Antimicrobial and Antioxidant Activities in 'Beluntas' (*Pluchea indica*), Turmeric (*Curcuma longa*) and Their Mixtures

(Aktiviti Antimikrob dan Antioksidan pada 'Beluntas' (*Pluchea indica*), Kunyit (*Curcuma longa*) dan Campurannya)

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ABSTRACT

*Traditional medicine provides us essential guidance on herbal remedies with approaching health preservation methods. In this paper, we present the relevant prescriptions related to 'Beluntas' (*Pluchea indica*) and Turmeric (*Curcuma longa*) ingredients mentions in Malay Manuscript Code MSS 2802. In this study, antioxidant and antimicrobial properties in the mixture of P. indica and C. longa and their individual plants were studied based on prescription captured. Antimicrobial activities were performed against* Staphylococcus aureus *and* Escherichia coli *in vitro by micro dilution method and disk diffusion method. The observation from MIC and MBC show that the mixture of both plants has the highest ability to inhibit* S. aureus *followed by* P. indica *and* C. longa*. Meanwhile, at concentration of 2000 mg/mL, the inhibition zone of* P. indica, C. longa, *and mixture of both plants showed significantly different (p <0.05) with the values of 12.67±0.58, 6.33±0.58 and 10.16±0.58 mm, respectively. In this study, the antioxidant activities of* P. indica, C. longa, *and their mixture was measured the DPPH scavenging activity, FRAP and TPC. The DPPH scavenging activity showed that* P. indica *has significantly highest antioxidant ability among all the samples as it IC$_{50}$ is the lowest with 379.822 µg/mL. In FRAP and TPC methods,* P. indica *also exhibited the significant highest antioxidant followed by their mixture and* C. longa*. In conclusion, this herb remedy may be considered as an alternative medicinal treatment that have competent antimicrobial and antioxidant properties. However, the formulation for the mixture of both plants need to further investigate.*

Keywords: Antimicrobial; antioxidant; Curcuma longa*; MSS 2802;* Pluchea indica

ABSTRAK

*Perubatan tradisi memberi kita panduan penting mengenai ubat-ubatan herba dengan pendekatan kaedah pemeliharaan kesihatan secara holistik. Dalam kajian ini, kami mengetengahkan ramuan berkaitan dengan Beluntas (*Pluchea indica*) dan Kunyit (*Curcuma longa*) yang terdapat dalam manuskrip Melayu berkod MSS 2802. Kandungan antimikrob dan antioksidan dalam campuran* P. indica *dan* C. longa *serta tumbuhan individunya telah dikaji. Aktiviti antimikrob ditentukan dengan menggunakan bakteria* Staphylococcus aureus *dan* Escherichia coli *secara* in vitro *melalui kaedah mikrodilusi dan resapan cakera. Hasil pemerhatian daripada penentuan MIC dan MBC menunjukkan campuran kedua-dua tumbuhan menpunyai kebolehan yang paling tinggi untuk merencatkan pertumbuhan* S. aureus *diikuti oleh* P. indica *dan* C. longa*. Manakala, pada kepekatan 2000 mg/mL* P. indica, C. longa *dan campuran kedua-dua tumbuhan, nilai zon perencatan berbeza secara bererti (p <0.05) dengan masing-masing adalah 12.67±0.58, 6.33±0.58 dan 10.16±0.58 mm. Dalam kajian ini, aktiviti antioksida* P. indica, C. longa *dan campuran kedua-duanya diukur melalui aktiviti skaveng DPPH, FRAP dan TPC. Aktiviti skaveng DPPH menunjukkan* P. indica *mempunyai aktiviti antioksida yang paling tinggi secara bererti dalam kalangan semua sampel dengan nilai IC$_{50}$ nya adalah paling rendah iaitu 379.822 µg/mL. Dalam kaedah FRAP dan TPC,* P. indica *juga menunjukkan aktiviti antioksida yang tinggi berbanding campuran ekstrak kedua-dua tumbuhan dan* C. longa*. Kesimpulannya, ubat herba ini boleh dijadikan sebagai rawatan perubatan altenatif yang mempunyai kandungan antimikrob dan antioksidan yang mencukupi. Walau bagaimanapun, ramuan penggunaan campuran daripada kedua-dua tumbuhan ini perlu dilakukan kajian lanjut.*

Kata kunci: Antimikrob; antioksidan; Curcuma longa*; MSS 2802;* Pluchea indica

INTRODUCTION

Malay manuscripts are made up of collection from several sheets of paper that had been bound into volume. Most of the manuscripts had been written in Arabic or *Jawi* using ink. The manuscripts contain knowledge about proper understanding of the cultural history of the Malay world (Jones 1999). There are many manuscripts originated

from Malay world and the total numbers of manuscript is estimated around 22,000 manuscripts. Out of 22,000 manuscripts, 4,000 of them are kept at Centre of Malay Manuscript at National Library of Malaysia while the rest are placed at various places including Indonesia, Brunei Darussalam, Sri Lanka, Europe, France, Belgium, Netherlands and more (Abdul Hamid & Fauzi 2012). With the introduction of press printing technology to Muslim world in early 19th century, the availability of this cultural practices have been expended and manuscripts rental has become evident before becoming family heritage and being in the hands of named individuals and spread over the world (Van 2017).

Among all Malay manuscripts, there are more than 100 manuscripts that are specific to medical field which mostly written within year 1820 to 1870 (Harun 2015). For instance, as recorded in one of the Malay manuscripts written by Haji Mustafa Bin Haji Ismail Pontianak, there are more than 100 type of herbs used to treat 15 diseases mentioned: garlic, nutmeg, caraway, eggplants, and galls of the *Quercus infectoria* (species of oak). The example of treatment for disease that have been mentioned in manuscripts are including abdominal pain, bloating, dementia, oral ulcers, and vaginal bleeding (Abdul Hamid & Fauzi 2012).

The use of herb plants as natural antioxidant and antimicrobial agents has caught some interests, considering that there are growing consideration about side effect of using synthetic compounds and increasing of bacterial resistant toward antimicrobial agents. However, the information about antimicrobial and antioxidant properties on the mixture of Indian Marsh Fleabane (*Pluchea indica*) or known as 'Beluntas' in Malay word and Turmeric (*Curcuma longa)* compare to each individual plant is not investigated yet. The exist evidence recorded in the Malay manuscripts about the benefit of this plants mixture which stated that these herbs can be used to treat stomach ache. Hence, the scientific inquiry of this particular claim must be carried out to prove the usability and legitimacy of this local wisdom in the light of modern methodology and paradigm.

The aim of this scientific research thus was to determine the antimicrobial and antioxidant activity of the herb remedy according to Malay Manuscripts Code MSS 2802. This research was performed by extracting leaf part of *P. indica* and rhizome of *C. longa* using methanol and analyse their antimicrobial and antioxidant activities.

MATERIALS AND METHODS

MALAY MANUSCRIPT CODE MSS 2802 (A: BINTANG DUA BELAS) (B: KITAB TIB 'CETERA LUQMAN AL HAKIM')

Manuscript study in the scope of medicine is a rarity which is very much neglected, albeit most studies that are done tends to furnish philological aspects and transliteration processes (Harun 2015). An interconnected

study of Malay codicology with scientific approach could prove a significant value to the medical industry, in which herbal-based medicine can be used to promote a safer, and healthier treatment.

A search team was established to locate and collate most Medicinal Malay Manuscript in Malaysia, the key term used was to include Malay language references such as *Kitab Tib, Petua-petua, rawatan,* and *ubatan*. The hand searching acquired sensitive and exhaustive search for eligible manuscripts. This includes other possible and indirect terms of medicine, treatment, and health preservation. The manuscripts were studied at National Malay Manuscript Centre (*Pusat Manuskrip Melayu*), National Library (*Perpustakaan Negara Malaysia*), National Archives (*Arkib Negara*) and Islamic Arts Museum (which houses the National Islamic Affairs Department collection of Malay Manuscripts).

DATA COLLECTION PROCESS

The original manuscripts were then viewed, read, extracted via scanning or copying, with its relevant parts of remedies classified according to each of their mentioned diseases. Colonial dated published dictionaries were used as the core reference during the process of reading the collected data from the manuscripts.

ANALYSIS AND SYNTHESIS

The various prescriptions were collected using a standard data collection form and stored in a unified database. These manuscripts used old Malay *Jawi* writings which personifies the Arabic alphabetization which is then transliterated into Roman alphabets, these data are then translated into English. One relevant prescription was identified and extracted to specifically examine its biochemical compounds for this study. This process is relatively one of the most novel approach, towards understanding local culture of health preservation and medication since the early works of GimLette (1923).

BIOLOGICAL SAMPLE PREPARATION

Leaves from three biological Indian Marsh Fleabane or 'Beluntas' (*Pluchea indica*) plants was obtained freshly from herbal garden located at Jitra, Kedah, Malaysia dated on September 2018. Meanwhile, three biological turmeric (*Curcuma longa*) was obtained from supermarket located at Nilai, Negeri Sembilan. The samples were washed with distilled water. Turmeric sample was dried at 60 °C in the drying oven (Binder) while the leaves of 'Beluntas' were dried at room temperature for two days, then all the samples were grinded and kept in air tight container (Srimoon & Ngiewthaisong 2015).

The percentage yield of *P. indica, C. longa* and its mixture were calculated based on initial weight of samples and weight of crude extract. Though the initial weight of all samples are within 30.0 g, different

percentage of yield was obtained. The highest yield of crude extract is from *P. indica* (33.81%), followed by *C. longa* (27.24%) and mixture of both plants (18.57%).

TEST BACTERIA

The bacterial strains which are *Escherichia coli* and *Staphylococcus aureus* were obtained from the Microbiology Laboratory, Faculty of Science and Technology, Universiti Sains Islam Malaysia, Nilai. Each bacterial strain was streaked onto Mueller Hinton Agar (MHA) and incubated at 37 °C overnight. The isolated single colony of each bacterial was cultured into Mueller Hinton broth (MHB) and incubated at 37 °C for 18-24 h. The turbidity of the resulting suspension was diluted with MHB to match with 0.5 McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0×10^8 CFU/mL (Singh et al. 2015). The density of bacterial growth was determined at 600 nm within range 0.08 - 0.12 using Bio Photometerplus (Eppendorf).

PLANT METABOLITE EXTRACTION

Three samples were prepared which are *P. indica* leaf, *C. longa* and mixture of *P. indica* and *C. longa* with 1:1 ratio. Each samples were prepared in three biological replicates. All the samples were then macerated with methanol (1:10 of w/v) for 24 h. The extracts were filtered using Whatman No.1 filter paper and concentrated using rotary evaporator (Eyela). The crude extracts were then stored at -18 °C for the further analysis (Srimoon & Ngiewthaisong 2015).

DETERMINATION OF MIC AND MBC OF PLANT EXTRACT

The dilution method for the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extract was carried out according to method by Chikezie (2017) and Wiegand et al. (2008) with modification. In MIC method, crude extract was diluted into six various concentrations by two-fold serial dilution in sterile Mueller Hinton broth (MHB) on microtiter plate. A 50 µL bacteria culture was then inoculated into well containing 50 µL of various concentrations of samples extract in MHB. The microtiter plate was incubated at 37 °C for 18 to 24 h and observed for growth or turbidity. The growth of bacteria in MIC method was detected by using 1 mg/mL of MTT (3-(4, 5- Dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide) assay as indicator.

The MBC of plant extracts were determined from the broth dilution of MIC tests by sub culturing to agar plates that did not contain the test agent. A loopful of broth from each well that showing no growth in MIC test was inoculated into MHA. Then, the growth of bacteria was examined after 18 - 24 h. The first concentration that did not show any growth of bacteria was determined as MBC values.

DISK DIFFUSION SUSCEPTIBILITY TEST

Bacteria with amount of 100 µL were spread uniformLy on the surface of MHA and the inoculum were allowed to dry for 5 min. Filter paper discs were treated with 15 µL sample and were placed on the agar surface. The diameters of inhibition zone were measured after 24 h of incubation. Streptomycin was used as positive control as it has spectrum activity suitable for gram positive and gram negative bacteria. The results were compared with the inhibition capacity of 50 mg/mL streptomycin (Srimoon & Ngiewthaisong 2015).

DPPH RADICAL SCAVENGING ACTIVITY ASSAY

The DPPH (2,2-diphenyl-1-picrylhydrazyl) analysis was adopted from Muhammad et al. (2017) with modifications. Firstly, 0.1 mL of five different concentrations (20, 40, 60, 80, and 100 µg/mL) of extracted samples were added into 2.9 mL of 0.1 mM DPPH and vortex to mix homogenously. The solutions were maintained in a dark condition at room temperature for 30 min. After the incubation, the absorbance of the samples was read at 517 nm through a UV- visible spectrophotometer (Varian Cary 50). Gallic acid was used as reference. The percentage of scavenging activities in the samples were calculated using the following formula:

$$\% \text{ DPPH scavenging activity} = ((Ac - As)/Ac) \times 100$$

where Ac is the absorbance of control; and As is the absorbance of sample

Then, the IC_{50} values were calculated using linear regression analysis and used to indicate antioxidant capacity. The experiment was repeated twice with three replicates for each measurement taken.

DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

The total phenolic content was determined using the Folin–Ciocalteu method adopted from Hatami et al. (2014) with modifications. Firstly, Folin's reagent was prepared by diluting with distilled water at ratio of 1:10. 1.5 mL of Folin's reagent was then added into 0.3 mL of the samples. After 5 min of incubation, 1.2 mL of 6% Sodium carbonate was added in the mixture and mixed homogenously by using vortex. The mixture was further incubated for 2 h to stabilize the color complex. The absorbance was measured at 765 nm through a UV- visible spectrophotometer (Varian Cary 50). A Gallic acid standard plot at varying concentrations (50-250 µg/ mL) were used to evaluate the total phenolic content. Total phenolic content was expressed as milligrams of Gallic acid equivalent per gram (mg GAE/g) of the extract by calculated using standard calibration curve. The experiment was repeated twice with triplicate for each measurement.

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STATISTICAL ANALYSIS

All experimental measurements were carried out in triplicate and were expressed as means ± standard deviation. Experimental data were analyzed using the analysis of variance (ANOVA) by MINITAB 17. The differences among samples were determined at a level of $p < 0.05$ of significance. The significant test between *P. indica, C. longa,* and mixture of both plants was based on Tukey Pairwise Comparisons.

RESULTS AND DISCUSSION

Remedy for a child who has stomach pain: Take leaf of *P. indica*, put in the tip of *C. longa*, grind altogether then badged upon the (patient's) stomach (Figure 1). The materials mention in this prescription are leaf of *P. indica* and tip of *C. longa.* Therefore, the scientific research was carried on with the metabolite extraction of *P. indica* and *C. longa* for the antimicrobial and antioxidant tests.
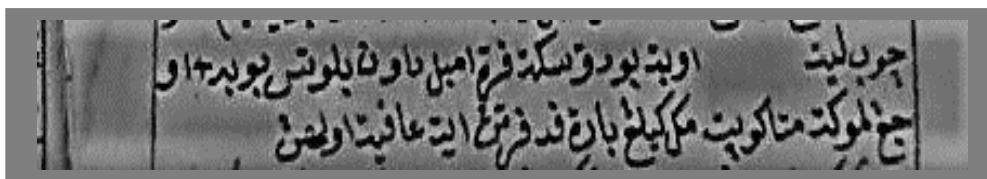

FIGURE 1. (Transliteration) *Ubat budak sakit perut: ambil daun belutas bubuh ujung lemukat mata kunyit maka giling barut pada perutnya itu* (*Page: 108, Line: 12, Folio: 56v*)

MIC AND MBC OF PLANT EXTRACTS

Determination of MIC has been conducted on microtiter plate. MTT assay was used as an indicator to determine the MIC values (Figure 2). As yellow MTT assay was reduced into purple formazan crystals by the activity of mitochondrial succinate dehydrogenase enzyme in viable cells, the MIC value was determined by observing the minimum sample concentration that did not cause a color shift as purple color indicating bacterial growth (Rao et al. 2014). Figure 2 indicates that *P. indica* has the lowest antimicrobial activity towards *E. coli* as the minimal sample concentration was 250 mg/mL, while minimal sample concentration of *P. indica* extract inhibit *S. aureus* growth was 62.5 mg/mL (Figure 2).
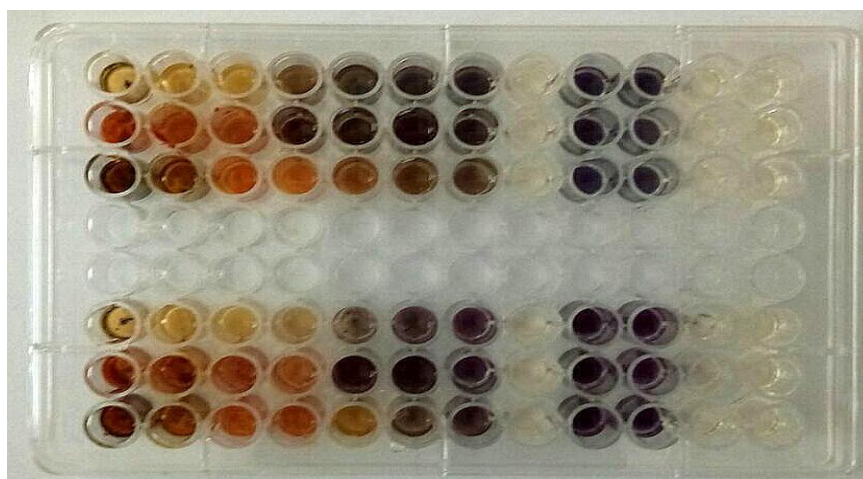

FIGURE 2. Antimicrobial test by MIC using MTT assay

Note: S = streptomycin, GC = growth control, M= methanol, A= *P. indica,* B = *C. longa*, C = Mixture

This result was consistent with the results from Srimoon and Ngiewthaisong (2015) that reported the dry leaves of *P. indica* has inhibition activity against *S. aureus* with MIC value of 83.04 mg/mL but no inhibition against *E. coli*. For *C. longa,* the minimal sample concentration was 250 mg/mL towards *E. coli* and *S. aureus*, while the minimal sample concentration for the mixture of both samples was 62.5 mg/mL towards both bacteria. This result contradicted with finding from Afrose et al. (2015) which stated that MIC for aqueous *C. longa* extract was 800 µg/mL against *S. aureus* and 2000 µg/mL against *E. coli*. This means the MIC value of *C. longa* against *E. coli* was higher than *S. aureus* in previous research. The lowest value of minimal samples concentration for the mixture of both samples indicates that it has the highest inhibition activity towards both bacteria followed by *P. indica* and *C. longa.*

Minimum bactericidal concentration (MBC) was performed based on the result obtained by MIC value. The well that had remained the original color after the addition of MTT reagent was considered that bacterial growth was inhibited. Hence, the lowest concentration of the extract in which there was no growth of colony after the incubation overnight was taken as MBC value. MBC values of *P. indica* and *C. longa* against *S. aureus* was 500 mg/mL, while MBC value of *P. indica, C. longa* and the mixture of both plants against *E. coli* was 1000 mg/mL. In addition, MBC value for the mixture of both plants against *S. aureus* was 250 mg/mL. This indicates that the ability of the herbs extract to inhibit bacteria were consistent with MIC results as the mixture of both plants showed the highest ability followed by *P. indica* and *C. longa.*

ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT

Disk diffusion test was conducted by using four different concentrations (2000, 1000, 500, and 250 mg/mL) by referring to concentration that show ability to inhibit based on MBC results. Streptomycin has been used as positive control and distilled water as negative control. After incubated, the diameter of inhibition of *P. indica, C. longa* and their mixture was measured including disk itself (Figure 3).
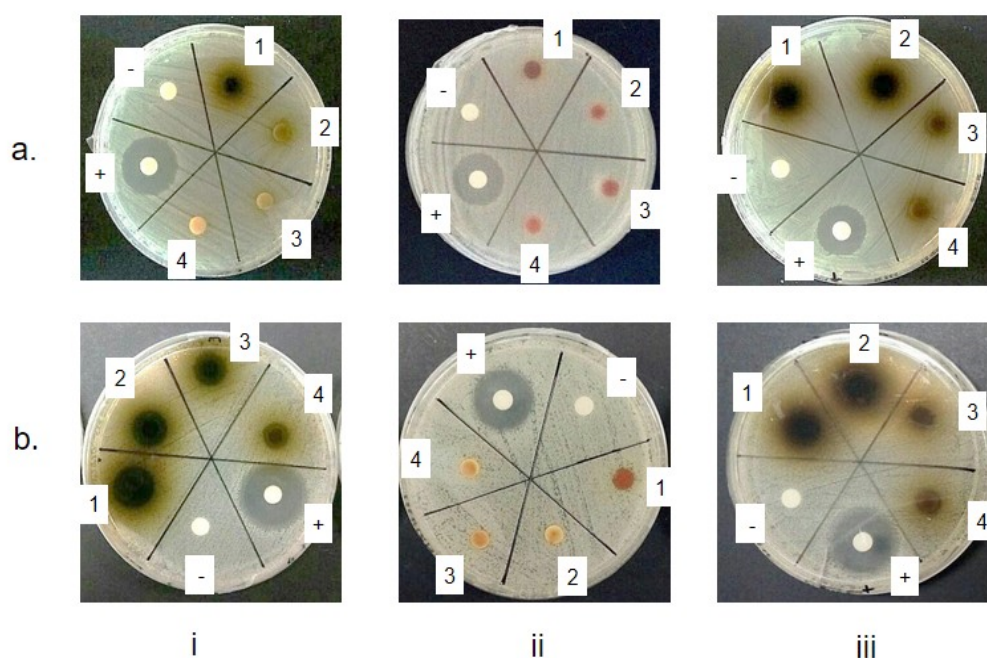

FIGURE 3. Results of disk diffusion for i) *P. indica*, ii) *C. longa* and iii) mixture of *P. indica* and *C. longa* against a) *E. coli* and b) *S. aureus*

Note: Concentration of sample, (1) = 2000 mg/mL, (2) = 1000 mg/mL, (3) = 500 mg/mL, (4) = 250 mg/mL, (+) = Streptomycin, (-) = Distilled water

There are significant in inhibition zone against *S. aureus* versus concentration of the *P. indica* and the mixture of *P. indica* and *C. longa* ($p < 0.05$) (Table 1). At concentration of 2000 mg/mL, the inhibition zone of *P. indica, C. longa* and mixture of both plants showed significantly different with the values of 12.67±0.58, 6.33±0.58 and 10.16±0.58 mm respectively. It indicates that *P. indica* has the largest inhibition zone against *S. aureus* and followed by the mixture of both plants. However, inhibition zone of *C. longa* against *S. aureus* did not showed any significant different in all concentrations. In contrast, Gupta et al. (2015) reported that methanolic extract of *C. longa* show highest inhibition zone with 19 mm at the concentration of 50 mg/mL.

TABLE 1. Antimicrobial activity of samples against *S. aureus* tested by disk diffusion method

Sample	Zone of inhibition (mm)				
	2000 mg/mL	1000 mg/mL	500 mg/mL	250 mg/mL	Positive control
P. Indica	12.67±0.58[Ab]	10.67±0.58[Ac]	9.00±1.00[Acd]	8.33±0.58[Ad]	19.67±0.58[a]
C. Longa	6.33±0.58[Bb]	6.00±0.00[Bb]	6.00±0.00[Bb]	6.00±0.00[Bb]	18.67±0.58[a]
Mixture	10.67±0.58[Cb]	10.16±0.76[Ab]	8.33±0.58[Ac]	7.5±0.50[Ac]	19.67±0.58[a]

Note: Values within a column with different uppercase letters (A, B, C) represent significant differences (p< 0.05). Values within a row with different lowercase letters (a, b, c, d) represent significant differences (p< 0.05). Significant test is using Tukey pairwise comparison. Inhibition zone (6mm) considered as no inhibition. All data are mean ± SD (n =3)

Meanwhile, there are no inhibition zone in all concentration of the samples against *E. coli* tested by disk diffusion method (Data not shown). This may due to the different type of bacteria as *E. coli* was a gram negative bacteria. *E. coli* has additional membrane called outer membrane that covers both the cytoplasmic membrane and the peptidoglycan layer (Martínez et al. 2012). In addition, there is periplasm space between the double membranes which contain enzymes which have ability to damage foreign molecules that come from outside cell. Gram-negative bacteria also have a hydrophilic coating on the outer membrane rich in lipopolysaccharide molecules that can act as a barrier against the entry of antimicrobial substances. Meanwhile, *S. aureus* is a gram positive bacteria which have outer membrane structure and different cell wall from *E. coli*. Apart from that, majority of gram positive bacteria are more sensitive to antimicrobial substance (Pargaputri et al. 2016). So it is acceptable that *S. aureus* has inhibition zone while no inhibition zone showed by *E. coli*.

Among all the samples, *P. indica* showed the highest ability to inhibit gram positive bacteria such as *S. aureus*, as it contains various compounds that have mechanism to damage cell wall and cell membrane such as tannin and flavonoids. Gallo and pirogallo group in tannin can react with protein membrane and cause protein leakage. This action causing damage to cell wall and resulted in bacteria death. Meanwhile, flavonoids can interact with DNA which can damage the hydrogen bonding of DNA resulting disruption of the stability in DNA thus affect the bacterial growth and metabolism (Pargaputri et al. 2016). Although *C. longa* lack of ability to inhibit bacteria, it still has competent antimicrobial properties. This may due to differences in percentage of compound that has antimicrobial ability between the samples. *P. indica* was reported containing tannin within range 1.93-3.12% while *C. longa* has 1.08% (Septiana et al. 2014). This can be one of the factors for different inhibition ability in both samples. For the mixture of both plants, the inhibition of bacteria became less than

P. indica but higher than *C. longa*. The synergistic effect between these two factors cannot be determine as this mixture only being conducted in 1:1 ratio.

DPPH SCAVENGING ACTIVITY

DPPH antioxidant assay can be determined by the ability of DPPH to decolorize in the presence of antioxidant. The DPPH will change the color from deep purple color to yellow when it accepted an electron donated by an antioxidant compound. This action can be measured from the change of absorbance at wavelength 517 nm (Widyawati et al. 2014).

A significant result was shown between concentration of the samples and percentage of scavenging activity but non-significant between the samples and scavenging activity ($p < 0.05$). All samples showed that it had radical scavenging activity at all concentrations. The IC_{50} which could imply the antioxidant activity of sample was calculated to determine the concentration of sample required to inhibit 50% of radical scavenging. The value of IC_{50} for Ascorbic acid standard was obtained at 22.399 µg/mL.

From Table 2, there were large difference between IC_{50} of all the sample and Ascorbic acid. *P. indica* show the highest antioxidant ability among all the samples as it IC_{50} is the lowest among all samples with 379.822 µg/mL. Research by Noridayu et al. (2011) indicated that methanolic extract of *P. indica* has the lowest IC_{50} value between all the samples tested which was 24.45±0.34 µg/mL. This showed that *P. indica* has strong antioxidant activity. Arya et al. (2015) reported that IC_{50} of DPPH for twenty *C. longa* from various places at India showed the result between range 64.38±1.89 µg/mL and 271.95±1.56 µg/mL. Both results from previous research were higher than the results in this experiment. There are several factors that may influence the results which are environmental conditions such as altitude, humidity, and temperature which can affect the bioactive compounds thus may affect its antioxidant activity (Arya et al. 2015). For mixture of both plants, it showed moderate antioxidant activity as compared with *P. indica,* and *C. longa* and its IC_{50} value was closer to *P. indica* when compared to *C. longa*.

TABLE 2. IC_{50} of DPPH scavenging activity

Sample	IC_{50}
P. indica	379.822 µg/mL
C. longa	717.5342 µg/mL
Mixture	400.9841 µg/mL
Ascorbic acid	22.399 µg/mL

TOTAL PHENOLIC CONTENT

Total phenolic content of the samples was expressed as milligrams of Gallic acid equivalent per gram (mg GAE/g). The Gallic acid standard curve was linear with $R^2 = 0.9986$ and the calibration equation was used to calculated total phenolic content of the samples. Phenolic compounds are major plant secondary metabolite and contribute to antioxidant and antibacterial activities (Pukumpuang et al. 2012).

The *p*-value of total phenolic content was significant with the samples ($p > 0.05$). *P. indica* showed the highest total phenolic content among all samples with 3.7830±0.001 mg GAE/g, follow by mixture of both plants and *C. longa* which are 1.6419±0.002 and 0.1618 ±0.002 mg GAE/g respectively (Figure 4).
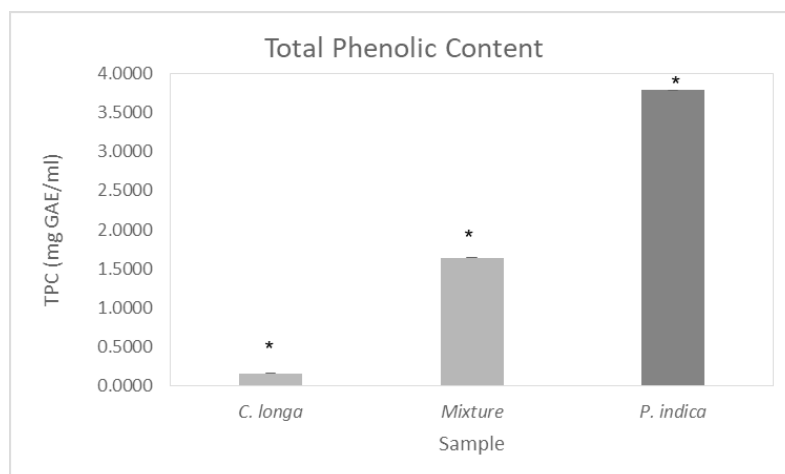
FIGURE 4. Graph of total phenolic content versus *P. indica, C. longa,* and their mixture. All data are mean ± SD (n =3), symbol (*) indicates a significant difference (*p* < 0.05)

As compared to result reported by Pukumpuang et al. (2012), local *P. indica* showed lower phenolic content than of aqueous extract which is 13.83±0.36 mg GAE/g. For *C. longa*, the result showed slightly higher than result by Damasius et al. (2007) which is 9.80±0.47 mg GAE/g. This differences may be due to extraction solvent they used which is ethanol.

Majority of compounds in *P. indica* leaves have polar properties. Methanol has been used to extract leaves as it can dissolve polar compound. Besides, phenolic compounds of methanolic extract were effective in donating hydrogen atomic to molybdenum ion in Folin-Ciocalteu reagent resulted in radical phenoxyl which then stabilized by delocalization (Widyawati et al. 2014). This affect the total phenolic content in *P. indica* to be highest among the samples. For mixture of both plants, total phenolic content showed the lowest among all samples. The mixture ratio of both plants may not be suitable to enhance or preserve total phenolic content as compared to individual plants.

Correlation of phenolic contents and IC_{50} of samples extract in DPPH scavenging activity Pearson correlation between total phenolic content equivalent to Gallic acid and IC_{50} of the samples in DPPH scavenging activity show negative results and there was no significant relation between the values. Relationship

between these two antioxidant tests are inversely proportional between each other which mean as total phenolic content in samples increase, the IC_{50} of samples decrease. Both antioxidant tests showed non- significant in the results. There must be several errors that affect the antioxidant test results such as multiple freeze-thaw cycles of the samples, storage time and temperature which can reduce the phytochemicals and antioxidant capacity of the samples (Louaileche & Djaoudene 2016).

FERRIC REDUCING/ANTIOXIDANT POWER ASSAY

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe3+-TPTZ) complex and producing a coloured ferrous tripyridyltriazine (Fe2+-TPTZ). Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom (Noor Atiqah et al. 2014).

Table 3 shows the mean FRAP values for *P. indica, C. longa,* and the mixture of both plants. The *P*-value of FRAP value was significant with the samples (*p* > 0.05). *P. indica* exhibited the highest antioxidant potential on the FRAP assay and followed by the mixture of both plants and *C. longa*.

TABLE 3. Mean FRAP values of samples

Sample	mg Fe (II)/mL
P. indica	3.2840±0.005[a]
C. longa	0.8352±0.000[b]
Mixture	1.7830±0.006[c]

Note: Values with different lowercase letters ([a, b, c]) represent significant differences (p< 0.05). Significant test is using Tukey pairwise comparison. All data are mean ± SD (n =3)

Dragovic-Uzelac and Boras (2007) stated that the higher phenolic content has shown to exert greater reducing power. Therefore, as the reducing power was determined with the Fe3+ to Fe2+ transformation, the reducing power increased with increasing concentrations of phenolics in the sample extracts. This is true for *P. indica* as previously it has shown to have the highest total phenolic content of 3.7830±0.001 mg GAE/g and having the highest antioxidant potential for FRAP assay. There was significant strong correlation between ferric reducing activity and total phenolic content with r = 1 and *p*-value= 0.016.

CONCLUSION

The present analysis was to test whether these traditional plants remedies boast a potential as antimicrobial and antioxidant or not. It had been discovered in this study that the herbs extract had more potent towards *S. aureus* than *E. coli*. In this study, the antioxidant activities of *P. indica*, *C. longa,* and their mixture was observed by measuring the free radical scavenging activity (DPPH scavenging activity), FRAP assay, and determination of TPC. From the three methods, it shows that *P. indica* leaves has the highest antioxidant activity followed by the mixture of both plants and *C. longa*.

REFERENCES

Abdul Hamid, F.F. & Fauzi, N.W. 2012. Perubatan melayu tradisional: Kitab tibb pontianak (Malay traditional medicine: kitab tibb pontianak). *Jurnal Al-Tamaddun* 7(1): 149-162.

Afrose, R., Saha, S.K., Banu, L.A., Ahmed, A.U., Shahidullah, A.S., Gani, A. & Ali, M.Y. 2015. Antibacterial effect of *Curcuma longa* (turmeric) against *Staphylococcus aureus* and *Escherichia coli*. *Mymensingh Medical Journal* 24(3): 506-515.

Arya, N., Prakash, O., Verma, A.K.V. & Pant, A.K. 2015. Variation in antioxidant potential of *Curcuma longa* L. collected from different ecological niches of Western Himalayan region. *International Journal of Pharmacy and Pharmaceutical Sciences* 7(7): 85-90.

Chikezie, I.O. 2017. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method. *African Journal of Microbiology Research* 11(23): 977-980.

Damasius, J., Skemaite, M., Kirkilaite, G., Vinauskiene, R. & Venskutonis P.R. 2007. Antioxidant and antimicrobial properties of caraway (*Carum carvi* L.*)* and cumin (*Cuminum cyminum* L.) extracts. *Veterinarija Ir Zootechnika* 40(62): 9-13.

Dragovic-Uzelac, V., Levaj, B., Mrkic, V., Bursac, D. & Boras, M. 2007. The content of polyphenols and carotenoids in three apricot cultivars depending on stage of maturity and geographical region. *Food Chemistry* 102(3): 966-975.

GimLette, J.D. 1923. *Malay Poison and Charm Cures.* London: J&A Churchill.

GimLette, J.D. & Thomson, H.W. 1903. *A Dictionary of Malayan Medicine.* Kuala Lumpur: Oxford University Press.

Gupta, A., Mahajan, S. & Sharma, R. 2015. Evaluation of antimicrobial activity of *Curcuma longa* rhizome extract against *Staphylococcus aureus*. *Biotechnology Reports* 6: 51-55.

Harun, M.P. 2015. Traditional Malay medicine from old Malay texts. *International Journal of the Malay World and Civilisation (Iman)* 3(3): 3-17.

Hatami, T., Emami, S.A., Miraghaee, S.S. & Mojarrab, M. 2014. Total phenolic contents and antioxidant activities of different extracts and fractions from the aerial parts of *Artemisia biennis* willd. *Iranian Journal of Pharmaceutical Research* 13(2): 551-559.

Jones, R. 1999. Malay manuscripts: Gatherings and soiled pages. *Archipel* 57(2): 97-108.

Louaileche, H. & Djaoudene, O. 2016. Impact of storage conditions on the bioactive compounds and antioxidant capacity of commercial orange jam. *Journal of Analytical, Bioanalytical and Separation Techniques* 1(1): 8-11.

Martinez de Tejada, G., Sanchez, Gomez, S., Razquin, O.I., Kowalski, I., Kaconis, Y., Heinbockel, L. & Brandenburg, K. 2012. Bacterial cell wall compounds as promising targets of antimicrobial agents I. Antimicrobial peptides and lipopolyamines. *Current Drug Targets* 13(9): 1121-1130.

Muhammad, D.R.A., Praseptiangga, D., Van de Walle, D. & Dewettinck, K. 2017. Interaction between natural antioxidants derived from cinnamon and cocoa in binary and complex mixtures. *Food Chemistry* 231: 356-364.

Noor Atiqah, A.A.K., Maisarah, A.M. & Asmah, R. 2014. Comparison of antioxidant properties of tamarillo (*Cyphomandra betacea*), cherry tomato (*SolanumLy copersicum* var. *cerasiform*) and tomato (*Lyopersicon esulentum*). *International Food Research Journal* 21(6): 2355-2362.

Noridayu, A.R., Hii, Y.F., Abas, F., Shaari, K. & Lajis, N. 2011. Antioxidant and antiacetylcholinesterase activities of *Pluchea indica* Less. *International Food Research Journal* 18(3): 925-929.

Pargaputri, A.F., Munadziroh, E. & Indrawati, R. 2016. Antibacterial effects of *Pluchea indica* Less. leaf extract on *E. faecalis* and *Fusobacterium nucleatum* (*in vitro*). *Dental Journal* 93(56): 93-98.

Pukumpuang, W., Thongwai, N. & Tragoolpua, Y. 2012. Total phenolic contents, antibacterial and antioxidant activities of some Thai medicinal plant extracts. *Journal of Medicinal Plants Research* 6(35): 4953-4960.

Rao, S., Timsina, B., Varalakshmi, A. & Nadumane, K. 2014. Antimicrobial effects of medicinal plants and their comparative cytotoxic effects on HepG2 cell line. *International Journal of Pharmacy and Pharmaceutical Sciences* 6(1): 101-105.

Septiana, A., Indrawati & Rustin. 2014. Analisis kadar alkaloid dan tanin tumbuhan Beluntas (*Pluchea indica* Less.) pada lahan salin di desa asingi kecamatan Tinanggea dan Non Salin di Desa Lambodijaya kecamatan lalembuu Sulawesi Tenggara. *Biowallacea* 1(2): 82-89.

Singh, R., Shushni, M.A.M. & Belkheir, A. 2015. Antibacterial and antioxidant activities of *Mentha piperita* L. *Arabian Journal of Chemistry* 8(3): 322-328.

Srimoon, R. & Ngiewthaisong, S. 2015. Antioxidant and antibacterial activities of Indian marsh fleabane (*Pluchea indica* (L.) Less). *Asia-Pacific Journal of Science and Technology* 20(2): 144-154.

Van der Putten, J. 2017. On the edge of a tradition. *Indonesia and the Malay World* 45(132): 179-199.

Widyawati, P.S., Dwi, T., Budianta, W., Kusuma, F.A. & Wijaya, E.L. 2014. Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* Less leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research* 6(4): 850-855.

Wiegand, I., Hilpert, K. & Hancock, R.E. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols* 3(2): 163-175.

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