Isolation and characterization of antibacterial compounds from *Euphorbia tirucalli* against *Xanthomonas axonopodis* pv. *citri*

Le T. M. Nguyen[1,2], Phong V. Nguyen[2], Minh T. L. Tran[2*], & Oanh T. T. Vo[3]

[1]Faculty of Fisheries, Nong Lam University, Ho Chi Minh City, Vietnam
[2]Faculty of Biological Sciences, Nong Lam University, Ho Chi Minh City, Vietnam
[3]Faculty of Agronomy, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: October 05, 2021
Revised: October 19, 2021
Accepted: November 01, 2021

Keywords

Ethyl acetate
Euphorbia tirucalli
Gallic acid
Scopoletin
Xanthomonas axonopodis

***Corresponding author**

Tran Thi Le Minh
Email: ttlminh@hcmuaf.edu.vn

ABSTRACT

Xanthomonas axonopodis pv. *citri* (*X. axonopodis* pv. *citri*) is the cause of canker disease on lime trees that negatively affect plant health and fruit quality. This study focused on the comparison of the extraction yield and antibacterial properties of *Euphorbia tirucalli* against *X. axonopodis* pv. *citri*, phytochemical screening, quantification of phenolic and flavonoid contents of the fraction extract. The results showed that the ethyl acetate fraction (EA) (7.5 mg/mL) of *E. tirucalli* from Dak Nong province had the best activity against bacteria with diameter of inhibition zone determined 15.50 ± 0.50 mm, and the minimum inhibition concentration was 0.312 mg/mL. Alkaloids, flavonoids, tannins, and terpenoids were found in the EA fraction extract of E. tirucalli, whereas saponin did not appear in the extract. The phenolic and flavonoid content was in the range of 14.46 - 98.63 mg GEA/g and 90.34 - 408.86 µg QE/g, respectively. Column chromatography followed Nuclear Magnetic Resonance spectra were performed and the three compounds were identified as scopoletin, gallic acid, and 3,3',4'-tri - O - methylellagic acid. This study suggests that the extract from E. tirucalli and the isolated compounds can be used for managing of citrus canker disease.

Cited as: Tran, T. K., Nguyen, L. T. M., Nguyen, P. V., Tran, M. T. L., & Vo, O. T. T. (2021). Isolation and characterization of antibacterial compounds from *Euphorbia tirucalli* against *Xanthomonas axonopodis* pv. *citri*. *The Journal of Agriculture and Development* 20(6), 1-8.

1. Introduction

Xanthomonas axonopodis pv. *citri* causes citrus canker disease that commonly occurs in citrus-growing regions. Bacterial colonisation causes lesions on fruits, leaves, and stems of citrus trees, including lime, orange, and grapefruit (Jalan et al., 2013). Using chemicals is the best strategy for preventing diseases and insect pests, but the overuse of chemicals with their residual toxicity is causing serious health hazards to humans, animal life, and the environment. To reduce or avoid the harmful effects of synthetic chemicals, there is an urgent need to find alternative methods for managing plant pathogens. Plants have developed effectively defense systems that consist of several complex secondary compounds. Antimicrobial compounds in plant tissues are an essential factor in disease resistance (Fawcett & Spencer, 1970), and used as fungicides, pesticides, and bactericides (Dubey & Kishore, 1987; Radcliffe et al., 1991). The development of plant-based compounds for pest control is a priority interest in sustainable agricultural production.

Euphorbia tirucalli, known as "san ho xanh" or "xuong ca" in Vietnam, is a popular herb known worldwide for its multiple uses. Stem latex is used in treating abdominal pain, asthma, cough, earache, intestinal worms, leprosy, rheumatism, skin

diseases, toothache, tumors, and warts (Ramesh et al., 2009). Previous studies have shown that active compounds from this plant, such as alkaloids, phenols, and tannins, contribute to medicinal treatments (Sugumar et al., 2010). The bacterial activity of *E. tirucalli* has reported on *Erwinia carotovora, Fusarium oxysporum,* and *Xanthomonas campestris* (Lirio et al., 1998); *Alternaria alternata, Aspergillus flavus, Aureobasidium pullulans, Bacillus subtilis, Drechslera oryzae, Escherichia coli, Pseudomonas aeruginosa, P. solanacearum, Staphylococcus aureus, Xanthomonas citri* (Jadhav et al., 2010); *Aspergillus fumigatus, A. flavus, A. niger, Bacillus subtilis, Candida albicans, C. tropicalis, Escherichia coli, Fusarium oxysporum, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella enteritidis,* and *Staphylococcus aureus* (Upadhyay et al., 2010). *Euphorbia tirucalli* is a good example, but little information is available on plant diseases, especially against phytopathogens in Vietnam. Therefore, this study was conducted on *E. tirucalli* with the following objectives: (i) to screen and evaluate the antibacterial activity of fraction extracts (hexan, ethyl acetate, and butanol) against *Xanthomonas axonopodis* pv. *citri*; (ii) to isolate and identify the bioactive compounds using Thin Layer Chromatography (TLC) analysis, Column Chromatography, and Nuclear Magnetic Resonance (NMR) spectra.

2. Materials and Methods

2.1. Biological materials

The whole fresh aerial parts of *E. tirucalli* (from two to four years old) was obtained from Dak Nong province (DNP) – MG (12°15'25" North latitude 107°42'06" East longitude) and Ho Chi Minh City (HCMC) – MG (10°48' North latitude 106°39' East longitude) on April, 2019.

The bacterial *X. axonopodis* pv. *citri* strain BLKQ1 (AN: MT328595) was isolated from canker disease on lime trees and preserved in Faculty of Biological Sciences, Nong Lam University Ho Chi Minh City, Vietnam.

2.2. Preparation of *E. tirucalli* extracts

Plant samples were thoroughly washed under running tap water, air-dried and subsequently dried in a hot air oven at 60°C for 72 h. Then, dried samples were milled to obtain 6.5 kg powder. The powder was drenched with 96% ethanol (EtOH) (5 × 15 L) for 24 h, to obtain the EtOH - soluble extract. After filtering, the EtOH - soluble was evaporated to obtain the crude ethanol extract. The ethanol extract was sequentially partitioned with n - hexane, ethyl acetate, and butanol to obtain the respective fractions (He, EA, and Bu). The percentage yield (%) was calculated as the ratio of the weight of the extract to the dried weight of the sample.

2.3. Antibacterial assay

The antibacterial properties of the fraction extracts were evaluated using the agar well diffusion method (Toda et al., 1989). Each fraction extract was dissolved in 10% dimethyl sulfoxide (DMSO), reaching final concentrations of 1.25, 2.5, 5.0, and 7.5 mg/mL for further testing (Upadhyay et al., 2010). Bacteria were cultured in nutrient broth (NB) for 20 - 24 h at 37°C and adjusting of the concentration to 0.5 McFarland Standard with sterile NB. Wells of 5.0 mm were punched in the agar medium and were filled with 60 µL of fraction extract. Streptomycin (0.01 mg/mL) was used as the positive control while 10% DMSO was used as a negative control. Inhibition zones (mm) were measured after 48 h of incubation at 37°C. Each assay was performed in triplicate.

2.4. Minimum inhibitory concentrations assay

Minimum inhibitory concentrations (MICs) were measured following the recommendations of the NCCLS (2003), and Devienne & Raddi (2002). Each extract was diluted to 5.0 - 0.078 mg/mL by dispensing 50 µL of extracts into each of the 96 wells of a standard microtiter tray containing 100 µL of Nutrient Broth followed by 2-fold dilutions. Bacterial cultures (100 µL) were added to each well. After bacterial growth, 20 µL resazurin (0.01%) was added to each well and incubated at 37°C for 2 h. The MIC was determined visually as the lowest concentration that led to growth inhibition after 2 h.

2.5. Phytochemical Screening

The phytochemical screening was performed according to Brain & Turner (1975) and Evans (1996) with minor modifications.

2.5.1. Alkaloids

H_2SO_4 5% (20.0 mL) was added to 2.0 g of fraction extract and filtered. Then, a few drops of NH3 were added to the filtrates until became alkaline. Chloroform (5.0 mL) was added for separating the aqueous and organic layers. Then, 2.0 mL of 1% HCl and 1 mL of Wagner's reagents were added to the organic layer. There was a brown/reddish - brown formation, indicating the presence of alkaloids.

2.5.2. Flavonoids

Ethanol 70% (20 mL) was added to 5 g of fraction extract and heated to boiling. After filtering, a few drops of H_2SO_4 were added to the solution. There was an orange colour formation, indicating the presence of flavonoids.

2.5.3. Saponins

About 10 mL of distilled water was added to 1 g of fraction extract, boiled, and cooled. The solution was vortexed for 15 sec. There was no foam formation, indicating the presence of saponins.

2.5.4. Tannin

Distilled water (100 mL) was added to 10 g of fraction extract. The solution was heated in a water bath for 3 min, cooled, and filtered. The ferric chloride was added to the solution. There was a dark green colour formation, indicating the presence of tannin.

2.5.5. Terpenoids

$CHCl_3$ (2 mL) and concentrated H_2SO_4 (3 mL) were added to 5 mg of fraction extracts. There was a reddish-brown colour formation in the inner face, indicating the presence of terpenoids.

2.6. Quantification of phenolic content

The total phenolic content of the extract was measured according to the method of Waterman & Mole (1994) using a UV - Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). First, either 0.5 mL of each extract or an appropriate gallic acid standard was added to 2.5 mL of 1/20 diluted Folin reagent and mixed for 4 min, after which 2 mL of 10% Na_2CO_3 solution was added and mixed. After standing 30 min and shaking, the absorbance of the mixture was measured at 758 nm. Total phenolic was expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g of the extract). All samples were analysed in triplicate.

2.7. Quantification of flavonoids content

The total flavonoid content of the extract was measured according to the method of Zhishen et al. (1999) using quercetin as a standard. Either 1 mL of each extract or an appropriate quercetin standard was added to 4 mL of distilled water, after which 0.3 mL of 5% NaNO2 solution was added. The solution was added 0,3 mL of 10% $AlCl_3$ (w/v) and 2 mL of 1 M NaOH and produced a total volume of 10 mL with distilled water. After 30 min, the solution was measured in a UV - Visible Ultraspec JANEWAY 7305 spectrophotometer at 320 nm. The contents were expressed as microgram equivalents of quercetin per gram of extracts (µg QE/g of extract). All samples were analysed in triplicate.

2.8. Column chromatography and isolation

Ethyl acetate (EA) fraction of *E. tirucalli* from DNP was run onto the silica gel column and eluted with different concentrations of n - hexane, ethyl acetate, and acetone (in the proportion 5:1:1 to 1:1:1, v/v/v). A total of 8 fractions had been collected (20 mL each) and marked as EA1 - 8. The fraction EA3 was adsorbed on a silica gel column and eluted with different concentrations of n - hexane, chloroform, ethyl acetate, acetone, and acetic acid (in the proportion 350:100:40:25:10, v/v/v/v/v). A total of 4 fractions had been collected (20 mL each) and marked as EA3.1 - EA3.4. EA3.2 was adsorbed on the silica gel column and eluted with methanol, and collected three fractions EA3.2.1 - EA3.2.3. EA3.2.1 (97.0 mg) was run onto the silica gel column and eluted with methanol and water (in the proportion 1:2 to 1:1, v/v). A total of three compounds had been collected and named as 1 (12 mg), 2 (3.7 mg), and 3 (7.6 mg).

2.9. The NMR profiling of compounds

The NMR spectra were recorded in CD_3COCD_3 on Bruker Avance III spectrometers at 500 MHz (1H) and 125 MHz (13C). The

signals of the residual solvents of CD_3COCD_3 at δH 2.05 and δC 29.4 were taken as reference points. The NMR temperature was set at 23°C.

2.10. Statistical analysis

The data were analysed using SPSS 20.0, where ANOVA (one way) or t-tests were used to show the statistical difference at a 95% confidence. The results were further subjected to Duncan test to separate the significantly different means.

3. Results and Discussion

3.1. Extraction yield

The extraction yield of the crude extract (EtOH) and fraction extracts (He, EA, and Bu) of *E. tirucalli* from DNP and HCM are given in Table 1. The extraction yield varied among the different solvents and regions to collect plants. The EtOH extract of sample from DNP showed a higher extraction yield of that from HCM. Compared to other fractions and regions obtained, *E. tirucalli* from DNP showed the extraction yield of EA fraction was highest (18.34%) while *E. tirucalli* from HCM showed that the extraction yield of n - hexan and Bu fractions were highest at 31.13% and 15.22%, respectively. This result showed that the composition and content of secondary compounds depended on geography, climate, age of trees (Upadhyay et al., 2010). According to Younes et al. (2018), the polarity and molecular weight of the chosen solvent effect phytochemical constituents in the extracts.

3.2. Antibacterial activity of fractions

Antibacterial activity of fractions The ability against bacteria of the fraction extracts from DNP and HCMC is presented in Table 2. At 5 mg/mL, all fractions exhibited antibacterial activity with inhibition zones ranging from 3.07 ± 0.60 to 12.23 ± 0.87 mm. The EA fraction (7.5 mg/mL) from DNP showed the highest inhibition diameter (15.50 ± 0.50 mm), and the MIC value was 0.312 mg/mL. Meanwhile, the He fraction (2.5 mg/mL and 1.25 mg/mL) from all two regions did not show antimicrobial activity. All fractions collected from DNP sample exhibited higher antibacterial activity than those obtained from the HCMC sample. The antimicrobial ability of EA fraction from DNP exhibited higher than those of neem and garlic extracts of Negi & Kuma (2015).

3.3. Phytochemical screening

The phytochemical composition of fraction extracts from DNP is shown in Table 3. Flavonoids are present in all fractions but any (not?) saponin, a toxic group. Alkaloids and tannins were positive in EA and Bu fractions, where terpenoids were positive in He and EA fractions. Compared to other fractions, EA fraction possessed more phytochemical constituents. Our results were similar to the report of Orlanda & Vale (2015). Controversy, Aleixo et al. (2018) reported that flavonoids present in the extract of *E. tirucalli* from Brazil, but not tannin, alkaloid, or saponin.

3.4. Phenolic and flavonoid content

The phenolic and flavonoid content of fraction extracts from DNP is shown in Table 4. Two linear equations ($y = 0.102x + 0.0075$, $R^2 = 0.9998$; $y = 0.0043x + 0.0118$, $R^2 = 0.9999$) were constructed based on the standardised gallic acid and quercetin amount used calculating the total phenolic and flavonoid content. All fractions of E. tirucalli from DNP contained varied phenolic and flavonoid content. Of the three fractions, EA has the highest phenolic and flavonoid content corresponding to 98.63 ± 4.14 mg of gallic acid equivalent (GAE)/g and 408.86 ± 7.67 µg quercetin equivalent (QE)/g. The lowest phenolic and flavonoid content were obtained with He, corresponding to 14.46 ± 1.53 mg GAE/g and 90.34 ± 2.90 µg QE/g. Besides chlorophyll and lipids, the He fraction also contained a few low polarity phenolic compounds (free-form flavonoids). The phenolic content of the EA fraction from DNP was higher than that of *E. tirucalli* from Brazil (Orlanda & Vale, 2015).

3.5. Antimicrobial compounds from *Euphorbia tirucalli* and its structure determination

Among fraction extracts, the EA fraction of DNP showed higher antibacterial activity was used to isolate antimicrobial compounds. The results of NMR showed the presence of scopoletin (Figure 1A), gallic acid (Figure 1B), and 3,3',4'-tri-O-methylellagic acid (Figure 1C). Data of NMR Spectra of three purified compounds are

Table 1. Extraction yield (%) by different solvents of *E. tirucalli* from Dak Nong province (DNP) and Ho Chi Minh City (HCMC)

No.	Solvents	Extraction yield (%)	
		DNP	HCMC
1	Ethanol (EtOH)	8.87 ± 0.05^{b}	8.67 ± 0.03^{a}
2	n-Hexan (He)	29.79 ± 0.30^{a}	31.13 ± 0.82^{b}
3	Ethyl acetate (EA)	18.34 ± 0.67^{b}	15.92 ± 1.07^{a}
4	Butanol (Bu)	13.14 ± 0.44^{a}	15.22 ± 0.57^{b}

[a-b]Values in the same row with different letters were significantly different at $P < 0.05$ (mean ± SD, n = 3).

Table 2. Antibacterial activity of fraction extracts of *E. tirucalli* from province (DNP) and Ho Chi Minh City (HCMC)

Sample	Fraction extracts	Inhibition zone diameter (mm)			
		Concentration (mg/mL)			
		1.25	2.5	5.0	7.5
DNP	n-Hexan (He)	-	-	3.07 ± 0.60	5.77 ± 0.75
	Ethyl acetate (EA)	8.67 ± 0.29	10.17 ± 0.29	12.23 ± 0.87	15.5 ± 0.50
	Butanol (Bu)	3.27 ± 0.25	5.00 ± 0.50	7.67 ± 0.76	10.03 ± 0.55
HCMC	n-Hexan (He)	-	-	2.67 ± 0.29	5.00 ± 0.50
	Ethyl acetate (EA)	6.67 ± 0.29	8.40 ± 0.36	9.97 ± 0.45	11.60 ± 0.36
	Butanol (Bu)	2.93 ± 0.40	4.67 ± 0.29	7.07 ± 0.60	8.33 ± 0.76
Streptomycin (0.01 mg/mL)		20.13 ± 0.61	20.13 ± 0.61	20.13 ± 0.61	20.13 ± 0.61
DMSO 10%		-	-	-	-

(mean ± SD, n = 3); "-" non-inhibition.

Table 3. Phytochemistry composition in fraction extracts of *E. tirucalli* from Dak Nong province

Fraction extracts	Alkaloids	Tannin	Saponin	Flavonoids	Terpenoid
n-hexan (He)	-	-	-	+	+
Ethyl acetate (EA)	++	+	-	++	+++
Butanol (Bu)	+	+	-	+	-

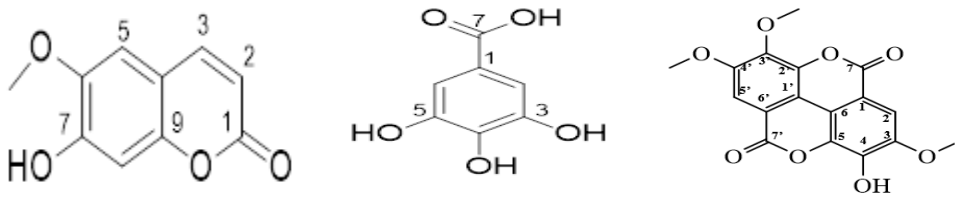
"-": negative; "+,++, and +++": Low, moderately present, highly present.

Table 4. Phenolic and flavonoid content in fraction extracts of *E. tirucalli* from Dak Nong province

Fraction extracts	Phenolic content (mg GEA/g)	Flavonoids content (µg QE/g)
n-hexan (He)	14.46 ± 1.53	90.34 ± 2.90
Ethyl acetate (EA)	98.63 ± 4.14	408.86 ± 7.67
Butanol (Bu)	50.94 ± 2.72	258.75 ± 2.82

shown in Table 5 and Table 6.

According to previous studies, scopoletin, gallic acid, and 3,3',4' - tri - O-methylellagic acid showed antimicrobial activity (Kuete et al., 2007). The mechanism of bacterial inhibition of scopoletin has been observed similar to antibiotic β - lactam. It inhibited cell wall formation and distorted bacterial morphology (Tiwtawat et al., 2018). For example, scopoletin inhibited Staphylococcus aureus (Natividad et al., 2019); *Salmonella typhimurium* (Wisnu & Risna, 2020); *Pseudomonas aeruginosa* (Tiwtawat et al., 2018); *Actinomyces naeslundii; A. israelii; Actinobacillus actinomycetemcomitans; Prevotella intermedia,* and *Porphyromonas gingivalis* (More et al., 2012). Gallic acid, a phenolic acid, against various bacteria such as *X. citri subsp. citri* (Silva et al., 2013); *Pseudomonas putida, P. fragile; P. fluorescens* (Elena et al., 2018); *Escherichia coli; P. aeruginosa, Staphylococcus aureus,* and *Listeria monocytogenes* (Anabela et al., 2013); *Stenotrophomonas maltophilia* (Navarro - Martinéz et al., 2005). According to Kuete et al. (2007), 3,3',4-Trimethylellagic acid from *Irvingia gabonensis* inhibited the growth of six Gram (+) and thirteen Gram (-) tested species. The results



(A) (B) (C)

Figure 1. (A) structure of Substance 1 (scopoletin); (B) structure of Substance 2 (gallic acid); (C) structure of Substance 3 (3,3',4'-tri-O-methylellagic acid).

Table 5. Data of NMR Spectroscopy of Substance 1 and Substance 2

Position	Substance 1 (Scopoletin)		Substance 2 (Gallic acid)	
	δ_H, J(Hz)	δC	δ_H, J(Hz)	δ_C
1				122.2
2		160.4	7.14 (1H, s)	110.4
3	6.17, d, 9.5	112.4		146.4
4	7.84, d, 9.5	143.7		139.6
5	7.19, s	109.1		146.4
6		144.9	7.14 (1H, s)	110.4
7		154.1		170.5
8	6.80, s	102.8		
9		150.9		
10		111.2		
11				
3-/5/6-OCH_3	3.90	55.7		
O-CH_2-O				

Table 6. Data of NMR Spectroscopy of Substance 3

Position	Type C	Substance 3		3,3',4'-tri-O-methylellagic acid	
		δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
1	>C<		111.70		111.62
2	>C<		140.91		141.23
3	>C<		140.71		140.26
4	>C<		153.68		152.23
5	-CH-	7.54 (1H, s)	107.38	7.73 (1H, s)	111.42
6	>C<		112.42		112.11
7	>C<		158.29		158.52
1'	>C<		111.78		111.65
2'	>C<		141.40		141.25
3'	>C<		140.22		140.23
4'	>C<		153.04		152.22
5'	-CH-	7.48 (1H, s)	107.38	7.61 (1H, s)	111.45
6'	>C<		113.34		112.12
7'	>C<		158.47		158.63
3-OMe	-CH_3	4.07 (3H, s)	61.25	4.19 (3H, s)	61.53
3'-OMe	-CH_3	4.06 (3H, s)	60.91	4.14 (3H, s)	61.26
4'-OMe	-CH_3	3.98 (3H, s)	56.66	4.04 (3H, s)	56.51

of this study provide the scientific basis to confirm the inhibitory activity of *X. axonopodis* pv. *citri* of EA extract of *E. tirucalli* from Dak Nong province.

4. Conclusions

The results showed that the extraction yield of sample from Dak Nong province is higher than that of Ho Chi Minh city. The phytochemical screening revealed that alkaloids, flavonoids, tannins, and terpenoids were in the ethyl acetate fraction, but not saponin in all fractional extracts. The ethyl acetate fraction produces higher amounts of phenolic and flavonoids and antimicrobial activity against Xanthomonas axonopodis than other fractions. From this fraction extract, scopoletin, gallic acid, and 3,3',4' - tri - O - methylellagic acid were identified.

References

Aleixo, T., Calmon, J., Falconi, A., Souza, A. R., Duarte, R. S., Fagundes, L. L., & Machado, R. R. P. (2018). Phytochemical screening and investigation of antimicrobial activity from derivatives of aveloz (*Euphorbia tirucalli* L.). *SOJ Microbiology & Injectious Diseases* 6(2), 1-7.

Anabela, B., Carla, F., Maria, J. S., & Manuel, S. (2013). Antibacterial activity and mode of action of ferulic and gallic acid against pathogenic bacteria. *Microbial Drug Resistance* 19(4), 256-265.

Brain, K. R., & Turner, T. D. (1975). *The practical evaluation of phytopharmaceuticals* (1st ed.). Bristol, UK: Wright Science Technical Publisher.

Devienne, K. F., & Raddi, M. S. G. (2002). Screening for antimicrobial activity of natural products using a microplate photometer. *Brazilian Journal of Microbiology* 33(2), 166-168.

Dubey, N. K., & Kishore, N. (1987). Fungitoxicity of some higher plants and synergistic activity of their essential oils. *Tropical Science* 27, 23-27.

Elena, S., Mariantonietta, S., Luca, T., Gianfranco, P., Lucia, M., Marina, S., Raffaele, C., & Patrizio, T. (2018). Antimicrobial activity of gallic acid against food related *Pseudomonas* strain and its use as biocontrol tool to improve the shelf life of fresh black truffles. *International Journal of Food Microbiology* 266(1), 183-189.

Evans, W. C. (1996). *Trease evans pharmacognosy* (14th ed.). London, UK: WB Saunders.

Fawcett, C. H., & Spencer, D. M. (1970). Plant chemotherapy with plant products. *Annual Review of Phytopathology* 8, 403-418.

Jadhav, D. M., Gawai, D. U., & Khillare, E. M. (2010). Evaluation if antibacterial and antifungal activity of *Euphorbia tirucalli* L. *Bionano Front* 3, 332-334.

Jalan, N., Kumar, D., Andrade, M. O., Yu, F., Jones, F. B., Graham, J. H., White, F. F., Setubal, J. C., & Wang, N. (2013). Comparative genomic and transcriptome analyses of pathotypes of *Xanthomonas citri* subsp. *citri* provide insights into mechanisms of bacterial virulence and host range. *BMC Genomics* 14, 551.

Kuete, V., Wabo, G. F., Ngameni, B., Mbaveng, A. T., Metuno, R., Etoa, F. X, Ngadjui, B. T., Beng, V. P., Meyer, J. J. M., & Lall, N. (2007). Antimicrobial activity of the methanolic extract, fractions and compounds from the stem bark of *Irvingia gabonensis* (*Ixonanthaceae*). *Journal of Ethnopharmacology* 112(3), 531-536.

Le, T. K. D., Bui, X. H., Nguyen, T. A. T., Pham, N. K. T., & Duong, T. H. (2019). Chemical constituents of *Euphorbia tirucalli* L. *Science and Technology Development Journal - Natural Sciences* 2(5), 76-82.

Lirio, L. G., Hermano, M. L., & Fontanilla, M. Q. (1998). Note antibacterial activity of medicinal plants from the Philippines. *Pharmaceutical Biology* 36(5), 357-359.

More, G., Lall, N., Hussein, A., & Tshikalange, T. E. (2012). Antimicrobial constituents of *Artemisia afra* Jacq. ex Willd. against periodontal pathogens. *Evidence Based Complementary and Alternative Medicine* 2012, 252758.

Natividad, G. D. L. C., Abrham, G. R., Patricia A. F., Elsa, V. Z., Ma, D. P. G., Margarita, A. F., Ana, S. G. R., & Manases, G. C. (2019). Antibacterial activity of *Morinda citrifolia* Linneo seeds against methicillin-resistant *Staphylococcus* spp. *Microbial pathogenesis* 128, 347-353.

Navarro - Martínez, M. D., Navarro - Perán, E., Cabezas - Herrera, J., Ruiz - Gómez, J., García - Cánovas, F., & Rodríguez - López, J. N. (2005). Antifolate activity of epigallocatechin gallate against *Stenotrophomonas maltophilia*. *Antimicrobial Agents and Chemotherapy* 49(7), 2914-2920.

NCCLS (National Committee for Clinical Laboratory Standards). (2003). *Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically*. Wayne, PA, USA: National Committee for Clinical Laboratory Standards.

Negi, A., & Kumar, P. (2015). Antibacterial effect of plant extracts and antibiotics on *Xanthomonas axonopodis* pv. *citri in vitro*. *Trends in Biosciences* 8(9), 2374-2376.

Orlanda, J. F. F., & Vale, V. V. (2015). Phytochemical analysis and photo-protective activity of theethanolic extract of *Euphorbia tirucalli Linneau* (*Euphorbiaceae*). *Revista Brasileira de Plantas Medicinais* 17(4), 730-736.

Radcliffe, E., Dunkel, F., Strzok, P., & Adam, S. (1991). Antifeedant effect of neem, *Azadirachta indica* A. Juss., kernel extracts on Kraussaria angulifera (Krauss) (Orthoptera:Acrididae), a Sahelian grasshopper. *Tropical Agriculture* 68(1), 95-101.

Ramesh, C. K., Prabha, M. N., Deepak, S. A., & Madhusudhan, K. N. (2009). Screening of antiviral property against tobamoviruses in latex of *Euphorbia tirucalli* L. *Indian Journal of Biotechnology* 3(1), 1-3.

Silva, I. C., Regasini, L. O., Petrônio, M. S., Silva, D. H. S., Bolzani, V. S., Belasque, J. Jr., Sacramento, L. V. S., & Ferreira, H. (2013). Antibacterial activity of alkyl gallates against *Xanthomonas citri* subsp. *citri*. *Journal of Bacteriology* 195(1), 85-94.

Sugumar, S., Karthikeyan, S., & Gothandam, K. M. (2010). Preliminary phytochemical and antibacterial investigations of *Euphorbia tirucalli* stem extracts. *Pharmacology Online* 3, 937-943.

Tiwtawat, N., Markus, B., Henrik, B., Kwankamol, T., Wichai, S., & Srunya, V. (2018). Scopoletin from *Lasianthus lucidus* Blume (Rubiaceae): A potential antimicrobial against multidrug-resistant *Pseudomonas aeruginosa*. *Journal of Applied Pharmaceutical Science* 8(9), 1-6.

Toda, M., Okubo, S., Ohnishi, R., & Shimamura, T. (1989). Antibacterial and bactericidal activities of Japanese green tea. *Japanese Journal Bacteriology* 44(4), 669-672.

Upadhyay, B., Singh, P. K., & Kumar, A. (2010). Ethnomedicinal, phytochemical and antimicrobial studies of *Euphorbia tirucalli* L. *Journal Phytology* 2(4), 65-77.

Waterman, P. G., & Mole, S. (1994). *Analysis of phenolic plant metabolites* (1st ed.). Boston, USA: Blackwell Scientific.

Wisnu, C. P., & Risna, A. (2020). Antibacterial activity of scopoletin from stem bark of *Aleurites moluccana* against *salmonella typhi*. *Journal of Tropical Pharmacy and Chemistry* 5(1), 29-32.

Younes, A. B., Salem, M. B., Abed, H. E., & Jarraya, R. (2018). Phytochemical screening and antidiabetic, antihyperlipidemic, and antioxidant properties of *Anthyllis henoniana* (Coss.) Flowers extracts in an alloxan-induced rats model of diabetes. *Evidence Based Complementary and Alternative Medicine* 2018, ID8516302.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and scavenging effects on superoxide radicals. *Food Chemistry* 64(4), 555-559.