ISOLATION AND CHARACTERIZATION OF ANTIBACTERIAL COMPOUNDS FROM *Euphorbia tirucalli*

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ABSTRACT

Medicinal plants constitute a natural reservoir for medicines worldwide. They serve mainstream therapeutics and are central in folklore medicine. In case of *Euphorbia tirucalli* belongs to the Euphorbiaceae family and is a very popular herb in traditional herbal medicine. Despite the traditional use of this plant, no scientific report or information was found in the literature regarding neither its biological activity nor its chemical constituents in Vietnam. This study was designed to determine the antimicrobial of different polarities crude extracts as well as the isolation and identification of the chemical constituents of this plant. The extracts and pure compounds of *E. tirucalli* were tested for antimicrobial activity against *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Staphylococcus epi* using agar well diffusion method. Compounds were isolated from EtOAC extract of *E. tirucalli* through column chromatography and their structures were determined by means of NMR. According to the antimicrobial assay, EtOAC extract showed the best antibacterial activity against all of bacteria test was submitted further separation and purification. This led to the isolation of three compounds identified as eriodictyol (1), quercitrin (2) and afzelin (3). Afzelin showed the best antibacterial activity against all of bacteria test with MIC values from 0.125–0.25 mg/mL. Our results revealed that *E. tirucalli* was a good source of antibacterial and could be used in the study area against enterocolitis and conjunctivitis.

Keywords: Euphorbia tirucalli, chemical constituents, antimicrobial activity, ethyl acetate extract.

1. INTRODUCTION

Euphorbia tirucalli L. belongs to the Euphorbiaceae family and is a very popular herb in traditional herbal medicine [1]. There are approximately 1600 species in the *Euphorbia* genus. Some species of this genus have long been used as herbal drugs in China, India, Brazil and Southeast Asia. *E. tirucalli* is universally known as Aveloz. It is a native of Africa and America. In the world, *E. tirucalli* is one of the most popular herbs that is known to have medicinal properties such as: a poultice of the roots or stems is applied to heal nose ulceration, haemorrhoids and swellings in Malaysia [2, 3]. The different parts of the plant such as the latex, leaves, stems and roots may have different medicinal purposes in Rajasthan and India [4]. Besides, the people used the plant to cure snake-bites, warts, sexual impotence, syphilis, broken bones, haemorrhoids, pains, warts, swellings and ulcerations [5]. In Brazil, it is also used for the treatment of scorpion bites, asthma, cancer, spasms and others [6]. In

Vietnam, *E. tirucalli* or known as San ho xanh, Xuong ca. This plant is used to treat gonorrhea, whooping cough, asthma, leprosy, enlargement of spleen, jaundice, tumors and bladder stones. Stem latex is used to treat warts, tooth ache, cough, asthma, ear ache, leprosy, abdominal pain, tumors, rheumatism, skin diseases and intestinal worms. Root is used for colic pains [7]. Various studies indicated that this plant is a valuable source of medicinal compounds such as: alkaloid, tannins and phenols and these compounds was contributed their effectiveness in medicinal treatment [8]. These active compounds could inhibit the bacteria growth due to its ability to form complex with extracellular proteins of cell wall and disrupt microbial membrane, or was toxic to microorganisms, inhibit bacteria by inactive microbial adhesion, enzymes and cell envelope transport proteins [9]. The presence of various bioactive compounds in this plant is significant evidence in relation to the effectiveness of the plant when employed as traditional medicine [10]. Therefore, screening of phytochemical constituents of the herb is important to correlate its therapeutic activity with the specific active constituents [11]. The aim of the present study was to isolate and identify the chemical composition of the stem of *E. tirucalli* and to assess their antimicrobial activity, in order to explain some of the traditional uses of the plant.

2. MATERIALS AND METHODS

2.1. General experimental procedures

NMR spectra were measured on Bruker Avance III (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometer. Proton chemical shifts were referenced to the solvent residual signal of CD_3COCD_3 at δ_H 2.05. The ^{13}C NMR spectra were referenced to the central peak of CD_3COCD_3 at δ_C 29.4. HR–ESI–MS were recorded on a Bruker microTOF Q-II. TLC analyses were carried out on pre-coated silica gel 60 F_{254} or silica gel 60 RP–18 $F_{254}S$ (Merck, Germany) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. Gravity column chromatography (CC) was performed using silica gel 60 (0.040–0.063 mm, Himedia, India).

2.2. Plant materials

The fresh whole plant of *Euphorbia tirucalli* (Euphorbiaceae) was collected from Đak Nong Province in July of 2018. The plant sample was identified by Dr. Pham Van Ngot, Department of Botany, Faculty of Biology, Ho Chi Minh City University of Education.

2.3. Extraction and isolation of compounds

The dried whole plant of *Euphorbia tirucalli* was milled to obtain 3.5 kg of powder. The powder was extracted with ethanol (EtOH) (2 × 10 L) at 70 °C, to obtain the EtOH-soluble extract. While the solution was being evaporated, a precipitate (P, 250.4 g) occurred and was filtered off. The remaining solution was evaporated until dryness to obtain crude ethanol extract (290.3 g). The resultant ethanol extract was sequentially partitioned with *n*-hexane, EtOAc, and *n*-BuOH to afford the extracts H (94.2 g), EA (61.8 g), Bu (27.0 g), respectively.

Extraction of EA was applied to normal phase silica gel CC and eluted with a solvent system of *n*-hexane: EtOAc with the ratio 8:2, 5:5, and 0:10 to afford 3 fractions, EA1 (10.32 g), EA2 (2.5 g), and EA3 (2.19 g), respectively. The fraction EA2 was fractionated by CC with the solvent system *n*-hexane: EtOAc (1:4) to afford three fractions EA2.1-3. Fraction EA2.1.3 (548.0 mg) was further applied to normal phase silica gel CC, eluted with *n*-hexane: EtOAc: EtOH: AcOH (5:1:0.2:0.1) to afford three sub-fractions EA2.1.3.1-3. Sub-fraction

EA2.1.3.2 (356 mg) was purified by preparative TLC using chloroform: MeOH: H_2O (4:0.38:0.02) to obtain three compounds 1 (6.5 mg), 2 (10.0 mg), and 3 (4.3 mg).

Eriodictyol (1). White amorphous powder; the ^1H and ^{13}C NMR (acetone-d_6) spectroscopic data, see Table 3.

Quercitrin (2). Light-yellow amorphous powder; the ^1H and ^{13}C NMR (acetone-d_6) spectroscopic data, see Table 3.

Afzelin (3). Light-yellow amorphous powder; the ^1H and ^{13}C NMR (acetone-d_6) spectroscopic data, see Table 3.

2.4. Antimicrobial activity

Tests were performed on fours species of selected bacteria such as *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 4028), *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epi* ATCC 12228 were commercially obtained from the American Type Culture Collection (ATCC, USA). The characteristics and diseases caused by these microorganisms were listed in Table 1. Antibacterial activity of different polarities crude extracts and the isolated compound of stem of *E. tirucalli* were measured against fours species of selected bacteria on nutrient agar plates using well diffusion method with modification [12]. Each stem crude extracts and the pure compound (1 mg/mL concentration) of *E. tirucalli* were prepared by addition of dimethyl sulfoxide (DMSO) solvent 10%. All the test bacterial species were maintained on nutrient agar medium. Old bacterial cultures were incubated into nutrient broth. After 18–20 hours of incubation, the bacterial suspension was centrifuged at 10000 rpm for 15 min. The pellet was resuspended in sterile distilled water and the concentration was adjusted to $1x10^7$ cfu/mL using UV Visible Spectrophotometer. By reading the OD of the solution to 0.8–0.9 (600 nm) it was used for further studies. Wells of 5.0 mm diameter were punched in to the agar medium and were filled 60 µL of prepared each stem crude extracts and the pure compound. Commercially Streptomycin (0.01 mg/mL concentration) was used as positive control while DMSO solvent 10% was taken as the negative control. These plates were allowed to stand for 5 minutes for the diffusion of extract to take place. The plates were then incubated at 37 °C for 48 hours. Antibacterial activity was evaluated by measuring the zones of inhibition (clear zone around each well) in millimeter (mm). Each method in this experiment was replicated three times.

Table 1. List of bacteria used in this study

Name of bacteria	Characteristic feature	Diseases caused by the organisms
Escherichia coli (ATCC 25922)	Gram - ve	Gastroenteritis, urinary tract disease
Salmonella typhimurium (ATCC 4028)	Gram - ve	Food poisoning, affects the intestinal tract.
Staphylococcus aureus (ATCC 25923)	Gram + ve	Chronic osteomyletis, meningitis, endocarditis
Staphylococcus epi (ATCC 12228)	Gram + ve	Chronic osteomyletis

2.5. Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined by using the modified agar-well diffusion method where six isolated compounds concentration ranged from 0.0625 to 1 mg/mL [13]. MIC was determined as the lowest concentration that showed a clear zone of inhibition after incubation for 48 hours at 30 ±1ºC.

2.6. Statistical analysis

The ethanol extract and it fractions were assayed for their antimicrobial activities. Each experiment was run in triplicate. For statistical analysis, the standard errors of the means were calculated and the means with a significant difference (p-value < 0.05) were compared using Duncan multiple range test in SPSS 20.

3. RESULTS AND DISCUSSION

3.1. Isolation of the antimicrobial compounds from *Euphorbia tirucalli* and its structure determination

Stems of *E. tirucalli* (3.5 kg of powder) were extracted with EtOH 96° (2 × 10 L) at 70 °C, followed by *n*-hexane, EtOAc, and *n*-BuOH or H_2O. Well diffusion method was used for the determination of antimicrobial activity of crude extracts and results were presented in Table 2. The zones of inhibition for all crude extracts showed activity within the range of 0-16.33 mm. All crude extracts showed moderate antimicrobial activity against *Escherichia coli*. On the other hand, butanol and hexane crude extract showed similar activity against *Staphylococcus aureus* and *Staphylococcus epi* and did not show any activity against *Salmonella typhimurium*. Among these extracts, the ethyl acetate extract showed the best antibacterial activity against all of bacteria test. Inside, the ethyl acetate extract displayed a moderate antibacterial activity against *E. coli* and *S. typhimurium* and antibacterial activity against *S. aureus* and *S. epi*. Therefore, the ethyl acetate extract was applied to a silica gel CC and eluted with a solvent system of *n*-hexane and ethyl acetate to afford 3 fractions, EA_1, EA_2, and EA_3. Among three fractions, EA_2 showed stronger activities with all of bacteria test. The zones of inhibition showed activity within the range of 14.67-15.33 mm. Therefore, fraction EA_2 was fractionated by CC with the solvent system *n*-hexane and ethyl acetate to afford three fractions $EA_{2.1}$, $EA_{2.2}$ and $EA_{2.3}$. Among three fractions, there are two fractions ($EA_{2.1.1}$ and $EA_{2.1.2}$) did not show antibacterial activity while the fraction $EA_{2.1.3}$ showed antibacterial activity within the range of 15.33-18.00 mm. The fraction $EA_{2.1.3}$ showed the best activity against *S. typhimurium* ATCC 4028. Therefore, fraction $EA_{2.1.3}$ was further applied to a silica gel CC eluted with *n*-hexane, ethyl acetate, ethanol and acid acetic to afford three sub-fractions $EA_{2.1.3.1}$, $EA_{2.1.3.2}$, $EA_{2.1.3.3}$. The sub-fraction $EA_{2.1.3.2}$ showed antibacterial activity (Figure 1) was purified by preparative TLC using chloroform:MeOH:H_2O (4:0.38:0.02) to obtain three compounds 1 (eriodictyol), 2 (quercitrin), and 3 (afzelin).

Table 2. Antimicrobial activity of different polarities stem crude extracts of *Euphorbia tirucalli*

Extracts	Zone of Inhibition (in mm)			
	Escherichia coli	*Salmonella typhimurium*	*Staphylococcus aureus*	*Staphylococcus epi*
EtOH	11.33 ± 1.53^{c}	11.67 ± 0.58^{c}	9.67 ± 1.15^{d}	15.67 ± 1.15^{de}
He	12.33 ± 1.15^{c}	0.00 ± 0.00^{a}	6.00 ± 1.00^{b}	9.33 ± 1.53^{c}
EA	**12.67 ± 1.15^{c}**	**13.33 ± 0.58^{d}**	**13.67 ± 0.58^{e}**	**14.67 ± 1.15^{d}**
Bu	7.67 ± 1.15^{b}	0.00 ± 0.00^{a}	8.33 ± 1.53^{cd}	7.67 ± 1.15^{b}
EA_1	8.67 ± 1.53^{b}	7.67 ± 0.58^{b}	7.00 ± 1.00^{bc}	8.00 ± 1.00^{bc}
EA_2	**14.67 ± 0.58^{d}**	**15.00 ± 1.53^{e}**	**15.33 ± 1.53^{ef}**	**15.33 ± 0.58^{d}**
EA_3	8.33 ± 0.58^{b}	0.00 ± 0.00^{a}	9.00 ± 1.00^{d}	7.33 ± 0.58^{b}
$EA_{2.1}$	**17.00 ± 1.00^{e}**	**17.00 ± 1.00^{f}**	**16.00 ± 0.00^{f}**	**15.67 ± 1.15^{de}**

Extracts	Zone of Inhibition (in mm)			
	Escherichia coli	*Salmonella typhimurium*	*Staphylococcus aureus*	*Staphylococcus epi*
$EA_{2.2}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
$EA_{2.3}$	0.00 ± 0.00a	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
$EA_{2.1.1}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
$EA_{2.1.2}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
$EA_{2.1.3}$	**17.33 ± 1.53^{e}**	**18.00 ± 1.00^{f}**	**15.33 ± 1.53^{ef}**	**17.00 ± 1.00^{ef}**
$EA_{2.1.3.1}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
$EA_{2.1.3.2}$	**21.67 ± 2.08^{f}**	**20.33 ± 1.53^{g}**	**16.33 ± 1.53^{f}**	**17.67 ± 0.58^{f}**
$EA_{2.1.3.3}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Control antibiotic	26.67 ± 1.53^{g}	24.00 ± 2.00^{h}	21.33 ± 2.08^{g}	24.67 ± 1.53^{g}

Values in the same column with different superscript letters were significantly different at p-value < 0.05 (mean ± SD, n = 3).

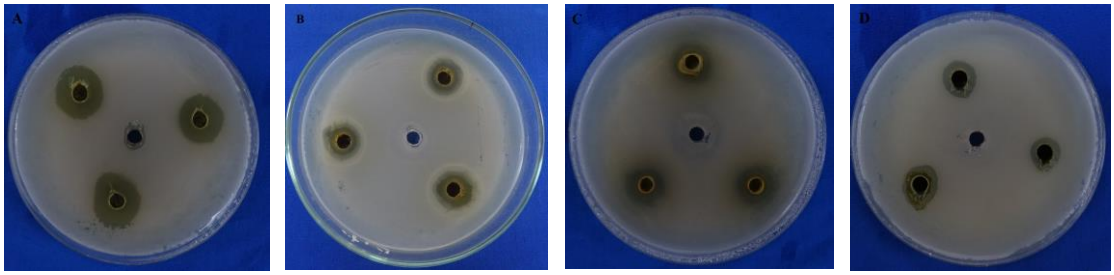

Figure 1. In vitro antibacterial evaluation of sub-fraction $EA_{2.1.3.2}$ through agar-well diffusion method. A: *E. coli*, B: *S. typhimurium*, C: *S. aureus* and D: *S. epi*

Compound 1 was obtained as a white amorphous powder. The ^{1}H NMR data of compound 1 showed the presence of two *meta* coupled aromatic protons at δ_H 5.96 and 5.94 (each 1H, d, J = 2.0 Hz), three aromatic protons of a 1, 2, 4 trisubstitutedbenzene moiety at δ_H 7.03 (1H, d, J = 1.5), 6.87 (1H, dd, J = 8.5, 1.5 Hz), and 6.86 (1H, d, J = 8.5 Hz), one methylene group at δ_H 3.14 (1H, dd, J = 17.0, 12.5, Hz) and 2.72 (1H, dd, J = 17.0, 3.0, Hz), one oxymethine moiety at δ_H 5.40 (1H, dd, J = 12.5, 3.0, Hz), and one chelated hydroxy group at δ_H 12.17 (Table 3). The ^{13}C NMR spectrum in accordance with HSQC spectrum showed fifteen carbons comprising five aromatic methine carbons, one oxymethine at δ_C 80.0, one methylene δ_C 43.6, one carbonyl δ_C 197.3 and seven aromatic quaternary carbons (including five oxygenated ones). These findings led to the identification of the flavanone skeleton of 2. In the A-ring, proton H-6 (δ_H 5.94) and H-8 (δ_H 5.96) showed HMBC correlations to signals at δ_C 167.4 (C-7) and δ_C 103.2 (C-10), to confirm their positions. In the B-ring, protons H-2' (δ_H 7.03), H-5' (δ_H 6.87), and H-6' (δ_H 6.86) showed HMBC cross-peaks to signals at δ_C 146.1 (C-3') and 146.5 (C-4'), to determine the two oxygenated carbons C-3' and C-4' (Fig. 1-1). Moreover, the HMBC showed correlation between proton H-2 and signals at δ_C 197.3 (C-4), 131.6 (C-1'), 114.8 (C-2'), and 119.2 (C-6'), to indicate the connectivity between the B- and C- rings at C-2. Comparison of NMR data of compound 2 and those of eriodictyol showed that they were identical, thus 1 was elucidated as eriodictyol [14]. Eriodictyol was isolated in *E. acanthothamnos* [15] and many plants but this is the first time found in *E. tirucalli*. This compound possesses antiinflamatory effects [16].

Compound 2 was obtained as a light-yellow amorphous powder. Analysis of 1D NMR data indicated that the compound 2 was a flavonoid glycoside with the presence of L-

rhamnopyranosyl moiety, comprising an amomeric proton signal at δ_H 5.19 (1H, d, J = 1.5 Hz, H–1"), four oxygenated proton signals in the ^{1}H zone of 3.1–4.0 ppm, and a characteristic methyl signal at δ_H 0.91 (3H, d, J = 6.0 Hz, H-6"). The NMR data of the aglycone moiety of 3 were similar with those of the compound 2 (Table 3), except for the absence of the methine CH-2 and the methylene CH_2-3 groups in compound 2 and the presence of a new double bond between C-2 and C-3. This finding was confirmed by the HMBC correlation of H-2' and H-6' and C-2 (δ_C 158.4) (Figure 2-3). The rhamnose moiety was linked to the aglycone moiety at its C-3, which was proved by the HMBC correlation of proton signal at δ_H 5.52 (H-1") and carbon C-3 (δc 135.9). All mentioned spectroscopic data were well matched with those of quercitrin reported in literature [17]. Quercitrin has isolated from this species [18].

Table 3. NMR spectral data of compounds 1 - 3

Position	Compound 1			Compound 2			Compound 3		
	δ_C	δ_H, m *J* (Hz)		δ_C	δ_H, m *J* (Hz)		δ_C	δ_H, m *J* (Hz)	
2	80.0	5.40	dd, 12.5, 3.0	158.4			158.4		
3	43.6	3.14 2.72	dd, 17.0, 12.5 dd, 17.0, 12.5	135.9			135.6		
4	197.3			179.4			nd		
5	165.3			163.2			163.2		
6	96.8	5.94	d, 2.0	99.3	6.26	d, 2.0	98.8	6.26	d, 2.0
7	167.4			164.9			164.9		
8	95.9	5.96	d, 2.0	94.7	6.46	d, 2.0	93.5	6.46	d, 2.0
9	164.4			158.0			157.5		
10	103.2			105.7			105.5		
1'	131.6			122.9			121.0		
2'	114.8	7.03	d, 1.5	116.1	7.51	d, 2.0	128.5	7.86	d, 8.5
3'	146.1			149.0			116.1	7.01	d, 8.5
4'	146.5			145.8			161.0		
5'	116.1	6.86	d, 8.5	116.8	6.98	d, 8.0	116.8	7.01	d, 8.5
6'	119.2	6.87	dd, 8.5, 1.5	122.6	7.40	dd, 8.5, 2.0	128.5	7.86	d, 8.5
1"				102.8	5.52	br	102.8	5.54	d, 1.5
2"				71.3	4.22	m	71.2	4.21	dd, 3.0, 1.5
3"				71.5	3.74	dd, 9.0, 3.0	71.4	3.74	dd, 8.5, 3.0
4"				73.0	3.41	m	73.2	3.33	m
5"				72.0	3.33	m	72.2	3.30	m
6"				17.8	0.91	d, 6.0	17.9	0.90	d, 5.5
5-OH	12.17		s	12.72		s	12.71		s

nd: not determined

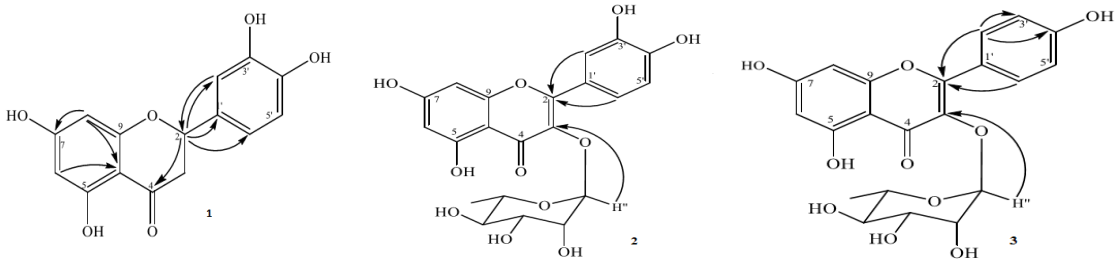

Figure 2. Selected HMBC correlations of 1, 2 and 3

Compound 3 was obtained as a light-yellow amorphous powder. Comparison of 1D NMR data of the compound 1 and 2 (both recorded in acetone-d_6) indicated that compound 3 was also a flavonoid glycoside with the presence of L-rhamnopyranosyl moiety, except for the absence of the hydroxy group in B-ring of the aglycone moiety (Table 2). This finding was confirmed by the presence of the 1,4-disubstituted benzene moiety in the compound 3 instead of the 1,2,4 trisubtitued benzene in B-ring. The HMBC correlations between H-2'/6' and C-2 (δ_C 158.4), C-3' (δ_C 116.1), and C-4' (δ_C 161.0) supported this finding (Figure 1-3). Comparison of NMR data of the compound 3 and those of afzelin showed that they are identical, thus the compound 3 was elucidated as afzelin [19]. Afzelin has been isolated in other *Euphorbia* plants such as *E. hirta* but this is the first time isolated in *E. tirucalli* [20].

3.2. Antibacterial activity of isolated compounds

The antibacterial activity of three pure compounds (eriodictyol, quercetrin and afzelin) was further evaluated by determining the minimum inhibitory concentration (MIC), which is the lowest concentration at which there is no growth. The MIC values of eriodictyol, quercetrin and afzelin were determined using a two-fold serial dilution method. As shown in Table 4, three pure compounds showed against the tested bacteria with the different MIC values from 0.125-0.5 mg/mL, which was significantly lower (*P-value* < 0.05) than the positive control. Afzelin showed the best antibacterial activity against all of bacteria test, followed by quercetrin and eriodictyol. The reason for antibacterial activity of afzelin was attributed to their phenolic structure. The structure of afzelin has the hydroxyl group at C3 of the C-ring of the flavone skeleton and without a rhamnose group. According to Lee *et al*., (2014), the hydroxyl group at C3 of the C-ring of the flavone skeleton negatively affects antibacterial activity, the rhamnose group contributes positively to the antibacterial activity [21]. Our results were similar with a report of Voukeng *et al.*, (2017) that afzelin and quercetrin inhibited the growth of all the test bacteria (*S. aureus* and *E. coli*) with MIC values from 128-64 μg/mL [22].

Table 4. Minimum inhibitory concentrations of isolated pure compounds

Bacterial	MIC (mg/mL)			
	Eriodictyol	Afzelin	Quercetrin	Control Antibiotic
Escherichia coli	0.5 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.125 ± 0.00
Salmonella typhimurium	0.25 ± 0.00	0.125 ± 0.00	0.25 ± 0.00	0.125 ± 0.00
Staphylococcus aureus	0.5 ± 0.00	0.125 ± 0.00	0.125 ± 0.00	0.0625 ± 0.00
Staphylococcus epi	0.5 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.125 ± 0.00

4. CONCLUSION

It is established from this study that most of the isolated compounds from the ethyl acetate extract of *Euphorbia tirucalli* have previously revealed potent antimicrobial activity. It could then

be concluded that the biological and phytochemical characterization of stem of *E. tirucalli* is supportive of the use of this plant for usage in the study area against enterocolitis and conjunctivitis.

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TÓM TẮT

PHÂN LẬP VÀ ĐẶC ĐIỂM CỦA CÁC HỢP CHẤT CÓ HOẠT TÍNH KHÁNG KHUẨN TỪ CÂY GIAO (*Euphorbia tirucalli)*

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Cây thảo dược là nuồn cung cấp các thành phần tự nhiên cho các loại thuốc trên thế giới. Chúng được ứng dụng trong y học dân gian. Trong đó, cây giao (*Euphorbia tirucalli)* thuộc họ Euphorbiaceae, là một loại thảo dược rất phổ biến trong y học cổ truyền. Mặc dù, cây thuốc này đang được sử dụng trong y học cổ truyền ở Việt Nam nhưng không có báo cáo khoa học nào liên quan về thành phần hóa học và hoạt tính sinh học hiện nay. Nghiên cứu này được thực hiện để xác định tính kháng khuẩn của các chiết xuất thô khác nhau cũng như phân lập và xác định thành phần hóa học của hợp chất có hoạt tính kháng khuẩn từ dịch chiết của cây *E.tirucalli*. Các dịch chiết và các hợp chất tinh khiết của *E. tirucalli* đã được thử nghiệm về hoạt tính kháng khuẩn chống lại vi khuẩn *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* và *Staphylococcus epi* bằng phương pháp khuếch tán giếng thạch. Các hợp chất được phân lập từ dịch chiết EtOAC của *E. tirucalli* bằng phương pháp sắc ký cột và cấu trúc của chúng được xác định bằng phương pháp NMR. Kết quả đánh giá kháng khuẩn, dịch chiết EtOAC có hiệu quả kháng khuẩn tốt nhất chống lại tất cả các vi khuẩn được thử nghiệm, dịch chiết EtOAC được dùng để phân tách và tinh sạch hợp chất. Kết quả tinh sạch được ba hợp chất và được xác định là eriodictyol (1), quercitrin (2) và afzelin (3). Trong đó, afzelin cho hoạt tính kháng khuẩn tốt nhất chống lại tất cả các vi khuẩn thử nghiệm với giá trị MIC từ 0,125-0,25 mg/mL. Điều này cho thấy, cây *E. tirucalli* có thể là một nguồn kháng khuẩn tốt và có thể được sử dụng trong nghiên cứu chống lại viêm ruột và viêm kết mạc.

Từ khóa: Euphorbia tirucalli, thành phần hóa học, hoạt tính kháng khuẩn, dịch chiết ethyl acetate.