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A Comparative Study on Antioxidation and Antibacterial Activities Triphala Herb Extracts from Chae Son, Lampang, Thailand

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Authors' contributions

This work was carried out in collaboration among all authors. Author WP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BW, AC and CU managed the analyses of anti-bacterial and antioxidant. Authors PU, PS and BW managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: Triphala, a herbal medicine which is a product from Chae Son, Lampang, Thailand**,** is an unequal-proportional mixture of fruits of three herbs, *Phyllanthus emblica*, *Terminalia chebula*, and *Terminalia bellirica* (2:1:1).
Materials and Methods: The contemporary study concentrated on extracts detection and comparative analysis between various annual year (A.D.2017/2018) activities in hexane, ethyl acetate, and methanolic extracts of Triphala. The determination of antioxidant was using by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). The broth dilution and agar well diffusion assay were determined antibacterial.
Results: Results exposed the valuable biological activity extracts from ethyl acetate and methanol solvents of *T. chebula* and *T. bellirica*, which strength is accountable for biological activities.

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Extracts displayed acceptable radical-scavenging activity analogous with ascorbic acid. The methanol extract of *T. bellirica* (0.0013 mg/mL), ethyl acetate extract of *P. emblica* (0.0009 mg/mL), in A.D. 2017 and the ethyl acetate extract of *P. emblica* (0.0090 mg/mL), the methanol extract of Triphala (0.0176 mg/mL) in 2018, were exhibited higher antioxidant activity. Also, extracts indicated hopeful antibacterial potential against tested strain comparable to chloramphenicol.

Conclusion: The results in A.D. 2017 showed that inhibition zone of *P. emblica* in ethyl acetate extract; *S. aureas* (8.67 mm), *E. aerogenes* (8.67 mm), *E. coli* (EPEC) (7.67 mm), *P. mirabilis* (7.73 mm) , *S. typhimurium* (8.33 mm), *S. flexneri* (8.33 mm) and *V. cholera* (7.33 mm) together with *P. emblica* methanol extract; *S. aureas* (6.67 mm), *E. aerogenes* (7.76 mm.) and *V. cholera* (4.0 mm). Therefore, it could be concluded that Triphala may be a talented candidate in pharmaceutical and future medicine.

Keywords: Triphala; Phyllanthus emblica; Terminalia chebula; Terminalia bellirica.

1. INTRODUCTION

Triphala is an ayurvedic herbal and Thai traditional medicine. The word Triphala has named by its formulation of three fruits, namely, *Phyllanthus emblica* Linn., *Terminalia chebula* Retz., and *Terminalia bellirica* (Gaertn.) Roxb., in a ratio of 1:1:1 or 2:1:1[1] ,each of which contains various structurally diverse chemicals with therapeutic potentials. Triphala contains major four phenolics chemical constituents such as gallic acid, tannic acid, syringic acid, chebulinic acid, and epicatechin along with ascorbic acid [2,3]. Fruits of *T. chebula*, that harvested in the spring are a rich source of tannins (30–40%), e.g. chebulic acid, neochebulinic acid, chebulinic acid, corilagin, chebulagic acid, gallic acid, punicalagin, ellagic acid, terchebin, and terflavin A [4]. *T. bellelica* fruits comprise mainly oils (35%) and proteins (40%), as well as omega-3 and -6 fatty acids (e.g. linoleic acid) [5]. Although the specific fillings of *P. emblica* are undecided, the fruits are high in vitamin C (ascorbic acid), triumph to 445 mg per 100 g [6,7]. Triphala investigation has establish the formula to be potentially current for several clinical uses such as reduction of hyperacidity, appetite stimulation, antioxidant, antifungal, antimalarial, antiviral activities, antiallergic [8], anticarcinogenic, antipyretic, analgesic, wound-healing, antistress, adaptogenic, anticancer [9,10], immunomodulating, anti-inflammatory, antibacterial, antimutagenic, adaptogenic, hypoglycemic, chemoprotective, antineoplastic, radioprotective effects together with prevention of dental caries [11]. Tarasiuk et al. also originate beneficial compounds in Triphala, such as vitamin C, polyphenols, and flavonoids. These compounds were afforded antioxidant and anti-inflammatory effects [4]. So, for quality assurance of herbal products, appropriate of formulation is required. In this study, information on compounds and extracts in herbal medicines of Triphala formula was acquired coming from Chae Son National Park, Lampang, a Province of Thailand, in January 2017 and 2018, were examined. With these multiple medicinal properties, especially antioxidation and antibacterial, this has been investigated to compare those stability properties of two years for the quality of Triphala Herbal Drink products.

2. MATERIALS AND METHODS

2.1 Plant Material

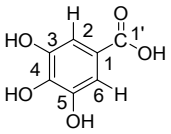
Triphala is an ayurvedic herbal formulation of dehydrated fruits from three herbal plants in 2:1:1 proportion: *Phyllanthus emblica* (BKF 113645), *Terminalia chebula* (BKF 170476) and *Terminalia bellirica* (BKF 188656).These fruits were collected from Chae Son National Park, Lampang, a Province of Thailand, in January 2017 and 2018 by Mr. Narong Nuntasaen. The plants were recognized and have been dropped in the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of National Resources and Environment, Bangkok, Thailand.

2.2 Extraction and Isolation

Dried fruits of *P. emblica* (2 kg), *T. chebula* (2 kg) and *T. bellirica* (2 kg) and Triphala herbal formula in 2:1:1 proportions: *P. emblica* (2.5 kg), *T. chebula* (1 kg) and *T. bellirica* (1 kg) were crushed into powder and formerly extracted successively with hexane, ethyl acetate, and methanol for 5 times each (5×7 L). Elimination of solvents from each extract under reduced pressure gave *P. emblica* (crude hexane ,33.55 g, crude ethyl acetate, 72.04 g) and crude methanol, 622.02 g) extracts; *T. chebula*(crude hexane, 24.0 g, crude ethyl acetate, 61.0g) and

crude methanol, 1083 g) extracts; *T. bellirica* (crude hexane, 63.83 g, crude ethyl acetate, 35.42 g) and crude methanol, 571.43 g) and Triphala herbal crude methanol, 1200 g), respectively. The crude ethyl acetate extract of three fruits and Triphala extract were nominated and parted by flash Column Chromatography (CC) over silica gel eluted with gradient systems of ethyl acetate:hexane and methanol:ethyl acetate to provide the brown precipitate which was filtered out and then recrystallized from ethanol to yield gallic acid.

2.3 Physical Properties of Gallic Acid



Pale brown needles, m.p. 259.2 – 260.52°C; IR (KBr) cm^{-1}: 3300 (OH), 1701 (C=O), 1623, 1541, 1452 (C=C), 1151(C-O-C); EIMS *m/z*: 170.12 ($[M]^+$, $C_7H_6O_5$), 153(100), 79(10)^{1}H NMR (400 MHz, CD_3OD): δ7.08 (1H, d, *J* = 2 Hz, H-2), 7.08 (1H, d, *J* = 2 Hz), 9.12 (1H, s, COO<u>H</u>).^{13}C NMR (100 MHz, CD_3OD): δ138.18 (C-1), 109.0 (C-2), 144.98 (C-3-OH), 120.62(C-4-OH), 144.98(C-5-OH), 109.0 (C-6), 168.98 (C-1′). HMBC correlations, H/C: 2/4, 3, 2, 1, 1′; 6/4, 5, 6, 1, 1′. COSY correlations, H/H: 2/6.

2.4 Antioxidant Activity

2.4.1 Ferric reducing antioxidant power assay (FRAP)

FRAP assay was done as described previously [12]. Firstly, 1 mL of crude extract (100 mg/mL) added to 1 mL of FRAP reagent, which confined sodium acetate buffer (300 mM, pH 3.6), ferric chloride in water (20 mM) and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ, 10 mM) at a 10:1:1 ratio and then incubated in a water bath for 30 minutes at 37°C. The absorbance was measured with a spectrophotometer at 595 nm. The results revealed the concentration of extract equivalents to 0.005 mg/mL ascorbic acid. All measurements were performed in triplicate.

2.4.2 DPPH radical scavenging capacity

DPPH radical scavenging activity of the crude extract was premeditated. Briefly, 1 mL of crude extract (100 mg/mL) was mixed with 1.0 mL of DPPH ethanolic solution (0.1 mM). After that, the mixture was shaken vigorously and incubated in the darkness at room temperature for 60 minutes. The absorbance was measured with a spectrophotometer at 520 nm. The DPPH radical scavenging activity was expressed as the concentration of extract equivalents to 0.005 mg/mL ascorbic acid.

2.4.3 Total phenolic contents

Total phenolic insides in the extracts were strong-minded by the modified Folin-Ciocalteu method [13]. The 0.1 mL of the extracts was assorted with 1 ml of Folin-Ciocalteu's reagent into 20 ml calibrated flask. After 1 minute, added 4 ml of sodium carbonate and volume made to 20 ml with distilled water. The flask was mixed well and allowed to stand for 30 min at room temperature in the dark to develop colour. Absorbance values of the sample were measured at 750 nm with UV-VIS spectrophotometer (Shimadzu 1601). Total phenolic content was stated as mg gallic acid equivalent (GA)/g, gained from a calibration curve of gallic acid standard solutions. All measurements were done in triplicate.

2.4.4 The total flavonoid contents

The total flavonoid contents of crude extract were determined by the aluminium chloride colourimetric method.[13] In brief, 0.5 mL of crude extracts (100 mg/mL) were made up to 1 mL with $MeOH/H_2O/CH_3COOH$ (14:5:1) and then mixed with 4 mL of $AlCl_3$ reagent solution (4 ml, 133 mg of $AlCl_3$ and 400 mg of CH_3COONa dissolved in 100 ml H_2O). After that, the mixture incubated for 5 min, and the absorbance was measured at 430 nm with UV-VIS spectrophotometer (Shimadzu 1601). The total flavonoid content was conveyed as mg quercetin equivalent (QE)/g, found from a calibration curve of quercetin standard solutions. All measurements were performed in triplicate.

2.5 Antimicrobial Susceptibility Test

In vitro antimicrobial studies were effective against 9 bacterial strains : *Staphylococcus aureas* ATCC 2593 DMST 8840 , *Enterobacter aerogenes* ATCC13048 DMST 8841, *Escherichia coli* 0157 : H7 DMST 12743, *Escherichia coli* (Enterotoxigenic ETEC) DMST 30543, *Escherichia coli* (Enteropathogenic EPEC) DMST 30546, *Proteus mirabilis* DMST 8212, *Salmonella typhimuriam* ATCC 13311 DMST 562, *Shigella flexneri* DMST 30543 and *Vibrio cholera* DMST

2873. Strains obtained from the Department of Medical Science, Bangkok, Thailand.

2.5.1 Preparation of bacterial strains suspension

Cell suspensions of 9 bacterial strains were prepared from stock culture, those cultured in trypticase soy broth (TSB) and incubated at 37°C for 15 hr. After that, subculture in the bacteriological Mueller-Hinton Broth (MHB) at 37°C for 4 hr for inhibition of microbial growth. Cell suspension turbidity was determined by using a spectrophotometer at OD 600 nm, which were diluted in sterile phosphate-buffered saline and then adjusted to a 0.5 McFarland standard at 600 nm (OD_{600}) of 0.132 was considered equivalent to a 0.5 McFarland standard. After that, the serial dilution of cell suspension in TSB was adjusted to approximately $1.5x10^8$ CFU/ml.

2.5.2 Effect of plant crude extract on *in vitro* inhibition of bacterial strains by Agar-well diffusion assay

The agar well diffusion method was screened antimicrobial activity of the 3 plant extracts [14]. An inoculum suspension, 0.1 ml of cell suspension from each strain, were evaluated by spread plate technique on solidified 25 mL Nutrient Agar (NA), and the inoculum was permitted to dry for 5 min. Holes of diameter were made in the agar using sterile cork borer (5 mm). Compounds of plant crude extracts were dissolved in 10% DMSO at the concentration of 100 mg/mL, and then 50 *µ*L from each plant crude extract was added into each well on the medium and allowed to stand on the bench for 1 h for proper diffusion then incubated at 37°C for 12 hr. Negative controls using 50 *µ*L 10% DMSO, Hexane and/or Ethanol:Dichloromethane was also run in the same manner and parallel to the treatments. Positive controls using 50 *µ*L of chloramphenicol (1 mg/ml) was also run in the same method and similar to the treatments. The clear zones were measured in millimeters (mm). These studies were planned as a Completely Randomized Design (CRD), and each treatment was done in triplicate.

2.5.3 Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined for the active plant extracts that displayed the widest spectrum of antimicrobial activity against test microorganisms. The methods were defined [14]. A culture of tested strains was used for the preparation of suspension (0.5 McFarland standard turbidity). Compounds of plant crude extracts were dissolved in 10% DMSO at the concentration of 50 mg/mL, and then 100 *µ*L from each plant crude extract was added into a 96-well microtiter plate with microbial inoculated, Mueller-Hinton Broth after that, incubated at 37°C for 24 hr, and 10 *µ*l of resazurin solution was added in each well and incubated further for 2 h. Positive controls using 50 *µ*L of chloramphenicol (1 mg/ml) were also route in the same manner and parallel to the treatments. The MIC, the lowest concentration of the sample that prohibited visible growth was deliberated. All samples were observed in triplicate.

2.6 Statistical Analysis

Statistical analysis: The mean values were showed as the Mean±Standard Deviation (SD) and were analyzed by one-way ANOVA (Duncan's Multiple Range) using the program SPSS version 19.0. Differences were measured significant at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity

The results of the antioxidant activity of the Triphala herb extracts in two years from Chae Son, Lampang, Thailand determined by the DPPH assay and FRAP assay (expressed as concentration of extract equivalents to 0.005 mg/mL ascorbic acid), total phenolic content (expressed as mg of acid equivalents per gram of dry extract) and total flavonoid (expressed as mg of quercetin equivalents per gram of dry extract).

The DPPH and FRAP results of this study showed in Table 1 that the methanol extract of *T. bellirica* (WP2447), the ethyl acetate extract of *P. emblica* (WP 2449), in A.D. 2017 and the ethyl acetate extract of *P. emblica* (WP 2319), the methanol extract of Triphala (WP 2321) in 2018 present the higher antioxidant activity than other extraction. The ethyl acetate extract of *P. emblica* established modest to high activity by DPPH and FRAP methods (in A.D. 2017: 0.0013 mg/mL, 0.0009 mg/mL and in A.D. 2018: 0.0090 mg/mL, 0.0176 mg/mL, respectively.

In the present study, total phenolic contents of the ethyl acetate extract of *P. emblica* was equivalent to 156 mg gallic acid/gram of dry

Table 1. Antioxidant activity in comparison of the year of Triphala herb by DPPH assay and FRAP assay

Ext.	A.D. 2017				Ext.	A.D. 2018			
	DPPH assay		FRAP assay			DPPH assay		FRAP assay	
	Conc. (mg/mL)	SD	Conc. (mg/mL)	SD		Conc.(mg/mL)	SD	Conc. (mg/mL)	SD
WP2442	5.3975	0.5215	0.4549	0.1022	WP2312	--	--	1.2586	0.0838
WP2443	0.0017	0.0013	0.6507	0.0065	WP2313	0.0193	0.0156	0.0376	0.0089
WP2444	0.0042	0.0014	0.3258	0.0501	WP2314	0.0207	0.0178	0.0689	0.0100
WP2445	1.2950	0.8909	0.0014	0.0003	WP2315	--	--	2.3153	0.4450
WP2446	0.0018	0.0001	0.0021	0.0006	WP2316	0.0250	0.0184	0.0421	0.0055
WP2447	0.0013	0.0002	0.0011	0.0000	WP2317	0.0265	0.0246	0.0364	0.0060
WP2448	3.0333	1.6950	0.0011	0.0002	WP2318	--	--	0.5033	0.1696
WP2449	0.0013	0.0006	0.0009	0.0000	WP2319	0.0090	0.0042	0.0176	0.0016
WP2450	0.0021	0.0002	0.0012	0.0003	WP2320	0.0110	0.0042	0.0310	0.0054
WP2451	0.0019	0.0001	0.0013	0.0003	WP2321	0.0080	0.0070	0.0458	0.0124
$FeSO_4$			0.0018	0.0004	$FeSO_4$			0.0018	0.0004

**WP2442=2312=T. chebula-hexane ext., WP2443=2313=T. chebula-ethyl acetate ext., WP2444=2314=T. chebula-methanol ext., WP2445=2315=T. bellirica-hexane ext., WP2446=2316 T. bellirica- ethyl acetate ext., WP2447=2317=T. bellirica- methanol ext., WP2448=2318=P. emblica-hexane ext., WP2449=2319=P. emblica-ethyl acetate ext., WP2450=2320=P. emblica- methanol ext., WP2451=2321=Triphala-methanol*

extract in A.D.2017 and 187 mg gallic acid/gram of dry extract in A.D.2018 (Fig. 1 and Fig. 2). Similarly, Naik et al. (2005) informed 33% gallic acid equivalents of the total phenolic content in the extract of this fruit [15]. The methanolic extract of *P. emblica* showed higher total flavonoid contents in A.D. 2017 and A.D. 2018 as equivalent to 1.94 mg (WP2440) and 1.78 mg (WP 2298) quercetin/ gram of dry extract, respectively (Fig. 3 and Fig. 4).

In the previous studies, fruits of *P. emblica* were known as a rich source of vitamin C and also contained a mixture of flavonoids and phenolic compounds. Moreover, it is an authoritative source of natural antioxidants, which have a free radical scavenging activity [16,17]. Therefore, it is important to note that the total flavonoid and phenolic content of *P. emblica* in this study positively correlated with their antioxidant properties, confirming their excellent natural antioxidant properties.

3.2 Antibacterial Activity

3.2.1 Antimicrobial susceptibility

Plant extracts were evaluated for antimicrobial activity against 9 bacterial strains using disc diffusion method. Evaluation of the antimicrobial activity of these plant extracts was recorded in Table 2 and Table 3. The results showed that in A.D. 2017, WP 2439 (*P. emblica*-ethyl acetate ext.) exhibited the highest antimicrobial activity against 7 bacterial strains (*S. aureas* , *E. aerogenes* , *E. coli* (EPEC), *P. mirabilis* , *S. typhimurium*, *S. flexneri* and *V. cholera*). Meanwhile, WP2433 and WP2437 of plant extracts were effective on suppressing microbial growth of 3 strains while WP2432, WP2434, WP 2435, and WP2441 could not inhibit microbial growth of 9 bacterial strains. In A.D. 2018, antimicrobial activity against 9 bacterial strains of plant extracts was showed in Table 3. WP2298 (*P. emblica*- methanol ext.) showed antimicrobial activity against 3 bacterial strains (*S. aureas* , *E. aerogenes* and *V. cholera*). Other plant extracts, WP 2291 and WP 2295 could inhibit microbial growth of 2 bacterial strains. The antimicrobial activity of these plant extracts was exhibited the inhibitory effect as well as found that the crude extract of *Lycium shawii* and *P. emblica* seeds exhibited the most active antimicrobials against the entire *S. aureas* strains [18]. In the same way, Nahor U reported that *P. emblica* against pathogenic *S. aureus* and aqueous extract of *P. emblica* larger zone of growth inhibition [19]. The total crude extract of *P. emblica* revealed a powerful antimicrobial activity that indicated the efficacy of methanol and ethyl acetate extracts [20] and aqua extract of *P. emblica*

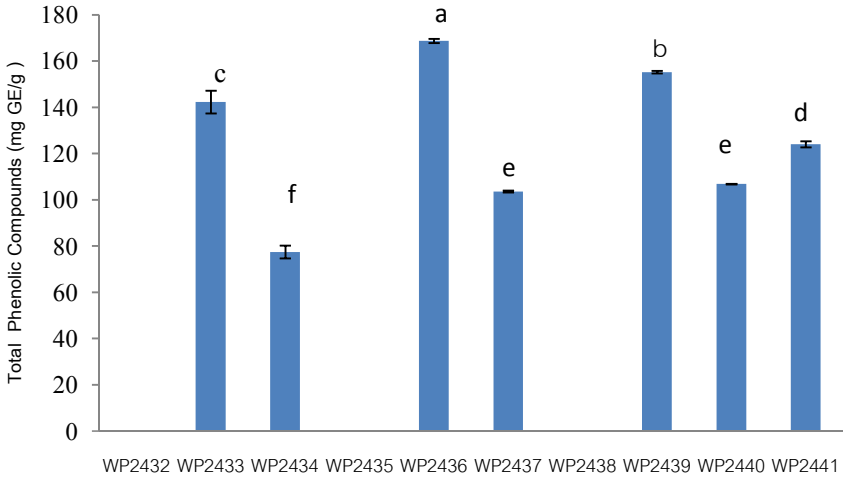


Fig. 1. Total phenolic contents of plant extract in 2017 (Total phenolic content was expressed as mg gallic acid equivalent (GA)/g) *Error bar represented standard error. Values with the same letter on the same color bars represented no significance differences (*P<0.05)

**WP2432=T. chebula-hexane ext., WP2433=T. chebula-ethyl acetate ext., WP2434=T. chebula-methanol ext., WP2435=T. bellirica-hexane ext., WP2436=T. bellirica- ethyl acetate ext., WP2437=T. bellirica- methanol ext., WP2438=P. emblica-hexane ext., WP2439=P. emblica-ethyl acetate ext., WP2440=P. emblica- methanol ext., WP2441=Triphala-methanol*

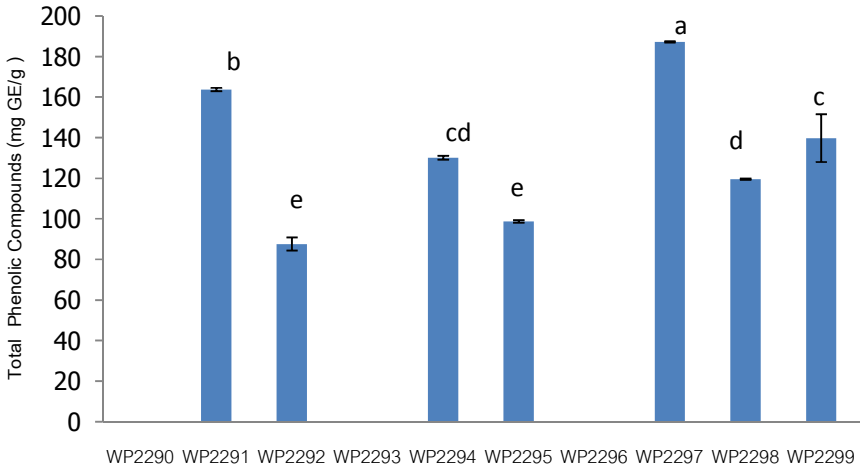


Fig. 2. Total phenolic contents of plant extract in 2018 (Total phenolic content was expressed as mg gallic acid equivalent (GA)/g) *Error bar represented standard error. Values with the same letter on the same color bars represented no significance differences (*P<0.05)

**WP2290=T. chebula-hexane ext., WP2291=T. chebula-ethyl acetate ext., WP2292=T. chebula-methanol ext., WP2293=T. bellirica-hexane ext., WP2294 T. bellirica- ethyl acetate ext., WP2295=T. bellirica- methanol ext., WP2296=P. emblica-hexane ext., WP2297=P. emblica-ethyl acetate ext., WP2298=P. emblica- methanol ext., WP2299=Triphala-methanol*

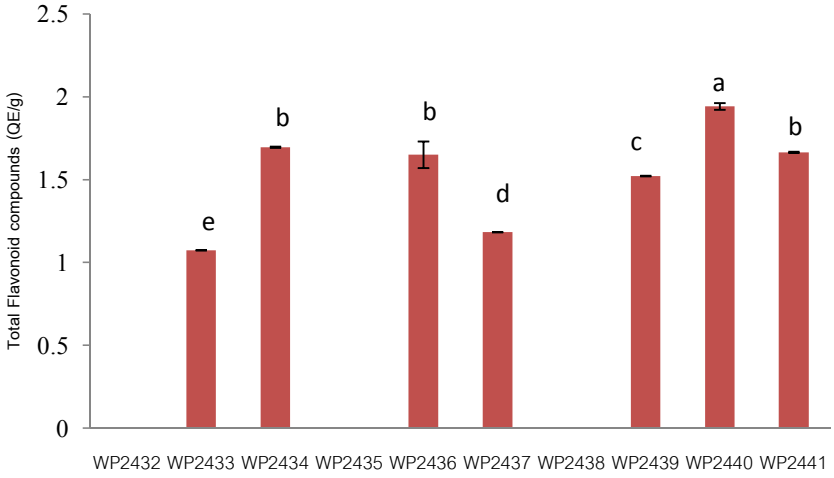


Fig. 3. Total flavonoid contents of plant extract in 2017 (Total flavonoid content was expressed as mg quercetin equivalent (QE)/g) *Error bar represented standard error. Values with the same letter on the same color bars represented no significance differences (*P<0.05)

**WP2432=T. chebula-hexane ext., WP2433=T. chebula-ethyl acetate ext., WP2434=T. chebula-methanol ext., WP2435=T. bellirica-hexane ext., WP2436=T. bellirica- ethyl acetate ext., WP2437=T. bellirica- methanol ext., WP2438=P. emblica-hexane ext., WP2439=P. emblica-ethyl acetate ext., WP2440=P. emblica- methanol ext., WP2441=Triphala-methanol*

against pathogenic *S. aureas* [21]. The investigation of *P. emblica* showed high relatively amount of bioactive compounds and secondary products such as phenol compounds, flavonoids, and tannins, which could be utilized as bactericidal agent [22]. The result of antimicrobial activity of plant extracts revealed *P. emblica* (WP2439) extracted was the most result in antimicrobialactivity. Hence, this research was determined their minimal inhibitory concentration (MIC) against 9 bacterial strains. It accustomed measure qualitatively the in *vitro* activity of an antimicrobial agent against the test bacteria.

Table 2. Antimicrobial activity against 9 bacterial strains of plant extracts in 2017 shown by inhibition zone (n=3)

Ext.	A.D. 2017								
	Diameter of inhibition zone (mm)								
	S. aureas	***E. aerogenes***	***E. coli* 0157 : H7**	***E. coli* (ETEC)**	***E. coli* (EPEC)**	***P. mirabilis***	***S. typhimurium***	***S. flexneri***	***V. cholera***
WP2432	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2433	0*	0*	0*	0*	0*	0*	4.67±4.04b	6.37±0.57b	6.33±0.57a
WP2434	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2435	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2436	0*	0*	0*	0*	0*	16.00±2.00ab	0*	0*	6.83±0.28a
WP2437	6.67±0.57b	0*	0*	0*	0*	13.33±4.93b	0*	0*	0*
WP2438	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2439	8.67±0.57a	8.67±2.51a	0*	0*	7.67±0.57a	11.00±0.00b	8.33±0.57a	8.33±1.15a	7.33±2.30a
WP2440	6.00±0.00c	0*	0*	0*	0*	0*	0*	0*	6.50±0.50a
WP2441	0*	0*	0*	0*	0*	0*	0*	0*	0*

[a]*Inhibition zone diameters are expressed as Mean±SD. The Mean±SD within column followed by the same letter are not significantly different (Duncan's new multiple range test (P≤0.05); *WP2432=T. chebula-hexane ext., WP2433=T. chebula-ethyl acetate ext., WP2434=T. chebula-methanol ext., WP2435=T. bellirica-hexane ext., WP2436=T. bellirica- ethyl acetate ext., WP2437=T. bellirica- methanol ext., WP2438=P. emblica-hexane ext., WP2439=P. emblica-ethyl acetate ext., WP2440=P. emblica- methanol ext., WP2441=Triphala-methanol*

Table 3. Antimicrobial activity against 9 bacterial strains of plant extracts in 2018 shown by inhibition zone (n=3)

Ext.	A.D. 2018								
	Diameter of inhibition zone (mm)								
	S. aureas	***E. aerogenes***	***E. coli* 0157 : H7**	***E. coli* (ETEC)**	***E. coli* (EPEC)**	***P. mirabilis***	***S. typhimurium***	***S. flexneri***	***V. cholera***
WP2290	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2291	0*	0*	0*	0*	0*	0*	0*	6.50±0.50a	10.67±0.57a
WP2292	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2293	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2294	0*	0*	0*	0*	0*	0*	0*	0*	11.00±0.00a
WP2295	4.00±0.00b	0*	0*	0*	0*	7.00±1.00a	0*	0*	0*
WP2296	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2297	0*	7.76±1.52a	0*	0*	0*	0*	0*	0*	9.33±2.51a
WP2298	6.67±1.15a	0*	0*	0*	0*	0*	0*	0*	6.00±0.00b
WP2299	0*	0*	0*	0*	0*	0*	0*	0*	0*

[a]*Inhibition zone diameters are expressed as Mean±SD. The Mean±SD within column followed by the same letter are not significantly different (Duncan's new multiple range test (P≤0.05); *WP2290=T. chebula-hexane ext., WP2291=T. chebula-ethyl acetate ext., WP2292=T. chebula-methanol ext., WP2293=T. bellirica-hexane ext., WP2294 T. bellirica- ethyl acetate ext., WP2295=T. bellirica- methanol ext., WP2296=P. emblica-hexane ext., WP2297=P. emblica-ethyl acetate ext., WP2298= P. emblica- methanol ext., WP2299=Triphala-methanol*

Table 4. Antimicrobial activity against 9 bacterial strains of plant extracts in 2017 shown by MIC (mg/ml)

Ext.	A.D. 2017								
	Diameter of inhibition zone (mm)								
	S. aureas	***E. aerogenes***	***E. coli* 0157 : H7**	***E. coli* (ETEC)**	***E. coli* (EPEC)**	***P. mirabilis***	***S. typhimuriam***	***S. flexneri***	***V. cholera***
WP2432	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2433	0*	0*	0*	0*	0*	0*	25	25	25
WP2434	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2435	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2436	0*	0*	0*	0*	0*	6.25	0*	0*	12.5
WP2437	6.25	0*	0*	0*	0*	12.5	0*	0*	0*
WP2438	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2439	12.5	25	0*	0*	12.5	12.5	12.5	12.5	6.25
WP2440	12.5	0*	0*	0*	0*	0*	0*	0*	6.25
WP2441	0*	0*	0*	0*	0*	0*	0*	0*	0*
Chloramphenicol 1 mg/ml	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03

**WP2432=T. chebula-hexane ext., WP2433=T. chebula-ethyl acetate ext., WP2434=T. chebula-methanol ext., WP2435=T. bellirica-hexane ext., WP2436=T. bellirica- ethyl acetate ext., WP2437=T. bellirica- methanol ext., WP2438=P. emblica-hexane ext., WP2439=P. emblica-ethyl acetate ext., WP2440=P. emblica- methanol ext., WP2441=Triphala-methanol*

Table 5. Antimicrobial activity against 9 bacterial strains of plant extracts in 2018 shown by MIC (mg/ml)

Ext.	A.D. 2018								
	Diameter of inhibition zone (mm)								
	S. aureas	***E. aerogenes***	***E. coli* 0157 : H7**	***E. coli* (ETEC)**	***E. coli* (EPEC)**	***P. mirabilis***	***S. typhimuriam***	***S. flexneri***	***V. cholera***
WP2290	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2291	0*	0*	0*	0*	0*	0*	0*	12.5	6.25
WP2292	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2293	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2294	0*	0*	0*	0*	0*	0*	0*	0*	12.5
WP2295	6.25	0*	0*	0*	0*	12.5	0*	0*	0*
WP2296	0*	0*	0*	0*	0*	0*	0*	0*	0*
WP2297	0*	12.5	0*	0*	0*	0*	0*	0*	12.5
WP2298	12.5	0*	0*	0*	0*	0*	0*	0*	12.5
WP2299	0*	0*	0*	0*	0*	0*	0*	0*	0*
Chloramphenicol 1 mg/ml	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03

**WP2290=T. chebula-hexane ext., WP2291=T. chebula-ethyl acetate ext., WP2292=T. chebula-methanol ext., WP2293=T. bellirica-hexane ext., WP2294 T. bellirica- ethyl acetate ext., WP2295=T. bellirica- methanol ext., WP2296=P. emblica-hexane ext., WP2297=P. emblica-ethyl acetate ext., WP2298=P. emblica- methanol ext., WP2299=Triphala-methanol*

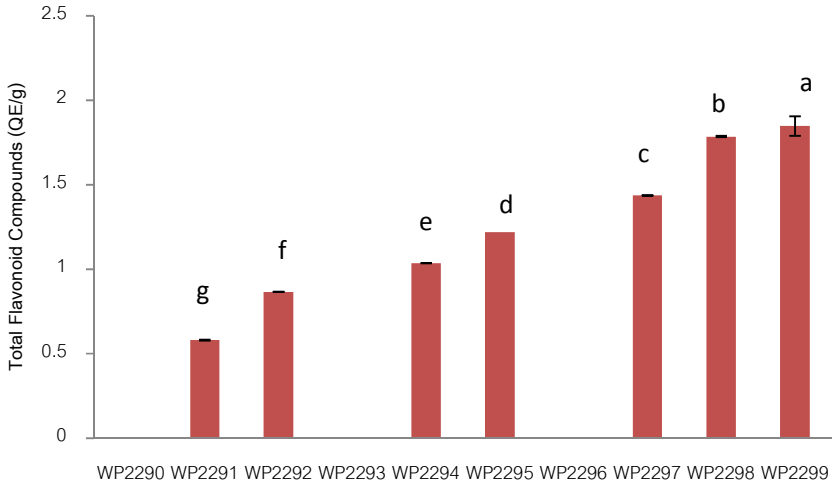


Fig. 4. Total flavonoid contents of plant extract in 2018 (Total flavonoid content was expressed as mg quercetin equivalent (QE)/g) *Error bar represented standard error. Values with the same letter on the same color bars represented no significance differences (*P<0.05)

**WP2290=T. chebula-hexane ext., WP2291=T. chebula-ethyl acetate ext., WP2292=T. chebula-methanol ext., WP2293=T. bellirica-hexane ext., WP2294 T. bellirica- ethyl acetate ext., WP2295=T. bellirica- methanol ext., WP2296=P. emblica-hexane ext., WP2297=P. emblica-ethyl acetate ext., WP2298=P. emblica- methanol ext., WP2299=Triphala-methanol*

3.2.2 Minimum inhibitory concentration

The MIC of plant extracts were presented in Table 4 and Table 5. Inhibitory effects of plant extract ranged from 6.25-25 mg/ml. In A.D. 2017, WP 2439 (*P. emblica*-ethyl acetate ext.) gave the result in inhibitory effect started at 6.25 mg/ml against *V. cholera.* And the concentration of 12.5 mg/ml against *S. aureas*, *E. coli* (EPEC)*, P. mirabilis*, *S. typhimurium,* and *S. flexneri* while the only concentration of 25 mg/ml against *E. aerogenes*. In A.D. 2018, The MIC of plant extracts was showed in Table 5. WP2298 (*P. emblica*- methanol ext.) showed the inhibitory effect started at 12.5 mg/ml against *S. aureas*, *E. aerogenes* and *V. cholera.* Other plant extracts, WP 2291 and WP 2295 showed the inhibitory effect at 6.25 mg/ml against *V. cholera S. aureas*, respectively, while 12.5 mg/ml against *S. flexneri* and *P. mirabilis***,** respectively. Ramalingam and Amaechi [23] reported that a mixed herbal powder extract (*Acacia arabica* 70%, *T. chebula* 10%, *T. bellirica* 10% and *Emblica officinalis* 10%) exhibited the highest inhibition zone of *S. mutans* at 50 µg/ml, and MIC of a mixed herbal powder extract ranged from 12.23-36.7 µg/ml. As well as Jamil K. [24] found that *P. emblica*, *T. chebula,* and *Eucalyptus globulus* had antibacterial activity against oral pathogens and suggested that *P. emblica* (MIC at 100 µg/ml) and *E. globulus* (MIC at 500 µg/ml) were more effective than against *S. aureas.*

4. CONCLUSION

Triphala is a potent polyherbal formula with many useful therapeutic usages for preserving immunity as well as the prevention and treatment of disease. Many researchers studies have reported evidence-based validation of various traditional uses of Triphala. Therefore, research for the determination of the natural antioxidants and antibacterial source is important. In this study, we concluded that ethyl acetate and methanolic of Triphala fruits extracts had satisfied antioxidant and antibacterial properties. From the two-year studies A.D. 2017 and 2018), it can be concluded that the three herbs in Triphala formula had stable active ingredients and can be routined as an easily accessible source of antibacterial substances and natural antioxidants and as a possible food supplement or in pharmaceutical industry, especially Triphala herbal drink water.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All protocols involving cell experiments were approved by the cell Ethics Committee of Lampang Rajabhat University, Thailand.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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