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Antimicrobial Study of Chloroform Fraction from the Leaves of *Entada spiralis* Ridl.

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ABSTRACT

Introduction: *Entada Spiralis* Ridl., or locally identified as Sintok, contains flavonoid, saponin, tannin, and glycoside, compounds that have antifungal and antibacterial activities. This research aims to identify bioactive compounds and determine the antimicrobial activity from crude and fraction of *E. spiralis* extract.

Methods: The crude extract was prepared by macerating the leaves with chloroform, and then proceeded to fraction it by vacuum liquid chromatography with Dichloromethane (DCM)/Hexane (Hex) (1/9) and Dichloromethane (DCM)/Methanol (MeOH) (9/1) solvent system. Disk Diffusion Test and Microdilution Assay evaluated the extracts' antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans*. The determination of bioactive compounds was done by Thin Layer Chromatography (TLC). Determination of Total Phenolic (TPC) and Flavonoid Content (TFC) were performed by Folin-Ciocalteu and AlCl₃ Colourimetric Assay

Results: The greatest inhibition zone against *C. albicans* was obtained from fraction Chloroform (CHCl₃) extract with an inhibition zone of 10.33 mm. DCM/MeOH (9/1) effectively killed *S. aureus* and *E. coli* with an inhibition zone of 11.67 and 12 mm, respectively. The minimum inhibitory concentration (MIC) of CHCl₃ crude extract were 1.563 mg/mL for both *E. coli* and *S. aureus*, and 0.781 mg/mL for *C. albicans*. The TLC revealed the presence of tannins, saponin, glycosides, phenol, flavonoid, triterpenoid, and aromatic compound in CHCl₃ crude extract. TPC of DCM/MeOH (9/1), CHCl₃, and DCM/Hex (1/9) were 50.56 ± 0.188, 51.913 ± 0.089, 24.16 ± 0.175 mg GAE/g extract.

Conclusion: In conclusion, *E. spiralis* leaves could be a source of active antifungal and antimicrobial agents used for food preservation by using a semipolar solvent for extraction.

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Introduction

Nowadays, researchers pay more attention to natural antimicrobials, such as extracts from plants, to preserve food and modern medicine. This issue is related to the increase of public concern over chemical and synthetic preservatives. About three-quarters of the world population was estimated to depend mainly on plants and plant extracts for their health care because synthetic products could cause postural hypertension, heart failure, and impotence (Zhang et al., 2013). One of the potential plants is *E. spiralis*. Ridley, (synonym, *Entada scheffleri*), locally known as Sintok, Sea Bean, or Beluru, from Leguminosae. Traditionally, it is used for hair treatment and as a cleaner for some skin disease. This plant is a tropical liana or woody climber that could go up to 25 m long (National Park, 2019), and it is commonly found in Australia and Southeast Asia. A previous study showed that *E. spiralis* stem bark contains flavonoid, saponin, tannin, and glycoside (Harun et al., 2014). These compounds have anti-inflammatory, antifungal, anti-yeast, and antibacterial activities (Arabski et al., 2012). Several researchers already found the potential of genus *Entada* as an antimicrobial agent. For example, *Entada rheedii* ethanol extract was reported to display moderate inhibitory against *S. aureus* and *C. albicans* (Ram et al., 2004). On the same species, the extract of methanol and fraction of *Entada spiralis* stem bark showed antifungal activity against *T. mentagrophytes*, *M. gypseum*, *T. mentagrophytes*, *S. aureus*, and *S. epidermis* (Harun et al., 2014). Meanwhile, the chloroform extract of *Entada spiralis* leaves showed flavonoid, phenol (Mohammad, 2017). According to the previous result related to the potential agent of *E. spiralis* leaves as an antimicrobial agent, we aimed to enlarge the potential of the crude chloroform extract of *E. spiralis* leaves as food preservation against *S. aureus*, *E. coli*, and *C. albicans*.

Methodology

Maceration

E. spiralis leaves were obtained from Tasik Chini, Pahang, with voucher specimen no. KMS-5228. The 900 grams of dried leaves were later milled into a fine powder and macerated with 4 L of chloroform for 48 h. Furthermore, the extracts were then filtered using a Whatman No. 1's filter paper. Chloroform was completely evaporated using a rotary evaporator at 40 °C. Once thoroughly dried, the extract was placed in a 5 L Erlenmeyer and stored in a fridge at a temperature of -4 °C before further analysis.

Phytochemical Screening Test – Thin Layer Chromatography (TLC)

TLC silica gel plate 60 F254 (Merck, Germany) was sized into 5 x 3 cm. The 5 µL of the extract was spotted in the middle bottom of the TLC plate. The DCM/Hex (9/1) and DCM/MeOH (9/1) solvent system solutions were used as mobile phases that were already tested from the preliminary research. The developing stage was conducted in a covered TLC developing chamber. The solvent was evaporated in a fume hood at 29 °C. Some colouring agents and wavelength, such as Vanillin, Dragendroff's, FeCl₃, UV 365 nm, UV 254 nm, iodine pearl, and concentrated H₂SO₄ was given onto the different developed plates to determine several bioactive compounds such as saponin, terpenoid, alkaloid, flavonoid, phenol, tannin, aromatic compound, and conjugated compound by R_f values calculations.

Fractionation of Extract by Vacuum Liquid Chromatography (VLC)

In the present study, a modified method from Mohammad (2017) was performed. It conducted a dilution of 10 grams of crude extract into 25 mL of chloroform solvent. The activated silica gel 60 PF254 (Merck, Germany) through a 24 h heating at 80 °C was dipped into the extract solution. Sample and silica gel were kept agitated in a Hotplate Stirrer (Lab Tech, Korea) at 70 °C until was mixed gently. The silica gel was loaded into the VLC column until the solvent front reached 5 cm of the column height. Hexane was utilized to rinse the column by pressed the layer with Aspirator A-1000S (EYELA, Japan). The silica gel was stabilized by setting it down overnight in the VLC column. Ten (10) grams of the crude extract was submitted into VLC, eluted using a gradient system of DCM/Hex (9/1) and DCM/MeOH (9/1) as much as 1 L for each. The solvent system's use depended on the result of R_f value in preliminary research by using TLC. Furthermore, every 200 mL of the fraction, or based on the UV lamp band result, was separated respectively in Erlenmeyer flask. The fractions were tested with the TLC profile to identify the specific compounds. The extract for each solvent system was combined and evaporated in the rotary evaporator IKA HB10 Basic (Buchi, Switzerland) to dryness at 40 °C.

Disk Diffusion Agar, Kirby-Bauer Method

The antimicrobial activity was conducted for crude (CHCl₃) and fractional (DCM/Hex (9/1), and DCM/MeOH (9/1)) extracts using a modified method from Harun et al. (2014) was performed in the present study. A 1600 mg/mL stock solution was formulated by dissolving 1.6 g of sample in 1 ml of the specific solvent system depend on the polarity of the fractions. The serial two-fold dilution was done to make the several concentrations ranged from 12.5 mg/mL - 1600 mg/mL. The 20 ml/µL of

each concentration was dipped onto paper discs (Whatman AA disc, 6 mm, United States of America) by using a micro-pipette. The discs were evaporated at the laminar airflow cabinet (Erla CMP Series, Malaysia) until the solvent was evaporated completely. All discs were stored at -5 °C in Chiller LC-213LD (Law Chain, Taiwan) for further analysis.

The positive control of antifungal, gram-negative bacteria and gram-positive respectively employed 100 µL Nystatin (Oxoid, United Kingdom), 10 µL ampicillin (Oxoid, United Kingdom), and 10 µL Gentamicin (Oxoid, United Kingdom). The Chloroform, DCM/Hex (9/1), and DCM/MeOH (9/1) (Merck, Germany) was used as a negative control. The pure culture of *E. coli*, *S. aureus*, and the fungal strain *C. albicans* were collected from the Basic Medicine Science of International Islamic University of Malaysia. Bacterial and fungi were incubated in 24 h at 37 °C in Mueller Hilton-Agar (Oxoid, United Kingdom) and Saboraud Dextrose Agar (Becton, United States of America). The OD of bacteria was 0.1 at 600 nm UV-Vis spectrophotometer (Secomam, United States of America). Thus, the standard inoculation of bacteria was standardized at 1.5×10^6 cell/mL and was swabbed on an agar plate using a sterile cotton bud. The sterile paper discs were aseptically transferred onto the inoculated agar plates' surfaces and were submitted to 24 h of incubation at 37 °C. The effect of the antimicrobial agent was indicated through a clear zone. The determination of this assay was conducted in triplicate.

Minimum Inhibitory Concentration (MIC), by Broth Microdilution Assay

The present study modified a method by Harun *et al.* (2014) and Mohamad (2012) by diluting the microbial stock into the Mueller Hilton-Broth (Oxoid, United Kingdom) to an absorbance of 0.11 at 600 nm for bacteria and Saboraud Dextrose Broth (Becton, United States of America) to the absorbance of 0.6 at 450 nm for fungi. The 1 mL inoculum was added to 100 mL of sterile broth and diluted until a reached number of OD 105 cells/mL for fungi and 1.5×10^6 4cells/mL for bacteria. The first row of the 96 well microtiter plate (Trueline, United States of America) was filled with 180 µL of 1/100 diluted microbial solution and 100 µL for the rest. Therefore, the 20 µL of two-fold serial dilution 50 mg/mL - 0.39 mg/mL concentration extract was diluted with DMSO. The microplates were incubated at 37 °C for 24 h. The 20 uL 0.5% (w/v) of MTT (3-(4,5-dimethylthiazol-2-yl) - 2,5 diphenyl tetrazolium bromide (Life Technologies, United States of America) and Phosphate Buffered Saline solution (Base, Singapore) was put to each well and re-incubation for 2 h for fungi and bacteria. The yellow color indicated the inhibition of microbial growth; meanwhile, the dark blue indicates microorganisms' presence.

Minimum Bactericidal Concentrations (MBC) and Fungicidal Concentrations (MFC)

The MBC and MFC were used for each concentration on the solvent system of all microorganisms tested. The MBC and MFC pursued the lowest concentration by calculating fewer than three colonies to achieve approximately 99% inhibition growth. 100 µL of the mixture from MIC that showed the positive result was incubated on SDA (for fungi) and MHA (for bacteria) at 37 °C for 24 h.

Total Phenolic Content (TPC)

The Folin-Ciocalteu assay was performed to identify the amount of TPC in *E. spiralis* leaves based on Mohamad's method (2012). 50 µL of the extract was formulated from 2 mg/mL with the original solvent. The standard of gallic acid solution (7.8 -1000 µL/mL) was diluted into 100 µL of methanol, where the blank was using methanol. The sample was diluted with a ratio of 4:1 (water: Folin- Ciocalteu phenol reagent) and 50 µL of 1 M Sodium carbonate (Na_2CO_3) solution in water. 25 µL sample or standard combined with 100 µL Folin Ciocalteu phenol reagent were filled into a 96-well plate and incubate for 5 min at 37 °C. 75 µL Na_2CO_3 was added and was kept from light for 1 h. The result's absorbance was analyzed at 765 nm against blank by a multi-detection microplate reader (Infinite M200 Nanoquant, Switzerland).

Results

Phytochemical Screening of Crude Extract

The phytochemical screening results of *E. spiralis* crude extract by TLC can be seen in **Table 1**. Crude extract showed several bioactive compounds such as; terpene, steroid, terpenoid, phenol, tannin, saponin, flavonoid, and aromatic compound, as shown in **Figure 1**.

Antimicrobial Activity Assay by Kirby-Bauer Test

The chloroform crude extract strongly inhibited the *E. coli* with an inhibition zone of 10.67 mm in **Table 2**, followed by *C. albican* and *S. aureus* with an inhibition zone of 10.33 and 9.67 mm, respectively. Moreover, the fraction DCM/MeOH (9/1) extract was strongly inhibited the *E. coli* with a 12.00 mm inhibition zone in **Table 3**, followed by *C. albicans* and *S. aureus* with an inhibition zone of 9.00 and 11.67 mm, respectively. Among all microorganisms that were used, *E. coli* was found to be the most susceptible, whereas *S. aureus* and *C. albicans* were less susceptible to all extracts.

Table 1: TLC Analysis of *Entada spiralis* Crude Extract

Reagent Sprayer	Spot on TLC plate			
	Total spot	Color	Rf	Compound
Nature	7	Yellow	0.1125	Neoxanthin
		Yellow	0.1625	Violaxanthin
		Dark Yellow	0.2	Lutein
		Blue Green	0.275	Chlorophyll b
		Grey	0.6875	Anthocyanin
		Dark Green	0.75	Pheophytin a
		Yellow	0.9375	Carotene
UV ₂₅₄ Light	7	Grey	0.1125	Aromatic compound
		Grey	0.1625	
		Dark Grey	0.2	
		Dark Grey	0.275	
		Dark Grey	0.6875	
		Dark Grey	0.75	
Sulphuric Acid	3	Green	0.25	Tertepenes
		Yellow	0.5	Steroid
		Pink	0.6875	Steroid
Iodine	3	Brown	0.25	Aromatic and organic compound
		Brown	0.5	
		Dark Brown	0.6875	
UV ₃₆₅ Light	1	Fluorescence	0.6875	Terpenoid
FeCl ₃	2	Green	0.1125	Phenol
		Green	0.357	Tannin
Vanillin	3	Dark Purple	0.175	Saponin
		Green	0.2625	Furastanol
		Violet	0.5375	Spirostanol
AlCl ₃	1	Orange	0.6155	Flavonoid

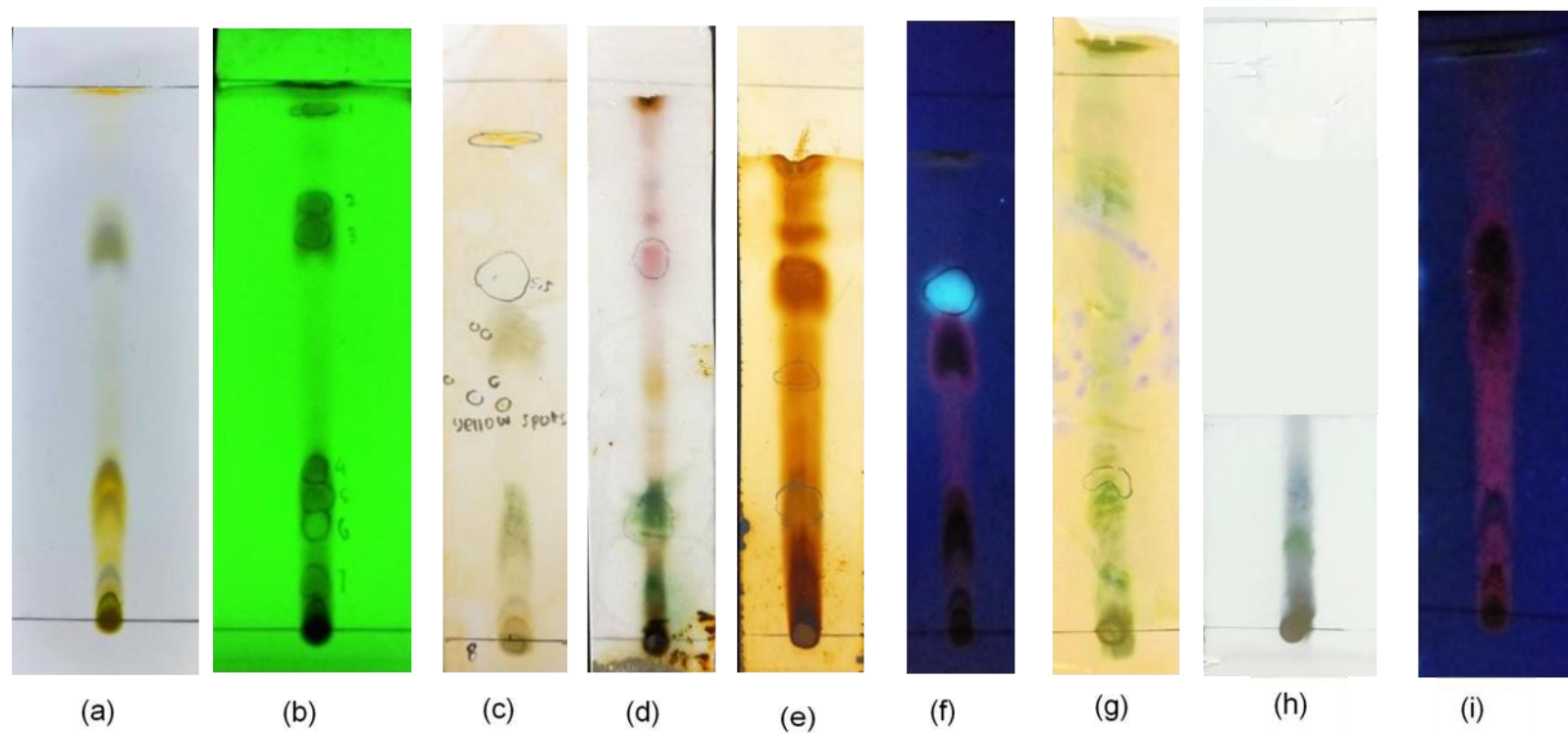


Figure 1: TLC result of *E. spiralis* Leaves Chloroform Extract with Several Conditions; (a) Nature, (b) UV₂₅₄ Light, (c) sprayed by Dragendorff reagent, (d) H₂SO₄, (e) sprayed by Iodine, (F) UV₃₆₅ Light, (g) sprayed by FeCl₃, (h) sprayed by vanillin reagent, (I) sprayed by AlCl₃

Table 2: *In vitro* Antifungal and Antimicrobial Activity of Fraction and Crude Extract of *E. spiralis* Leaves

Extract	Concentration (mg/mL)	Inhibition zone (mm) ^a		
		EC	SA	CA
DCM/Hex	12.5	-	-	-
	25	-	-	-
	50	-	-	-
	100	-	-	-
	200	6.67 ± 0.577 ^c	-	-
Negative control DCM/Hex		-	-	-
Chloroform	12.5	7.33 ± 1.155 ^{bc}	7.17 ± 0.288 ^b	-
	25	7.67 ± 0.577 ^{bc}	7.33 ± 0.577 ^b	-
	50	7.67 ± 0.577 ^{bc}	7.67 ± 1,443 ^{ab}	-
	100	8.33 ± 0.577 ^{abc}	7.77 ± 1,328 ^{ab}	-
	200	9.00 ± 1 ^{ab}	7.67 ± 0.577 ^{ab}	-
Negative control Chloroform		-	-	-
DCM/MeOH	12.5	8.33 ± 0.577 ^{abc}	7.33 ± 1,527 ^b	6.33 ± 0.577 ^{bc}
	25	8.67 ± 0.577 ^{ab}	7.67 ± 0,527 ^{ab}	6.67 ± 0.577 ^{bc}
	50	9.00 ± 1 ^{ab}	8.33 ± 0,577 ^{ab}	7.0 ± 0 ^b
	100	9.00 ± 0 ^{ab}	9.167 ± 0,763 ^a	7.0 ± 0 ^b
	200	9.67 ± 0.577 ^a	9.00 ± 1 ^a	7.0 ± 0 ^b
Negative control DCM/MeOH		-	-	-
Nystatin	100			24.0 ± 1
Ampicillin	10		24.33 ± 1.527	
Gentamicin	10	32.67 ± 0.577		

- No activity; EC; *E. coli*, CA; *C. albicans*, SA; *S. aureus*; ±, Standard Deviation (SD); ^aMean of triplicates. Statistical significance was determined using ANOVA. Differences were analyzed significant (P<0.05) on the same microbes. The different notation means significantly different values.

Table 3: *In vitro* Antimicrobial and Antifungal Activity of DCM:MeOH Fraction and Crude Extract of *E. spiralis* Leaves

Extract	Concentration (mg/mL)	Inhibition zone (mm) ^a		
		EC	SA	CA
Chloroform	400	9.33 ± 0.577 ^b	7.67 ± 0.577 ^b	8.67 ± 0,577 ^{ab}
	800	10.33 ± 1.154 ^{ab}	8.833 ± 1,607 ^{ab}	8.67 ± 1,155 ^{ab}
	1600	10.67 ± 1.154 ^{ab}	9.667 ± 2,081 ^{ab}	10.33 ± 2,309 ^a
Negative control Chloroform		-	-	-
DCM / MeOH	400	9.00 ± 1 ^b	8.00 ± 0 ^b	7.33 ± 0,577 ^b
	800	11.33 ± 0.577 ^a	9.67 ± 0,577 ^{ab}	8.33 ± 1,155 ^{ab}
	1600	12.00 ± 1 ^a	11.67 ± 2,887 ^a	9.00 ± 0 ^{ab}
Negative control DCM/MeOH		-	-	-
Nystatin	100			24.0 ± 1
Ampicillin	10		24.33 ± 1.527	
Gentamicin	10	32.67 ± 0.577		

- No activity; EC; *E. coli*, CA; *C. albicans*, SA; *S. aureus*; ±, Standard Deviation (SD); ^aMean of three replicates Statistical significance was determined using ANNOVA. Differences were analyzed significant (P<0.05) on the same microbes. The different notation means significantly different values.

Determination of MIC, MFC and MBC

The MIC results of the positive result fractions against bacteria and fungi are presented in **Table 4**. The Chloroform crude extract showed the MIC value of 1.563 mg/mL against both *E. coli* and *S. aureus*, and 0.781 mg/mL against *C. albicans*. Meanwhile, fraction DCM/MeOH (9/1) extract showed the MIC value of 1.563 mg/mL against *E. coli* and 3.125 mg/mL against both *S. aureus* and *C. albicans*.

According to the result of Minimum Bactericidal Concentration (MBC), the chloroform extract had the lowest MBC result against *E. coli*, *S. aureus*, with 0.39 and 0.781 mg/mL compared to fraction DCM/MeOH (9/1) with MBC results of 0.781 and 1.563 mg/mL and positive control 0.39 mg/mL for Gentamicin ($\mu\text{g/mL}$) and 0.39 mg/mL for Ampicillin ($\mu\text{g/mL}$). On the other hand, chloroform extract also gave lower MFC result (0.781 mg/mL) compared to DCM/MeOH (9/1) with an MFC result of 1.563 mg/mL, but still fell behind the positive control Nystatin ($\mu\text{g/mL}$) with an MFC result of 0.39 mg/mL. In conclusion, the chloroform extract has a promising role as an antimicrobial agent against significant microorganisms, especially bacteria.

Table 4: Minimum Inhibitory Concentration, Minimum Fungicidal (MFC) and Bactericidal Concentration (mg/mL) of *E. spiralis* Crude Extract

Extract	MBC		MFC		MIC	
	EC	SA	CA	EC	SA	CA
Chloroform crude extract	0.390	0.781	0.781	1.563	1.563	0.781
Fraction (DCM/MeOH)	0.781	1.563	1.563	0.781	3.125	3.125
Gentamicin ($\mu\text{g/mL}$)	0.390	-	-	0.390	-	-
Nystatin ($\mu\text{g/mL}$)	-	-	0.390	-	-	0.390
Ampicillin ($\mu\text{g/mL}$)	-	0.390	-	-	0.390	-

Total Phenolic Content

Total phenolic content of *E. spiralis* leaves extracted by chloroform, fraction DCM/MeOH (9/1), and fraction DCM/Hex (1/9) were 50.56 ± 0.089 , 51.913 ± 0.188 , 24.16 ± 0.175 the concentration of phenolic was in terms of equivalent (mg GAE/g of the dry weight of extract), separately.

Discussion

In the Kirby-Bauer method, the restraint zone's size expressed the compound's competences wherein the highest the zone, the more powerful the compound. Higher active compounds from the extracts could cause the higher inhibition zones recognized at a higher concentration of all extracts. Fractionation in several plant extracts brought about improved movement, yet others brought about the loss of the action. For instance, in an extract of fraction DCM/Hex (1/9), the inhibition was only detected at a concentration of 200 mg/mL against *E. coli* with zone inhibition of 7. Since the CHCl_3 (100%) and DCM/MeOH (9/1) extract showed promising anti-dermatophytes and antibacterial activity, thus it was chosen to undergo fractionation and was further assayed to investigate the effectiveness of the fractions, except DCM/Hex (1/9). The greatest inhibition zone against *C. albicans* was obtained from fraction Chloroform (CHCl_3) extract with an inhibition zone of 10.33 mm. DCM/MeOH (9/1) effectively killed *S. aureus* and *E. coli* with an inhibition zone of 11.67 and 12 mm, respectively. Those results were determined by the polarity of the solvent's polarity that may be affected by the bioactive compound after the fractionation process and the chemical nature of its bioactive constituents.

The antifungal and antibacterial potency of several plants was related to secondary metabolites established in some fractionations of the unrefined concentrate, permits the appropriation of bio-active compounds into solvents as per their polarity. The current investigation's perception showed that the antifungal and antibacterial activity of the fraction DCM/MeOH (9/1) and CHCl_3 (100%) extract of *E. spiralis* leaves maybe showed that the active compound was either modestly nonpolar or polar. This may fill into the natural dissolvable that can be utilized in removing dynamic elements of *E. spiralis* leaves. The phytochemical compound can be isolated. For example, the related movement against contagious and antibacterial action may be associated with the nearness of phytochemicals, such as alkaloid, flavonoid, saponin, sterol, and tannins in the individual portion that previously controlled by TLC profile in past study.

The inhibitions were supported by microdilution assay in which the chloroform crude extract gave the highest MIC value of 1.563 mg/mL against *E. coli*, 1.563 mg/mL against *S. aureus*, 0.781 mg/mL against *C. albicans*. As mentioned before, the polarity of the fraction will affect the compound that, in turn, will affect the efficiency of antimicrobial activity. For an extract of fraction, DCM/Hex (1/9) has nonpolar properties. The nonpolar solution contains only a few bioactive compounds such as; flavonoid, sterol, phenol, alkaloid (Widyawati, 2014), terpenoid (Liu et al., 2011), and diterpenoid (Hidayat, 2014) that dissolved by hexane solvent. Meanwhile, in

semipolar - polar extract of fraction DCM/MeOH (9/1) has more bioactive compounds such as sterol, phenol, flavonoid, and alkaloid (Widyawati, 2014). In Semipolar - polar extract of DCM/MeOH (9/1) may also have polar properties such as alkaloid, phenolic, saponin, carotenoid, tannin, amino acid, and glycoside (Elfirta, 2018).

Terpenoids, tannins, phenolics, and flavonoids are secondary metabolites compounds that naturally exist in several types of plants. The secondary metabolites realized with pharmacological significance are commonly known as bioactive compounds (Swabha, 2018). These bioactive compounds will increase lymphocyte activity, such as macrophage cells, especially compounds such as phenolics and its derivatives. Phenolics play several important functions in plants as an immunomodulator and antioxidant. They give opposition against microbes and predators, other than that phenolics protect cellular membranes and tissues from lipid peroxidation, and fix DNA by electron transfer responses (Shankar et al., 2007).

Flavonoids, particularly catechins, have been known for their antimicrobial activity in Gram-positive and Gram-negative microorganisms (Tsuchiya 2015). Catechins will have collaborate with the cell layer of microbes by authoritative to the lipid bilayer and by inactivating or hindering the union of intracellular and extracellular compounds (Reygaert, 2014). Tannins also fill as guards against microorganisms. Their antimicrobial activity method might be identified with their capacity to idle microbial adhesin, cell envelope transport protein, catalyst several enzymes, that properly known as astringency (Bobbarala, 2012). They are fit for restricting metal particles related to the development of microscopic organisms. In this manner, by consolidating with Ca²⁺ particles engaged with Gram-negative bacteria's structure, dense tannins influence the bacterial wall cell, disrupting the retention of minor components fundamental for bacterial development (Joseph, 2016).

Conclusion

The CHCl₃(100%) extract of *E. spiralis* uncovered several bioactive compounds such as tannins, saponin, glycosides, phenol, flavonoid, triterpenoid, and aromatic compound through Thin Layer Chromatography (TLC). The highest zone inhibition zone for *E. coli* was obtained from DCM/MeOH (9/1) extract with a diameter of 12.00 ± 1 mm. The highest inhibition zone for *S. aureus* was obtained from DCM/MeOH (9/1) extract with a diameter of 11.67 ± 2.887 mm. The greatest inhibition zone for *C. albicans* was obtained from CHCl₃(100%) extract with a diameter of 10.33 ± 2.309 mm. The inhibitions were supported by microdilution assay in which the chloroform crude extract gave the highest MIC value of 1.563 mg/mL against *E. coli*, 1.563 mg/mL against *S. aureus*, 0.781 mg/mL against *C. albicans*. This study suggests that the leaf extract of *Entada spiralis* has great potential to become

a natural preservative for foods, replacing chemical preservatives. A future study is needed to distinguish the isolated specific bioactive compounds that can be done by utilizing an LC-MS or GC-MS examination.

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Conflict of Interest

Authors have no conflicts of interest with this publication.

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