. sumatranum* yielded 24.2974 g (17.3%). Thus, *C. sumatranum* provided 4.1 percentage points more crude extract under the applied procedure (Table 1).

Table 1. Moisture factors and ethanolic extraction yields of the two leaf materials

Plant material	Initial mass (g)	Extract mass (g)	Yield (%)
<i>T. cannabina</i>	133.20	17.6110	13.2
<i>C. sumatranum</i>	140.20	24.2974	17.3

Moisture factors from 1 g fresh samples were 0.45 for *T. cannabina* and 0.48 for *C. sumatranum*.

The antibacterial response differed clearly between the two extracts and between target bacteria (Table 2). Against *Ralstonia solanacearum*, *Trema cannabina* relative inhibition increased from 9.8% at 2,500 ppm to 29.4% at 5,000 ppm and 33.5% at 10,000 ppm. The 10,000 ppm treatment produced a 7.2 mm inhibition zone and was therefore in the moderate category. *Cratoxylum sumatranum* showed stronger activity against the phytopathogen at every concentration, with relative inhibition of 36.1%, 43.9%, and 60.0% at 2,500, 5,000, and 10,000 ppm, respectively. The highest treatment produced a 22 mm zone, classified as very strong.

Against *Propionibacterium acnes*, the contrast was larger. *Trema cannabina* produced no detectable inhibition at any tested concentration. *Cratoxylum sumatranum* produced 68.6% relative inhibition at 2,500 ppm, 77.3% at 5,000 ppm, and 90.7% at 10,000 ppm. The highest concentration generated a 20 mm inhibition zone, indicating strong to very strong activity under the classification applied. The concentration-response pattern was monotonic for all active extract-bacterium combinations except that the original *T. cannabina*-*P. acnes* series remained at zero throughout.

Table 2. Relative antibacterial inhibition (%) of ethanolic leaf extracts

Target bacterium	Extract	2,500 ppm	5,000 ppm	10,000 ppm
<i>R. solanacearum</i>	<i>T. cannabina</i>	9.8	29.4	33.5
<i>R. solanacearum</i>	<i>C. sumatranum</i>	36.1	43.9	60.0
<i>P. acnes</i>	<i>T. cannabina</i>	0	0	0
<i>P. acnes</i>	<i>C. sumatranum</i>	68.6	77.3	90.7

Values are inhibition-zone diameters expressed relative to the 100 ppm chloramphenicol control (100%). The 40% ethanol negative control produced no reported inhibition.

Both extracts reduced DPPH absorbance, and the percentage inhibition increased with concentration (Table 3). *Trema cannabina* rose from 46.18% at 25 ppm to 62.50% at 50 ppm and 76.24% at 100 ppm, an increase of 30.06 percentage points across the tested range. *Cratoxylum sumatranum* was less concentration-sensitive but consistently high, increasing from 74.17% to 75.21% and 78.31%. At 25 ppm, *C. sumatranum* exceeded *T. cannabina* by 27.99 percentage points; at 100 ppm, the difference narrowed to 2.07 points. Vitamin C remained the strongest antioxidant reference, with inhibition between 96.90% and 98.24%.

Considering all measured endpoints, the highest tested concentration produced the maximum observed value for every active extract-assay combination. *Cratoxylum sumatranum* ranked first in both antibacterial comparisons and at each antioxidant concentration. *Trema cannabina* ranked second in the *R. solanacearum* and DPPH assays and remained below the detection threshold in the *P. acnes* diffusion assay. The difference between the plant extracts was largest at the low DPPH concentration and in the *P. acnes* test, whereas their antioxidant values became similar at 100 ppm. These rankings are descriptive because the available summary dataset did not include variance estimates or inferential comparisons.

Table 3. DPPH radical-scavenging inhibition (%) at three concentrations

Sample	25 ppm	50 ppm	100 ppm
<i>T. cannabina</i>	46.18	62.50	76.24
<i>C. sumatranum</i>	74.17	75.21	78.31
Vitamin C	96.90	97.42	98.24

DPPH inhibition was calculated from the decrease in absorbance at 514 nm. Vitamin C was the positive control.

DISCUSSION

The higher extraction yield of *Cratoxylum sumatranum* suggests that the applied ethanol maceration recovered a larger soluble fraction from its leaves than from *Trema cannabina*. Yield alone does not establish biological potency because it includes both active and inactive constituents, but it affects the practical amount of crude material available for screening. Extraction outcomes depend on plant tissue, drying, particle size, solvent polarity, extraction time, temperature, and agitation [13,14]. Ethanol was suitable for this exploratory work because it can dissolve a broad range of moderately polar and polar secondary metabolites while limiting heat exposure during maceration.

Cratoxylum sumatranum was the more active antibacterial extract, especially against *Propionibacterium acnes*. Its 90.7% relative inhibition and 20 mm zone at 10,000 ppm approached the chloramphenicol reference in the relative metric used. This result is biologically plausible because *Cratoxylum* species contain xanthenes, benzophenones, flavonoids, terpenoids, and other phenolics [6,7]. These classes include compounds capable of perturbing membranes, precipitating or denaturing proteins, and interfering with cellular enzymes. However, the present experiment tested a crude extract and did not perform phytochemical profiling, bioassay-guided fractionation, minimum inhibitory concentration testing, or compound identification. The mechanism must therefore remain a hypothesis rather than a demonstrated explanation.

The organism-specific response is also important. *Trema cannabina* inhibited *Ralstonia solanacearum* moderately at the highest concentration but did not inhibit *P. acnes*. This selectivity agrees with the broader observation that medicinal-plant extracts can be active against some bacteria while inactive against others, even within the same Gram group [5,9]. Differences may arise from the permeability of bacterial envelopes, efflux, enzymatic detoxification, growth requirements, diffusion of extract constituents through agar, and the concentration of target-active molecules. For *R. solanacearum*, the stronger activity of *C. sumatranum* supports continued screening of plant-derived antibacterials. Recent studies have shown that individual plant-derived compounds can inhibit growth, biofilm formation, or virulence-associated processes in this pathogen [2,15]. Indonesian leaf-extract studies have likewise reported activity against *R. solanacearum* and other bacterial targets

[19,20], although direct crop-protection efficacy must still be demonstrated in planta.

The agar-well diffusion results should be interpreted as screening evidence. Zone size is influenced not only by antimicrobial potency but also by solubility, molecular mass, polarity, and diffusion through agar. Chloramphenicol provides a useful reference because it inhibits bacterial protein synthesis and acts against a broad range of bacteria [16], yet expressing extract activity as a percentage of the antibiotic zone does not substitute for minimum inhibitory concentration or minimum bactericidal concentration. The zero result for *T. cannabina* against *P. acnes* likewise means no detectable zone under these conditions, not necessarily absence of every possible anti-acne effect at other concentrations, with other solvents, or in non-diffusion assays.

Both plants demonstrated substantial DPPH radical-scavenging activity. The concentration-dependent response of *T. cannabina* is consistent with later evidence that its ethanolic leaves contain antioxidant constituents and can scavenge DPPH radicals [5]. *Cratoxylum sumatranum* maintained more than 74% inhibition even at 25 ppm, suggesting that effective reducing constituents were already abundant at the lowest tested level. Phenolic hydroxyl groups can donate hydrogen atoms or electrons to stabilize radical species, while tannins, flavonoids, and related compounds often contribute jointly to the response [17,18]. Nevertheless, DPPH is a chemical assay. It does not measure bioavailability, metabolism, cellular protection, toxicity, or efficacy in an organism, and it should be followed by complementary antioxidant and biological assays.

The combined antibacterial and antioxidant profile gives *C. sumatranum* priority for further investigation, while *T. cannabina* remains relevant as an antioxidant source and as a selective inhibitor of *R. solanacearum*. Next steps should include authenticated voucher specimens; qualitative and quantitative phytochemical analysis; solvent fractionation; chromatographic and spectrometric identification of active compounds; determination of MIC, MBC, and IC₅₀ values; and statistical analysis of independent biological replicates. For agricultural use, greenhouse and field assays should examine phytotoxicity, formulation stability, persistence, and disease suppression. For any human-health application, cytotoxicity, skin compatibility, anaerobic susceptibility testing, and appropriate ethical and regulatory evaluation are essential before efficacy claims are made.

The main limitations were the descriptive analysis, absence of variance estimates in the summarized tables, restricted concentration range, reliance on one extraction solvent, and lack of phytochemical confirmation. Species-level bacterial strain information was also not recorded in the source dataset. These constraints limit generalization, but they do not negate the study's value as a comparative primary screen of two underexplored Bornean plant resources tested under a common protocol.

CONCLUSION

Ethanolic leaf extracts of *Trema cannabina* and *Cratoxylum sumatranum* showed distinct in vitro bioactivity. *Cratoxylum sumatranum* was the stronger and broader antibacterial extract, reaching 60.0% relative inhibition against *Ralstonia solanacearum* and 90.7% against *Propionibacterium acnes* at 10,000 ppm. *Trema cannabina* reached 33.5% against *R. solanacearum* but produced no detectable inhibition of *P. acnes* under the tested conditions. Both extracts scavenged DPPH radicals, with 100 ppm inhibition of 76.24% and 78.31%, respectively. The results support *C. sumatranum* as the priority candidate for bioassay-guided antibacterial fractionation and identify both species as promising sources of antioxidant constituents. Confirmatory work should quantify active compounds, establish MIC/MBC and IC₅₀ values with statistical replication, assess safety, and test efficacy in biologically relevant systems.

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REFERENCES

- [1] Ahmed, W., Yang, J., Tan, Y., Munir, S., Liu, Q., Zhang, J., et al. (2022). *Ralstonia solanacearum*, a deadly pathogen: Revisiting the bacterial wilt biocontrol practices in tobacco and other Solanaceae. *Rhizosphere*, 21, 100479. <https://doi.org/10.1016/j.rhisph.2022.100479>
- [2] Xia, H., Huang, Y., Wu, R., Tang, X., Cai, J., Li, S., Jiang, L., & Wu, D. (2023). A screening identifies harmine as a novel antibacterial compound against *Ralstonia solanacearum*. *Frontiers in Microbiology*, 14, 1269567. <https://doi.org/10.3389/fmicb.2023.1269567>
- [3] Perry, A. L., & Lambert, P. A. (2006). *Propionibacterium acnes*. *Letters in Applied Microbiology*, 42(3), 185-188. <https://doi.org/10.1111/j.1472-765X.2006.01866.x>
- [4] Hossain, H., Jahan, I. A., Islam, H. S., Kanti, D. S., Arpona, H., & Arif, A. (2013). Phytochemical screening and anti-nociceptive properties of the ethanolic leaf extract of *Trema cannabina* Lour. *Advanced Pharmaceutical Bulletin*, 3(1), 103-108.
- [5] Shah, V. K., & Rahman, M. A. (2022). Antioxidative and animal model-based pharmacological properties evaluation of leaves of *Trema cannabina* Lour. *Bioscience and Bioengineering Communications*, 4(1), 152-161. <https://doi.org/10.18801/bbc.040122.18>
- [6] Vieira, L. M. M., & Kijjoo, A. (2005). Naturally occurring xanthenes: Recent developments. *Current Medicinal Chemistry*, 12, 2413-2446.
- [7] Laphookhieo, S., Maneerat, W., & Koysomboon, S. (2009). Antimalarial and cytotoxic phenolic compounds from *Cratoxylum maingayi* and *Cratoxylum cochinchinense*. *Molecules*, 14(4), 1389-1395. <https://doi.org/10.3390/molecules14041389>
- [8] González-Lamothe, R., Mitchell, G., Gattuso, M., Diarra, M. S., Malouin, F., & Bouarab, K. (2009). Plant antimicrobial agents and their effects on plant and human pathogens. *International Journal of Molecular Sciences*, 10(8), 3400-3419. <https://doi.org/10.3390/ijms10083400>
- [9] Choi, H.-A., Ahn, S.-O., Lim, H.-D., & Kim, G.-J. (2021). Growth suppression of a gingivitis and skin pathogen *Propionibacterium* (*Propionibacterium*) *acnes* by medicinal plant extracts. *Antibiotics*, 10(9), 1092. <https://doi.org/10.3390/antibiotics10091092>
- [10] Vanselow, K. H., Marxen, K., Lippemeier, S., Hintze, R., Ruser, A., & Hansen, U.-P. (2007). Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. *Sensors*, 7(10), 2080-2095. <https://doi.org/10.3390/s7102080>
- [11] Davis, W. W., & Stout, T. R. (1971). Disc plate method of microbiological antibiotic assay. *Applied Microbiology*, 22(4), 659-665. <https://doi.org/10.1128/am.22.4.659-665.1971>
- [12] Arung, E. T., Kusuma, I. W., Kim, Y.-U., Shimizu, K., & Kondo, R. (2012). Antioxidative compounds from leaves of Tahongai (*Kleinhovia hospita*). *Journal of Wood Science*, 58(1), 77-80. <https://doi.org/10.1007/s10086-011-1217-7>
- [13] Febrina, L., Rusli, R., & Muflihah, F. (2015). Optimization of extraction and secondary metabolite testing of Libo (*Ficus variegata* Blume). *Journal of Tropical Pharmacy and Chemistry*, 3(2), 74-81.
- [14] Arifianti, L., Oktarina, R. D., & Kusumawati, I. (2014). Effect of extraction solvent on sinensetin content in *Orthosiphon stamineus* Benth. leaf extract. *E-Journal Planta Husada*, 2(1), 1-4.
- [15] Han, S., Yang, L., Wang, Y., Ran, Y., Li, S., & Ding, W. (2021). Preliminary studies on the antibacterial mechanism of a new plant-derived compound, 7-methoxycoumarin, against *Ralstonia solanacearum*. *Frontiers in Microbiology*, 12, 697911. <https://doi.org/10.3389/fmicb.2021.697911>
- [16] Cavalieri, S. J., Rankin, I. D., Harbeck, R. J., Sautter, R. S., McCarter, Y. S., Sharp, S. E., Ortez, J. H., & Spiegel, C. A. (2005). Manual of antimicrobial susceptibility testing. *American Society for Microbiology*.
- [17] Desmiaty, Y., Ratih, H., Dewi, M. A., & Agustin, R. (2008). Determination of total tannins in *Guazuma ulmifolia* and *Excoecaria bicolor* leaves by Prussian blue colorimetry. *Ortocarpus*, 8, 106-109.
- [18] Malangngi, L., Sangi, M., & Paendong, J. (2012). Determination of tannin content and antioxidant activity of avocado (*Persea americana* Mill.) seed extract. *Jurnal MIPA*, 1(1), 5-10.
- [19] Dewi, M. K., Ratnasari, E., & Trimulyono, G. (2014). Antibacterial activity of *Crescentia cujete* leaf extract against *Ralstonia solanacearum*, the causal agent of bacterial wilt. *LenteraBio*, 3(1), 51-57.
- [20] Nurjanah, S., Isbiyantoro, & Fadhillah, H. (2018). *Tithonia diversifolia* leaf extract as an antibacterial against *Streptococcus mutans* and *Streptococcus sanguinis*. *Jurnal Farmasi Lampung*, 7(1), 33-40.