

**Original Article**

**PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIMICROBIAL ASSESSMENT OF *PLUCHEA INDICA* (L.) LESS EXTRACT AS AN ACTIVE INGREDIENT IN NATURAL LOTION BAR**

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**ABSTRACT**

**Objective:** To screen phytochemical compounds and study antioxidant and antimicrobial activities of *Pluchea indica* (L.) Less (PI) leaves extracts for assessment the moisturizing lotion bar formulation containing it's as a bioactive ingredient.

**Methods:** PI leaves were extracted in different solvents. Crude extracts were screened phytochemical compounds and evaluated antioxidant and antimicrobial activities before formulating to antibacterial moisturizing lotion bar.

**Results:** 95% ethanolic extract displayed high antioxidant activities. DPPH radical scavenging capacity with IC<sub>50</sub> was 6.80±0.04 µg/ml (61.37±0.41%) and the ferric reducing ability power was 381.20±0.10 mg AAE/100 gDW. Additionally, 95% ethanolic extract served as a broad spectrum bacteriostatic agents against gram-positive and gram-negative species with an inhibition zone of 6.50±0.00-46.67±0.58 mm. It also was simultaneously as a broad spectrum bactericidal agents (MIC and MBC value of 25-200 mg/ml) by broth micro-dilution method. The screened phytochemical constituents (saponins, tannins, flavonoids, terpenoids, steroids, and alkaloids) in PI leaves extract may be responsible for high biological activities. Thus, 95% ethanolic extract was the most appropriate bioactive ingredient for formulated the product development.

**Conclusion:** The developed moisturizing lotion bar with 0.0041% of 95% ethanolic extract was the most eligible formula that showed good appearance, odor, color, homogeneity, after feel and stability. This extract-containing lotion bar formulation achieved a high level of customer satisfaction by sensory evaluation and development of this specific formulation should be subject to further clinical investigation.

**Keywords:** *Pluchea indica*, Phytochemical compounds, Antioxidant and antimicrobial activities, Extraction solvents, Natural lotion bar

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**INTRODUCTION**

Nowadays, many people's behaviors in all genders and ages are paying more attention to their skincare. Skin is the external organ of human that is most exposed to the various foreign matters in the diverse environment, which may cause various skin-related disorders [1]. In order to prevent skin disorders, it is always necessary to well protect, keep and maintain your skin. There are a wide variety of popular skincare products available today, such as moisturizing, anti-aging, sunscreen and skin whitening products in term of lotions and creams. Skincare products that have been currently very popular are natural lotion products. Because it can reduce the side effects or skin irritation symptoms of consumers. The herbal trend has now received a leapfrogging interest. As a result, there are continuing efforts to study and research the bioactive ingredients from plants with antioxidant and antimicrobial activity as components for developing product formulations to meet the growing demand of consumers.

*Pluchea indica* (L.) Less (name as Khlu in Thailand) is a member of *Asteraceae* family and a well-known medicinal plants that grows naturally in many Asian countries. It could be found Thailand, India, China, Philippines, Malaysia and Myanmar [2, 3]. In Thailand, *P. indica* (L.) Less leaves are used as tea products because they are believed to play role an indigenous remedy due to their anti-diabetic properties [4]. It contains many biochemical compounds like sesquiterpenes, flavonoids, tannins, saponin, sterols, alkaloids and thiophenes [5]. It presented the various pharmacological activities such as antioxidant [6, 7], antimicrobial [8, 9], anti-inflammatory [10], anti-tumor [11], anti-cancer [12], antiproliferation [13], anti-diabetic [14], anti-diuretic [15] activities. Thus, moisturizing lotion products, especially lotion bar was the skincare products that will be developed in this research. It is not only increase the moisturizing and strengthening for the skin to fight various pollutions, it is also a valuable addition to the antioxidant and antimicrobial activity from *P. indica* (L.) Less extracts that used as a bioactive ingredient in the development of natural lotion bar.

**MATERIALS AND METHODS**

**Plant material and preparation of extracts**

The leaves of *P. indica* (L.) Less (PI) in this study were obtained from the Thai Pharmacy Store, Mueang District, Saraburi Province. The plant material was dried in a hot air oven at 50°C for 3-5 d after cleaning with water (2-3 times). All dried leaves were extracted in 95% ethanol, 75% acetone and 99.8% dichloromethane in the ratio of 1:20 (plant: a solvent; (w/v)) by maceration for 7 d. Crude aqueous extract of PI leaf was prepared by boiling into distilled water (ratio of 1:17.5) at 60°C for 1 h. Then, the filtrates were concentrated using a rotary vacuum evaporator under vacuum condition. The concentrated extracts of PI leaves were stored at-20 °C under dark condition until future use.

**Preliminary phytochemical screenings**

A small portion of the crude extracts was subjected to qualitative phytochemical analysis using standard procedure [16] with little modification.

**Estimation of total phenolic content (TPC)**

The quantitative of TPC was determined with Folin Ciocalteu (FC) reagent following prescribed methods [17] with a little modification. 750 µl of 10% Folin-Ciocalteu reagent was thoroughly mixed with 100 µl of plant extracted solution at a proper concentration (0.2 mg/ml) and then incubated at room temperature for 5 min. After that, 750 µl of Na<sub>2</sub>CO<sub>3</sub> (6% w/v) was added to the mixture solution. The resulting mixture was incubated and allowed to for 90 min under dark condition for the development of color. The absorbance of the samples was measured at 725 nm using spectrophotometer (Hanon instruments, Japan). The phenolic concentration was expressed as mg of gallic acid (0.025-0.500 mg/ml) equivalent per 100 g of dry weight.

### Estimation of total flavonoid content (TFC)

The amount of flavonoid in each crude extracts was analyzed by aluminum chloride colorimetric assay in accordance with the previous method [18]. A total of 200  $\mu$ l of sample extracts (0.2 mg/ml) was mixed with 2.3 ml of methanol solution (30% w/v), 100  $\mu$ l of 0.5 M NaNO<sub>2</sub> and 100  $\mu$ l of 0.3 M AlCl<sub>3</sub>, respectively. The resulting solution was thoroughly mixed and remained at room temperature for 5 min in the dark condition. The absorbance of mixture solution was measured spectrophotometrically at 506 nm against blank sample. The flavonoid concentration was expressed as mg of rutin (0.25-5.00 mg/ml) equivalent per 100 g of dry weight.

### In vitro antioxidant activities

#### DPPH radical scavenging activity

The evaluation of scavenging ability of crude extract against free radical was conducted by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [19] with a little modification. 100  $\mu$ l of sample extracts (0.2 mg/ml) was mixed to 900  $\mu$ l of 0.1 mmol DPPH in methanolic solution under vigorous shaking and allowed to stand for 20-30 min in dark at room temperature. The absorbance of solutions was then read at 517 nm. The IC<sub>50</sub> values (scavenge 50% of the radicals) of plant extract were calculated using linear regression graph as antioxidant activity. DPPH scavenging effect was calculated using the following formulation:

$$\text{DPPH radical scavenging (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of sample extracts

#### Ferric reducing/antioxidant power assay (FRAP)

The ferric reducing antioxidant power (FRAP) of plant extracts was analyzed using tripyridyltriazine (TPTZ) solution with modified from the previous method [20]. FRAP reagent was prepared in acetate buffer (300 mmol, pH 3.6) and used freshly. After that, 2.7 ml of the working FRAP reagent (warmed to 37 °C before use) was thoroughly mixed to 300  $\mu$ l of sample extracts (0.2 mg/ml) and then incubated for 30 min. the absorbance of the reaction mixtures was read at 596 nm. Ascorbic acid (0.01-0.25 mg/ml) as standard solution was prepared the standard curve. The antioxidant capacity was expressed as mg of ascorbic acid equivalents/100 g dry weight of the plant samples.

### Determination of antimicrobial activity

#### Microorganisms and culture condition

Crude extracts were evaluated the antagonistic activities against 2 bacterial groups; 1) Gram-positive bacteria (*Bacillus cereus* DMST

5040, *Enterococcus faecalis* DMST 4736, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis*) and 2) Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* DMST 8212 and *Salmonella typhimurium* ATCC 13311). Bacterial strains were source from them Department of Biotechnology, King Mongkut's University of Technology North Bangkok, Thailand. All tested microorganisms were cultured and maintained on brain heart infusion (BHI, Difco) broth/agar medium under 37°C for 18-24 h.

#### Agar disc diffusion assay

Antimicrobial activity was performed by agar disc diffusion assay at the concentration of 50, 100, 200, 300 and 400 mg/ml against nine tested bacterial strains. In brief, the overnight cultures of tested bacterial strains were adjusted to 1.0 x 10<sup>8-9</sup> CFU/ml (OD<sub>600</sub> = 0.2) in BHI broth by spectrophotometer. After that, the inoculums of pathogenic strains were spread on agar surface by sterile cotton swab and allowed to stand for 10-15 min. Then, sterile paper discs (6 mm in diameter) were placed on the surface of previous plates and followed by impregnation of extracts (10-20  $\mu$ l) at different concentrations. The diameter (mm) of the inhibition zones (IZ) was measured after 1, 3 and 5 d incubation at 37°C. [18]. The positive controls used were ampicillin (Amp, 10  $\mu$ g/ml) and ciprofloxacin (CIP, 10  $\mu$ g/ml) discs.

#### Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assay

The MIC values of the crude extracts was conducted by micro-dilution method [21]. The serial two-fold dilutions of the crude extracts were prepared at the concentrations ranging from 200 to 0.195 mg/ml with BHI broth in 96-well plate. Then, 5  $\mu$ l of bacterial suspension (10<sup>8-9</sup> CFU/ml; OD<sub>600</sub> = 0.2) was added to each wells. The micro-dilution trays were incubated at 37 °C for 24 hrs. Bacterial growth was detected by observing the first clear well which was considered as the MIC values. Then, all of the clear MIC well was transferred to the agar plates via loop to evaluate the MBC values. The growth controls (medium+inoculums) and sterility controls (medium+plant extracts) were performed simultaneously in this study.

#### Development of lotion bar

The based ingredients of lotion bar, including emulsifying agents, nourish agents, and oil phase ingredients were adjusted in order to produce an optimized lotion bar formulation (table 1). Each ingredient phases were prepared under heat condition (70°C) and continuously stirred until a smooth uniform lotion (viscous solution). Then, the viscous solution was slowly poured into molds and allowed to stand until firm at room temperature.

Table 1: Ingredients of natural lotion bar formulations in this study

Ingredients	Formulation (%w/w)				Functions
	Basic formulas		Developed formulas		
	1	2	1	2	
<b>Phase A</b>					
Coconut oil	32.67	31.42	31.41	31.39	Emulsifying/moisturizer
Beeswax	32.67	31.42	31.41	31.39	Emulsifying
Shea butter	-	31.42	31.41	31.39	Emulsifying/moisturizer
<b>Phase B</b>					
Vitamin B5 (Pantothenic Acid)	-	0.50	0.50	0.50	Nourish agent (helping to soothe a dry and irritated skin)
Plant extract	-	-	0.004	0.041	Skin conditioning/Active ingredient
<b>Phase C</b>					
Biocide	2.00	0.25	0.25	0.25	Preservative
Fragrance	-	5.00	5.00	5.00	Fragrance
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	

### Evaluation of lotion bar

#### Physical evaluation

Five grams of each formulated lotion bar were prepared for studying the physical appearance by organoleptic properties in terms of clarity, color, and odor. The pH of 1% solution of lotion bar was measured using the digital pH meter.

#### Stability of the formulation

All the formulated lotion bars were evaluated the stability at various storage conditions (placing at 5±2, 25±2, and 40±2 °C (six cycle; 75±5% relative humidity) for 6 w. The each formulation was examined for changes in appearance, color, odor, homogeneity and pH.

**Homogeneity test**

Each formulated lotion bar was tested the homogeneity by visual appearance and touch.

**Type of smear**

Formulated lotion bars were checked the type of smear formed on the skin after lotion bar application.

**Removal test**

Formulated lotion bars were tested the ease of removal by rinsing the part of applied lotion bar with tap water and minimal force to remove.

**After feel test**

The emollient and slipperiness of lotion bar formulation after the applying of lotion bar (0.1 g) was checked.

**Customer satisfaction survey**

Panelists (n = 30) used a 5-point hedonic scale (rate quality from dislike extremely to like extremely) to assess the consumer satisfaction.

**Microbial examination of lotion bar**

One gram of materials was aseptically transferred into the Erlenmeyer flask containing 50 ml of sterile phosphate buffer (pH 7.2). The tested solution was evaluated the microbial growth on BHI

(for bacteria strains), PDA (for fungal strains) by pour plate technique. The microbial limits test were performed to detect the *P. aeruginosa* (Cetrimide agar), *E. coli* (MacConky agar), *S. aureus* (Mannitol salt Agar) and *Salmonella* sp. (Xylage lysine deoxycholate Agar) on selective mediums in formulated lotion bars. Average number of colonies (CFU/ml) was determined after incubating at 37°C for 24-72 h and 25°C for 3-7 d for bacteria and fungi, respectively.

**Statistical analysis**

Results were presented as mean±SD (n=3). Statistical analyses were performed by one-way ANOVA and pair t-tests at 0.05 of significant level (p≤0.05).

**RESULTS AND DISCUSSION****Extracts yields (%) and qualitative phytochemical analysis**

The yields of crude leaves extracts from *Pluchea indica* (L.) Less (PI) in this study obtained by maceration in different solvents including distilled water, 95% ethanol, 75% acetone and 99.8% chloroform. The highest yield of was observed for the aqueous (18.37±0.56%; w/v) crude extract followed by 95% ethanolic (12.30±0.58; w/v), 75% acetonetic (9.39±0.52; w/v) crude extracts, respectively. The results of preliminary phytochemical analysis in crude extracts of PI leaves were given in table 2. Steroids, terpenoids and alkaloids as secondary metabolites were present in all the solvent extracts of PI leaves while anthraquinone and cardiac glycosides were absent from all the extracts (table 2).

**Table 2: Preliminary phytochemical analysis of *P. indica* (L.) less leaves**

Phytochemical compounds	Distilled water	95% Ethanol	75% Acetone	99.8% Chloroform
Saponins				
Froth Test	+++	-	-	-
Tannins				
10% FeCl <sub>3</sub>	+++	+	-	+
Flavonoids				
Shinoda Test	+	+	+	-
10% Lead (IV) acetate	+++	+++	++	-
Anthraquinones	-	-	-	-
Steroids				
Libermann Test	+	+	+	+
Keller-Kiliani Test	+++	+	+	+
Terpenoids				
Salkowski Test	+	++	+++	++
Cardiac Glycosides				
Kedde reagent	-	-	-	-
Keller-Kiliani Test+10% FeCl <sub>3</sub> Test	-	-	-	-
Alkaloids				
Dragendroff's reagent	+	++	+	+
28% NH <sub>4</sub> OH	-	-	-	-
Wangner's reagent	+	++	++	+

-= absent present; += trace (1-30%); += moderately present (31-50%); +++= highly present (>50%)

In addition, it has noted that saponins were not detected in the aqueous, 75% acetonetic and 99.8% chloroform extracts of PI leaves whereas flavonoids were found in all solvent extract except only 99.8% chloroform extract. PI leaves extracts in aqueous and 95% ethanolic contained the wide variety of secondary metabolites but in acetone and chloroform solvents showed the less variety of phytochemical compounds. Research proceeded by Kao *et al.* (2015) showed the similar results to this study, that is PI contains phenolics, flavonoids alkaloids and tannins [13]. Moreover, previous study found cardiac glycosides in PI leaves extracts [14], but not found in this study. These screened phytochemical compounds in this study are well known to support bioactive activities in medicinal plants which will be assessed next in this study.

**Quantitative analysis of the total phenolic and flavonoid contents**

The total phenolic content (TPC) of the PI extracts in various solvents were analyzed using the Folin-Ciocalteu reagent. The

results indicated that TPC of 95% ethanolic extract of PI leaves (547.40±1.60 mgGAE/100g DW) was displayed higher than that of other solvent extracts followed by aqueous (517.3±1.40 mgGAE/100g DW), 75% acetone (390.10±1.60 mgGAE/100g DW) and 99.8% chloroform extracts (77.00±0.40 mgGAE/100g DW), respectively (table 3). TPC of PI crude extracts (177.00±0.40-547.40±1.60 mgGAE/100g DW) obtained in this study was also different from other previous reports. It was lower than those of reported in 95% ethanolic extract (164.8 µg GAE/mg) [22] and methanolic extract (37.99±1.39 µgGAE/mg) [23] of PI, but it was higher than in methanol (0.421±0.0026 mg/ml) and water (0.382±0.0269 mgGAE/ml) extract of PI from Poland [24]. The total flavonoids content (TFC) was high in 95% ethanolic extract (381.20±0.10 mg RE/100 g DW) followed by aqueous and 75% acetone extracts of PI leaves (293.20±1.42 and 245.40±0.26 mg RE/100 g DW, respectively) using aluminum chloride assay which was higher than TFC from Nonthaburi province, Thailand (7.67±0.19 mg CE/100g DW) from the previous study [25].

Table 3: Total phenolics and flavonoids contents and antioxidant activities of *P. indica* (L.) less leaves extracts

Tests	Solvent extraction			
	Distilled water	95% Ethanol	75% Acetone	99.8% Chloroform
Total Phenolic Content (mg GAE/100g DW)	517.3±1.40	547.40±1.60*	390.10±1.60	177.00±0.40
Total flavonoids Content (mg RE/100g DW)	293.20±1.42	381.20±0.10*	245.40±0.26	107.60±0.12
Antioxidant Capacity				
FRAP assay (mg AAE/100 g DW)	15.40±0.50	98.00±1.72*	87.20±1.91	62.00±0.12
DPPH assay (mg AAE/100g DW)	58.20±0.20	128.40±0.40*	81.20±0.40	75.71±0.38
% Inhibition (DPPH)	57.71±0.38	61.37±0.41*	48.61±0.32	43.99±0.29
IC <sub>50</sub> Sample; µg/ml	11.00±0.40	6.80±0.04*	73.40±0.20	17.40±0.30

GAE = Gallic Acid Equivalent; RE = Rutin Equivalent; AAE = Ascorbic acid Equivalent; IC<sub>50</sub> = 50% Inhibitory Concentration; \* = P≤0.05

The results of quantitative antioxidant activities revealed the TPC and TFC were consistent in the same direction, but their result was different in various solvents due to the varied solvent polarities. This suggested that the phenolic compounds of this plant might primarily contain flavonoids. The polarity of the solvent is very important in enhancing the solubility of phenolic compounds [26]. Furthermore, differences in the number and concentration of plant secondary metabolites were depending on these factors; the species of plant, parts of plant, stage of development, seasons and external factors such as light conditions, temperature and water stress [27, 28].

#### Antioxidant activity

The ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity results were given in table 3. The solvent extracts of PI leaves with the highest antioxidant activity was 95% ethanol with 98.00±1.72 mgAAE/100 g DW and the lowest antioxidant activity was distilled water with 15.40±0.50 mgAAE/100 g DW by FRAP assay. Regarding of DPPH radical scavenging assay, higher DPPH radical scavenging activity has a lower IC<sub>50</sub> values. About 95% ethanolic extract of PI leaves significantly revealed the highest DPPH activity with the IC<sub>50</sub> value of 6.80±0.04 µg/ml (%inhibition of 61.37±0.41). This value was rather close to the previous study which had IC<sub>50</sub> of 4.34±0.42 µg/ml [23], while IC<sub>50</sub> of standard ascorbic acid in this study was 0.166±0.010 µg/ml. Previous studies have shown that phenolic compounds contribute to the antioxidant activity of plants. There was the significantly positive correlation between antioxidant activity (DPPH assay) and TPC with 95% ethanolic extract (p≤0.05) in this study. Because phenolic compounds are good electron donors

(hydrogen atoms to free radicals) [29]. Aqueous extract displayed a significantly negative correlation between total flavonoids content and antioxidant capacities by FRAP assay in this study (p≤0.05).

#### Antimicrobial activity

All crude leaves extracts of PI were screened for antagonistic activity by agar disc diffusion method against nine pathogenic strains. The different degrees of the antimicrobial activities of the PI extracts were showed in table 4. The most potent antimicrobial activities against *S. aureus* ATCC 25923 is attributed to 95% ethanolic extract which exhibited a very strong inhibitory activity at 200 mg/ml with inhibition zones of 46.67±0.58 mm (p≤0.05) while showed weak inhibitory effect on *S. epidermidis*, *E. coli* ATCC 25922, *K. pneumoniae*, *P. aeruginosa* ATCC 27853, *P. mirabilis* DMST 8212 and *S. typhimurium* ATCC 13311 at 100 mg/ml with IZs of 7.00±0.00-8.00±0.00 mm. *S. aureus* ATCC 25923 was the most sensitive strain to all extract of PI leaves, whereas *B. cereus* DMST 5040 and *P. mirabilis* DMST 8212 was significantly resistant to all solvent extracts of PI leaves except 95% ethanolic extract for *P. mirabilis* DMST 8212 (p≤0.05). Similar bacteriostatic activity in aqueous extract of PI leaves, especially against *E. coli* (concentrations of 25 to 100 mg/ml; IZs of 14.6-21.0 mm) and *K. pneumoniae* (concentrations of 50 to 100 mg/ml; IZs of 11.7-18.6 mm) was recorded in previous study [30]. Ampicillin (15.67±0.58-41.33±0.58 mm) and ciprofloxacin (21.67±0.58-41.67±0.58 mm) used as a positive control was effective against all the bacteria except *E. coli* ATCC 25922 and *S. typhimurium* ATCC 13311 for ampicillin and *S. typhimurium* ATCC 13311 for ciprofloxacin.

Table 4: Antibacterial activities of *P. indica* (L.) Less leaves extracts against some bacterial pathogens

Concentration (mg/ml)	Inhibition zone (IZ; Ø = 6 mm); mm±SD									
	Gram positive strains				Gram negative strains					
	BC	EF	SA	SE	EC	KP	PA	PM	ST	
Distilled Water	50	R	R	20.33±0.58 <sup>l</sup>	6.50±0.00 <sup>s</sup>	7.83±0.29 <sup>qr</sup>	7.00±0.00 <sup>r</sup>	6.50±0.00 <sup>s</sup>	R	10.33±0.58 <sup>n</sup>
	100	R	R	28.00±0.00 <sup>i</sup>	6.50±0.00 <sup>s</sup>	8.00±0.00 <sup>pq</sup>	7.00±0.00 <sup>r</sup>	6.50±0.00 <sup>s</sup>	R	9.67±0.58 <sup>n</sup>
	200	R	R	40.50±0.50 <sup>c</sup>	6.50±0.00 <sup>s</sup>	8.00±0.00 <sup>pq</sup>	7.00±0.00 <sup>r</sup>	6.50±0.00 <sup>s</sup>	R	8.17±0.29 <sup>op</sup>
	300	R	R	34.33±0.58 <sup>f</sup>	6.50±0.00 <sup>s</sup>	7.67±0.29 <sup>qr</sup>	7.00±0.00 <sup>r</sup>	6.50±0.00 <sup>s</sup>	R	8.00±0.00 <sup>pq</sup>
95% Ethanol	50	R	R	31.67±1.15 <sup>sh</sup>	7.00±0.00 <sup>r</sup>	8.00±0.00 <sup>pq</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>
	100	R	R	37.00±0.00 <sup>e</sup>	7.00±0.00 <sup>r</sup>	8.00±0.00 <sup>pq</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>
	200	R	R	46.67±0.58 <sup>a</sup>	7.00±0.00 <sup>r</sup>	8.00±0.00 <sup>pq</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>
	300	R	R	44.00±1.00 <sup>b</sup>	7.00±0.00 <sup>r</sup>	8.00±0.00 <sup>pq</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>
75% Acetone	50	R	7.00±0.00 <sup>r</sup>	46.33±1.15 <sup>a</sup>	8.00±0.00 <sup>pq</sup>	8.00±0.00 <sup>pq</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>	7.00±0.00 <sup>r</sup>
	100	R	7.00±0.00 <sup>r</sup>	21.67±0.58 <sup>k</sup>	R	R	7.00±0.00 <sup>r</sup>	6.50±0.00 <sup>s</sup>	R	R
	200	R	7.00±0.00 <sup>r</sup>	38.00±0.00 <sup>d</sup>	R	R	7.00±0.00 <sup>r</sup>	6.50±0.00 <sup>s</sup>	R	R
	300	R	7.00±0.00 <sup>r</sup>	30.67±1.15 <sup>h</sup>	R	R	7.00±0.00 <sup>r</sup>	6.50±0.00 <sup>s</sup>	R	R
99.8% Chloroform	50	R	7.00±0.00 <sup>r</sup>	30.00±0.00 <sup>h</sup>	R	R	7.00±0.00 <sup>r</sup>	6.50±0.00 <sup>s</sup>	R	R
	100	R	7.00±0.00 <sup>r</sup>	39.33±1.53 <sup>c</sup>	7.00±0.00	R	7.00±0.00 <sup>r</sup>	6.50±0.00 <sup>s</sup>	R	R
	200	R	R	19.33±1.15 <sup>l</sup>	7.00±0.00 <sup>r</sup>	R	R	7.00±0.00 <sup>r</sup>	R	7.00±0.00 <sup>r</sup>
	300	R	R	16.67±0.58 <sup>m</sup>	7.00±0.00 <sup>r</sup>	R	R	7.00±0.00 <sup>r</sup>	R	7.00±0.00 <sup>r</sup>
	400	R	R	28.33±0.58 <sup>j</sup>	7.00±0.00 <sup>r</sup>	R	R	7.00±0.00 <sup>r</sup>	R	7.00±0.00 <sup>r</sup>
	200	R	R	24.33±1.15 <sup>j</sup>	7.00±0.00 <sup>r</sup>	R	R	7.00±0.00 <sup>r</sup>	R	7.00±0.00 <sup>r</sup>
	300	R	R	31.67±0.58 <sup>sh</sup>	7.00±0.00 <sup>r</sup>	R	R	7.00±0.00 <sup>r</sup>	R	7.00±0.00 <sup>r</sup>
	400	R	R							

Abcdefghijklmnopqrs = significant at the 0.05 level (p≤0.05). BC = *B. cereus* DMST 5040, EF = *E. faecalis* DMST 4736, SA = *S. aureus* ATCC 25923, SE = *S. epidermidis*, EC = *E. coli* ATCC 25922, KP = *K. pneumoniae*, PA = *P. aeruginosa* ATCC 27853, PM = *P. mirabilis* DMST 8212 and ST = *S. typhimurium* ATCC 13311; R: Resistance

Interestingly, crude aqueous and 95% ethanolic extracts of PI leaves had a bactericidal effect on both Gram-positive and Gram negative stains with the MBC values of 50-200 mg/ml (table 5). At the concentration of 50 mg/ml of plant extract, *S. aureus* ATCC 25923 and *B. cereus* DMST 5040 (Gram-positive bacteria) were destroyed by 95% ethanolic extracts while was only *S. aureus* ATCC 25923 in aqueous extracts. Among gram negative bacterial strains, the bactericidal concentration of 95% ethanolic extract at 100 mg/ml (MBC values) was found in *K. pneumoniae*, *P. aeruginosa* ATCC 27853; and *S. typhimurium* ATCC 13311 but found in *K. pneumoniae* with aqueous extract. The previous studies presented the similar result of potential antibacterial activities of ethanol PI extract against *B. cereus* (MIC and MBC of 500 µg/ml), *S. aureus* (MIC and MBC value of 500 µg/ml) and *E. coli* (MIC value of 2,000 µg/ml),

but it is no potential against *P. aeruginosa*, which this study had anti-*P. aeruginosa* by broth micro-dilution method [31].

Comparisons of antibacterial activities all solvent extracts on both assays, the result of MIC part of the crude aqueous and 95% ethanolic extracts was in conformity with their inhibition zone of antimicrobial activity in only the case of *S. aureus* ATCC 25923, which showed the strong inhibitory effect its growth. In this study, gram-negative bacteria had more tolerance to PI leaves extracts than gram-positive bacteria due to the presence of an outer membrane (lipopolysaccharide) in gram-negative bacteria. Moreover, plant active compounds (phenolics, flavonoids, tannins, steroids, terpenoids and alkaloids) is capable of damage protein of cell membrane (lipid bilayer) by damaging the proteins and penetrating into bacterial cells to inhibit and destroy bacterial cells [32].

**Table 5: The MIC and MBC values of *P. indica* (L.) Less leaves extracts against some pathogenic strains by broth micro-dilution method**

Solvents extraction	Microorganisms								
	Gram positive strains				Gram negative strains				
	BC	EF	SA	SE	EC	KP	PA	PM	ST
<b>Distilled water</b>									
MIC (mg/ml)	100	50	25	100	200	50	100	200	100
MBC (mg/ml)	200	100	50	200	>200	100	200	>200	200
<b>95% Ethanol</b>									
MIC (mg/ml)	50	100	25	200	100	100	100	100	100
MBC (mg/ml)	50	200	50	>200	>200	100	100	200	100
<b>75% Acetone</b>									
MIC (mg/ml)	>200	>200	200	>200	>200	>200	100	200	>200
MBC (mg/ml)	>200	>200	200	>200	>200	>200	200	200	>200
<b>99.8% Chloroform</b>									
MIC (mg/ml)	>200	>200	200	>200	>200	>200	>200	>200	>200
MBC (mg/ml)	>200	>200	200	>200	>200	>200	>200	>200	>200
<b>Antibiotics</b>									
<b>Ampicillin</b>									
MIC (µg/ml)	0.039	0.009	0.156	0.009	0.156	0.009	0.019	0.019	0.078
MBC (µg/ml)	0.039	0.009	0.156	0.019	0.156	0.009	0.019	0.039	0.078
<b>Ciprofloxacin</b>									
MIC (µg/ml)	0.078	0.313	0.019	0.001	0.019	0.313	0.009	0.004	0.078
MBC (µg/ml)	0.156	0.625	0.039	0.002	0.019	0.625	0.019	0.039	0.156

BC = *B. cereus* DMST 5040; EF = *E. faecalis* DMST 4736; SA = *S. aureus* ATCC 25923; SE = *S. epidermidis*, EC = *E. coli* ATCC 25922; KP = *K. pneumoniae*; PA = *P. aeruginosa* ATCC 27853; PM = *P. mirabilis* DMST 8212 and ST = *S. typhimurium* ATCC 13311; All results ± 0.00 (SD).

#### Lotion bars formulation

Crude 95% ethanolic extracts of PI leaves was chosen to apply in formulation development of natural lotion bar as an active ingredient based on its higher antioxidant (IC<sub>50</sub> of 6.80±0.04 µg/ml) and antimicrobial actions (broad spectrum). Developed formula 1 and formula 2 contain 95% ethanolic extract of PI leaves at concentration of 10 (0.0041%) and 100 (0.0410%) times of IC<sub>50</sub> value, respectively (table 1).

#### Evaluation of natural lotion bars

This study was aimed to developed the lotion bar formulation containing *P. indica* (L.) less extract. Comparisons of the effects of

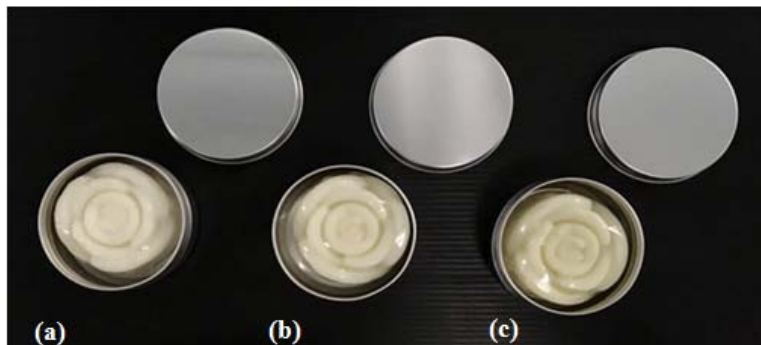
each formulation on stability and acceptance are important factors that must be studied for the good quality of the upcoming products. The results of the evaluation of formulated lotions bar indicated that the color, odor and homogeneity of the lotion bar were stable during the freeze-Thaw (6<sup>th</sup> cycles) of observation (table 6). The pH values of the both developed formula 1 and 2 remained constant with a slight decrease in the value. The color of developed lotion bar (1) and (2) was yellowish-white and slightly yellowish, respectively (fig. 1). The pH values of the lotion bar was presented in range of 5.1 to 5.7. Developed formula 1 of lotion bar shows the pH value in the range that is better than developed formula 2 of that for human skin pH (average pH = 5.5).

**Table 6: Stability of lotion bar containing *P. indica* (L.) less leaves extract**

Storage condition/Test	Developed formula 1		Developed formula 2	
	Initial	Final	Initial	Final
25±2 °C/75% RH				
Color	yellowish-white	unchanged	slightly yellowish	unchanged
Odor	Pleasant	unchanged	Pleasant	unchanged
Homogeneity	Satisfactory	unchanged	Satisfactory	unchanged
pH	5.6±0.02	5.7±0.02	5.1±0.02	5.2±0.02
Freeze-Thaw (6 <sup>th</sup> cycles)				
Color	yellowish-white	unchanged	slightly yellowish	unchanged
Odor	Pleasant	unchanged	Pleasant	unchanged
Homogeneity	Satisfactory	unchanged	Satisfactory	unchanged
pH	5.6±0.02	5.7±0.02	5.1±0.02	5.1±0.02

Both developed lotion bar formulations had a homogeneous distribution of extracts with the other components that was asserted by the appearance and touch when both formulations were kept for long time. After the application of both developed lotion bar (0.1 g) on skin, emollient and slipperiness of lotion bar was found and easily removed by washing with tap water. Satisfaction test of developed lotion bar formula 1 and 2 was evaluated in terms of appearance, odor,

color, after feel, packaging and overall acceptability by 5-point hedonic scales (n = 30 persons). The acceptability scores of lotion bar formulate 1 showed higher score in all terms compared with lotion bars formulate 2. The overall acceptability scores from customer satisfaction of lotion bar formula 1 and 2 were  $4.23 \pm 0.57$  (like moderately) and  $3.80 \pm 0.66$  (like moderately), respectively while commercial lotion bar was as  $4.47 \pm 0.57$  (like moderately).



**Fig. 1: Physical appearance of formulated natural lotion bars; based formula (a), Developed formula 1 (b; 0.0041% of extract) and Developed formula 2 (c; 0.0410% of extract)**

## CONCLUSION

The crude extract of *Pluchea indica* (L.) Less leaves contains the many phytochemical constituents including saponins, tannins, flavonoids, steroids, terpenoids and alkaloids. The types of secondary metabolites from plant materials depended on the polarity of the solvent used for extraction. 95% ethanolic extract of PI leaves noticeably displayed stronger antioxidant and antimicrobial activities than other solvents (distilled water, 75% acetone and 99.8% chloroform). It showed broad spectrum of inhibiting (nine bacterial strains) and killing (seven bacterial strains) for both Gram-negative and Gram-positive bacteria especially *S. aureus* ATCC 25923 with IZ of  $16.67 \pm 0.58$ - $46.67 \pm 0.58$  mm (MICs and MBCs of 25 and 50 mg/ml, respectively). Furthermore, 95% ethanolic extract showed the highest total of phenolic and flavonoid contents and antioxidant activities (DPPH and FRAP assay) as  $61.37 \pm 0.41\%$  of inhibition and  $IC_{50}$  value of  $6.80 \pm 0.04$   $\mu$ g/ml by DPPH assay. Accordingly, the PI leaves extract in 95% ethanol was as a rich source of antioxidant and antibacterial compounds that had reasonable component as a bioactive ingredient to apply for development of lotion bars formulation. Based on organoleptic properties, stability and consumer satisfaction test, PI (moisturizing) lotion bar formula 1 is a suitable formula for further study in the field of allergic and cytotoxicity testing for further commercial development.

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## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICTS OF INTERESTS

The authors report no conflicts of interest.

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