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Post-Cold Storage Shelf Life of Organic Chili Pepper (*Capsicum annum* cv. 'Superhot') Applied with Hot Water Dip and Active Packaging

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Abstract

Cold storage could increase shelf life of chili pepper (*Capsicum annum*) but too long a period of storage could adversely affect shelf life during subsequent holding at ambient. This study determined the effects of different durations of cold storage on the shelf life during subsequent ambient holding of organic chili cv. 'Superhot' applied with hot water dip and active packaging. Organic chili pepper at the full-red stage were dipped in 50°C water for 4 min and then packed in 32-34 µm-thick polyethylene (PE) bag with micro-perforations (Active PACK™). The fruit were cold-stored at 10°C with 95% RH for 4-16 days and then held at ambient (25°C, 75% RH) to simulate the distribution and marketing period. Cold storage of 12 days is the best duration in prolonging fruit shelf life at ambient by about four days more than that of the control. Longer duration of cold storage shortened the shelf life due to increased weight loss and decay.

Introduction

Chili pepper (*Capsicum annum* cv. 'Superhot') is an important domestic and export vegetable in Thailand. Chili peppers are usually harvested and marketed as whole fruit at the red ripe stage and have a short post-harvest life (7-10 days) even under proper temperature management due to decay, shriveling and softening (Rodoni et al., 2015). At temperatures below 7°C, peppers develop chilling injury (González-Aguilar et al., 2004).

Several postharvest techniques can be applied in combination in order to extend shelf life of fruit and vegetables including organic product which is a product that the market needs high due to the emphasis on environment-friendly food production and human healthcare. In organic green and red peppers, Rodoni et al. (2016) combined the use of hot water dip (HWD) and cold storage and found that HWD at 45°C for 3 min resulting in lower fruit spoilage than the control fruit. The treatment reduced soft rot, shriveling, weight loss, color changes and respiration rate; delayed pectin solubilization and softening; and prevented membrane leakage during storage at 4°C. It did not alter sugar content, acidity and antioxidant capacity. The research

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concluded that HWD is a simple and safe non-chemical approach to supplement low temperature management, extending the shelf life of organic peppers.

Modified atmosphere packaging (MAP) is another non-chemical method to improve shelf life of fruit and vegetables. It is exemplified by the use of polymeric films to create an atmosphere of low O_2, high CO_2 and high water vapor content which can effectively slow the rates of respiration, water loss and ripening or senescence (Kader & Watkins, 2000). In recent years, many research studies have focused on the application of active packaging on a broad array of food systems (Atar & Chiralt, 2016). In an earlier study in organic chili pepper cv. Superhot, active MAP using a propriety product (Active PAK™) of 32-34 µm-thick polyethylene (PE) bag with micro-perforations combined with HWD at 50°C for 4 min applied before packaging was found to effectively reduce weight loss and decay during 16-day cold storage (10°C, 95% relative humidity) as compared to passive MAP (62 µm-thick PE) combined with 45°C or 55°C HWD (Krongyut & Duangsi, 2015). However, fruit shelf life during subsequent holding at ambient conditions was not improved. The present study determined the duration of cold storage that could improve fruit shelf life during subsequent ambient holding.

Materials and methods

1. Preparation of fruit samples

Freshly harvested chili pepper cv. 'Superhot' at the full-red stage were obtained from a local organic farm in Ubon Ratchathani Province and brought to the Post-harvest Laboratory of the Faculty of Agriculture, Ubon Ratchathani Rajabhat University, Ubon Ratchathani, Thailand. The fruit were sorted and only defect-free and uniformly sized fruit were used as experimental samples.

2. Application of treatments

The fruit were treated first with HWD, followed by active packaging before cold storage and subsequent ambient holding. The chili pepper were dipped in 50°C water for 4 minutes and then air-dried at room temperature for 30 min (Krongyut & Duangsi, 2015). The HWD-treated fruit weighing 100 g per replicate were placed in a fruit tray and packed in active MAP using Active PAK™ from the National Metal and Materials Technology Center (MTEC), Thailand. The active MAP has micro-perforations and anti-fog agent to prevent water condensation and is essentially made of PE of 32-34 µm thickness and 8 x 12 inches size, with O_2 transmission rate of 10,000-12,000 $cm^3/m^2/day \cdot atm$, CO_2 transmission rate of 29,000-32,000 $cm^3/m^2/day \cdot atm$, and water vapor transmission rate of 10 $g/m^2 \cdot$ day. The packed fruit were then stored in a cold chamber at 10°C temperature and 95% RH at different durations - 0 (control), 4, 8, 12 and 16 days. After cold storage, the fruit were transferred to a 25°C room with 75% RH for 5 days for ambient shelf life simulation.

3. Data measurement

Peel color was measured using a CR-300 Chromameter (Minolta, Hong Kong) taking the a* (reddening) and L* (lightness) values. Surface color was taken from each 10 fruit samples, recording the average of five readings from the middle part of the fruit.

Weight loss was determined as percentage of the initial weight.

Decay incidence was obtained by taking the number of fruit that showed sign of decay which was then expressed as percentage of the total number of fruit samples per replicate.

Titratable acidity (TA) was analyzed using the titration method. Pulp tissues (10 g) were homogenized using a blender with 40 ml of distilled water. The mixture was then filtered through cotton wool. The filtrate (5 ml) was added with one to two drops of 0.1% phenolphthalein indicator and was titrated using 0.1 N NaOH to an endpoint of faint pink color (pH 8.1). The results were expressed as percentage citric acid per 100 g fresh weight.

Shelf life was determined as the number of days to reach the limit of marketability based on the visual quality of the fruit scored by eight trained panelists using a 5-point hedonic scale as previously described (Gonzalez-Aguilar et al., 2004).

4. Statistical analysis

The experiments were conducted in a completely randomized design (CRD) with three replications. Results were analyzed by performing the analysis of variance (ANOVA) and treatment mean comparison by the Least Significant Difference (LSD) test at 95% confidence intervals using Statistica 7 software (StatSoft, Tulsa, OK, USA). All data reported as means ± standard deviations (SD).

Results and discussion

Peel red color (a* values) during ambient holding for five days was not significantly affected by the duration

of cold storage (Table 1). In general, a* values ranged from 21.4-24.4 regardless of the holding period at ambient. Data measurement was terminated earlier in some treatments because the fruit became unmarketable. Similarly, surface lightness (L* values) was not significantly affected by the duration of cold storage and ranged from 39.5-41.4 throughout the five-day ambient holding period (Table 2).

Table 1 Peel color a* values during ambient storage of hot water-treated and active MAP-held organic chili pepper subjected to different durations of cold storage.

Cold storage duration (days)	Days at ambient					
	0[ns]	1[ns]	2[ns]	3[ns]	4[ns]	5
0	23.75±0.32	22.69±1.56	22.49±0.29	ND	ND	ND
4	24.12±0.75	22.42±1.67	22.49±0.29	22.20±0.36	21.99±1.08	21.45±0.99
8	23.40±0.50	22.81±1.25	22.48±1.62	22.09±1.33	21.78±0.81	ND
12	23.31±0.20	22.43±0.11	22.44±0.45	22.18±0.14	22.20±0.09	ND
16	23.34±0.12	22.87±0.65	22.50±1.16	22.29±0.91	ND	ND

Remark: Each value is presented as mean standard deviation (n=3).
The letters ns are displayed in column. There was no significant different ($p>0.05$).
ND is not detectable.

Table 2 Peel color L* values during ambient storage of hot water-treated and active MAP-held organic chili pepper subjected to different durations of cold storage.

Cold storage duration (days)	Days at ambient					
	0[ns]	1[ns]	2[ns]	3[ns]	4[ns]	5
0	40.78±0.46	40.41±0.41	40.11±0.49	ND	ND	ND
4	40.78±1.12	40.67±1.11	40.52±0.99	39.95±0.56	39.87±0.54	39.77±0.55
8	41.38±1.45	41.13±1.78	40.55±1.19	39.49±0.62	39.57±0.49	ND
12	40.31±0.95	40.43±0.98	40.32±0.91	39.49±0.62	39.90±0.71	ND
16	40.82±0.72	40.08±0.28	39.85±1.12	39.65±1.11	ND	ND

Remark: Each value is presented as mean standard deviation (n=3).
The letters ns are displayed in column. There was no significant different ($p>0.05$).
ND is not detectable.

Weight loss increased with increasing period of ambient holding (Table 3). The duration of cold storage had a significant effect (Table 3). After one day of ambient holding, the fruit that was not held under cold storage (0 day, control) and those cold-stored for 16 days had markedly higher weight loss of 1-2% than fruit cold-stored for 4-12 days which had less than 1% weight loss. After three days of ambient holding when only cold-stored fruit remained, fruit cold-stored for 16 days had much higher weight loss (about 4%) than fruit cold-stored for 4-12 days (less than 2%). Differences in weight loss of fruit cold-stored for 4-12 days were not significant.

Decay set in faster in the control fruit than in fruit subjected to cold storage (Table 4). After 2 days of ambient holding, more than 20% of control fruit had decay while cold-stored fruit had less than 20% decay. Among cold-stored fruit, fruit stored for 16 days had significantly higher decay incidence after 1-3 days of ambient holding than fruit stored for 4-12 days. Fruit held in cold storage for 12 days had the lowest decay after 3 days of ambient holding.

Table 3 Weight loss (%) during ambient storage of hot water-treated and active MAP-held organic chili pepper subjected to different durations of cold storage.

Cold storage duration (days)	Days at ambient					
	0	1	2	3	4[ns]	5
0	0.00	1.14±0.47[b]	1.93±0.02[a]	ND	ND	ND
4	0.00	0.76±0.18[b]	1.30±0.07[b]	1.38±0.01[b]	1.73±0.76	1.76±0.77
8	0.00	0.65±0.35[b]	1.79±0.18[a]	1.69±0.06[b]	1.91±0.08	ND
12	0.00	0.67±0.05[b]	1.34±0.03[b]	1.75±0.60[b]	1.80±0.56	ND
16	0.00	1.96±0.03[a]	1.94±0.05[a]	3.98±0.10[a]	ND	ND

Remark: Each value is presented as mean standard deviation (n=3).
Mean with ns in the same column are not significantly different ($p>0.05$).
Mean with a-b in the same column are significantly different ($p\leq0.05$).
ND is not detectable.

Table 4 Decay (%) during ambient storage of hot water-treated and active MAP-held organic chili pepper subjected to different durations of cold storage.

Cold storage duration (days)	Days at ambient					
	0	1	2	3	4	5
0	0.00	14.67±0.58[a]	22.67±2.52a	ND	ND	ND
4	0.00	6.33±0.58[c]	12.67±0.58[c]	15.41±0.52[c]	17.24±0.41[b]	18.41±0.52
8	0.00	6.87±0.32[c]	13.15±0.26[c]	17.00±0.23[b]	18.13±0.23[a]	ND
12	0.00	6.50±0.62[c]	11.22±1.01[c]	13.67±1.53[d]	17.34±0.59[b]	ND
16	0.00	10.66±0.77[b]	16.12±0.19[b]	20.16±0.06[a]	ND	ND

Remark: Each value is presented as mean standard deviation (n=3).
Mean with a-c in the same column are significantly different ($p\leq0.05$).
ND is not detectable.

Titratable acidity (TA) slightly increased during ambient holding and was significantly affected by the treatments only after 3 days at ambient when only cold-stored fruit remained marketable (Table 5). However, TA did not widely vary as it ranged only from 0.20-0.24% citric acid regardless of the duration of cold storage.

Ambient shelf life was shortest (less than two days) in control fruit (no cold storage) (Fig. 1). Cold storage increased shelf life but prolonged storage for 16 days shortened the shelf life at ambient to less than three

days. This was apparently caused by the higher weight loss and decay than that of fruit cold stored for 4-12 days which lasted for more than three days at ambient. Based on the results, cold storage of 4-12 days is a safe holding period that has no adverse effect on shelf life of chili pepper during subsequent holding at ambient.

Table 5 Titratable acidity (%) during ambient storage of hot water-treated and active MAP-held organic chili pepper subjected to different durations of cold storage.

Cold storage duration (days)	Days at ambient					
	0[ns]	1[ns]	2[ns]	3	4[ns]	5
0	0.16±0.04	0.19±0.01	0.20±0.01	ND	ND	ND
4	0.16±0.00	0.18±0.00	0.24±0.04	0.22±0.01[ab]	0.21±0.01	0.19±0.00
8	0.18±0.01	0.19±0.01	0.25±0.02	0.24±0.02[a]	0.23±0.02	ND
12	0.16±0.04	0.21±0.02	0.22±0.01	0.23±0.01[a]	0.22±0.02	ND
16	0.15±0.05	0.20±0.03	0.22±0.01	0.20±0.01[b]	ND	ND

Remark: Each value is presented as mean standard deviation (n=3)
Mean with ns in the same column are not significantly different ($p>0.05$).
Mean with a-b in the same column are significantly different ($p\leq0.05$).
ND is not detectable.

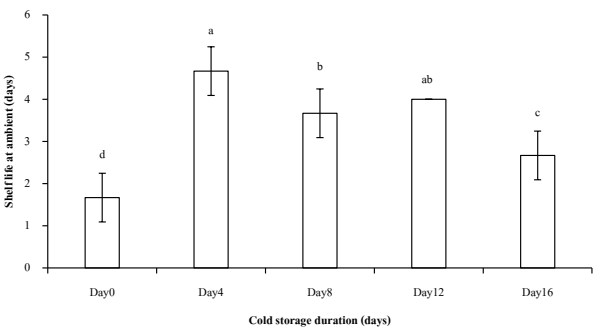

Fig. 1 Shelf life at ambient of hot water-treated and active MAP-held organic chili pepper subjected to different durations of cold storage. Error bars indicated standard deviation from mean values (n=3). Bar with different letters are significantly different ($p\leq0.05$) according to the Least Significant Difference (LSD) test.

Chili peppers have a very short postharvest life and proper temperature management in combination with other techniques is important to improve the shelf life. In the present study, fruit shelf life without cold storage was less than two days because of high weight loss indicating rapid water loss and high decay incidence indicating rapid rotting and tissue breakdown associated with fruit senescence. Cold storage remarkably increased shelf life. Using 12-day cold storage duration, shelf life increased to 16 days, i.e. 12 days in cold storage plus 4 days at ambient. Extending the cold storage duration to 16 days had detrimental effect as it shortened ambient shelf life to less than three days due to high weight loss and decay, thereby limiting post-cold storage distribution and marketing at ambient. This supported earlier results in which 16-day cold storage did not improve ambient shelf life of organic red chili despite the application of HWD and active MAP (Krongyut & Duangsi, 2015) which were similarly applied in the present study.

Cold storage for at most 12 days seemed to maintain normal metabolism which was sustained during subsequent ambient holding. The rate of metabolic processes after cold storage for 4-12 days and during ambient holding was slowed as the fruit lasted for more than three days at ambient whereas fruit held immediately at ambient (without cold storage) lasted for less than two days. Prolonged cold storage for 16 days seemed to induce non-visible chilling damage as there was no chill-induced fruit discoloration seen based on peel color parameters. This non-visible chilling injury may include alteration in physicochemical processes leading to more rapid quality loss at ambient evidenced as high weight loss and decay. Cold-induced injury could increase the susceptibility of fruit to decay. The cold-induced metabolic alteration seemed to be physical in nature as TA analysis did not suggest abnormality in chemical composition of the fruit. Chilling injury is known to be a result of physical alteration of cell membranes and chili peppers developed visible chilling injury such as peel discoloration at temperatures below 10°C (González-Aguilar et al., 2004). The development of chilling injury in chili peppers and other tropical produce is a major limitation of cold storage which is considered as the single most effective method in prolonging postharvest life of fruit and vegetables (Rodoni et al., 2015).

It is recommended to elucidate the mechanism underlying the adverse effect of longer cold storage duration (e.g. 16 days) on ambient shelf life of organic chili pepper. Changes in cell membrane structure and enzymes associated with chilling injury development can be determined. Understanding these changes can provide clues to maximize the utility of cold storage without compromising ambient shelf life of the fruit. Other safe techniques to increase the resistance of the fruit to chilling storage can be tested, such as calcium treatment (pre- and/or post-harvest application) and surface coating. It is also worth applying conditioning treatments, such as gradual exposure to ambient temperature, in order to improve fruit shelf life at ambient. Alternatively, higher temperatures (e.g. 13°C) that will not induce chilling

injury can be used and supplemented with other postharvest techniques that can prolong shelf life during and after cold storage.

Conclusion

Cold storage for 12 days was the most effective and longest duration that did not adversely affect subsequent shelf life at ambient of chili pepper pre-treated with HWD and then packed in active MAP. Longer cold storage duration seemed to induce non-visible chilling injury which consequently shortened ambient shelf life thereby limiting post-cold storage marketing and holding at ambient.

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Multiplex PCR for Detection of *Staphylococcus aureus* and *Listeria monocytogenes* in Ready-to-eat Foods

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Abstract

Foodborne diseases are common problems worldwide. *Staphylococcus aureus* is one of the most important food-borne pathogens. *Listeria monocytogenes* is widely found in contaminated foods, especially in refrigerated and ready-to-eat foods. Rapid detection and identification are needed to prevent and control the food contamination by these infectious microorganisms. For the objective of the research, multiplex PCR technique has been developed for rapid detection of *S. aureus* and *L. monocytogenes* in ready-to-eat foods. In this study, two-pair of primers were designed within conserved regions of the virulence genes, *coa* gene of *S. aureus* and *prfA* gene of *L. monocytogenes* and then were used for detection of those bacteria. The results showed that this multiplex PCR could detect at least 1 ng of *S. aureus* DNA and 150 pg of *L. monocytogenes* DNA. Investigation into the artificially contaminated foods, this multiplex PCR was able to detect less than 10^4 cells/g of *S. aureus* and 1 cell/g of *L. monocytogenes* in foods. In addition, there were no amplifications of nucleic acids from other food related-pathogens, indicating the specificity of this test. Detections in thirty ready-to-eat food samples from local markets in Chonburi province, Thailand, showed that none of them were contaminated with *S. aureus* and *L. monocytogenes*. Therefore, this finding indicated good hygiene in production of ready-to-eat foods in these areas. Consequently, this multiplex PCR can be further developed and employed for monitoring of *S. aureus* and *L. monocytogenes* in contaminated foods.

Introduction

Foodborne disease is defined by the World Health Organization as a disease of infectious or toxic nature caused by the consumption of contaminated food or water. It is still a major concern worldwide (Ananchaipattana et al., 2012; Laaksonen et al., 2017; Mercado et al., 2012; Paudyal et al., 2017). Staphylococcal food poisoning is one of the most common food-borne diseases resulting from the ingestion of enterotoxigenic strains of *Staphylococcus aureus*. *S. aureus* is a facultative anaerobic gram-positive coccus found in the air, dust, sewage, water and environmental surfaces. *S. aureus* belongs to the normal flora found on the skin and mucous

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membranes of mammals and birds. Some strains of *S. aureus* are able to produce enterotoxin which is the causative agent of staphylococcal food poisoning. The foodstuff or ingredients can be contaminated with toxins in various temperatures that allow the growth of *S. aureus.* However, staphylococcal enterotoxins are highly heat resistant. Many foods can be a good medium growth for *S. aureus* including milk, butter, ham, sausages, cheese, meat, salad, and cook meals. Pasteurization can kill *S. aureus* but has no effect on enterotoxin. The symptoms of staphylococcal food poisoning are abdominal cramps, nausea, vomiting followed by diarrhea. *Listeria monocytogenes* is a gram positive facultative intracellular bacterium found in soil, silage, groundwater sewage and vegetation. *L. monocytogenes* has become an important food-borne pathogen distributed in dairy products, undercooked meat, poultry, seafood, vegetables, ice cream, and especially refrigerated and ready-to-eat foods (Chen et al., 2016; Wilson, 1995). Various ready-to-eat foods contaminated with *L. monocytogenes* have been linked to outbreaks and illnesses (Garner & Kathariou, 2016). In a healthy person, *L. monocytogenes* causes gastroenteritis which can resolve itself without treatment. Whereas immunocompromised individuals, the bacterium can cause systemic infections that lead to meningitis, encephalitis (Barocci et al., 2015). *L. monocytogenes* synthesizes a number of secreted virulence factors whose expression is regulated by the transcriptional activator *prfA*. Moreover, it has become a serious issue to the food industry due to its ability to survive in most common food processing conditions such as high pH, high salt concentration, low water content, and refrigeration temperature. The microbiological food testing is necessary to prevent food contamination and outbreaks of foodborne illness. Commonly food products are usually contaminated at low levels (i.e. <100 CFU/g). However, up to now there is still not a specific method for testing foods.

Conventional microbiological methods for detection and identification of the pathogens in foods involve culture methods regarding enrichment and biochemical tests, which are usually very sensitive. However, these methods need a long time such as 3 to 5 days. Molecular methods based on multiplex polymerase chain reaction (PCR) technology for rapid detection of these pathogens have been developed (D'Amico & Donnelly, 2008, 2009; Kumar et al., 2009). These methods can reduce the time for detection of contaminated foods. In this study, multiplex PCR based on using primers for *coa* gene which encode coagulase, a major virulent factor of *S. aureus* and *prfA* gene encoding a transcriptional activator and is required for expression of virulence gene of *L. monocytogenes,* were developed and used for detection of those bacteria in ready-to-eat foods. Therefore, the aim of this research is to develop the multiplex PCR for detecting foodborne pathogens, *L. monocytogenes* and *S. aureus.* Several local ready-to-eat foods were tested for contamination by using this method.

Materials and methods

1. Microorganisms

S. aureus, L. monocytogenes, Escherichia coli and *Vibrio cholerae* used in this study were obtained from Faculty of Allied Health Sciences, Burapha University. Bacteria were grown in LB medium at 37°C for 18 h.

2. Primer design

The primers targeting of *coa* and *prfA* gene were designed by Bioedit (version 7.0.4) based on the National Center for Biotechnology Information (NCBI). Primers were analyzed by Oligos (version 9.1) and Standard Nucleotide BLAST for the specification. The forward and reverse primers; F-5'GGGATAACAAAG CAGATGCG-3', R-5'-ACGTTGATTCAGTACCTT GTG-3', amplify a 1,353 bp fragment of *coa* gene of *S. aureus.* The forward and reverse primers; F-5'GAG TATTAGCGAGAACGGGA-3', R-5'-TAACAGCT GAGCTATGTGCG-3', amplify a 420 bp fragment of *prfA* gene of *L. monocytogenes.*

3. DNA extraction

Bacterial DNAs were extracted by commercial DNA extraction kit (GF-1 Bacterial DNA Extraction Kit) and tested for the sensitivity of monoplex PCR and multiplex PCR.

4. Monoplex PCR

Each primer pair was used as a specific primer for detection of each bacterial specie. The primer pair for *coa* gene was used to amplify *S. aureus* DNA. The primer pair for *prfA* gene was used to amplify *L. monocytogenes* DNA. The PCR reaction consisting of *Taq* buffer with KCl, 0.2 mM of dNTPs, 1 µM of primer for *coa* gene or primer for *prfA* gene, 0.5 U of *Taq* DNA polymerase (DyNAzyme II DNA polymerase, Finnzymes Oy, Finland) and 1 µl of DNA template, in a final volume of 25 µl. Negative control of amplification was performed with nuclease-free water instead of the DNA template. Each PCR reaction were done in a thermal cycler

(GeneAmp PCR System 9700 (PE Applied Biosystems, Norwalk, CT, USA). Amplification condition including initial denaturation at 94 °C for 2 min and 30 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min. A final extension was performed for 5 min at 72 °C.

5. Multiplex PCR

The multiplex PCR was carried out using specific two-pair of primers. The forward and reverse primers; F-5'-GGGATAACAAAGCAGATGCG-3', R-5'- AC GTTGATTCAGTACCTTGTG-3', amplify a 1,353 bp fragment of *coa* gene of *S. aureus*. The forward and reverse primers; F-5'-GAGTATTAGCGAGAACGG-GA-3', R-5'-TAACAGCTGAGCTATGTGCG-3', amplify a 420 bp fragment of *prfA* gene of *L. monocytogenes*. PCR amplification was conducted in a solution containing *Taq* buffer with KCl, 0.2 mM of dNTPs, 1.2 µM of each primer for *coa* gene and 1 µM of each primer for *prfA* gene, 0.5 U of *Taq* DNA polymerase (DyNAzyme II DNA polymerase, Finnzymes Oy, Finland) and 1 µl of DNA template, in a final volume of 25 µl. Negative control of amplification was performed with nuclease-free water instead of the DNA template. Reactions were carried out in a GeneAmp PCR System 9700 (PE Applied Biosystems, Norwalk, CT, USA) thermal cycler. Amplification following initial denaturation at 94 °C for 2 min was performed in 30 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min. A final extension was performed for 5 min at 72 °C.

6. Artificially contaminated food samples

Sensitivity assays were carried out for artificially inoculated sample. *S. aureus* and *L. monocytogenes* were grown in LB medium at 37°C for 18 h, after cell counting by haemacytometer, the cells were 10-fold diluted in LB medium ranging from 1 to 10^6 cells/ml. Cell counting was confirmed by colony plate count. One milliliter of each dilution or LB medium only as negative control was added into 5 grams of ready-to-eat food (sushi) in 13 ml of LB medium. The foods were sterilized by autoclaving before being artificially contaminated. The samples were homogenized and enriched by incubation at 37°C for 6 h. One milliliter of the enriched sample was taken and centrifuged at 500 rpm for 5 min to eliminate food particles and then at 6000 rpm for 5 min to collect bacterial cells, finally the pellets were washed twice with 0.8% NaCl and subjected to DNA extraction with Qiagen DNA extraction kit.

7. Naturally contaminated food samples

Thirty ready-to-eat foods including sushi, sausage, undercooked meat, dairy products, refrigerated and ready-to-eat foods were randomly purchased from local markets in Chonburi province during January to February 2015 and freshly processed. Before the investigation of bacterial contamination by the multiplex PCR, samples were homogenized, enriched and prepared by the process as described above.

8. DNA analysis by gel electrophoresis

Amplified products were analyzed by electrophoresis through 1% agarose gel. One microliter of the PCR product was stained with SYBR gold and analyzed by gel electrophoresis in 1% agarose gel at 110 V for 25 min. GeneRuler 100 bp (MBI Fermentas, St. Leon-Rot, Germany) was used as a DNA marker.

Results

1. Detection of *S. aureus* and *L. monocytogenes* by specific primers

The primer pair synthesized for *S. aureus* allowed PCR amplification of a 1,353 bp. Figure 1 shows the result obtained when *S. aureus* DNA was amplified and tested in the PCR using specific primer for *coa* gene of *S. aureus*. Amplification of *L. monocytogenes* DNA using the designed primer pair results in presence of 420 bp product. Therefore, both *S. aureus* and *L. monocytogenes* strains can be amplified while negative control were not amplified (Fig. 1). Moreover, other bacteria such as *E. coli* and *Vibrio* sp. did not presence the amplification (data not shown).

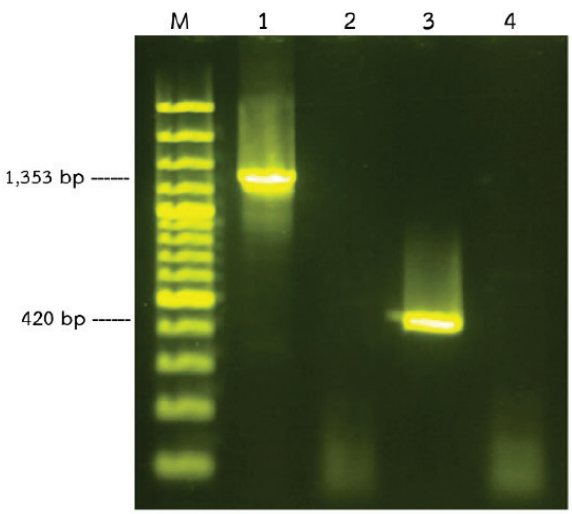

Fig. 1 Amplified products from purified *S. aureus* and *L. monocytogenes* DNA by using its specific primer. (lane M: 100 bp DNA marker, lane 1: *S. aureus* DNA, lane 3: *L. monocytogenes* DNA, lane 2 and 4: negative control (use DNAase-free water instant of bacterial DNA)

2. Detection of *S. aureus* and *L. monocytogenes* by multiplex PCR

Specific primer pair for *coa* gene of *S. aureus* and *prfA* gene of *L. monocytogenes* were used for multiplex PCR. The conditions of the multiplex PCR were optimized as described in the method. When both purified *S. aureus* and *L. monocytogenes* DNA were used as template, it showed that PCR products of 1,353 bp and 420 bp were amplified from *S. aureus* and *L. monocytogenes,* respectively (Fig. 2, lane 1).

Specificity of the multiplex PCR was tested with other food pathogens including *E. coli* and *V. cholerae. S. aureus, L. monocytogenes* and *E. coli* or *V. cholerae.* They were performed with the multiplex PCR in the same condition. The result showed that there was no amplification from *E. coli* or *V. cholerae* (Fig. 2, lane 2 and 3). Therefore, these *coa* and *prfA* primer pairs have more specificity for *S. aureus* and *L. monocytogenes.*

The investigation of sensitivity was tested by different amounts of each DNA, the results showed that the multiplex PCR can detect at least 1 ng of *S. aureus* DNA (Fig. 3) and 150 pg of *L. monocytogenes* DNA (Fig. 4).

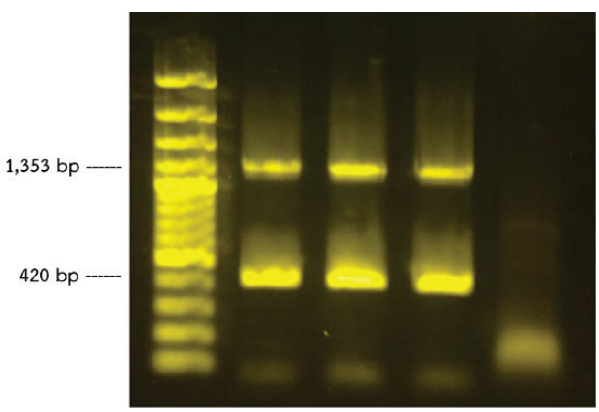

Fig. 2 Amplified products from *S. aureus* DNA (1,353 bp) and *L. monocytogenes* DNA (420 bp) by using the multiplex PCR. (lane M: 100 bp DNA marker, lane 1 : PCR products from *S. aureus* and *L. monocytogenes* DNA; lane 2: PCR products from *S. aureus, L. monocytogenes,* and *E. coli* DNA; lane 3: PCR products from *S. aureus*, *L. monocytogenes*, and *V. cholerae* DNA; lane 4: negative control)

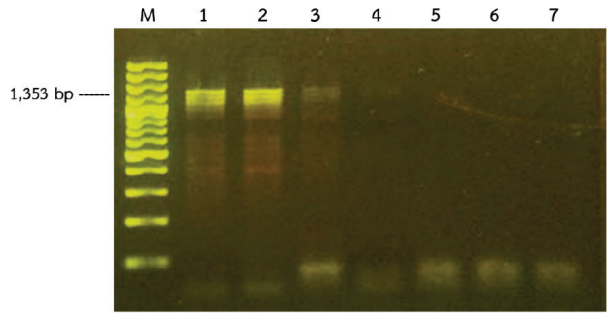

Fig. 3 Sensitivity of the multiplex PCR for detecting of *S. aureus* DNA. Different amounts of *S. aureus* DNA from 1 µg to 10 pg was used as template for the multiplex PCR. (lane M: 100 bp DNA marker, lane 1-7: 1 µg, 100 ng, 10 ng, 1 ng, 100 pg, 10 pg, and negative control, respectively)

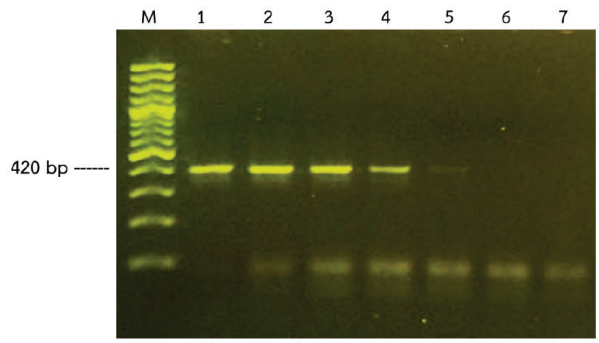

Fig. 4 Sensitivity of the multiplex PCR for detecting of *L. monocytogenes* DNA. Different amounts of *L. monocytogenes* DNA from 1.5 µg to 15 pg was used as template for the multiplex PCR. (lane M: 100 bp DNA marker, lane 1-7: 1.5 µg, 0.15 µg, 15 ng, 1.5 ng, 150 pg, 15 pg, and negative control)

3. Investigation of multiplex PCR in artificially contaminated food

Food samples were added with *L. monocytogenes* or *S. aureus* cells in different concentrations. After enrichment and DNA extraction, the multiplex PCR was able to detect *L. monocytogenes* less than 1 cell/5 g of food (1 cell/g) and 10^5 *S. aureus* cell/5 g of food (2 x 10^4 cells/g) (Fig. 5). Furthermore, there was no amplification for detection of related food pathogens such as *E. coli* and *Vibrio cholera,* represented specificity of the multiplex PCR.

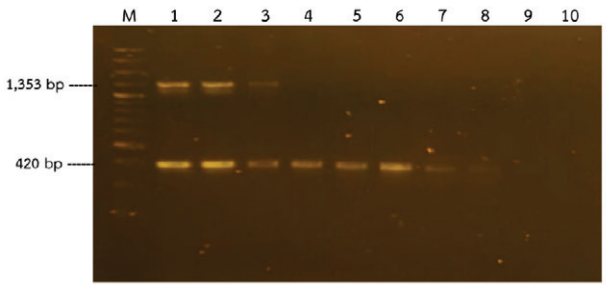

Fig. 5 Sensitivity of the multiplex PCR for detecting of *S. aureus* and *L. monocytogenes* cells in artificially contaminated food. (lane M: 100 bp DNA marker, lane 1: positive control, lane 2-8: 10^6,10^5, 10^4, 10^3, 10^2, 10, 1 cells of each *S. aureus* and *L. monocytogenes*, lane 9-10: negative control)

4. Detection of food samples by using the multiplex PCR

The multiplex PCR developed was used as a tool to detect contaminated foods in local markets. Thirty samples of several types of ready-to-eat foods such as sushi, sausages, undercooked meat, dairy products and refrigerated ready-to-eat foods were randomly purchased and tested. Sample preparation, DNA extraction and detection by multiplex PCR was done as described. The results showed none of them were contaminated with *L. monocytogenes* and *S. aureus.*

Discussion

Contaminated foods represent a risk of infection. Some types of contamination may be caused by infection existing in animals such as products from meat, pork, and chicken. Moreover, food might be contaminated during processing. Contamination of *S. aureus* and *L. monocytogenes* in food is regarded as poor hygiene of food manufacturing. Bacterial identification by conventional method based on culturing and biochemical testing requires several days. Moreover, negative result may be due to presence of antimicrobial agents which can be found in some foods. The development of PCR-based method in this study provides more rapid identification and is able to identify more than one specie.

In this study, *S. aureus* and *L. monocytogenes* were identified using primer for *coa* gene and *prfA* gene, respectively. Coagulase production is a common characteristic for identification of *S. aureus*. *PrfA* gene is and transcriptional activator, required for expression of virulence gene of *L. monocytogenes*. Many studies have developed PCR using various primers (Akineden et al., 2001; Alarcon et al., 2006; Kearns et al., 1999; Li et al., 2008).

Our multiplex PCR showed high sensitivity for detection of *S. aureus* and *L. monocytogenes,* which allowed the detection of 1 ng and 150 pg of the bacterial DNA, respectively. Investigation in artificial contaminated food revealed that 10^4 cell/g of *S. aureus* cells and 1 cell/g of *L. monocytogenes* cells were detected by the multiplex PCR. Infectious dose of *S. aureus* and *L. monocytogenes* is 10^5 - 10^6 cells and 10^3 cells, respectively (Schmid-Hempel & Frank, 2007). Consequently, our multiplex PCR is appropriate to detect those bacteria underneath the range of the infectious doses.

There are many problems when using PCR as a tool for detecting of bacteria directly from foods. They may be due to various factors in foods that can inhibit PCR reaction including inappropriate DNA extraction, low number of bacteria cells, and other unforeseen issues (Aznar & Alarcon, 2003). A variety of substances in foods such as thermonuclease enzyme in milk has been described in inhibiting the PCR and they usually cause false negative results (Wilson et al., 1994). Therefore, increased levels of sensitivity of the PCR are required. Sensitivity of identification of bacteria in food can be improved by a suitable extraction of bacteria. The commercial DNA extraction kit was used in this study, which provided high yield of DNA rather than another method such as the boiling method.

From our investigation, the randomly sampling of ready-to-eat foods in local markets for detecting of *L. monocytogenes* and *S. aureus,* showed that no contaminated foods were detected by using the developed PCR. However, it assessed only pathogens that are mostly found in ready-to-eat foods. Other food-borne pathogens, including *Escherichia coli, Salmonella enteritis, Bacillus cereus, Campylobacter jejuni* and their toxins were not assessed in this study. The determination of ready-to-eat foods in this study was indicated good for microbiological quality, while also revealing a good quality for processing, nevertheless the foods may present preservatives, including acids and salt. Further investigation of contaminated foods should be performed in more samples and locations, which will be adequate to represent food safety in the area.

In many cases of listeriosis outbreaks from foods, the amount of the cells are significantly higher than 1,000 CFU/g (Thevenot et al., 2006). Detection of bacterial contamination in 137 raw food samples from open-markets in Thailand during 2010 to 2011, 5% of samples were contaminated with *L. monocytogenes* and were obtained from meat and fish samples, 39% of samples were

contaminated with *Staphylococcus* spp. which were found in vegetables and fish samples (Ananchaipattana et al., 2012). However, our study investigated the ready-to-eat foods and found no evidence of *S. aureus* or *L. monocytogenes* contamination, indicating a good food processing practice.

Conclusion

The developed multiplex PCR in this study can be used as a rapid, high sensitive and specific method for detection of *S. aureus* and *L. monocytogenes* in contaminated ready-to-eat foods. Investigation of the food pathogens in the ready-to-eat foods also indicates the degree of good hygiene of the foods in these areas. Currently, there are many new techniques that have been developed such as real time-PCR, however multiplex PCR method is still appropriate in laboratory routines and where the other new techniques are not available. Furthermore, multiplex PCR is cost effective and still rapid for the detection. Consequently, this multiplex PCR assay can be further developed for monitoring of *S. aureus* and *L. monocytogenes* contaminated foods. Moreover, it could be a benefit in terms of public health and surveillance of disease outbreaks.

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Complementary Cancer Treatment at Wat Khampramong: Thai Traditional Healthcare

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Abstract

The research documents the origin, practices and cultural format of Thai traditional healthcare in the Buddhist temple, Wat Khampramong, in Sakon Nakhon province and to study the cultural format of Thai traditional healthcare at that temple. The research is a qualitative study and the data analyzed through data triangulation method from data gathered from document analysis, field research data obtained from 25 research informants through the use of survey forms, interview forms, observation forms and from focus group discussions. The results found that the complementary healthcare treatment at Wat Khampramong consists of: 1. Modern medical treatment by licensed nurses who perform weekly blood samples of cancer patients, 2. Treatment by alternative methods, cancer treating herbal medicine, acupuncture and nutritional therapy, 3. Utilizing Buddhism principles through meditation, prayers, Chong Krom pacing therapy, Qigong aerobics, music and nature therapy and 4. Provide transfer service for critical patients to hospitals. The healthcare personnel at the temple include Phra Paponpatchara Jirathamo the abbot of Wat Khampramong working together with doctors, nurses, pharmacists and public health volunteers. The herbal medicine remedy for cancer treatment at the temple was created by Phra Paponpatchara Jirathamo during meditation in 2003. The complementary treatment at Wat Khampramong Temple provided opportunities for both the patient and their family to participate in religious activities at the temple and they also receive spiritual treatment which is an important factor affecting both the patients' physical and mental fitness. The Buddhist prayers and sermons help create the proper and correct environment for patients in terminal situations and will lessen the impact of the fear of dying so that the patients are at peace. The complementary treatment also provides the patient with the strength to face the reality of death and live their final moments in peace and happiness.

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Introduction

Wat Khampramong is located in Tambon Sawang, Amphoe Phanna Nikhom in Sakon Nakhon Province. The Buddhist temple is a complementary healthcare center for cancer patients that integrates Thai traditional herbal medicine and modern medical practices. Phra Paponpatchara Jirathamo, the abbot of the temple, invented the herbal cancer remedy called "Yot Mareng" (cancer apex) which is derived from an ancient Thai cancer remedy called "Phet Nam Nueng". Phra Paponpatchara Jirathamo, first tried the Yot Mareng remedy on himself to treat his maxillary sinus cancer and through continued treatment of his cancer through Yot Mareng was successful and he is now cancer free. Phra PaponpatcharaJirathamo created the Thai Herbal Nursing Center at Wat Khampramong Temple and initial care for late stage cancer patients in 2003 and would later become the temple's Arokhayasarn hospice in 2005, providing palliative care to late stage cancer patients. The temple's Arokhayasarn hospice still continues to offer cost free palliative care to cancer patients up to the present-day (Khampramong, 2019). The center does not reject modern medical practices or treatment but the primary cancer treatment at the temple relies on the Yot Mareng herbal remedy. The patient's relatives participate in providing nursing care and support the medical team and healthcare volunteers at the temple. Patients travel to the hospice from close and far communities in Thailand and from abroad. The main factors for their decision to come to the hospice as established by Poonthananiwatkul et al. (2015) include a positive attitude towards herbal medicines and their own previous use of them, dissatisfaction with conventional treatment, the home environment and poor relationships with hospital doctors. There have been more than 5,200 cancer patients tended at Wat Khampramong since 2003 which includes Thai citizens and foreign patients. The treatment is without clause and provided to everyone, free of cost. The temple relies on donations for funding and donations have surpassed more than 100 million baht in 2016. The continued success and popularity of palliative cancer treatment through complementary methods at Wat Khampramong has provided an alternative and supplemental treatment. Success and acceptance of complementary/alternative medicine has increasingly become popular for cancer treatment (Richardson et al., 2016; Cassileth & Chapman, 1996; Tascilar et al., 2006). The research is therefor aimed to document the origin, practices and cultural format of Thai traditional healthcare in the Buddhist temple, Wat Khampramong, to study the cultural format of Thai traditional healthcare through herbal medicine which offers benefits to patients, and to add to the knowledge and practicality of complementary cancer treatment to communities in Thailand and abroad.

Materials and methods

The research was conducted between April 2015 and September 2016. Qualitative methodology was applied which focused on the origin, Thai traditional healthcare practices and activities at Wat Khampramong in Sakon Nakhon Province.

The population and research informants consist of 25 individuals. Key informants include 3 individuals who are experienced traditional healthcare practitioners and licensed nursing professionals at Wat Khampramong. Casual informants included 7 individuals. 1 individual is the supervisor of the Thai traditional healthcare treatment, 1 individual from the pharmaceutical at the temple and 5 volunteer caregivers at the temple. General informants include 15 former patients and family caregivers at the temple.

The research tools included basic surveys to gather information on the basics of the Thai traditional healthcare at the temple, participatory and non-participatory observation forms were utilized during joint activities with the sampling group to record information on the format and detailed practices of Thai traditional healthcare at the temple.Structured interviews and non-structured interview forms were used to collect data on the origin and background of the Thai traditional healthcare and the activities and the processes involved in nursing and caring for patients. Focus group discussions were recorded during data collection, questioning and interviews with the research informants to debate and discuss the research findings and to also exchange knowledge on the cultural format of Thai traditional healthcare applied at the temple. The research findings from field research and data from document analysis from documents and published papers were verified through data triangulation.

Results

Wat Khampramong is recognized for palliative care and nursing of late stage cancer patients through complementary treatment which consists of 4 methods:

1. Modern medical treatment by licensed nurses who perform weekly blood samplings of cancer patients. 2. Treatment by alternative methods, cancer treating herbal medicine, acupuncture and nutritional therapy. 3. Utilizing Buddhism principles through meditation, prayers, Chong Krom pacing therapy, Qigong Aerobics, music and nature therapy and 4. Provide transfer service for critical patients to hospitals. The healthcare officials at the temple include Phra Paponpatchara Jirathamo, the abbot of Wat Khampramong working together with doctors, nurses, pharmacists and public health volunteers. Herbal medicine treatment at the temple was crafted by Phra Paponpatchara Jirathamo during meditation that resulted in the hospice's herbal medicine remedy for cancer called "Yot Mareng" (cancer apex). The Yot Mareng cancer remedy was based on the herbal medicine remedy called "Phet Nam Nueng" which is derived from a basic herbal remedy described in Treatises on Thai traditional medicine and Pharmacognosy book. The Yot Mareng herbal remedy ingredients are boiled with water and portioned down to 250 milliliter doses for cancer patients to take before breakfast, lunch and dinner.

Wat Khampramong cancer treatment procedures

1. Types of cancer patients that are accepted

1.1 Final stage cancer that has been terminally diagnosed through modern medicine such as liver cancer or gall bladder cancer patients.

1.2 Cancer patients that desire to extend their Thai traditional treatment.

1.3 Cancer patients who prefer Thai traditional treatment.

1.4 Patients who are at risk of cancer such as individuals who are infected with Hepatitis B.

1.5 Patients who cannot afford the cost of modern medical facilities.

2. Patient preparation

2.1 The patient or relative must submit their medical records such as: biopsy, blood parameters, cancer tissue count, surgical records, chemotherapy records, x-rays, MRI, CT scan or ultrasound.

2.2 The patient must have at least 1 caregiver such as a relative who can assist them during their treatment at the temple.

2.3 Patients must notify the staff if they have any congenital diseases and also prepare their own medicine for any congenital diseases they might have. They must also alert the staff to their allergies, food or medicine that they are allergic too.

3. Items that the patient must bring with them.

3.1 Set of cooking pots with handles that is appropriate for boiling or warming herbal remedy prescribed by the temple. The pots are a necessity, because the patient is responsible for warming their herbal medicine before taking it on a daily basis at the temple and also at home.

3.2 Patients are encouraged to stay at least 2 weeks for their treatment so that they may understand and replicate the treatment at their residence.

3.3 Relatives or friends who are accompanying the patient should be healthy and strong enough to help patients through exercises, help with the preparation of the herbal medicine, food preparation and also understand the treatment and practices that are to be performed at their residence.

Thai traditional cancer treatment

1. The orientation process is a necessary step in helping patients and their relatives to understand the constraint and purpose of the temple's Thai traditional nursing. Patients that are too frail to complete the schedule, must rely on relatives or friends that accompany them as family caregivers (Table 1).

Table 1 Wat Khampramong healthcare treatment schedule

Morning Session	
7.00 a.m.	Patients and relatives are opted to participate in alms giving, mediation and breathing practices and attend Buddhist sermons.
8:00 a.m.	New patients will receive appointments for herbal remedy detoxification that is in accordance with the patient's essence through Traditional Thai Healthcare classifications.
8:30 – 11:00 a.m.	Measurement of fever temperature, blood pressure and heart rate readings, diagnosis and receive medication.
Afternoon - Evening Session	
19:00 – 22:00 p.m.	Meditation and prayer training, receive herbal medicine, receive medication as prescribed by visiting doctors, receive appointments for approved herbal remedy detoxification, receive appointment on how to make and prepare herbal cures.

2. Verification of the patient's condition and treatment is based on Buddhism and is part of the complementary healthcare treatment which integrates the knowledge and methods of modern medicine and Thai traditional medicine and cultural practices. The complementary healthcare treatment is a supplementary treatment which will provide cancer patients with the most benefits and is focused on the mind, social and spirit. The goal of the temple is to provide holistic care for treating the patient's

illness and also activities and knowledge that will enable the patient and relatives to emulate and continue the treatment practices if they have to return home.

3. Wat Khampramong cancer treatment and healthcare activities is as follows:

3.1 Prayers: Prayers is the communication between an individual and a higher power as according to an individual's religious belief. Buddhist will pray in remembrance of the Buddha. Christians pray to the Holy Spirit and Muslims pray to Allah. Prayer activities at Wat Khampramong is open to patients of different worships and together they perform prayers of their worship together in the morning and evening. The activity is not segregated and there is also an area allocated for those who are not bound by religious belief to meditate during the prayers in peace.

3.2 Meditation: Meditation therapy at Wat Khampramong is for cancer patients to learn how to meditate and gain hope, how to walk and meditate through the Doen Chong Krom pacing technique, teach how to regularly perform Charoen Mora Na Nu Sati prayers (remembrance of death). The treatment techniques through mediation are to create an understanding of the cycle of life and to accept the changes in life from birth, aging, sickness and death. Accepting the truth of the eventuality of death is the beginning of mental health treatment so the mind is at peace. This is necessary for terminal cancer patients to have strength to live in peace and in consciousness for the remainder of their life. Cancer patients are directly taught by Phra Paponpatchara Jirathamo in which the abbot teaches to pray for hope through a sermon called Thamma 9 Nathi Kon Tai (9 minute prayer before dying). The Thamma 9 Nathi Kon Tai sermon, teaches oneself to be in constant relevance of death and not underestimate the importance of life.

3.3 Exercise: Cancer patients are suggested to perform light exercises that they are physically capable of and include flexible activities with a duration of 15-30 minutes each. These exercise include such as yoga, body exercises and Thai qigong. Qigong exercises is a holistic system of coordinated body posture and movement, breathing, and meditation used for the purposes of health, and spirituality.

3.4 Art Therapy: Art therapy was derived from the idea that the creative process in making art will nourish and create a self-healing process, promoting a higher quality of life (Cutler et al., 2011). The treatment is utilized at the temple to nurse the patient's feeling and help them to understand themselves. Art therapy is affective in removing negative thoughts and feelings. The elimination of negativity from the body will open up the mind to change and creativity, leading to a more focused meditation and relaxation of the mind.

3.5 Laughing Therapy: Laughing therapy is a group treatment at Wat Khampramong which helps to elevate the mood and feelings of patients and their relatives. Voluntary laughter or forced laughter is a mind exercise and is an important part in social bonding (Kataria, 2002).

3.6 Music Therapy: Music therapy affects the physical and mental health of patients and can be used together with medical treatment. Music therapy is affective against pain, lowering anxiety and fear, increase motor functions and entices consciousness, thoughts, and feelings promoting a healthy spirit.

3.7 Nutrition: Nutrition therapy at Wat Khampramong for cancer patients focuses on a healthy nutritional diet that is flexible and appropriate to the condition of the patient. Patients are given simple suggestions towards nutritional habits that include both suggested and prohibited foods and nourishments while receiving treatment. Prohibited foods include protein from meats, frogs, turtles, ray fish, ducks, geese, bird eggs, fermented fish, fermented shrimp, pickled fish, fermented sour sausages, pickled crabs, sea crabs, sea foods, crustaceans, beef, buffalo meat, fish organs, pickled seafood, chicken eggs, dairy products, animal fats, sweet fruits, jack fruit, longan, durian, banana, sapodilla plum, coconut milk or cream, whitened or polished rice, white sugar, watermelons, coconut, bamboo shoots, cucumbers, piper sarmentosum leaves, guava's, green beans, mung beans, peanuts, soybeans, tofu, mushrooms, sesame oil, soybean oil, flavorings and spices with preservatives, seasoning powder with monosodium glutamate, fish sauce, shrimp paste, pickled vegetables, food coloring, tea, coffee, sweetened birds nest, chicken extracts and malt coco drinks. Suggestions also extends to the use of utensils, prohibiting the use of aluminum and plastics pots and pans. Patients and their relatives are supported in using cookware and using utensils made from earthenware, stainless steel, enamelware and wood. Foods that should be eaten include proteins from garden peas, sprouts, cow-peas, string beans, fresh vegetables, fresh fruits that are not too sweet, and vegetable juice. Vegetable and fruits should be submerged in water with activated charcoal to absorb toxins from the vegetables and fruits.

3.8 Continual Care: Wat Khampramong keeps in contact and extend their nursing care to patients even after they have left the temple. Most patients stay for the

minimal requirement of two weeks for their treatment and are allowed to go home after they have recuperated. Temple staff and volunteers maintain contact through social media channels, email, internet or communicate through the telephone. The continued nursing and advice promotes good relationships between the temple, volunteers, patients and their relatives to get the best care and advice for their treatment.

3.9 Symptoms Management: Symptoms management at Wat Khampramong is focused on relieving physical and mental pain induced from nausea, vomiting and food boredom which are addressed through Buddhist dharma and complementary treatment. Meditation principles are directed at lowering stress and fear in the mind to make both mental and physical aspects of the body to be at peace. Using Dharma and meditation in healthcare treatment is a current trend that is affective, cost saving and creates no additional physical complications or side effects. The effectiveness of self help in dealing with pain through the Buddhist principle of Ariyatsat Si (the four truths) can lower pain in cancer patients that have been through chemotherapy.

3.10 Spiritual Care: Spiritual treatment is essential and affects the mental and physical health of an individual. Spiritual treatment at Wat Khampramong is carried out through religious activities. Buddhist activities include giving alms, water pouring ceremony to offer kindness and compassion to karma. Learning how to boil or make the Yot Mareng herbal remedy is a part of the spiritual remedy and a mandatory activity for all new cancer patients and their family caregiver. Making the herbal remedy is an important process of the cancer treatment at Wat Khampramong and serves the purpose so that the patient's family can replicate the process back at home through delivered ingredients of the herbal remedy in which they will receive from the temple. Additional spiritual treatment include a written confession of their sins that they have done in the past. The confessions are handed directly to Phra Paponpatchara Jirathamo before a sermon and prayers are performed. The confessions must include a recollection of their feelings and lingering regrets that they have not told anyone else such as, attempted suicides, theft, abortions, and abuses.

Discussion

The holistic approach to cancer treatment at Wat Khampramong encompasses physical treatment, modern medical practices, Thai traditional medicine, mental and social treatment and spiritual healing. Treatment through traditional herbal medicine requires the patient to drink the Yot Mareng cancer remedy which is used to adjust the body's equilibrium and strengthen the body's natural immunizing agents. The Yot Mareng remedy will also adjust the body's elemental factors. Mental exercises such as laughing therapy is used to nurture a strong mental fitness. Laughter can help patients cope with cancer by reducing the psychological impact of cancer patients' experiences and shift perspective on a stressful situation (Kuiper et al., 1993). Laughter also decreases serum levels of cortisol, epinephrine, growth hormone, and 4-dihydrophenylacetic acid, indicating a reversal of the stress response (Yim, 2016). A variety of physical exercise at the temple include yoga and Qigong aerobics. Qigong aerobics is also the most recommended aerobic exercise for coronary artery disease and can significantly reduce systolic and diastolic blood pressure (Xiong et al., 2015) and has physiological effects that indicate stabilization of cardiovascular system (Zhao et al., 2018). Volunteer groups also provide supplementary physical fitness activities such as painting and music courses to support the patient's physical rehabilitation. Cancer patients that have undergone chemotherapy are particularly vulnerable to depression and anxiety. Art Therapy can substantially help cancer patients cope and reduce depression (Bar-Sela et al., 2007). Nutritional diets include programs such as, giving up meats, limiting the consumption of dairy products, soymilk, yogurts, egg yolk, vegetable oil, concentrated fats, deep fried foods, seasonings and fruits with high concentration of sugar. The temple also has an in-house garden that grows hydroponic vegetables that are made available to the patients and their families to use in their cooking. Nutritional therapy is also a prominent treatment in Chiang Mai province (Khattiya, 2006), because tribal communities in Northern Thailand believe that many illness and disease stem from improper foods and eating habits. Similar results were also observed by Chimsut (1998), where villagers in rural Tak province believed that nutrition was one of three main causes of illnesses along with physical and supernatural causes.

Mental and social treatments at the temple include, meditation, performing prayers and Chong Krom pacing remedy. These treatments help patients to realize and be mindful of their health and to understand and accept the cycle of life. The social treatments are just as important as the physical treatments which are necessary in helping patients to be aware about the changes to their life and

accept the final stages, which are illness and death. Meditation creates empathy and nurture building good relationships with others. Meditation will also help lower high blood pressure and lower high heart rates. Meditation is knowledge and is the root of knowledge. Meditation creates mental and spiritual freedom, lowers anxiety, leading to a healthy body and mind (Wasi, 2009) and the mental fitness and health of Buddhist practitioners is higher in those who regularly practiced meditation (Moore & Malinowski, 2009). The majority of patients and their spouses were satisfied with the cancer treatment and palliative care at Wat Khampramong (Piew-on, 2011) and they also confirmed that they felt physically better and could eat more food. Consistent results were confirmed by Poonthananiwatkul et al., (2016), that cancer patients reported a significant reduction in symptoms after their stay at the hospice. Many patients have left the temple feeling more pleasant and were not as stressed as before. Terminal patients were able to embrace their fate and were content to live as long as they can and be at peace for the remainder of their lives. The social aspect of healthcare at the temple plays an important part for patients and relatives in coping with their illness and condition with a clear mindset. The mental and social treatments focuses on the positive useful of life and encourages patients not to be mentally tormented by their condition and fate. Patients and their relatives are provided with the opportunity to share and discuss their experiences with others. The surrounding environment and lodgings are adjusted to resemble the patient's home as much as possible. Patients and relatives are provided with opportunities to participate in alms offerings to Buddhist monks and participate in the water pouring ceremony. An important social treatment is to have terminal patients, their relatives and friends exchange their final farewells to each other and forgive each other with Dharma prayers performed in the background.

Conclusion

The treatment at Wat Khampramong provide opportunities for the patient and their family to participate in religious activities at the temple and also prayers according to their individual beliefs. Buddhist practitioners can participate in merit making through alms offerings and water pouring ceremonies to wish happiness to karma. Brewing and boiling herbal medicine is mandatory for all new patients and is crucial step that patients and relatives know how to produce and duplicate the herbal medicine at home through ingredients distributed from the temple to their homes. During the brewing process, the patient is instructed to confess their sins through their written biography and confession. The patient's biography must detail the immoralities and regrets that they still have. Patients are encouraged to write down truths that they cannot tell others such as, abortions, attempted suicide and murder. The biography is followed by the Bangsakul prayer hymns to renounce past sins. The acknowledgment of sins and prayers for forgiveness is essential for cancer patients. Patients are trained to be prepared and face death through acknowledgement and sermons from Phra Paponpatchara Jirathamo, the abbot of Wat Khampramong . The prayers and sermons, help create the proper and correct environment in terminal situations and will lessen the impact of the fear of dying so that the patients are at peace. The treatment is also to provide the patient with the strength to face the reality of death and final moments in peace and happiness, which in Buddhist terms is called tai dee (a good death). Complementary healthcare is essential for terminal cancer treatment at Wat Khampramong. The treatment and care at the temple followed by continual treatment at their residence in supporting the patient until their death so that they can die with dignity is crucial for modern society. The testimonials from the majority of patients and relatives at Wat Khampramong have all looked for alternative options when they are told by hospitals that the disease is in the terminal stages. Cancer treatment through modern medicine is very effective and is the primary choice, but when the disease is in the final stages, many patients are left with limited options and the complementary treatment at Wat Khampramong is a better alternative than just solely transporting the patient to the Intensive Care Unit (ICU) and wait for their passing.

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Utilization of Pineapple Residue for Pineapple Paste and Gluten-free Pie

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Abstract

This research was conducted to optimize the formula to produce pineapple paste and gluten-free pie products. The seven formulas of the pineapple paste consisting of pineapple residue (70-100%), pineapple juice (0-30%) and sugar (0-30%) were studied using Mixture Design. The properties and sensory evaluation by untrained panelists were investigated. The results showed that the pH of pineapple paste tended to decrease when the amount of pineapple juice increased. Apparently, the total soluble solid and brightness of pineapple paste varied directly as the amount of sugar. An appropriate formula of pineapple paste was 75% pineapple residue, 5% pineapple juice and 20% sugar, respectively. This formula had the highest score for all attributes. Next, the nine formulas of gluten-free pie products consisting of mixed flour (60-70%), salted butter (20-40%) and pineapple residue (0-10%) were studied using Mixture Design. The properties and sensory evaluation by untrained panelists were investigated. The results indicated that weight loss of pies varied directly with the amount of butter. The hardness and the brightness of pies varied directly with the amount of mixed flour. Obviously, a suitable formula of gluten-free pie product was 70% mixed flour, 20% salted butter and 10% pineapple residues, respectively. This formula had the highest score for all attributes. In conclusion, utilization of pineapple residue helps provide value-added on agricultural by-products in gluten-free foods.

Introduction

Pineapple is economically the most important crop in Thailand. At present, Thailand is the world's largest exporter of canned pineapple and pineapple juice concentrate in the world. Apparently, Thailand's the major pineapple exporter to markets include the European Union, the United States, Japan and the Middle East. The Thai pineapple sector-generates income into the country at about 23,000-25,000 million baht/year. There are three main products including fresh consumption (18%), canned pineapple and pineapple juice (80%) and other (2%.) (Office of agricultural economics, 2018). Generally, by-products from the processing industry consist of peel, crown, core, stem, and pulp or residue of flesh. By-products may have a particular part that is very different depending on the processing of each factory. However, if it is not used as a benefit, it will turn into fresh waste. Moreover, it becomes a major problem for the environment, and it has high cost for destroying

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the waste. Pineapple residues are by-products from squeezing or removing water from the pineapple flesh. Obviously, these can be consumed including core, and flesh in the shell. The chemical compositions of pineapple residues are protein (3.2-3.6%), fat (1.2-1.3%), fiber (4.7-8.9%) and ash (3.8-4.2%) (Thamkaew & Susirirut, 2017). At present, there are many studies on the utilization of pineapple residues, such as chicken sausage (Mapaya et al., 2016), body scrub (Susirirut et al., 2013), broiler chicken food (Chalermsan et al., 2011) and cookies (Utama-ang & Tepjaikad, 2001) etc. Specifically, the utilization of pineapple residue that can be consumed is very interesting; it not only reduces the amount of waste, but also has value added for by-products or waste from the pineapple processing industry.

Interestingly, pineapple residues are raw materials for food product. Pie is a bakery product which is made from wheat flour or wheat flour mixed with other types of flour. Other ingredients including water, fat, salt, sugar and milk are added in appropriate proportion. These are mixed together until homogeneous. Then, it is pressed into pie molds and baked. In terms of pie filling, it is made with various ingredients such as chicken, ham, preserved fruit, young coconut, corn, custard that can filled before or after baking (Thai industrial standards institute, 2012). However, some consumers have gluten allergic from a protein in wheat flour. The need of gluten-free product sales are forecast to increase by a compound annual growth rate of 10.4% between 2015 to 2020. Moreover, as the clinical utilization and the popularity of the gluten-free diet increase, consumer demands righteously continue to influence the food market and labelling standards of gluten-free products (Khoury et al., 2018). Wheat flour has viscosity and elasticity properties that able to form into the structure of dough by forming a disulfide bond between the amino acid molecules. Gluten contains glutenin and gliadin which has 30% of wheat protein (Surojanametakul, 2013). Utilization of low grade and pineapple residues for production of gluten-free pie product is a new product that can respond to needs of a hereditary intolerance consumer. The objective of this research is to study the prototype formula that is suitable for producing pineapple paste and gluten-free pie products from pineapple residues. Physical properties and sensory evaluation of consumers were investigated. These are keys for selecting the prototype formula. The knowledge gained from this research can be used as a guideline for the production of gluten-free foods and value-added from agricultural by-products.

Materials and methods

1. Raw materials

Low-grade pineapples (*Ananas comosus* cv. Smooth Cayenne) was used in this study which were small in size and of low cost. It was purchased from the local market in Sakaeo province. The ingredients used in pineapple paste and gluten-free pie formulas were composed of sugar (Mitrphol, Suphan Buri, Thailand), salt (Prungthip, Nakorn Ratchasima, Thailand), rice flour (Erawan brand, Nakhon Pathom, Thailand), cassava flour (Fish brand, Nonthaburi, Thailand), potato flour (McGarrett, Bangkok, Thailand), xanthan gum (Tookdee-chemipan, Bangkok, Thailand), salted butter (Orchids, Bangkok, Thailand), fresh eggs (Betagro, Bangkok, Thailand), cream cheese (Philadelphia, Bangkok, Thailand), icing (Imperial, Bangkok, Thailand) and whipping cream (Foremost, Bangkok, Thailand).

2. Pineapple residues and juice preparation

In terms of pineapple residues and juice preparation, low-grade pineapples were washed. Next, they were peeled and cut into small size. All edible parts of pineapple including core and flesh were used. These were blended by juice blender (Tefal DPA 130 La Moulinette, China). Then, it was squeezed and separated into two parts including residues or pulp (solid) and juice (liquid). In terms of residues, the moisture content was not over 10% which is close to residues or pulp from canned pineapple and pineapple juice production in the industry.

3. Pineapple paste preparation

The appropriate ratio of pineapple paste was studied by using mixture designs. The independent factors were the proportions of different components of pineapple residues (70-100%), pineapple juice (0-30%) and sugar (0-30%), respectively. The points on the designated triangle area were selected for studying. The seven formulas of pineapple paste from mixture design were investigated (Table 1). The pineapple residues, pineapple juice and sugar were weighed. Salt was added at 2% of all ingredients (consisting of pineapple residues, pineapple juice and sugar) in all formulas. In terms of pineapple paste production, all ingredients were mixed and stirred at 75 °C for 10 min. Then, it was kept in glass bottles.

Table 1 Formula of pineapple paste by a 3-component* mixture design

Formulas	1	2	3	4	5	6	7
Pineapple residue (%)	70	100	80	75	90	70	75
Pineapple juice (%)	0	0	10	5	5	30	20
Sugar (%)	30	0	10	20	5	0	5

Remark: * a 3-component mixture (100% in the mixture design) was 100% of the total formula.

4. Properties and sensory evaluation of pineapple paste

The appearance of pineapple paste from seven formulas was observed. Total soluble solid was investigated by hand refractometer (ATAGO MASTER-M, China). The pH value was measured by a pH meter (PH Meter 0.01, China). The color was investigated by color meter (Minolta colorimeter CR–400, Japan). The CIE system was evaluated by L * or brightness (0 = black, 100 = white), a * (+ a = red, -a = green) and b * (+ b = yellow, -b = Blue). Sensory evaluation by 30 untrained panelists were investigated. The importance of liking of appearance, flavor, taste, texture and overall liking were expressed by 9-point hedonic scale. The suitable formula was selected for the developmental pineapple paste product. The contour plot was overlapped to find the right ratio of the amount of pineapple residues, pineapple juice and sugar. The qualities of pineapple paste composed of total soluble solid, pH, color and sensory evaluation score were determined for selecting the appropriate formula of pineapple paste. It was produced as a pie filling in the next step.

5. Gluten-free pineapple pie preparation

The prototype formula of gluten-free pie product was studied. Specifically, mixed flour consisting of 65% rice flour, 25% potato flour, 10% cassava flour and 1.5% xanthan gum of mixed flour were used (Charoenphun & Kwanhian, 2018). The nine formulas of gluten-free pie products consisting of mixed flour (60-70%), salted butter (20-40%) and pineapple residue (0-10%) were determined by Mixture Design. The points on the designated triangle area were selected for studying. The nine formulas of pineapple paste from mixture design were determined (Table 2). Mixed flour, salted butter and pineapple residues were weighed. In other ingredients, cream cheese, icing, egg yolk and whipping cream were added at 15, 20, 25 and 20% all ingredients (consisting of mixed flour, salted butter and pineapple residue), respectively. In terms of pies production, salted butter and cream cheese were beaten by a food mixer (FRY KING, FR-089B, China) at medium speed until homogeneous. Icing, egg yolk, pineapple residues and whipping cream were added and mixed. Next, mixed flour was added and mixed until homogeneous. These were mixed and put in a plastic bag by rolling into a cylinder. It was chilled in refrigerator at 4°C for 30 min. Then, it was cut into small sizes at 20 g per piece, and was rolled onto a round sheet. After that, 20 g of pineapple paste that was selected from previous steps was added and molded. It was pressed into pie molds and was chilled in refrigerator at 4°C for 10 min. It was baked at 160°C for 10 min and then flipped over, and baked at 160°C for 10 min again.

Table 2 Formula of pie with pineapple paste by a 3-component* mixture design

Formulas	1	2	3	4	5	6	7	8	9
Mixed flour (%)	67.5	67.5	62.5	62.5	70	60	65	70	60
Salted butter (%)	25	30	30	35	30	30	30	20	40
Pineapple residue (%)	7.5	2.5	7.5	2.5	0	10	5	10	0

Remark: * a 3-component mixture (100% in the mixture design) was 100% of the total formula.

6. Properties and sensory evaluation of gluten-free pineapple pie

The appearance of pies from nine formulas was observed. Baking loss of cookies was investigated. The pies before baking and after baking were weighed by balance (Zepper EPS-3001, China). Baking loss was defined as follows: Baking loss (%) = [(Weigh of pies before baking- Weigh of pies after baking)/ Weigh of pies before baking] x 100 (Kotoki & Deka, 2010). The hardness of pies was measured by hardness instrument (Daiichi FG 520K, Japan). The unit of force was newton (N). Color of pies was measured by color meter (Colorimeter, WR10QC, China). The CIE system was defined by L * or brightness (0 = black, 100 = white), a * (+ a = red, -a = green) and b * (+ b = yellow, -b = Blue). Moreover, sensory evaluation by 30 untrained panelists were investigated. The number of panelists was appropriate that they had the proper qualifications in accordance with human research ethics. In the smaller tests, the 9-point hedonic scale of testing generally requires 25–50 participants, but will depend on the variability within the sample, as well as the test objective (Moskowitz et al., 2012). The importance of liking of appearance, flavor, taste, texture and overall liking were expressed by 9-point hedonic scale. The suitable formula was selected for developing gluten-free pie products. The contour plot was overlapped to find the right ratio of the amount of mixed flour, salted butter and pineapple residues. The quality of pies composed of weight loss, hardness, color and sensory evaluation score were used for selecting the appropriate formula of the pie. Chemical composition of pie including moisture content, protein, fat, carbohydrate and ash were investigated (AOAC, 2000).

7. Statistical analysis

The statistical technique one-way ANOVA was used for calculating. Duncan's new multiple-range Test (DMRT) was used to compare the difference in the average values at the 95% confidence level (Duncan, 1995).

Results and discussion

1. Properties and sensory evaluation of pineapple paste

The appearance of pineapple paste for seven formulas by a 3-component mixture design were observed. It was found that all seven formulas of pineapple paste had a different appearance and characteristics. The pineapple pastes in formula 1 was overly sweet, orange-brown color, non-adhesion and over dry of texture. The pineapple pastes in formulas 2, 3, 5, 6 and 7 were very sour, yellow color, and non-adhesion. Interestingly, number 4 was the best formula which was similar to the general pineapple paste in the market. It was yellow color, appropriate texture and adhesion. In addition, it had a moderately sweet taste. As a result, the appearance of pineapple pastes is caused by the ratio of the main ingredients and the unit operation during production. Free water was removed during stirring due to heat transfer. Therefore, changing the appearance of pineapple pastes including flavor, color, and texture were found.

The pH, total soluble solids content and color of pineapple paste with a 3-component mixture design are shown in Fig. 1. The results show that pH from seven formulas had significant difference of the 95% confidence interval when $P \leq 0.05$. Apparently, pH value was decreased when the amount of pineapple juice increased. The amount of pineapple juice in formulas 4 and 5 are the same. Obviously, the pH in formula 4 higher than formula 5. However, the amount of pineapple residue in formula 5 is higher than formula 4. Presumably, there are left over of organic acids in pineapple residue. There were many organic acids in pineapple juice including oxalic acid, tartaric acid, malic acid, ascorbic acid, acetic acid, citric acid and succinic acid etc (Pongjanta et al., 2011). These had an effects on the pH value of pineapple paste. In this case, the pineapple species Pattawia (or Smooth Cayenne) was the most favorite among packers used for processing canned pineapple and also popular for fresh consumption. The distinctive characteristics of this pineapple species were sweet and juicy which were used for producing canned pineapple and pineapple juice in the industry. It had a total acidity in the range of 1.16-1.94%. Specifically, citric acid is the main acid which had a high volume of 70%, followed by acetic acid and malic acid, respectively (Pongjanta et al., 2011). The total soluble solid from seven formulas had significant difference of the 95% confidence interval, $P \leq 0.05$. It was found that the total soluble solids content varied directly as the amount of sugar. The major sugar substances that contribute to sweetness are glucose and fructose that playes a major role in taste. A strong positive correlation is observed between trained panel response to sweetness and total soluble solids content (Majidi et al., 2011). The amount of sugar in formulas 5 and 7 are the same. Apparently, the total soluble solids content in formula 5 is higher than formula 7. Nevertheless, the amount of pineapple juice that were sour in formula 7 is higher than formula 5. Presumably, the organic acids in pineapple juice may be related with total soluble solids. The total soluble solid was low and the total soluble solid/the total organic acid ratio was also low (Ikegaya et al., 2019).

The color of pineapple pastes from seven formulas had significant difference of 95% confidence interval when $P \leq 0.05$. The results show that L* (lightness) tendes to decrease when the amount of sugar increases. The a * value is the green range when pineapple residues increases. Furthermore, the b* is was the yellow range when pineapple juice increases. Obviously, the chemical composition is the main cause of color appearance in pineapples. There are many pigments in pineapples such as chlorophyll and carotenoid. Normally, chlorophyll in the pineapple peel changes to faded green color while carotenoid was develops into a yellow color according to the stage of maturity. In pineapple flesh, high amounts of carotenoids were found which were yellow-orange pigments depending on the varieties of pineapple species (Panyamongkol, 2017). It corresponds to the a* and b* values of pineapple paste which were measured in green and yellow ranges. Chlorophyll in pineapple causes the green color in pineapple paste. In particular, thermal processing induces structural and chemical variations to the tissue of vegetables that often result in color changes. Moreover, the reason for the green color loss during processing is mainly attributed to the conversion of chlorophylls to pheophytins by the influence of pH (Erge et al., 2008). Changing the lightness of pineapple paste occurred from many factors such as quality of raw materials, temperature and processing time, etc. In general, pineapple have a chemical composition consisting of

moisture, protein, fat, carbohydrates, dietary fiber, vitamins, minerals, organic acids, sucrose, glucose and fructose with different according to varieties and harvesting periods. Moreover, chemical composition of pineapple

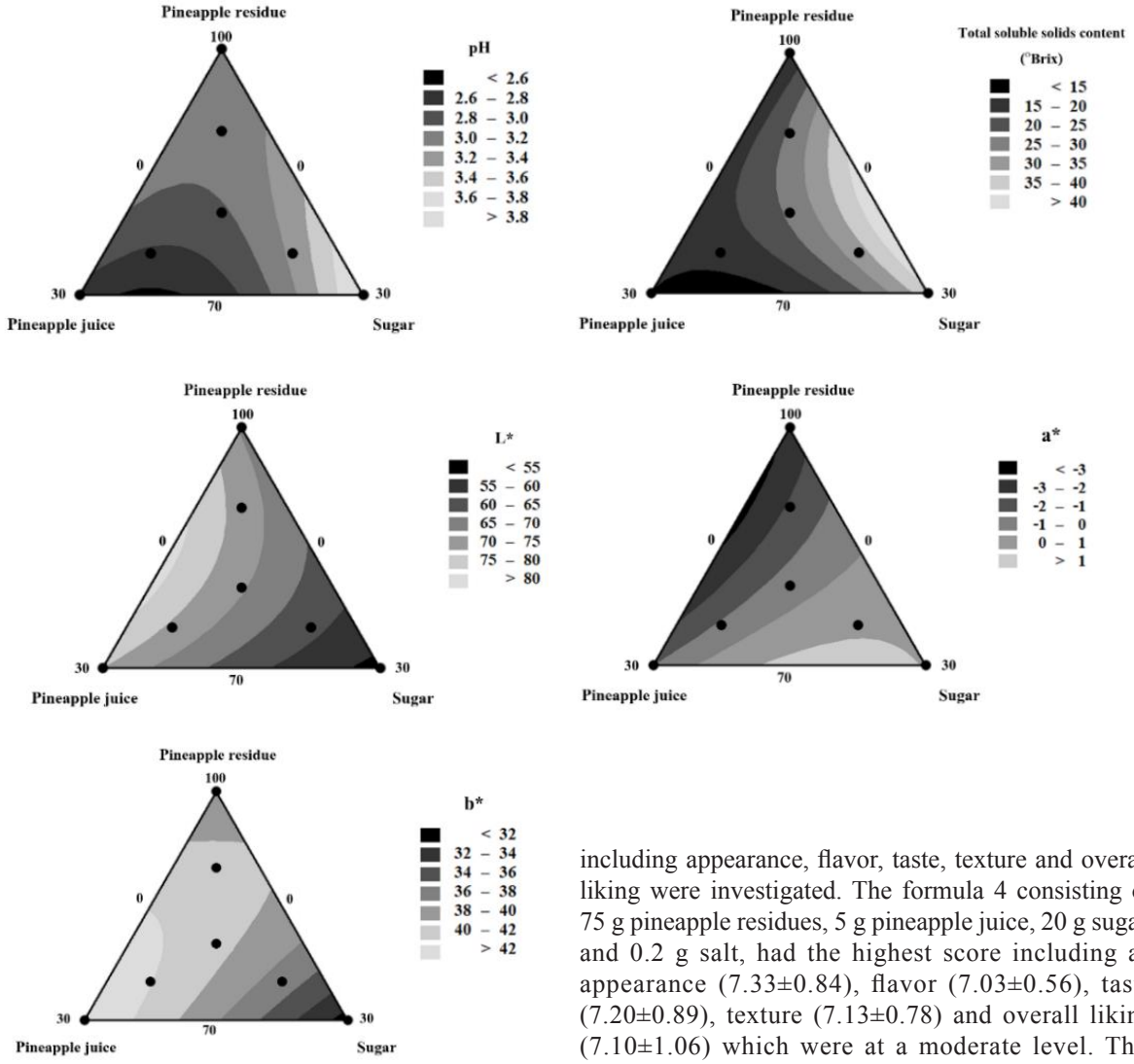

Fig. 1 pH, total soluble solids content and color of pineapple paste by a 3-component mixture design

and sugar is the main factors which have an effected on the color changes of the pineapple paste. Basically, the Maillard reaction was a form of non-enzymatic browning. It is a chemical reaction between an amino acid and a reducing sugar, usually requiring the addition of heat (Rattanapanone, 2006). Probably, the decrease of lightness value is caused by browning reaction.

The effects of pineapple residues, pineapple juice and sugar on the liking score for seven formulas of pineapple paste were shown in Table 3. The average score from 30 untrained panelists by 9-point hedonic scale including appearance, flavor, taste, texture and overall liking were investigated. The formula 4 consisting of 75 g pineapple residues, 5 g pineapple juice, 20 g sugar, and 0.2 g salt, had the highest score including an appearance (7.33±0.84), flavor (7.03±0.56), taste (7.20±0.89), texture (7.13±0.78) and overall liking (7.10±1.06) which were at a moderate level. This corresponds with the pH, total soluble solid, color and appearance of pineapple paste investigated. The formula 4 is similar to the general pineapple paste in the market. It had appropriate texture and adhesion. In addition, it is a moderately sweet taste.

Table 3 The liking score (n = 30) for seven formulas of pineapple paste

Formulas	Appearance	Flavor	Taste	Texture	Overall liking
1	5.90±0.71^{b}	5.90±0.66^{b}	4.20±0.71^{c}	4.67±0.76^{b}	4.73±0.78^{b}
2	3.40±1.90^{d}	5.20±1.40^{c}	3.13±1.25^{d}	2.80±1.45^{d}	3.13±1.20^{c}
3	4.23±1.52^{c}	5.37±1.13b^{c}	4.10±0.96^{c}	3.80±1.13^{c}	4.67±1.12^{b}
4	7.33±0.84^{a}	7.03±0.56^{a}	7.20±0.89^{a}	7.13±0.78^{a}	7.10±1.06^{a}
5	4.63±1.67^{c}	5.53±1.22^{bc}	5.10±1.18^{b}	4.50±1.48^{b}	4.93±1.34^{b}
6	4.20±1.88^{c}	5.53±1.22^{bc}	3.63±1.13^{cd}	3.10±1.56^{d}	3.60±1.22^{c}
7	4.33±1.83^{c}	5.67±0.96^{bc}	3.93±0.94^{c}	3.37±1.52^{cd}	3.70±1.21^{c}

Remark: mean ±SD with different superscripts in each column indicate significant differences ($P \leq 0.05$).

The pH, total soluble solid, color and sensory score were created the contour plot for optimum overlapping (A) of pineapple paste formulas (Fig. 2). The suitable formula was selected from the contour plot. The contour plot for optimum overlapping, pH (3-4), total soluble solid (25-35 0Brix), L * values (50-70), a * values (0-2), b *values (20-40) and sensory score (6-9) were the criteria for selecting suitable formula. The different factor levels (low-high) was the optimal range of pineapple paste that obtained from testing and compared with commercial samples. The results show that optimum overlapping (A) is a suitable area for pineapple paste production (Fig. 2). Formula 4 is in the overlapping area. The optimum proportion of pineapple residues, pineapple juice and sugar are 75, 5 and 20%, respectively. Obviously, pH, total soluble solid, color and the average score of sensory evaluation were taken into consideration together. All in all, the formula 4 was selected to produce gluten-free pie products in the next step.

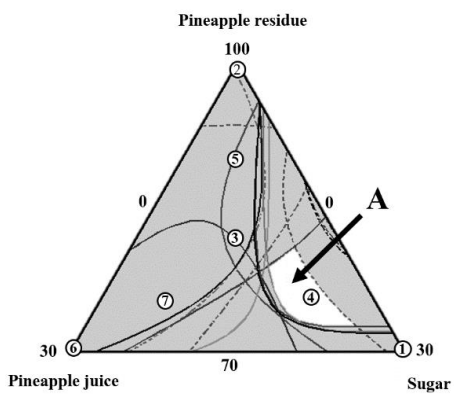

Fig. 2 Contour plot for optimum overlapping (A) of pineapple paste formulas

2. Properties and sensory evaluation of gluten-free pineapple pie

Weight loss, hardness and color of gluten-free pie products with a 3-component mixture design is shown in Fig. 3. The results shown that color of gluten-free pineapple from seven formulas have significant differences of the 95% confidence interval when $P \leq 0.05$. The weight loss varied directly with the amount of salted butter. Obviously, the moisture or free water in the structure of pies which decreased during baking was the main cause of weight loss of pies. The heat transfer, expansion, changing of structure and texture occurred during pie production (Kotoki & Deka, 2010). In this case, a large amount of salted butter was used of pie production. Therefore, the mixed composition of pie products had a high starting moisture content before baking and the moisture or free water in the structure of pies was removed. During baking of pies, the mass was transferred from the surface of pies and especially the surface area of pie was high temperature. Consequently, the free water in the pie structure evaporated which resulted in weight loss during baking.

The hardness of pies after baking from the surface mixture response surface contour plots is shown in Fig. 3. The hardness of the pies increased with an increase in the amount of mixed flour. Obviously, if the solid ingredients in the mixture increased, the hardness of pies increased. As a result, mixed flour was a dry powder that had the ability to absorb water well. At this point, if the mixed flour was added at the optimum ratio, pies had appropriate texture. However, there was a small number of mixed flours in the formula. It absorbed lots of water and had difficulty forming. In contrast,when a large number of mixed flours was in the formula, the amount of water in the mixture was limited. This resulted in the flour not absorbing the water fully. Thus, pies had very high hardness. The results corespond with the results of Luangsakul et al. (2012). Where the effects of wheat flour on the quality of fortune cookies was reported. The hardness of fortune cookies varied directly with the amount of wheat flour.

The color of pies after baking from the surface mixture response surface contour plots is shown in Fig. 3. The L* value varied directly with the amount of mixed flour. The a* value was the red range, and b* value was the yellow range. The a* and b* value varied directly with the amount of pineapple residues. Moreover, the tendency of gluten-free pie product was yellow-red color. These occurred during the baking of pies in the oven. In terms of heat transfer, both convection and radiation were transferred from the oven to the surface of pies. Consequently, it was transferred to inside of pies by conduction during baking. At the same time, mass

transfer from the surface of pies caused a high temperature in the structure of pies, and the free water in pies structure evaporated. Subsequently, changing the quality of pies occurred including gelatinization of starch, protein denaturation, expansion of pies, hardness of pies crusts, air holes of pies, and brown color. Color changes of pies may occur by Maillard reaction that was non-enzymatic browning reaction. In theory, it was a reaction between proteins or amino acids and sugar under high temperature conditions (Rattanapanone, 2006). Moreover, the color of pies also depends on the composition of pigment in raw material that are used to produce the pies. Chlorophyll and carotenoid pigments presented in the pineapple as described earlier (Panyamongkol, 2017). Therefore, the b * value was the yellow range. Furthermore, the yellow color of salted butter was observed that it was the dairy product. The color of salted butter appears to be dictated by the concentrations of β-carotene and other compounds and pigments, deposited into the lipid phase of the milk from the cow's ingestion of green, grassy materials (Paine, 2013).

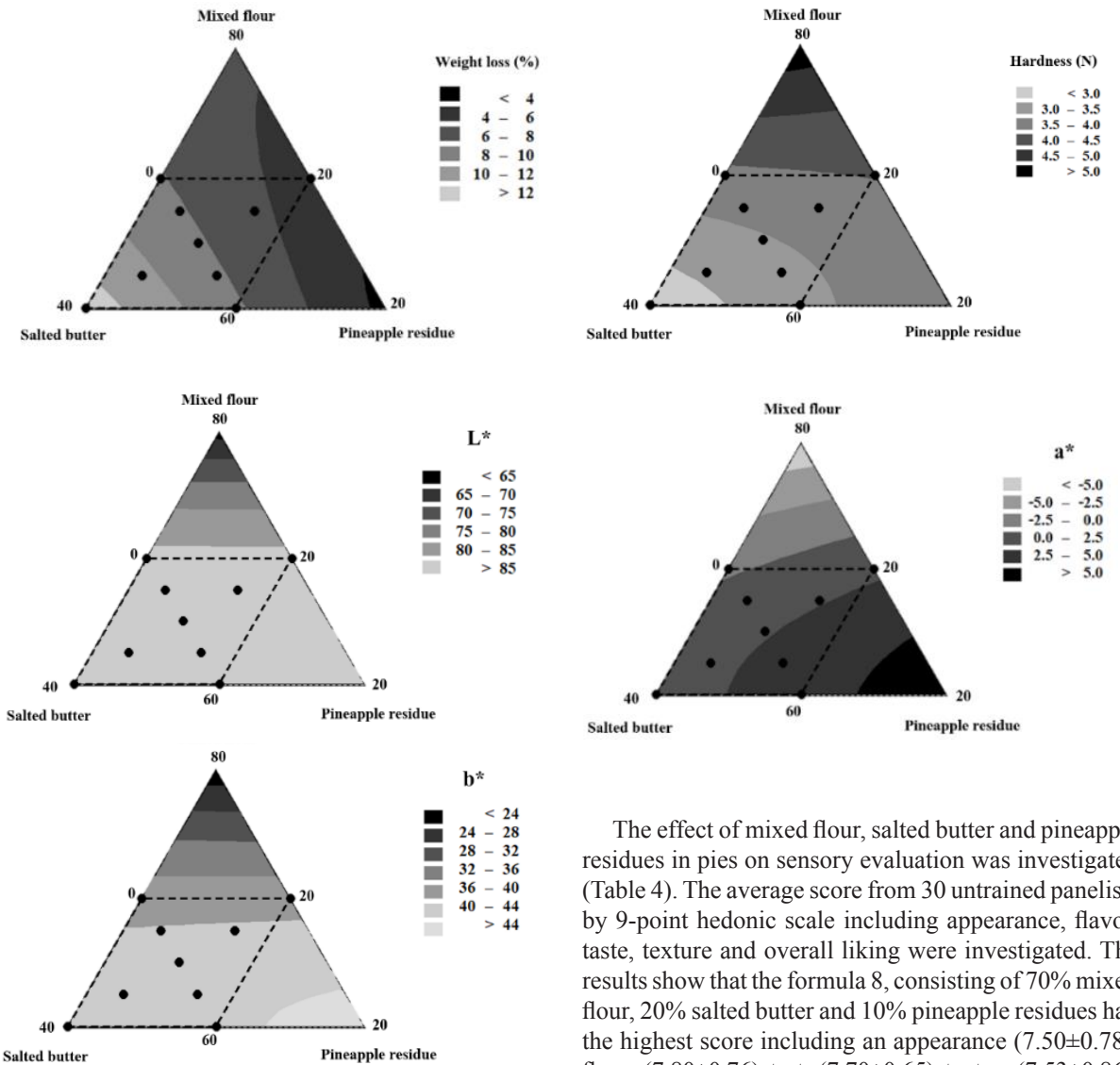

Fig. 3 Weight loss, hardness and color of gluten-free pie products by a 3-component mixture design

The effect of mixed flour, salted butter and pineapple residues in pies on sensory evaluation was investigated (Table 4). The average score from 30 untrained panelists by 9-point hedonic scale including appearance, flavor, taste, texture and overall liking were investigated. The results show that the formula 8, consisting of 70% mixed flour, 20% salted butter and 10% pineapple residues had the highest score including an appearance (7.50±0.78), flavor (7.80±0.76), taste (7.70±0.65), texture (7.53±0.86), and overall liking (7.63±0.85) which were at a moderate level.

Table 4 The liking score (n = 30) for nine formulas of gluten-free pie products

Formulas	Appearance	Flavor	Taste	Texture	Overall liking
1	6.20±0.41^{d}	7.10±0.71^{bcd}	7.20±0.71^{abc}	6.23±0.97^{c}	6.37±0.49^{c}
2	6.33±0.61^{cd}	6.70±1.12^{cde}	7.00±0.98^{bc}	6.37±0.85^{c}	6.57±0.68^{bc}
3	6.40±0.67^{cd}	7.00±0.64^{bcd}	7.13±0.68^{bc}	6.37±1.13^{c}	6.57±0.68^{b}
4	6.40±0.62^{cd}	6.57±1.19^{de}	7.10±0.92^{bc}	6.43±0.90^{c}	6.70±0.75^{bc}
5	5.63±1.03^{e}	6.17±1.32^{e}	6.33±1.56^{d}	4.07±1.41^{d}	4.53±2.00^{d}
6	6.33±0.61^{cd}	7.23±0.77^{bc}	7.37±0.76^{abc}	6.50±1.14^{bc}	6.57±0.68^{bc}
7	7.00±0.91^{b}	7.27±0.69^{b}	7.50±0.68^{ab}	7.00±0.91^{b}	7.03±0.85^{b}
8	7.50±0.78^{a}	7.80±0.76^{a}	7.70±0.65^{a}	7.53±0.86^{a}	7.63±0.85^{a}
9	6.73±0.83^{bc}	6.30±1.29^{e}	6.90±0.92^{c}	6.23±0.86^{c}	6.57±0.86^{bc}

Remark: mean ±SD with different superscripts in each column indicate significant differences ($P \leq 0.05$).

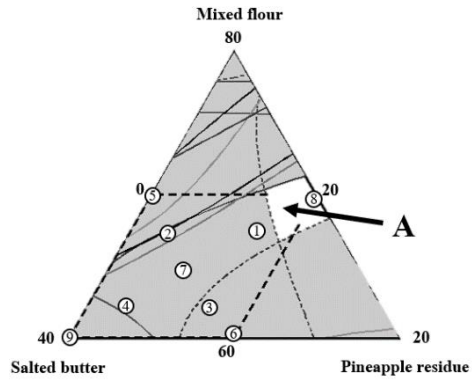

Fig. 4 Contour plot for optimum overlapping (A) of gluten-free pineapple filled pie formulas

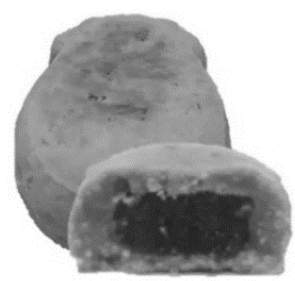

Fig. 5 Appearance of gluten-free pie products

The weight loss, hardness, color and sensory evaluation were created to contour plot for optimum overlapping (A) of cookie formulas (Fig. 4). The suitable formula was selected from the contour plot. The contour plot for optimum overlapping, weight loss (0-6), hardness (3-5 N), L* values (70-90), a* values (1-3), b* values (30-59) and sensory score (6-9) were the criteria for selecting suitable formula. The different factor levels (low-high) was the optimal range of pies that obtained from testing and compared with commercial samples. The results showe that the optimum overlapping (A) was a suitable area for pies production (Fig. 4). Formula 8 was in the overlapping area. Mixed flour, salted butter and pineapple residues were 70%, 20% and 10%, respectively. Obviously, weight loss, hardness, color and the average score of sensory evaluation were taken into consideration together. All in all, the formula 8 was selected for gluten-free pie products (Fig. 5). In addition, the chemical composition of pies in formula 8 was analyzed. It was found that the gluten-free pie products had a moisture content (30.5±0.3%), protein (6.1±0.2%), fat (12.9±0.1%), carbohydrate (49.0±0.2%) and ash (1.5±0.2%), respectively. Specifically, the changes for adding pineapple residue in mixed flour was observed. Pineapple residue helps increase dietary fiber of pies. The following are the characteristics of gluten-free pie products. It is easily broken, if the very high content of pineapple residue was added. Presumably, chemical composition in pineapple residue may decrease the strength of mixed flour structure. However, the properties of gluten-free pies in this research are similar to the original pies that are made from wheat flour. It is an alternative product for consumers who want to avoid gluten-containing foods.

Conclusion

The pineapple residue is the by-products of the pineapple processing industry that contains nutrients such as fiber and vitamin. Specifically, it is a good choice of low-cost ingredient for producing pineapple paste and gluten-free pie products that add value to the agricultural industry. The appropriate formula for pineapple pastes production from pineapple residues is a formula consisting of pineapple residues, pineapple juice and sugar in the range of 75%, 5% and 20%, respectively. In terms of pies, the suitable formula for producing gluten-free pie product is a formula with a mixture of flour, salted butter and pineapple residues, 70%, 20% and 10%, respectively. In total, the knowledge gained from this study can be used as a guideline for value adding process of pineapple residue in healthy food products that can be extended to commercial production in the future.

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The Screening Balance Ability to Identify Fall Risk Using the Mini-BESTest in the Pre-elderly

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Abstract

A fall is a common problem in all ages. The age-related physiological changes of various systems. (e.g., cardiovascular system, nervous system, musculoskeletal system, etc.). Especially, the balance control is the main factor that affects the fall in elderly and can lead to the death of elderly. The preparation of fall prevention in pre-elderly is needed. Mini-BESTest is important screening tool for evaluating fall risk and dynamic balance. The Mini-BESTest has the sensitivity of 68% and specificity of 65% in the pre-elderly people who are at a risk to fall. The scores of the Mini-BESTest are less than 22.5 that represented a risk to fall. The Mini- BESTest is suitable for screening risk to fall in pre-elderly due to the accuracy, sensitivity and specificity. The evaluation of sensitivity and specificity of the Mini-BESTest screen falls in the pre-elderly. Healthy male and female pre-elderly participants were divided into two groups; non-fall group and fall group (History of past 6-month of falls) (n=64 per group). Their balance abilities were assessed by using the Mini-BESTest, BBS, and TUG. An analysis of the resulting receiver operating characteristic curves was performed to calculate the area under the curve (AUC), sensitivity, specificity, cutoff score, and post-test accuracy. The results show that the Mini-BESTest had the highest AUC (0.71) compared with the BBS (0.59) and TUG (0.62). It demonstrates that the Mini-BESTest has the highest accuracy for identifation of pre-elderly with history of falls. At the cutoff score of 22.5 (out of 28), the Mini-BESTest demonstrated a post-test accuracy of 66% with a sensitivity of 64.06% and specificity of 68.75%. The Mini-BESTest has the highest post-test accuracy, with the others having results of 57% (BBS), and 52% (TUG).

Introduction

A fall is defined as "to come or drop down suddenly to a lower position, especially to leave a standing or erect position suddenly". Slips and trips are the causes of falls and unexpected changes during walking that lead to injury and death. (Onla-or et al., 2004). A fall in the elderly is considered as a major health problem. Since the world population is entering to the aging society,a fall is the cause of injury and leading to death in the elderly (Lausawatchaikul, 2000).

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falls in pre-elderly aged 50 to 59 years was 66% in developing countries and 34 % in high-income developed countries (Williams et al., 2015). Previous study found that the prevalence of falls increased 27% in 55-59 years old (Peeters et al., 2018). The result showed that the fall rates of Thai pre-elderly people were similar to the Thai older elderly people (Sorysang et al., 2014).

One of the majority consequences of fall is fracture (Sorysang et al., 2014). Therefore, the preparation and prevention of a fall are need. The balance assessments were used to examine the risks of fall in clinical and research. However, the lack of the balance ability during external perturbations or sensory conflicts assessment were limitations of balance assessments. Balance Evaluation Systems Test (BESTest) is a comprehensive clinical tool for evaluating postural control systems that focus on static and dynamic balance. The BESTest can identify the underlying postural control systems (Horak et al., 2009). Franchignoni et al., (2010) developed a shorter version of the BESTest. The Mini- Balance Evaluation Systems Test (Mini-BESTest) designed as a comprehensive clinical tool for evaluating dynamic balance that is related with a fall. The Mini-BESTest requires less administration time and less equipment. (Franchignoni et al., 2010). In addition, the Mini-BESTest has excellent interrater (ICC $\geq$ 0.91) and test-retest (ICC $\geq$ 0.88) reliability, compared to the Berg Balance Scale (BBS), the Mini-BESTest test lacks the ceiling effects (Leddy et al., 2011b) and has better sensitivity and specificity to identify people with Parkinson who have more of a chance to fall. The previous studies in individuals with PD showed the sensitivity 62-89% and the specificity 74-81%. In individuals with stroke showed a cut off 17.5 out of 28, sensitivity 64% and specificity 64.2%. (Tsang et al., 2013). The Mini-BESTest was used to assess the balance performance. It may be accurate in screening of the pre-elderly people who have balance problems and people who are at a risk of falls.

Therefore, the purpose of the present study was to determine the sensitivity and the specificity of Mini-BESTest for predicting the pre-elderly with having risk of a fall.

Participants and methods

Participants were recruited from the Pathum Thani of the Thailand between which October 2016 and which February 2017. Participants aged between 55 to 59 years were asked to participate in the present study (mean age 56.6 ± 1.4). There were 128 participants and were divided into two groups; non-fall (n=64) and fall groups (n=64) (history of fall in past 6-months) by convenience sampling. The research design is cross sectional study. This study defined a faller as a subject who fell at least once. Participants were included in the study if they met the following inclusion criteria: 1) with and without history of fall of their 6 months fall history 2) independence basic activities daily 3) able to walk 6 meters without using gait aids 4) able to communicate and follow instructions 5) being in good health and not affected with diseases such as stroke, spinal cord injury, Parkinson's and severe musculoskeletal problem disease that impact on movement and balance in the day of assessment 6) giving informed consent. Participant characteristics and others related information were gathered using the questionnaire and assessment through physical examinations. The cognitive functions and comprehensions were assessed by using the Mini-Mental State Examination Thai version 2002 (MMSE-Thai). The fear of falling was assessed by using the fear of falling scales. Randomly assessment balance ability was assessed by using the Mini- Balance Evaluation Systems Test (Mini-BESTest), Berg Balance scale (BBS) and Timed up and go test (TUG). Vital signs and blood pressure were measured before and after the testing. Participants were allowed to rest as long as they needed for muscle fatigue prevention during the test. This study was conducted using a cross-sectional approach. This study was approved by the Human Research Protection Committee, Rangsit University, Thailand (RSEC18/2559).

The statistical analysis was performed using SPSS version 11.5. A descriptive statistical analysis of the baseline characteristics of the participants was conducted. The Mann-Whitney U test was used to compare mean balance scores between the pre-elderly with and without a fall history 6 months. A statistically significant considered was p-value less than 0.05. Receiver operating characteristic curves was performed to calculate the area under the curve (AUC), sensitivity, specificity, cutoff score and post-test accuracy. The receiver operating characteristic (ROC) curves were used to determine the relative performance of the Mini-BESTest score, the BBS scores and the TUG scores for classifying pre-elderly into fallers and non-fallers. ROC curve used plots graph between true positive rate (sensitivity) and false positive rate (1-Specificity). Cutoff score selected the score that demonstrated the best balance between high sensitivity and high specificity.

Locating the cut-off point that requires a compromise between sensitivity and specificity. A method of determining the cutoff was used to calculate from post-test accuracy whether the selected cutoff score could correctly screen the pre-elderly fallers, the percentage accuracy of the pre-elderly who actually fell was calculated using the cutoff score (McHorney et al., 1994). Area under the curve (AUC) assessed accuracy of each balance test to discriminate the fallers and the non-fallers. If AUC closely was the 1 which representing the test corresponds to a perfect classification the fallers and the non-fallers (Akobeng, 2007).

Results and discussion

Subject characteristics data from pre-elderly in the community totaled 128 people and were categorized at entry into 64 participants per group based on their one fall history within the last 6 months. The characteristics data of the groups with no history of falls reported average age was 56.67 ± 1.35 years, Proportion of female and male was 56/6, the average score of the Mini mental state examination was 25.75 ± 9.19 and the average score of Fear of falling scale was 25.75 ± 9.19. Body mass index showed 23.92±3.76. Twenty-six participants with no history of falls reported chronic diseases such as hypertension, diabetes and dyslipidemia.

The characteristics data of the groups with a history of falls in 6 months reported average age was 56.49 ± 1.41 years, Proportion of female and male was 47/17, the average score of the Mini mental state examination was 24.03 ± 5.65 and the average score of Fear of falling scale was 30.89 ± 7.97. Body mass index showed 23.44±4.03. Thirty participants with history of falls reported chronic diseases such as hypertension, diabetes and dyslipidemia. The causes of falls in pre-elderly were mostly tripping, slipped and Postural transition was 25.69%, 13.49% and 1.92%, respectively.

Table 1 Score of Mini-BESTest, BBS and TUG between pre-elderly who had a fall history in 6 months and those who had no history of falls in 6 months

Balance assessments	pre-elderly who had no history of fall in 6 months; N=64	pre-elderly who had fall history in 6 months; N=64	*p* value
Mini-BESTest (/28)	23.47 ±2.68	21.14±2.82	0.021*
Berg Balance scale (/56)	54.81 ±1.50	54.08±1.76	0.081
Time up and go test (/12)	8.97 ±1.70	10.45±1.59	0.042*

Remark: * Significant difference between fallers and non-fallers at p <0.05

There were statistically significant differences in the scores of the Mini-BESTest ($p<0.05$) and TUG ($p<0.05$) between the pre-elderly with and without history of fall groups. Nonetheless, there were no statistically significant differences in the BBS between both groups ($p>0.05$). The results showed that the BBS had a trend of ceiling effect as 15 participants from the pre-elderly who had a history of falls in past 6 months and showed the maximum score at 56 points (Table 1).

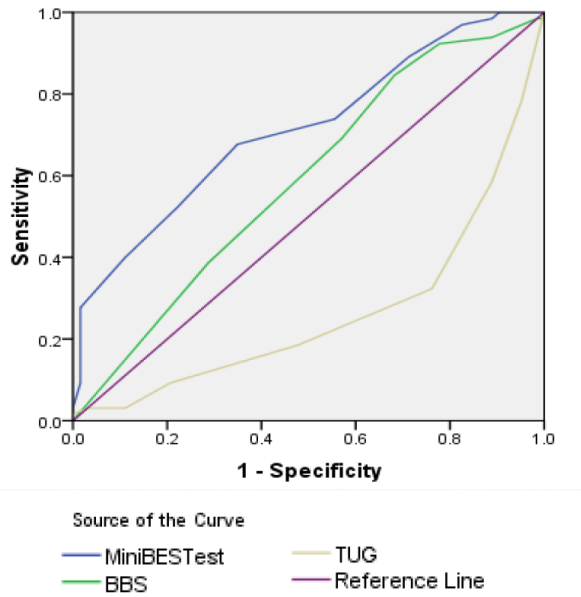

Fig. 1 Receiver Operating Characteristic (ROC) Curves of measurements (Mini-BESTest, BBS and TUG)

Fig. 1 illustrates that the area under the curve (AUC) of the Mini-BESTest is 0.71, which is closer to 1 than the BBS and TUG, which was 0.59 and 0.26, respectively. Moreover, the results show the cutoff score of the Mini-BESTest at 22.5 (68% sensitivity and 65% specificity) in predicting fallers in the pre-elderly. The findings show that the BBS, TUG have the cutoff score at 54.5 (69% sensitivity and 43% specificity) and 8.5 seconds (59% sensitivity and 11% specificity), respectively. The Mini-BESTest shows that the accuracy of predicting a fall was 66 %, which is higher than the BBS (57%) and the TUG (52%).

The Mini-BESTest could predict fall and has the ability to assess balance deficit that causes a fall. Because the Mini-BESTest had a comprehensive composition of postural control during walking (Franchignoni et al., 2010). Approximately, 10-25% of individuals with

history of a fall occurred due to poor postural stability (Shumway-Cook et al., 1997). The falls during a slip or a trip were caused by lack of automatic postural responses. The Mini-BESTest could capture these abilities as the test included the automatic postural response items (Yingyongyudha et al., 2016). This result shows the causes of a fall, including tripping 25.69 % and slipping 13.49% and the hazard environment in and outside places lead to falls. The results of the present study are consistency with the findings of the previous studies. They reported that the causes of fall, tripping 41.8% and slipping 38.2%, led to injury, such as fracture (Sorysang et al., 2014). The results demonstrate the Mini-BESTest has the accuracy tool for predicting a fall in pre-elderly more than BBS and TUG with the AUC of 0.71, sensitivity of 68% and specificity of 65%. The cutoff point was 22.5 score. The AUC in the present study is similar to the range in the previous study. Compared with the Mini-BESTest, the accuracy for fall prediction in healthy elderly with a history of a fall of the BBS and the TUG was 1 time or more within 12 months. They reported that the AUC of the Mini-BESTest was 0.84, sensitivity of 85% and specificity of 75% (Yingyongyudha et al., 2016).

In addition, the Mini-BESTest evaluated the accuracy for predicting falls in people with neurological problems, with a history of falls more than 2 times in the past 6 months. It was found the sensitivity of 88% and specificity of 78% (Leddy et al., 2011b). The previous study investigated the sensitivity and specificity of the Mini-BESTest in individuals with Parkinson's disease that had a history of falls in the past 6 months. The results showed the sensitivity of 79% and the specificity of 69% (King et al., 2012). Even though the BBS was considered as a reference standard for assessing the balance in the elderly, as it is one of the most commonly used balance assessments in the clinic and in research (Leddy et al., 2011a).

However, the BBS had 77% sensitivity, specificity 42% for predicting a fall in the elderly (Yingyongyudha et al., 2016). The present study shows that 11.72% of pre-elderly demonstrated a trend of ceiling effect. Therefore, BBS was unable to differentiate the postural control in the elderly. In contrast, the Mini-BESTestdid not show the ceiling effect (King et al., 2012). Hence, it could be an appropriate tool for identification for the risk of falls in the elderly.

There were two limitations to the present study. Firstly, the other fall risk factors, such as psychological aspects, medications and co-morbidity, was not assessed. Therefore, further study is needed to examine the other fall risk factors. Secondly, the fallers in this study had fall experience for only one time. Fall risks may have several factors, internal factors and consideration of the nature of occupation. Thus, subsequent studies is needed to compare three different groups, the additional group is the group of pre-elderly subjects with 2-times or more in history of falls.

Conclusion

Mini-BESTest is suitable evaluating postural stability and screening falls in the pre-elderly. Mini-BESTest has accuracy for screening falls in the pre-elderly. In clinical, Mini-BESTest could be helpful in directing treatment and prepare a suitable fall prevention strategy for the pre-elderly.

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Antimicrobial Activity of Edible Plant Extracts Against Skin Infection Pathogens

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Abstract

Antimicrobial activity of four edible plant extracts including ginger, galangal, lemongrass and tree basil have been investigated. For this purpose, the extract of plants was acquired using ethanol and distilled water. The inhibitory effect on six skin infection pathogenic microorganisms, i.e., *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Propionibacterium acnes* and *Candida albicans* were performed by disc diffusion, agar well diffusion and microdilution methods. From the results, ethanolic extract of galangal showed the best antimicrobial activities against all test pathogens in terms of the minimum inhibitory concentration and minimum bactericidal concentration, ranging from 0.49-15.62 mg/ml and 0.49-62.5 mg/ml, respectively. Therefore, the aqueous extract has lower antimicrobial activity compared with the ethanolic extract. The ethanolic extracts of ginger, galangal and lemongrass could inhibit *P. acnes* at the concentrations lower than 1 mg/ml. The outcome of this study suggested that edible plant extracts could possibly be applied as a natural antimicrobial agent and combined with other materials for further applications.

Introduction

Skin infection pathogens are important problems in both hospital and community. Skin and skin-structure infections aetiology is dominated by *Staphylococcus aureus* and other common bacteria such as *Pseudomonas aeruginosa* (Lipsky et al., 2007; Livermore et al., 2015). *Propionibacterium acnes* is the major bacteria causing acne, which is one of the most common and chronic skin problems (Vora et al., 2018). This group of microorganisms is often mentioned in health and beauty products. Synthetic drugs or antibiotics induced mutations in the genetic composition of these microorganisms, leading them to be resistant to drugs or antibiotics (Cohen, 1992). In recent years, an increasing number of human pathogenic microorganisms poses a concern on antibiotic resistance strains. For this reason, bioactive compounds isolated from plants may offer a new solution to this problem by being a novel antimicrobial agent. The search for new antibacterial agents should be conducted and studied among various plant families. Due to their therapeutic properties, plants have been widely used in many pharmaceutical industries. Medicinal plants have been known to produce compounds with therapeutic properties such as antidiabetic, antioxidant, antibacterial, anti-inflammatory, antipyretic and gastroprotective

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effects (Gupta et al., 2016).

There was a report on seven Cameroonian dietary plants that display their inhibitory effect on the multidrug-resistant Gram-negative bacteria (Djeussi et al., 2013). Various Thai edible plants should be studied on their biological activities. Ginger *(Zingiber officinale* Roscoe) is an edible plant that has been widely used all over the world. It belongs to the Family Zingiberaceae. It possesses antimicrobial activity and can be used to treat bacterial infection (Tan & Vanitha, 2004). Chemical composition of ginger shows that it contains over 400 different compounds. The major constituents in ginger rhizomes are carbohydrates, lipids, terpenes and phenolic compounds (Grzanna et al., 2005). Ginger contains terpene components including zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene α-curcumene, phenolic compounds including gingerol, paradols, and shogaol. The antimicrobial activity of methanol and n-hexane extract of ginger may be due to the gingerol and shogaol as active ingredients (Hasan et al., 2012). Galangal (*Alpinia galanga* (L.) Willd.) belongs to the Family Zingiberaceae and has also been used as a traditional medicine for treatment of stomachache, a carminative and diarrhea (Oonmetta-aree et al., 2006). The essential oil from *A. galanga* consists of cineole, 4-allylphenylacetate, α-farnesene, (2, 6-dimethylphenyl) borate and α-pinene (Hamad et al., 2016). Lemongrass (*Cymbopogon citratus* (DC.) Stapf) belongs to the Family Gramineae and is commonly used in folk medicine for treatment of nervous and gastrointestinal disturbances, and as an antispasmodic, analgesic, anti-inflammatory, anti-pyretic, diuretic and sedative (Santin et al., 2009). The major constituents of *C. citratus* essential oils are geranial, neral and myrcene (Bassolé et al., 2011). Tree basil (*Ocimum gratissimum* L.) is an herbaceous plant that belongs to the Labiatae family. Chemical analysis of the essential oil demonstrated the presence of Eugenol and other compounds such as 1, 8-cineole and β-selinene (do Nascimento Silva et al., 2016). Since a large number of different chemical compounds is presented in this extract, their combined mechanism of actions can affect multiple target sites against the bacterial cells. Therefore, due to their availability and affordable production cost, Thai edible plants with medicinal properties should be further investigated for antimicrobial applications.

In Thailand, studies of the biological activity of plants have been widely reported (Komutiban, 2014; Sritubtim et al., 2014; Junsathian et al., 2018). Some Thai medicinal plants were already screened and observed for their antibiofilm activity (Teanpaisan et al., 2017) and anti-protozoa effect (Leesombun et al., 2017). However, the role of Thai edible plants that can provide useful medicinal properties especially the antimicrobial effect is not quite clear and still needs to be elucidated. The purpose of the present study was to evaluate the antimicrobial activity of the extracts from some edible plants including ginger (*Zingiber officinale* Roscoe), galangal (*Alpinia galanga* (L.) Willd.), lemongrass (*Cymbopogon citratus* (DC.) Stapf), tree basil (*Ocimum gratissimum* L.) against some skin infection pathogens.

Materials and methods

1. Preparation of crude extract

Four plants were purchased from local market in Phayao province, Thailand, from July to December 2017 including ginger (*Zingiber officinale* Roscoe), galangal (*Alpinia galanga* (L.) Willd.), lemongrass (*Cymbopogon citratus* (DC.) Stapf), tree basil (*Ocimum gratissimum* L.). Information on the plant material is shown in Table 1. Fresh plants were washed and dried at 40ºC. After dried completely, the material was powdered for further extraction. Twenty grams of powdered plant material was extracted with 200 ml of 95% ethanol and distilled water at room temperature for 24 h maceration without shaking; this extraction process was repeated 3 times. The extract was then filtrated through Whatman No.4 filter paper and concentrated using a rotary evaporator. The extract yield was determined on a weight basis and the yield percentage was calculated as follows:

% extraction yield = (mass of extract/mass of dry matter) x 100

Table 1 Edible plant materials and their extract yield percentage

Scientific name	Common name	Part of sample	% extraction yield	
			Aqueous extract	Ethanol extract
Zingiber officinale Roscoe	Ginger	Rhizome	17.16	10.66
Alpinia galanga (L.) Willd.	Galangal	Rhizome	18.06	14.80
Cymbopogon citratus (DC.) Stapf	Lemongrass	Stem	17.40	11.14
Ocimum gratissimum L.	Tree basil	Leaf	13.04	8.38

The crude extract was stored in a freezer until further use. The samples were re-dissolved using 95% ethanol or distilled water depending on their solvent. Three concentrations of the plant extract (100, 200 and 300 mg/ml) were used to test antimicrobial activity by disc diffusion and agar well diffusion methods.

2. Bacterial strains and growth conditions

Escherichia coli TISTR 117, *Staphylococcus aureus* TISTR 746, *Staphylococcus epidermidis* TISTR 518, *Pseudomonas aeruginosa* TISTR 1287 and *Candida albicans* TISTR 5554 were purchased from the culture collection of the Thailand Institute of Scientific and Technological Research (TISTR). *Propionibacterium acnes* was provided by Division of Microbiology and Parasitology, School of Medical Sciences, University of Phayao, Thailand. Bacteria (except *P. acnes*) and fungus were cultured on Mueller Hinton broth (MHB) for 24 h at 37ºC and yeast extract-malt extract broth (YMB) for 48 h at 30ºC, respectively. *P. acnes* was cultured on Brain Heart Infusion broth (BHI broth) for 48 h at 37ºC under anaerobic condition. Before each experiment, cultures of the test microorganisms were suspended in MHB and YMB and the optical density of the suspension was adjusted to obtain the viable cell count of 10^6 CFU/ml.

3. Antimicrobial activity

3.1 Disc diffusion method

Disc diffusion method was carried out according to the standard method by Bauer et al. (1966). The microbial inocula were spread using the sterile cotton swab on Mueller Hinton agar (MHA) for bacteria and yeast extract-malt extract agar (YMA) for fungus. Sterile filter paper discs (6 mm) added with 20 µl of the extracts were placed on the cultured agar before incubation at 37ºC for 24 h. *P. acnes* and *C. albicans* were incubated for 48 h at 37ºC and 30ºC, respectively. The experiments were performed in triplicate. Diameters of the inhibition zones were measured in millimeter. Antimicrobial activity of the controls (ethanol, tetracyclin and Amphotericin B) against all the tested isolates were also determined.

3.2 Agar well diffusion method

Similar to the procedure used in the disc diffusion method, the fresh inocula were spread using a sterile cotton swab on MHA and YMA. A hole with a diameter of 6 mm is punched with a sterile cork borer on the medium. Fifty microliters of the extract were added into each hole. The bacteria were grown for 24-48 h at 37 ºC whereas the fungus was grown for 48 h at 30 ºC. Inhibition zones were measured in millimeter.

3.3 Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The minimal inhibitory concentrations (MICs) of the plant extracts were determined according to Clinical and Laboratory Standard Institute (CLSI) (2016) guidelines. Plant extracts were prepared at different concentrations, ranging from 0.49 to 500 mg/ml in a 96-well microtiter plate. The microbial cells of 10^6 CFU/ml were inoculated in the microtiter plate followed by incubation at 37 ºC for 24 h. After incubation, 10 µl of resazurin was added and incubated further for 2 h to evaluate the growth inhibition. Resazurin is a blue dye that can be reduced to pink color by viable cells. The lowest concentration causing the color change was considered as the MIC. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values were tested by subculturing the microorganisms from the MIC well onto the agar. The MBC and MFC values were defined as the lowest concentration of plant extract at which no bacteria and fungus growth was recorded.

4. Statistical analysis

Data are expressed as mean ± standard deviation (SD). The data are subjected to analysis of variance (ANOVA) and Duncan's New Multiple range Test (DMRT). The difference level of $p < 0.05$ is considered significant.

Results and discussion

1. Plant extraction yield

Both of ethanolic and aqueous extracts of four edible plants including ginger, galangal, lemongrass and tree basil were examined for the antimicrobial activity against the skin infection pathogens. The extraction yields of plant materials with ethanol and water ranged from 8.38 to 14.80 % and 13.04 to 18.06 %, respectively. The highest yield of the plant extracts was obtained from galangal while tree basil gave the lowest extraction yield.

2. Antimicrobial activity of plant extract

The antimicrobial activities of edible plants at different concentrations against six microorganisms including three Gram-positive bacteria, two Gram-negative bacteria and one fungus were shown using the disc diffusion method (Table 2) and the agar well diffusion method (Table 3). Both methods present different ability of

Table 2 Antimicrobial activity of edible plant extracts using disc diffusion method

Crude extract		Concentration (mg/ml)	Diameter of inhibition zone (mm) (mean±SD)					
			E. coli	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*P. acnes*	*C. albicans*
Ginger	Aqueous	100	6.00±0.00^{e}	7.00±0.00^{e}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
		200	10.33±0.58^{b}	8.33±0.58^{d}	6.00±0.00^{d}	6.00±0.0^{0e}	6.00±0.00^{i}	6.00±0.00^{d}
		300	10.67±0.58^{b}	8.67±0.58^{d}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
	Ethanol	100	7.33±0.58^{d}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	9.00±0.00^{g}	6.00±0.00^{d}
		200	9.33±1.15^{c}	6.00±0.00^{f}	6.00±0.00^{d}	8.33±0.58^{c}	11.00±0.00^{e}	6.00±0.00^{d}
		300	10.67±0.58^{b}	6.00±0.00^{f}	6.00±0.00^{d}	8.00±0.00^{c}	12.33±0.58^{c}	6.00±0.00^{d}
Galangal	Aqueous	100	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
		200	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	7.00±0.00^{d}	6.00±0.00^{i}	6.00±0.00^{d}
		300	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	10.00±0.00^{b}	6.00±0.00^{i}	6.00±0.00^{d}
	Ethanol	100	6.00±0.00^{e}	19.33±0.58^{c}	21.67±0.58^{c}	6.00±0.00^{e}	10.33±0.58^{f}	6.00±0.00^{d}
		200	6.00±0.00^{e}	21.00±1.00^{b}	24.33±1.15^{b}	6.00±0.00^{e}	11.33±0.58^{d}	8.33±0.58^{c}
		300	6.00±0.00^{e}	21.33±0.58^{b}	24.33±0.58^{b}	6.00±0.00^{e}	15.00±0.00^{b}	9.33±0.58^{b}
Lemongrass	Aqueous	100	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
		200	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
		300	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
	Ethanol	100	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00d^{d}
		200	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
		300	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	7.00±0.00^{h}	6.00±0.00^{d}
Tree basil	Aqueous	100	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
		200	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
		300	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
	Ethanol	100	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
		200	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
		300	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
Ethanol			6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{d}	6.00±0.00^{e}	6.00±0.00^{i}	6.00±0.00^{d}
Tetracycline (30 µg/ml)			27.33±1.53^{a}	26.67±0.58^{a}	29.33±1.15^{a}	23.33±1.53^{a}	32.00±0.00^{a}	ND
Amphotericin B (10 µg/ml)			ND	ND	ND	ND	ND	21.00±2.00^{a}

Remark: Diameter of inhibition zone including disc diameter of 6 mm.
The different letters within the same column indicate statistically significant difference at 0.05 probability level.
ND; not determined.

diffusions. The disc diffusion method shows the capacity of antimicrobial agent to adsorb and diffuse through paper discs into the agar medium and inhibits the growth of the microbial strain whereas the agar well diffusion method shows the capacity of diffusion to agar medium.

As shown in Table 2 and 3, the ethanolic extract of galangal showed the most effectiveness of antibacterial activity against all microorganisms tested by both methods. The inhibition zone of the extracts ranged from 7 to 24 mm for the disc diffusion method and 7 to 29 mm for the agar well diffusion method. The results indicated that the inhibition zone variation was correlated with the concentration of the crude extracts.

At the concentration of 100 mg/ml, the microbial growth was inhibited by the ethanolic extracts of four plants using agar well diffusion method. While, at the same concentration using disc diffusion method, only the ethanolic extracts of ginger and galangal could inhibit the microbial growth. The aqueous extracts of lemongrass and tree basil extract could not inhibit all tested microorganisms using disc diffusion method. From the results, the antibacterial activity depended on the concentration of the extract and the extraction solvent. Generally, high antibacterial activity was observed in high concentration of the extract. Accordingly, an increase in the extract concentration produced a relative increase in the diameter of inhibition zone. This effect could be from the major and minor chemical components of the extract, including the interactions between them. Our results agree with the study of Er et al. (2018) who demonstrated that inhibition zone diameter significantly indicated an increase parallel to the application dose.

From these results, the edible plant extract had more potential of antibacterial activity against Gram-positive than Gram-negative bacteria. In accordance with previous findings, higher antimicrobial activity against Gram-positive bacteria than Gram-negative bacteria may be due to the fact that the cell wall of Gram-negative bacteria is multilayered structure and composed of the outer membrane. Accordingly, antibacterial agents could not easily penetrate Gram-negative cells and inhibit their growth (Parekh & Chanda, 2011; Meeprathom et al., 2018).

Table 3 Antimicrobial activity of edible plant extracts using agar well diffusion method

Crude extract		Concentration (mg/ml)	Antimicrobial activity					
			E. coli	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*P. acnes*	*C. albicans*
Ginger	Aqueous	100	6.00±0.00^{h}	6.00±0.00^{h}	6.00±0.00^{l}	6.00±0.00^{h}	6.00±0.00^{j}	6.00±0.00^{h}
		200	6.00±0.00^{h}	6.00±0.00^{h}	6.00±0.00^{l}	6.00±0.00^{h}	6.00±0.00^{j}	6.00±0.00^{h}
		300	6.00±0.00^{h}	6.00±0.00^{h}	7.00±0.00^{kl}	6.00±0.00^{h}	6.00±0.00^{j}	6.00±0.00^{h}
	Ethanol	100	12.67±0.58^{d}	10.67±0.58^{e}	11.33±1.53^{fg}	9.33±0.58^{de}	13.67±0.58^{c}	8.00±0.00^{f}
		200	13.67±0.58^{c}	11.67±1.15^{e}	12.33±0.58^{ef}	9.67±0.58^{cd}	14.33±0.58^{c}	8.00±0.00^{f}
		300	14.33±0.58^{b}	13.67±1.53^{d}	13.00±0.00^{de}	10.33±0.58^{bc}	15.33±0.58^{d}	8.00±0.00^{f}
Galangal	Aqueous	100	6.00±0.00^{h}	6.00±0.00^{h}	10.67±0.58^{gh}	6.00±0.00^{h}	6.00±0.00^{j}	6.00±0.00^{h}
		200	6.00±0.00^{h}	6.00±0.00^{h}	11.67±0.58^{fg}	6.00±0.00^{h}	7.00±0.00^{i}	6.00±0.00^{h}
		300	6.00±0.00^{h}	6.00±0.00^{h}	13.67±1.15^{d}	6.00±0.00^{h}	10.00±0.00^{g}	6.00±0.00^{h}
	Ethanol	100	9.67±0.58^{f}	20.67±1.53^{c}	26.33±1.15^{c}	9.33±0.58^{de}	14.33±0.58^{c}	20.00±0.00^{c}
		200	12.00±0.00^{c}	23.33±1.15^{b}	28.33±0.58^{b}	9.67±0.58^{cd}	21.67±1.15^{c}	20.33±0.58^{c}
		300	13.33±0.58^{c}	24.33±1.15^{b}	29.00±1.00^{b}	11.00±0.00^{b}	23.33±1.53^{b}	22.00±1.00^{b}
Lemongrass	Aqueous	100	6.00±0.00^{h}	6.00±0.00^{h}	6.00±0.00^{l}	6.00±0.00^{h}	6.00±0.00^{j}	6.00±0.00^{h}
		200	6.00±0.00^{h}	6.00±0.00^{h}	7.67±0.58^{k}	9.33±0.58^{de}	6.00±0.00^{j}	6.00±0.00^{h}
		300	6.00±0.00^{h}	6.00±0.00^{h}	8.33±0.58^{jk}	10.67±0.58^{b}	6.00±0.00^{j}	6.00±0.00^{h}
	Ethanol	100	8.67±0.58^{g}	8.67±0.58^{fg}	8.00±1.00^{jk}	8.00±0.00^{f}	9.00±0.00^{h}	9.67±0.58^{c}
		200	9.33±0.58^{f}	9.33±0.58^{f}	9.33±0.58^{ij}	8.33±0.58^{f}	9.67±0.58^{gh}	9.67±0.58^{c}
		300	9.67±0.58^{f}	9.33±1.53^{f}	9.67±0.58^{hi}	8.66±0.58^{f}	11.00±0.00^{f}	11.00±0.00^{d}
Tree basil	Aqueous	100	6.00±0.00^{h}	6.00±0.00^{h}	6.00±0.00^{l}	6.00±0.00^{h}	6.00±0.00^{j}	7.00±0.00^{g}
		200	6.00±0.00^{h}	6.00±0.00^{h}	6.00±0.00^{l}	6.00±0.00^{h}	6.00±0.00^{j}	7.00±0.00^{g}
		300	6.00±0.00^{h}	6.00±0.00^{h}	6.00±0.00^{l}	6.00±0.00^{h}	6.00±0.00^{j}	7.00±0.00^{g}
	Ethanol	100	8.00±0.00^{g}	7.67±0.58^{g}	7.67±1.53^{k}	7.00±0.00^{g}	6.00±0.00^{j}	6.00±0.00^{h}
		200	8.00±0.00^{g}	7.67±0.58^{g}	7.67±0.58^{k}	7.00±0.00^{g}	6.00±0.00^{j}	6.00±0.00^{h}
		300	8.00±0.00^{g}	8.00±0.00^{g}	8.00±0.00^{jk}	7.00±0.00^{g}	6.00±0.00^{j}	6.00±0.00^{h}
Ethanol			6.00±0.00^{h}	6.00±0.00^{h}	6.00±0.00^{l}	6.00±0.00^{h}	6.00±0.00^{j}	6.00±0.00^{h}
Tetracycline (30 µg/ml)			35.00±1.00^{a}	35.33±0.58^{a}	31.67±1.53^{a}	32.33±1.53^{a}	34.00±0.00^{a}	ND
Amphotericin B (10 µg/ml)			ND	ND	ND	ND	ND	23.00±2.00^{a}

Remark: Diameter of inhibition zone including well diameter of 6 mm.
The different letters within the same column indicate statistically significant difference at 0.05 probability level.
ND; not determined

3. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

MIC is the lowest concentration of the extract that can inhibit the microbial growth after incubation. The MIC of four edible plants against skin infection pathogens were determined using the microdilution method. From this study, the observed MIC, MBC and MFC of the extracts ranged from 0.49 to 500 mg/ml. The MIC value of galangal for the ethanolic extract was 0.49 to 15.63 mg/ml and the MIC on *S. aureus* was 0.98 mg/ml. This finding is consistent with the studies from Oonmetta-aree et al. (2006) and Mayachiew & Devahastin (2008), which indicates that galangal ethanolic extract has the strongest antibacterial activity. The ethanolic extract of ginger shows the highest antibacterial activity on *P. acnes* with the value of 0.49 mg/ml.

According to the results from this study, the aqueous extract has lower antimicrobial activity compared with the ethanolic extract. It is possible that the ethanolic extract consists of more active compounds than the aqueous extract due to higher potency of the antimicrobial activity. The previously study showed that the main compound of galangal ethanolic extract was D,L-10-acetoxychavicol acetate, which has high antibacterial effect against *S. aureus* (Oonmetta-aree et al., 2006). Moreover, the minor compounds of crude extract were identified by GC-MS, which are defined as p-coumaryl diacetate, palmitic acid, acetoxyeugenol acetate, 9-octadecenoic acid, eugenol, b-bisabolene, b-farnesene and sesquiphellandrene. This ethanolic extract demonstrated both outer and inner membrane damages and disruption of the cytoplasmic membrane function (Oonmetta-aree et al., 2006).

The extracts were calculated for possibility to have a bactericidal or bacteriostatic effect. The extracts show bactericidal activity when the ratio of MBC/MIC ≤ 4 and bacteriostatic activity when the ratio is of MBC/MIC > 4 (Djeussi et al. 2013; Noumedem et al., 2013). Interestingly, bactericidal effect was obtained with the ethanolic and aqueous extracts from ginger, galangal, lemongrass and tree basil against all tested bacteria. This confirms that the studied extracts from edible plants exhibits bactericidal effect.

Generally, the inhibitory activity of essential oils was greater than that of ethanolic extracts. It has been shown

Table 4 MIC, MBC and MFC of edible plant extracts

Crude extract		Concentration of extract (mg/ml)											
		E. coli		*S. aureus*		*S. epidermidis*		*P. aeruginosa*		*P. acnes*		*C. albicans*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Ginger	Aqueous	62.5	62.5	62.5	125	7.81	15.63	62.5	125	250	500	250	500
	Ethanol	0.49	0.49	0.98	1.95	0.49	0.49	31.25	125	0.49	0.49	15.63	31.25
Galangal	Aqueous	0.98	3.91	31.25	62.5	31.25	125	62.5	250	250	250	500	500
	Ethanol	0.98	0.98	0.98	1.95	0.98	0.98	15.63	62.5	0.98	0.98	0.49	0.49
Lemongrass	Aqueous	62.5	125	15.63	62.5	15.63	125	125	125	125	250	500	500
	Ethanol	1.95	3.91	31.25	125	7.81	7.81	31.25	125	0.98	0.98	15.63	62.5
Tree basil	Aqueous	250	500	250	250	250	250	250	250	125	500	62.5	500
	Ethanol	31.25	62.5	125	125	125	125	62.5	250	125	250	31.25	500

that the essential oil of galangal showed antimicrobial activities against bacteria, fungi, yeast and parasite (Farnsworth & Bunyapraphatsara, 1992). However, this result also supports that the ethanolic extract inhibited growth of bacteria and yeast. Moreover, the process to obtain ethanol extract by maceration extraction is simple and requiring less equipment. These support that aqueous and ethanolic extracts from plants are probably good alternative antimicrobial agents.

Conclusion

This study shows that the antimicrobial activity against all tested pathogens is highest for the ethanolic extract of galangal, followed by ginger and lemongrass, respectively. Furthermore, the extracts of ginger, galangal, lemongrass and tree basil exhibits bactericidal effects on all tested bacteria. The findings of this study suggest that edible plant extracts could possibly be used as a natural antimicrobial agent for further applications.

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Thai Traditional Medicine at Wat Nong Ya Nang Buddhist, Uthai Thani Province

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Abstract

The research aims to study the origin of the healthcare treatment, application of Thai herbal medicine and the indigenous health practices at Wat Nong Ya Nang in Uthai Thani province. The data was collected by focus group between April, 2015 to November, 2016. Data analysis used content analysis. Phrakhru Up Kan Phat Kit was born in 1946 in Uthai Thani. In 1971, he was ordained as a Buddhist monk at Wat Thamma Sophit in Nakhon Sawan province. In 1974 he started treatment of bone injuries at Wat Nong Ya Nang temple by using herbal medicine and massage therapy which has evolved into a mix treatment by utilizing Thai traditional herbal medicine together with Buddhist principles, meditation, spiritual rituals, massages, indigenous knowledge, modern physical therapy and prescription medication. This mix treatment format is consistent with the culture of Thai communities. The combined treatments have been affective in helping the recovery of patients with bone injuries, cancer and paralysis. Research results conclude that the holistic method of treatment had to be on both body and soul and majority of patients were favorable towards the mixed treatment method at the hospice. This will build the strength of the community, making people feel sympathy to each other. This also will help to support and promote traditional Thai medicinal practices

Introduction

Buddhist monks who are Thai traditional healers are called Maw Pra (monk healer) and they provide treatment to the public at the Buddhist temple where they reside. Thai communities have long respected and trusted indigenous healers (Ganjanapan, 2000) and Maw Pra healers up to modern times, even when the primary healthcare services are at community clinics and hospitals. Monks in Thailand have extensively been healers throughout the history of Thailand and have treated the public and the community through the use of herbal medicine (Tiyavanich, 1997). Buddhist monks who became Maw Pra, did not have any initial interest in becoming a healer before they were ordained. Most had little or no knowledge at all of traditional medicine and healing remedies. It was only after being ordained, that they comprehended the fact that they wanted to help their fellow human being and decided to become a healer through traditional methods and customs. The

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Maw Pra healers provided medical treatment for illnesses and provided spiritual healing and guidance to patients and to their family and friends. Basic herbal remedies and medicinal properties of plants and ingredients are described in the Buddhist scripture of Vinaya Pitaka, which is a chapter the Tripitaka Buddhist scriptures. The scriptures also provide a guide to supplemental spiritual treatment through Buddhist principles and practices which strengthen and revitalize the mental health of patients to be mindful, to be at peace, to have strength in fighting the illness and to be prepared for the eventuality of death (Pannapajato, 2013). The adaptation of traditional physical treatment, mental healthcare, herbal medicine, Thai traditional medicine and contemporary practices have been offered to the public long before modern medicine became widespread and they are still an affective medical and healthcare service. Maw Pra, Thai traditional medicine and indigenous practices provide healthcare alternatives and is still provided by dedicated Buddhist temples in Thailand.

Many patients with terminal or incurable illnesses turn to alternative treatment and to Buddhist temples which might provide them with a better solution then just yielding to their sickness. The development of Thai traditional medicine and treatment at Buddhist temples continues to be developed and Maw Pra's are working together with doctors, hospitals and clinics to provide an alternative option for society. The integration of Thai traditional medicine, indigenous knowledge, and herbal medicine and Buddhism principles has been able to provide patients and their families with spiritual rehabilitation, peace and preparation for death (Chantraket et al., 2007). Healthcare and treatment of patients can be strengthened and enhance through the development and integration of allopathy and traditional methods (Kurup et al., 1993). There are still many Thai's who seek alternative methods and other possibilities when faced with a terminal diagnosis and Wat Nong Ya Nang in Uthai Thani province in central Thailand is a respected treatment center in providing healthcare free of costs through Thai traditional medicine and indigenous practices.

Wat Nong Ya Nang is a Buddhist temple located in rural Uthai Thani province and was established during the Ayutthaya Kingdom (1350-1767). The temple is a renowned traditional healing center through the efforts of Phrakhru Up Kan Phat Kit who created the hospice in 1974 for treating cancer and bone injuries by using Thai herbal medicine and indigenous health practices. The treatment continues to be popular with patients from other provinces and from other countries. Phrakhru Up Kan Phat Kit continued to heal and supervise treatment services at the temple up until his death in 2013. Phrakhru Palat Sutthi Phong is currently the acting abbot of Wat Nong Ya Nang after the passing of Phrakhru Up Kan Phat Kit. The treatment is cost-free for patients and the temple relies on donations for funding. The herbal medicine used at the temple is collected from the forest, bought from merchants and are also grown in the temple gardens.

The accomplishment and reception of Wat Nong Ya Nang hospice is significant and is the motive for this research because the practices and treatment at the hospice demonstrates the positive integration of Thai herbal medicine, indigenous health practices and modern medical practices that is effective in helping the recovery of patients with bone injuries, cancer and paralyses.

The research aims to study the origin of the healthcare treatment, application of Thai herbal medicine and the indigenous health practices at Wat Nong Ya Nang in Uthai Thani province.

Materials and methods

The study was conducted at the Wat Nong Ya Nang in Uthai Thani province from April 2015 to November 2016. The temple was purposively chosen because the temple is recognized for paralysis treatment and Thai massages. The temple is a traditional treatment center to the local community and utilizes modern medicine, Buddhist principles and Thai traditional herbal medicine.

A qualitative method through focus group interview was used. Two basic reasons led to the adoption of this technique. First, gathering the essential information on Thai traditional medical practices at the temple. Second, no previous research is reported from Wat Nong Ya Nang to identify the use of Thai traditional medicine in the management of chronic diseases. The individual open-ended interview guide was used to interview participants with information on the history and practice of Thai traditional medicine. Participants were categorized into three group; 1) key informants were local indigenous healers who are the supervising medical examiners at the temple (n=3); 2) casual informants included Thai traditional medicine practitioners, pharmaceutical staff and caregivers (n=7);

3) general informants are former patients at Wat Nong Ya Nang (n=15). Participants who were able and willing to describe to describe information on history and practice of Thai traditional medicine were consented for the interview.

Results

1. Content analysis of the interviews identified three major themes

These include familiarity and understanding of history of Thai traditional medicine at Wat Nong Ya Nang; and service procedures and facility at Wat Nong Ya Nang; and Thai traditional medicine. Each theme with illustrative excerpts from patients' transcripts is described below.

1.1 History of Thai Traditional Medicine at Wat Nong Ya Nang

Phrakhru Up Kan Phat Kit was born in 1946 in Uthai Thani. His father and mother were farmers. His father was also a maw ya (local healer) and cured illness through herbal medicine. The knowledge of using herbal remedies and herbal medicine to cure illness was practiced by his father and his uncle which was a knowledge passed down to them through the generations. In 1967 he was drafted into the Thai army and stationed at the Jiraprawat military base in Nakhon Sawan province. After discharge from the army in 1971, he was ordained as a Buddhist monk at Wat Thamma Sophit in Nakhon Sawan province. In 1973, he was transferred to Wat Nong Ya Nang as the acting abbot. It was at Wat Nong Ya Nang that he revived his knowledge and practice of healing through herbal medicine and also massage therapy. As he continued to perform his duties as the acting abbot, he also pursued knowledge and practice in herbal medicine through the guidance and knowledge from his uncle and started treating patients at Wat Nong Ya Nang in 1974. The first healthcare service provided at the temple is treatment of patients with bone injuries such as bone fractures, dislocations and sprains. Phrakhru Up Kan Phat Kit continued his pursuit of traditional healing methods to include bone injuries using herbal medicine of sesame oil, foot massage therapy to apply high pressure to reach deep nerves and to penetrate thick skin and mass. The temple also uses herbal compression balls to relieve stress and pain. The treatment was very popular in the community and patients traveled from other provinces and also from abroad. The services were extended to include patients that suffer from paralysis, dry beriberi and diabetes. Phrakhru Up Khan Phat Kit received the title of abbot of Wat Nong Ya Nang in 1977. In 1993, Phrakhru Up Kan Phat Kit was recognized as an accomplished individual with outstanding work in cultural indigenous knowledge in disease therapy by the cultural committee of Thailand. In 2003, the Uthai Thani Provincial Public Health Office established an education center for Thai traditional medicine at Wat Nong Ya Nang.

1.2 Service Procedures and Facilities

The current medical staff at the temple include Mr. Sam-ang Yao Man who is the leading supervisor and primary medical examiner, 3 male masseuses, 12 female masseuses and 2 volunteers. There are dedicated treatment facilities separated from the religious buildings and patients can register and receive treatment from 8 a.m. to 4:30 p.m. Massage therapy is performed in a dedicated facility which is segregated to male and female patients and the herbal sauna building is segregated into 3 saunas for male patients and 2 saunas for female patients. Each sauna can accommodate 5 individuals.

Treatment for bones disease and injuries at the temple have increased since services started in 1974. The majority of patients suffer from bone and muscle injuries, Cerebro-Vascular accidents and poisoning from contact or ingesting of agricultural chemicals. Many of the patients that come to the temple have partial paralysis. Patients that register for treatment at the temple come for additional treatment after being treated at local hospitals in the community from word of mouth from family and friends. The treatment that the temple utilizes Thai traditional medicine, Buddhism, indigenous ritual practices and physical therapy.

All patients are initially screened and have their history documented during registration. The patient's medical history and condition is evaluated. The the patient is questioned about their; current condition and illness, the cause of their illness, body motor function, daily livelihood, their nutritional diet, bowel and bladder control, sleeping disorders, mental fitness, feelings, frame of mind, known congenital diseases, current and past medication prescribed by the hospital, (they must present the prescribed medicine that they currently take), past medical treatment from the hospitals or other alternative medical facilities, past surgeries if any, past accidents and if they suffered from any handicapped issues and have specific symptoms and allergies.

Physical checkup is performed by the temple's masseuse which evaluates the patient through observation of their posture, stance, and ability to walk, sit, lay down and ability to toss from side to side. They also assess the

patient's ability in handling and holding items with their hands, ability to swallow, ability to chew food, vocal ability, communication ability, eye movement and respiration. Pulse checking is a mandatory procedure which the temple examiner will compare the pulses of the upper and lower body. Pulse checking is performed on blood veins on the neck, wrists and instep. Recording the number of pulses is performed and compared with the heart rate of the patient to determine if they also suffer from heart disease. If the patient's heartbeat is low, it will indicate possible pain from deep ligaments and might induce muscle fatigue. Examiners will test the patient's skin surface tension, elasticity and temperature of ligaments by using fumbling presses. If the patient's skin temperature is high, it will indicate fever or high blood pressure. If the skin temperature is cold, it will indicate problems with the body's ligaments or that they might have low blood pressure. Observation of facial features, mouth, tong and eyes are also recorded. If the patient is unable to move their eyelids, it might indicate a neurologic or heart problem. If patients are unable to open their eyes, then a massage will be prohibited and the patient will be provided instead with herbal medicine to take together with their prescription medicine. The final physical checkup is to observe the patient's motor function by having them move their arms, legs and elevate their foot to see if one side is more heavily effected than the other.

1.3 Thai Traditional Medicine

The Thai traditional medicine at the temple follows the guidelines detailed in the Treatises on Traditional Thai Medicine and Pharmacognosy book. Healthcare and treatment are primarily based on herbal medicine, remedies, bolus, compression ball, herbal sauna, herbal oils and Thai massage. This is consistent with Sridharmma et al. (2009), in which maw ya (indigenous healer) in communities in northeast Thailand relied on herbal medicine and remedies made from plants, animals, minerals and also massage therapy which is also a popular healthcare and traditional medical treatment in Asian communities abroad (Salguero, 2019). Buddhist principles are utilized as a mental treatment so patients are able to gain back their morality and continue to practice the 5 Buddhist precepts, learn and practice on meditation to increase the strength of the spirit and of the body. Meditation and prayers also aid in a more affective recovery which help patients focus on the truths of their condition and for their minds to be at peace and can significantly reduce stress and depression and anxiety (Boelens et al., 2012). Astrology and traditional spiritual rituals are also utilized to calm the patient and relatives to be at peace and not live in vain.

The last theme suggested that there are a variety of Thai traditional medicine at Wat Nong Ya Nang. This information illustrative from participants and documents are described below

2. Herbal Medicine

The completion of the medical evaluation is followed by the planning of the treatment program and selection of the required herbal medicine. The herbal medicine given to patients at Wat Nong Ya Nang is categorized into 9 types which are targeted at the different systems or symptoms of the body; 1. digestive system, 2. respiratory, 3. urinary, 4. skin, 5. fever, 6. malaria, 7. pain relief, 8. eye infections and 9. general health. The transformation of herbal medicinal plants into herbal medicine at Wat Nong Ya Nang uses dried medicinal plants and herbs because they can be conveniently stored. The herbal medicine is transformed through simple processes resulting in herbal medicine remedies in various forms such as boiled remedies, herbal bolus, herbal potions, herbal compression balls, and herbal sauna or aromatherapy. Herbs and medicinal plants that have had scientific research applied to them have received positive outcomes in the area of fighting cancer (Chavan et al., 2013).

3. Boiled Herbal Remedies

Boiled herbal remedies can either use fresh or dried medicinal plants and herbs which are boiled proportionately with water. Different parts of the medicinal plants and herbs such as the stem, bark, seeds and roots are boiled accordingly to Wat Nong Ya Nang's herbal formula. There are 9 various boiled herbal remedies at Wat Nong Ya Nong: 1. Five remedies for treating temporary paralysis patients. 2. Two remedies for nausea and the nervous system. 3. Two remedies to treat ligaments and beriberi, 4. One remedy for treating gout and bone joint pains. 5. One remedy for diabetes. 6. One remedy for stiff tongue symptoms. 7. One remedy for treating pustule cancer. 8. One remedy for treating rectal bleeding and 9. One remedy to treat the heart. The amount of boiled remedies that are prescribed to patients are in pots or portions. Larger portions will be determined by the supervising examiner if the patients' symptoms are severe and substantial. If the patient doesn't respond or doesn't start to heal, then the portions will be increased and the boiled remedy consumed until the patient has recovered or is cured.

4. Herbal Bolus Remedies

Bolus remedies are used when the patient's symptoms require a remedy with herbs and medicinal plants that are difficult to ingest, such as plants that are nauseating in taste, odor and difficult to dissolve in water. The herbal bolus are balls of herbal medicine mixed with natural honey and are sometimes referred to as honey balls. The herbal balls have a desired characteristic in that it slowly dissolves and can gradually distribute the healing herbal medicine into the patient's body. The herbal balls are prepared by grinding up the proportioned herbal plants and herbs into a fine powder medicine. The powdered medicine is then combined with natural honey so that it can be rolled into a ball and is stored for at least 1 night. The mixture of powdered medicine and honey makes the herbal bolus have a long lifespan because the added honey has more density than water and is more resistant to moisture and yeast than mixtures that are mixed water. The final stages include drying the bolus balls in sunlight and baking it until it becomes firm and solid.

5. Herbal Potions

Herbal potions are prepared through simmering a mixture of herbal medicinal plants, herbs and ingredients until the medicinal properties are extracted as a liquid. The main ingredient for the herbal potions at Wat Nong Ya Nang as well as water and medicinal ingredients is coconut oil. The finished potion can be applied through various methods such as spraying, rubbing, coating, eye droplets and used as a massaging agent.

6. Herbal Compression Balls

Herbal compression balls at Wat Nong Ya Nang are made from fresh herbal plants and herbs. The ingredients are proportioned accordingly to Wat Nong Ya Nang's formula and crushed into a fine mix and enveloped with a clean cloth. The compression balls are put in a steamer until they are firm enough to be applied to the various parts of the body to relieve pain and are also used in massages to relax ligaments, muscles and pain which will help restore health and strength to the respiratory and strengthen the blood circulation in the body.

7. Herbal Sauna and Aromatherapy

The herbal sauna and aromatherapy of Wat Nong Ya Nang is prepared by boiling all the required herbal plants and herbs into a big pot and piping the steamed extracts into the sauna room. The sauna room at Wat Nong Ya Nang is a standard sauna which can accommodate 5 individuals at a time. The sauna therapy and aromatherapy is so that the steamed medicine can penetrate the skin, body and be inhaled to relieve stress and clear up the respiratory system. The mixture of medicinal plants and herbs can cure and aid in the recovery of many diseases and symptoms.

8. Massage Therapy

Massage therapy at the temple is focused on temporary or periodic paralysis which are due from accidents or is inherited and can come from family history. (Dissanayake & Padmaperuma, 2018). Herbal oils and lotions are used in massages along with sauna therapy. Patients are required to ingest herbal medicine prior to the massage and the therapist will determine at a later stage if a herbal sauna or aromatherapy is required. If the patient is physically strong enough and can adequately help themselves, then one herbal sauna per day will be authorized. The herbal sauna is a 30-minutes process and will help increase the patients' blood recirculation and help stimulate nerves and muscles. Herbal compression balls and massages are focused and applied only to the stiff muscles and joints. Sesame oil or hot herbal balls is a choice which will help stimulate deep muscle aches. Limp muscles are massaged by hands and sometimes accompanied with hot herbal balls. Massage therapy for paralysis start with light finger massages to prompt responsiveness of the muscles. This is performed through light clasps and touches to the arms and legs or pressing clasps to muscles with the aid of herbal oils to relieve tension to the muscles and tendons. Applying herbal boils, hot or cold is to increase stimulation to the nerves and initiate the patient's responsiveness which will increase efficient blood circulation. The relief of stress and pain in the body will give patients more confidence and will also increase their cooperation in their own treatment. Massage therapy is more effective for temporary paralysis than for permanent paralysis, because paralysis patients can respond to the treatment and provide feedback that is used to determine what additional treatments should follow. The initial movement of fingers or limbs are a signifier of the patient's responsiveness. When the therapist verifies that it is adequate, then the next step is to perform massages with the patient in a face up sleeping position. This massage is orderly applied to the legs, arms, shoulders, neck, head and face. The massage is applied to the limb or body part that is paralyze or applied to the part of the body that is causing the most pain. Further massages will be applied while the patient is in an inclined position and also when the patient is able to flip over by themselves. The massage in the incline position will start from the

lower back and emphasize on muscles surrounding the waist. Face massages will start from the head and neck when the patient is able to sit upright by themselves. The steps will initially start at the side or muscle that is normal first and gradually move to the muscle that is hurting the most.

9. Physical Therapy

Temporary or partial paralysis patients that have re-gained slight control of their motor functions will be assessed by their therapist to see if they are able to proceed to the next step which is their self-balance and walking capabilities. Patients will be taught how to regain their walking ability through exercises and specialized tools devised by the temple. The physical tools and exercises include exercising with the help of human aids, parallel bars, walking sticks and four legged walkers. The exercise area is located in the backyard of the temple. Other utilities and tools include arm reels and slings, steps with rail bars and shoulder movement guide plates. The tools and equipment are all created at the temple by the Buddhist monks, relatives and volunteers. The physical therapy is available and practiced on a daily basis. Assistants and caregivers at the temple are usually the patient's relatives and friends who stay at rooms provided by the temple. Volunteers also assist patients that do not have relatives or spouses to take care of them during their treatment at the temple.

Discussion

This qualitative analysis revealed results that were not previously available and documented from other temples. Buddhist monks as healers is a phenomenon not only in Thailand because belief in Buddhism is related to healing of the mind, body and spirit. Buddhist monk healers were believed to have outstanding healing powers and the medical skills of Buddhist priests were valued above those of the indigenous physicians in Japan (Shinmura, 2006). Buddhist monks perform many functions in society and many are healers, practitioners and modern psychiatrists (Cheam & Keo, 2018).

The integration between modern and traditional medical practices is evident in rural communities in Thailand and also in Africa. Wat Nong Ya Nang healthcare services and medical treatment operates by combining indigenous knowledge, cultural practices Buddhism and modern medicine. It is similar to rural communities in Africa in which rural African communities healthcare treatment consisted of three practices of; 1) Medicinal Substance, 2) Cintanqible forces which combines religion, ritual, social practices and psychology, and 3) Medical treatment of combined medicinal and Cintanqible treatments (Tompkins & Bird, 1973). Wat Nong Ya Nang's formula of steaming together a variety of medicinal plants and herbs together and using the steamed medicinal properties to cure many diseases all at once is consistent with the findings of Berlin et al. (1974) in which the medicinal properties of herbal plants are different according to their size and place of growth. Medicinal plants have contributed a rich health to human beings (Pawar et al., 2018) and the medicinal strength of each plant will also depend on the geography in which the plants grow creating a variety of healing properties that can be applied as different cures. Sauna therapy or the herbal steam baths at Wat Nong Ya Nang utilizes the different healing properties of many types of herbal plants and herbs to create a universal treatment of diseases which is delivered to patients through skin penetration and inhalation.

Alternative medical treatment and herbal cures have been expanding in Thailand and have increased significantly at Wat Nong Ya Nang since treatment services began in 1974. The popularity of meditation, herbal medicine and indigenous methods is also apparent in the United States of America. Upchurch & Chyu (2005) research into the factors that influence the choice of holistic and alternative medicine in American females revealed that within one year, 33.5% of American females have used alternative treatment methods. Most of the alternative methods include psychological treatment, meditation and herbal medicine. Meditation has also been demonstrated to aid in the recover and cure from high blood pressure, alcoholism, drug addiction and stress as confirmed by Wallace & Benson (1972) where medication elevates higher alpha brain waves which was documented with electroencephalographs.

The health caring practices at Wat Nong Ya Nang is different from other health care in terms of the use of both physical and spiritual cares.

The experimental results of Wat Nong Ya Nang show that it isa temple that has outstanding care for ailments includingparalysis, paralysis and beriberi. The temple has monks, folk doctors collaborating in the treatment. Patients are treated by using herbal medicine, oil, stepping on red iron, massage with a herbal steam. There is a healing process that combines magic rituals, spells, herbal medicine, massage with meditation or chanting. All patients are satisfied with the treatment

results. The Provincial Health Office of Uthai Thani Province have selected Wat Nong Ya Nangas a center of learning Thai folk medicine of Uthai Thani Province. Objective: To be the knowledge center of the community in promoting learning. The transfer and exchange of knowledge and experience in Thai traditional medicine for people in the community.

The results showed that in the herbal care of Wat Nong Ya Nang there is a treatment similar to the research of Yomna (2002) who studied the process of transferring knowledge of local orthopedic doctors: a case study of Wat Yukon Rat Samakkhi, Phan Thong District, ChonBuri Province The study indicated that Wat Yukkarat Rat Samakkhi. Is a temple that has monks to treat the disease with "ancient foot massage" methods. And do not collect any treatment fees and medication at all by foot massage. Is a treatment for patients with osteoporosis, paralysis, without patients with fresh wounds or open wounds, using a method of massage and stepping on the heel on the area that needs treatment, such as arms, legs, trunk. The method of treatment would include monks and pupils using the feet to dip the medicine. Is coconut oil mixed with ancient herbs, set on a gentle fire. Then use the heel to dip the oil into the injured area. When the oil is cool. Then dip the feet in the oil to massage again. Do 1 hour once a day if there are many symptoms. Must massage for 20-30 days or until the symptoms improve.

And herbal care of Wat Nong Ya Nang Differences with the research results of Chanket et al. (2008). Studied the integration of Thai traditional medicine: the role of temple and community health care. The study indicated that Community health care of Wat Huay Kiang, Chiang Mai Province, which is a rehabilitation center for paralysis patients. With alternative medicine processes, rehabilitation with alternative and fair medicine. There is a physical therapist. To convey the correct methods to patients' relatives and patients to understand. For the patient to relax and heal to live like a normal person in society with quality, healthy body.

Conclusion

Wat Nong Ya Nang is recognized for paralysis treatment and massages. Healthcare and treatment at Wat Nong Ya Nang in Uthai Thani province are healthcare and medical treatments that combines Thai traditional medicine together with Buddhist principles, meditation, massages, herbal medicine and spiritual rituals in a format that is consistent with the culture of Thai communities. The combined treatments have been affective in helping the recovery of patients with cancer, paralysis and bone injuries. The services at Wat Nong Ya Nang has also provided patients with mental and spiritual healing which increased the patient's strength in dealing with their illness and situation. The treatment also affects the patient's relatives, spouses and friends to be united through tradition and culture. The holistic approach in both treating the body and the spirit creates a strong relationship within the community and promotes empathy and compassion in helping others and living with each other in peace. Supporting and promoting traditional medicine and indigenous healthcare treatment is a sustainable format in proper management of natural resources in the community (Cartledge, 1994). It is also a means to create social norms and nurture the respect for natural resources within the community.

The development of traditional healthcare through herbal medicine and traditional healing at Buddhist temples can be enhanced by supporting research in holistic healthcare through traditional methods and integration of scientific and modern medical practices. Suggested research topics should be extended to other illnesses such as AIDS, dysfunctional immune system, hearing disabilities, detoxification, chiropractic, hydrotherapy and blindness. The funding for research and development of supplementary traditional or indigenous healing centers in Thailand should be included in the national budget. Government support will also create an organized and systematic training system and seminars to disseminate the knowledge and practices of indigenous knowledge and cultural healthcare practices.

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Nitrogen-fixing Bacteria and Trends in Agricultural Applications

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Abstract

Bacteria that can increase the number of nutrients in the soil are important to plants, especially nitrogen-fixing bacteria that fix atmospheric nitrogen and change into the form that plants can use. In recent years, the use of nitrogen-fixing bacteria in agriculture has received a lot of attention because it offers an economically attractive and environmentally friendly method. Many species of nitrogen-fixing bacteria, symbiotic and non-symbiotic, that promote plant growth are used on a regular basis in order to improve crop yields. In addition to agricultural benefits, there are also potential benefits for environmental applications. Many nitrogen-fixing bacteria which grow and multiply within plant tissues are called endophytes. They illustrate the tight association with the plant tissues without causing damage. Therefore, different types of endophytes which produce plant growth hormone provide benefit for many plants.

Introduction

Nitrogen is one of the most abundant elements in the Earth's atmosphere. Air is composed of 78 percent nitrogen, predominately in the form of nitrogen gas (N_2). Nitrogen is an essential element for all living organisms. Nitrogen exists either in the reduced or oxidized forms in the global nitrogen cycle as shown in Fig. 1. In plants, nitrogen is a major element of amino acids which are the building blocks of proteins such as enzymes, cell membranes, transport proteins, hormones, nucleic acids and ATP (energy currency of the cell). Moreover, nitrogen is also an essential element in chlorophyll, which is the most crucial pigment for photosynthesis. When plants lack nitrogen, the leaves will appear yellow and/or pale green because plants are unable to produce the chlorophyll. Plants will also develop and grow more slowly than we would normally expect. Although the atmosphere contains a large amount of nitrogen, plants cannot use that nitrogen gas. Plants can use nitrogen in the form of nitrogen compounds in the soil, but it is tiny amount (Graham & Vance, 2000). About 90 percent of the nitrogen compounds in the soil are in the form of organic matter or organic nitrogen. When they are transformed into inorganic nitrogen, nitrate ion (NO_3^-) and ammonium ion (NH_4^+), they will be beneficial to the plants. Most plants prefer nitrogen in the form of NO_3^- rather than NH_4^+. Plants can absorb NO_3^- better than NH_4^+, which is toxic for plants (Ruan et al., 2007; Babourina et al. 2007). However, both NH_4^+ and NO_3^- contained in the soil are limited and easily lost by processes such as leaching and other procedures such as

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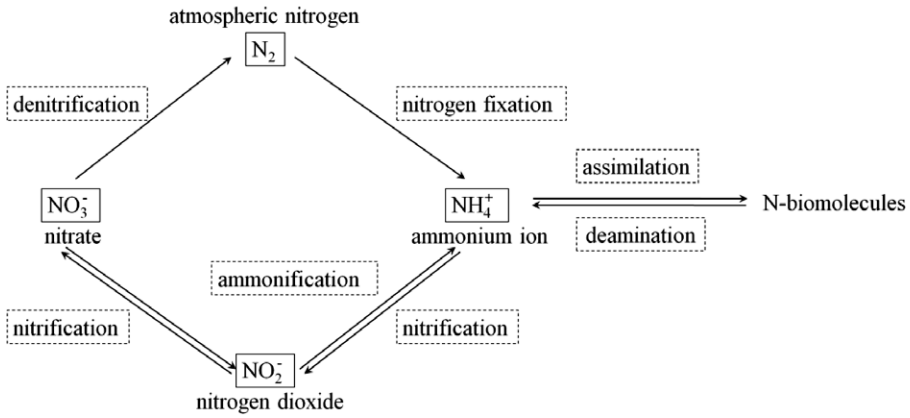

Fig. 1 Steps involved in global nitrogen cycle (modified from Igarashi & Seefeldt, 2003).

biological reduction of NO_3^- (denitrification). Therefore, it can be explained that nitrogen is an element that limits the growth of plants. Even though nitrogen is the most abundant element in the world, plants can only utilize combined nitrogen or reduced forms of nitrogen. Plants acquire these forms of nitrogen from two main sources, first is from the soil through adding fertilizers such as ammonia, manure and/or decomposition of organic substances to the soil. The second source is the conversion of atmospheric nitrogen through nitrogen fixation, such as chemical fixation via the Haber-Bosch process, which reduces N_2 to ammonia using both high temperature and pressure. In other cases, chemical nitrogen fixation can be achieved by oxidation reaction which fixes N_2 by oxidation to nitrate. In nature, lightning strike transforms N_2 into oxide of nitrogen in various forms (finally to NO_3^-). The other nitrogen fixation is symbiotic nitrogen fixation or biological nitrogen fixation by free-living or plant-associated bacteria (Sprent, 2007; Santi et al., 2013). Beijerinck (1901) discovered the biological nitrogen fixation (BNF) for the first time in 1901. It was found that the operation was carried out by a group of prokaryotes including cyanobacteria (*Anabaena* and *Nostoc*) and some bacteria. They have the nitrogenase (EC 1.18.6.1, EC 1.19.6.1), enzymes that catalyze the reduction of N_2 to produce ammonia (NH_3), which is the key step in nitrogen fixation processes. Then, NH_3 will be modified by bacteria to create their organic compounds. The overall reaction of nitrogenase is described by the equation below (Morrison et al., 2017).

$$N_2 + 10H^+ + 8e^- + 16ATP \rightarrow 2NH_4^+ + H_2 + 16ADP + 16Pi$$

Some prokaryotes are able to convert nitrogen in the atmosphere to ammonia which can be used by plants through a biological nitrogen fixation (BNF) which are called diazotrophs (Lam et al., 1996; Franche et al., 2009). These prokaryotes include aquatic organisms, free-living soil bacteria and bacteria that interactions with plants, legumes and other legumes (Postgate, 1982). Nitrogen-fixing bacteria can be divided into three broad categories based on the degree of intimacy and interdependency between them. The first is the free-living bacteria (nonsymbiotic), such as *Azotobacter, Beijerinckia* and *Clostridium.* The second category is endophytic bacteria that can colonize interior the plant tissues and provide benefits to the plant. The third is the endosymbiosis bacteria or mutualistic bacteria (symbiotic), such as *Rhizobium* which are associated with leguminous plants.

Endosymbiosis bacteria

Symbiotic nitrogen fixation is a fixation of nitrogen from the air that relies on microorganisms and plant roots. Many diazotrophs can be found in the rhizosphere, the region of soil around plant roots that influence growth, respiration and nutrient exchange of a plant. The diazotrophs show a tremendous competitive advantage over other bacteria in the rhizosphere, mainly when nitrogen in the soil is limited due to their ability to fix nitrogen from the atmosphere (Döbereiner & Pedrosa, 1987). The habitat of diazotrophs are not only in the rhizosphere, area around the root plant but also in the phyllosphere which are all the above-ground parts of

plants (Ruinen, 1956). The well-known mutualistic relationship between bacteria and plant is the association between legume and *Rhizobium* (Fig. 2). The leguminous plants produce the energy from photosynthesis to drive the nitrogen-fixing process and the *Rhizobia* fix the N_2 from the atmosphere, supplying both the bacteria and the plants. Most of the leguminous plants such as soybeans, peanuts, alfalfa, clover and lentils can fix N_2 in association with the *Rhizobia.* The *Rhizobia* have been classified on the species of legume that they nodulate. This type of grouping is called "cross-inoculation". The plants were divided into cross-inoculation groups and *Rhizobium* species would inoculate plants in the same group (Table 1). The interactions between plants and nitrogen-fixing bacteria are the purest form of nitrogen-fixing symbiosis. Plants create the root areas for the symbiotic nitrogen-fixing bacteria which are embedded into the root hairs of plants (generally do not invade the plant tissues). The symbiotic

Table 1 The cross-inoculation groups

Cross-inoculation Groups	Leguminous plant	*Rhizobium* species
Clover group	*Trifolium* sp. (clovers, trefoil)	*Rhizobium trifolii*
Alfalfa group	*Medicago* sp. (alfafa, burclover) *Melilotus* sp. (melilot, sweet clover, kumoniga)	*Rhizobium meliloti*
Bean group	*Phaseolus* sp. (bean, wild bean)	*Rhizobium phaseoli*
Lupine group	*Ornithopus* sp. (lupines, serradella)	*Rhizobium lupine*
Pea group	pea, sweet pea, lentil, vetch	*Rhizobium leguminosarum*
Soybean group	*Glycine max* (soybean)	*Rhizobium japonicum*
Cowpea group	cowpea, pegionpea, lespedza, groundnut, kudz	*Rhizobium* sp.

Remark: modified from Somasegaran, 1994.

Fig. 2 Nitrogen-fixing bacteria (Rhizobium) nodules on peanut roots.

bacteria stimulate the formation of roots nodules when they grow and multiply. In the root nodules, bacteria can turn free nitrogen into ammonia which the plant can absorb and utilize for growth and development. Typically, *Rhizobia* have two sets of genes, *nod* genes for nodulation and *nif* genes for nitrogen fixation (Masson-Boivin et al., 2009). The *Rhizobia* secrete the Nod factors to stimulate the re-orientation of cell wall of the growing root hairs lead to curled root hairs and induce the formation of infection threads. The *Rhizobia* use these tubular structures to enter leguminous plants (Fig. 3). In the actinorhizal plant, a group of angiosperms, is associated with *Frankia* acts similar to legumes and *Rhizobium* (Benson & Silvester, 1993).

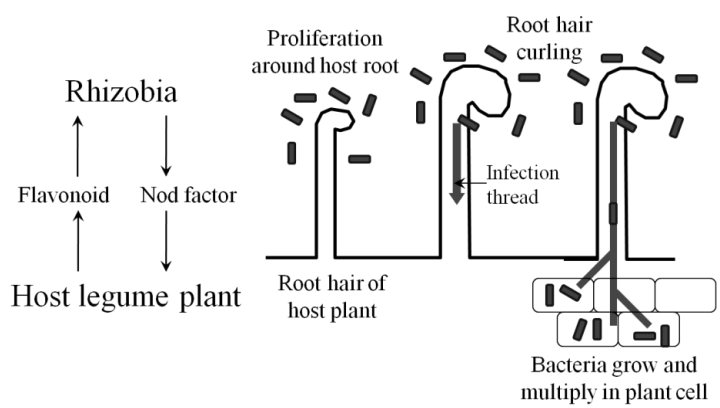

Fig. 3 Scheme of chemical signal exchanges and infection processes involving rhizobia. (modified from Okasaki et al., 2004)

Endophytic bacteria

Although biological nitrogen-fixation using endosymbiosis bacteria is very beneficial for a plant, not all plants have acquired a symbiosis with the *Rhizobia* due to the specificity between *Rhizobia* and host plant (Mylona et al., 1995). The *Rhizobia* require nodules to fix nitrogen from the atmosphere, but in case of some bacteria, endophytic, they not need nodules. It has been reported that the plant-bacteria association can enhance the nitrogen-fixing efficacy of both legume and non-legume plants (Udvarte & Poole, 2013; Santi et al., 2013). In 1961, the diazotrophs in the non-legume plant were first reported by Döbereiner J., Brazilian researcher, and he found the diazotrophs in the rhizosphere of sugarcane (*Saccharum officinarum*) (Döbereiner J., 1961). In subsequent researches, many diazotrophs were isolated from the rhizosphere of sugarcane such as *Azospirillum lipoferum, A. amazonense, Bacillus azotofixans, Enterobacter cloacae, Erwinia herbicola,* and *Paenibacillus polymyxa* (Puri et al., 2017). Many diazotrophic bacteria have evolved to grow, spread and multiply within plant tissues without causing damage or causing plant defense responses such as *Azoarcus, Herbaspirillum* and *Gluconacetobacter.* These bacteria illustrate the tight association with plant tissues and they are classified as endophytes (Pedraza, 2008). They enter to plant tissues through stomata on leaves or through the lateral root (Fig. 4).

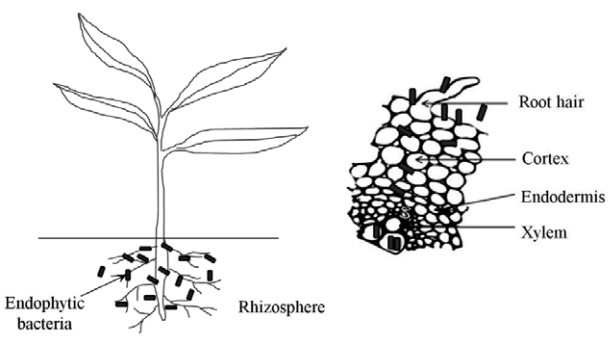

Fig. 4 Plant colonization routes by endophytic bacteria (modified from Audi pudi et al., 2017)

Endophytes are often found as epiphytes, suggesting that endophytes may also colonize surrounding environments of host plants. Many endophytes originate from the rhizosphere and move into the plant cell due to the presence of root exudates and through root colonization. Besides, inside the surface of the stem and leaf can produce exudates that attract microbes as well. Then, bacteria can be found in both areas as well. However, UV light, lack of nutrients and desiccation generally reduce the colonization of the leaf surface.

The most studied association system in the non-legume plant is the association between sugarcane and *Gluconoacetobacter diazotrophicus*. It has been reported that living *G. diazotrophicus* can produce some molecules that activate the sugarcane defense response, protecting the plant against *Xanthomonas albilineans* (pathogenic) attack (Arencibia et al., 2006). This relationship has resulted in increasing of sugarcane production without adding nitrogen fertilizers. There are many experiments which have illustrated that nitrogen-fixing bacteria are beneficial to plants. In 2014, Szilagyi-Zecchin et al. (2014) isolated and identified six endophytic strains from roots of corn growing in the southern Brazilian region of Campo Largo, PR. Out of these six endophytic isolates were found the presence of *nifH* gene and shown nitrogen-fixing activity. Interestingly, they found that two strains, identified as *Bacillus* sp., showed other plant growth–promotion (PGP) characteristics, like production of indole-3-acetic acid (IAA) or auxin plant hormone. It stimulates plant growth, cell division and cell elongation, siderophores and lytic enzymes and antagonism against pathogenic fungi. In 2013, Gupta and coworkers isolated the endophytic diazotrophic strains from pearl millet plants growing in a nutrient-deficient sandy clay loam soil located in Rajasthan. They found that the most dominant *diazotrophic* strain in pearl millet plants was *Pseudomonas aeruginosa* strain PM389. This strain can migrate and live in the stem tissues. *P. aeruginosa* strain PM389 has the ability not only on nitrogen fixation, but also possesses other PGP characteristics such as mineral phosphate solubilization, siderophore production, and antagonistic activity against many pathogenic bacteria and fungi. Moreover, when *P. aeruginosa* strain PM389 was inoculated into wheat, it can promote wheat growth by increasing the, seed germination rate, and root and shoot length (Gupta et al., 2013). The association of endophytic diazotrophic bacteria and their host plants, especially agricultural crops are shown in Table 2.

Because Thailand is the largest rice producer and exporter in the world, then a lot of research focuses on endophytic diazotrophic bacteria and rice. Koomnok et al. (2007) reported that they can be isolated the diazotrophic bacteria which are composed of *Azospirillum,*

Table 2 List of endophytic diazotrophic bacteria recently isolated and associated with agricultural plants.

Endophytic diazotrophic bacteria	Isolated from	Reference
- *Paenibacillus kribbensis* HS-R01, HS-R04; *Bacillus aryabhattai* HS-S05; *Bacillus megaterium* KW7-R08; *Klebsiella pneumonia* KW7-S06, KW7-S22, KW7-S27, KW7-S33; *Bacillus subtilis* CB-R05; *Microbacterium binotii* CB-S18; *Microbacterium trichotecenolyticum* SW521-L21, SW521-L37	Rice (*Oryza sativa* var. *Japonica* c.v. ilpum)	Ji et al. (2014)
- *Enterobacter dissolvens; Brevundimonas aurantiaca; Pantoea agglomerans; Pseudomonas* spp.	Rice (*Oryza sativa* L. cultivar KDML-105)	Prakamhang et al. (2009)
- *Bacillus* sp. BPSAC3, BPSAC6, BPSAC14; *Paenibacillus* sp. BPSAC45; *Bacillus thuringiensis* BPSAC46; *Lysinibacillus sphaericus* BPSAC60; *Pseudomonas* sp. BPSAC75; *Pseudomonas stutzeri* BPSAC43; *Staphylococcus* sp. BPSAC18, BPSAC155	East Indian glory bower (*Clerodendrum colebrookianum*)	Passari et al. (2016)
- *Bacillus amyloliquefaciens* MBL_B26; *Bacillus subtilis* MBL_B4; *Bacillus firmus* MBL_B5; *Brevibacterium* sp. MBL_B7 ; *Micrococcus* sp. MBL_B10, MBL_B11; *Bacillus pumilus* MBL_B12; *Bacillus subtilis* MBL_B13; *Bacillus* sp. MBL_15, MBL_16, MBL_17, MBL_20, MBL_21; *Micrococcus luteus* MBL_B18; *Micrococcus lylae* MBL_B1; *Kocuria* sp. MBL_19; *Pseudomonas psychrotolerans* MBL_B23, MBL_27; *Pseudomonas monteilii* MBL_B24; *Ralstonia solanacearum* MBL_B6; *Staphylococcus arlettae* MBL_B2, MBL_B3, MBL_B14, MBL_B22; *Staphylococcus hominis* MBL_B8; *Staphylococcus saprophyticus* MBL_B9; *Staphylococcus warneri* MBL_B25;	Jute (*Corchorus olitorius*)	Haidar et al. (2018)
- *Bacillus aryabhattai; Pantoea cypripedii; Bacillus licheniformis; Klebsiella* sp.; *Pantoea dispersa; Klebsiella variicola; Pantoea* sp.; *Agrobacterium larrymoorei; Bacillus* sp., *Bacillus amyloliquefaciens; Lactococcus lactis; Bacillus cereus; Staphylococcus homini*	Maize (*Zea mays* L.)	Marag & Suman (2018)
- *Ancylobacter* sp. UT3R1; *Ochrobactrum* sp. C7HL1; *Novosphingobium sediminicola* C2HL2; *Novosphingobium capsulatum* C34MR1	Sugarcane (*Saccharum officinarum* L.)	Muangthong et al. (2015)
- *Mycobacterium* spp.; *Streptomyces thermolineatus; Micromonospora endolithica; Micromonospora peucetica; Gordonia polyisoprenivorans*	Wheat (*Triticum aestivum*)	Conn & Franco (2004)

Herbaspirillum, Beijerinckia and *Pseudomonas* from cultivated rice (khao dawk mali 105, purple glutinous rice kum doi saket and bue polo) and wild rice (*Oryza granulata, O. rufipogon, O. rufipogon* 18883 and *O. nivara* 18852). In 2016, Raweekul et al. reported that 126 endophytic bacteria isolated from rice (*Oryza sativa*) roots and stems consist of the members of phyla *Firmicutes, Proteobacteria, Bacteroidete* and *Actinobacteria*. From theses phyla, 12 members of isolated in genera *Bacillus, Micrococcus* and *Acinetobactere* showed the increased fresh weight of rice seedlings when compared to the water-treated control group. Theses genera contain *nifH* gene, siderophore production, IAA synthesis and ACC-deaminase activity which represented their potential application as biofertilizers (Raweekul et al., 2016). Many studies showed that endophytic diazotrophic bacteria have the ability to nitrogen-fixation and can act as biofertilizer, especially for highly N-demanding crops such as sugarcane, corn, and rice. Moreover, they present PGP characteristics which can enhance plant growth and antagonistic activity against many pathogenic microorganisms.

Free-living bacteria

There are many free-living microorganisms can fix N_2 from the atmosphere. The free-living nitrogen-fixers live in soil or on soil surfaces such as *Cyanobacteria, Proteobacteria, Archaea* and *Firmicutes*. The free-living nitrogen-fixing bacteria is a group of bacteria that lives independently in soil or other environments. Nitrogen can be fixed from the air without using carbohydrates or energy sources from plants. These microbes rely on energy sources from organic matters in the soil. Therefore, the activity of these microorganisms does not require coexistence with plants (non-symbiotic). The vital enzyme in atmospheric nitrogen fixation is nitrogenase. There are many factors affect the nitrogenase activity, enzymes which convert N_2 to NH_3 or NH_4^+, the composition of free-living nitrogen-fixer communities including nutrients in soil, soil pollution, plant rhizosphere, plant species and temperature (Zhan & Sun, 2012). This nitrogenase enzyme complex is composed of three subunits Nitrogenase 1 which encoded by the *nif* gene and it is dependent on iron and molybdenum. Nitrogenase 2 which encoded by *vnf* gene and it is dependent on vanadium. Nitrogenase 3 which encoded by *anf* gene and it is dependent on iron. In free-living nitrogen-fixing bacteria, the *nif* genes are responsible for encoding highly conserved subunits (Franche et al., 2009). Due to the high conservation of *nif* gene, it has been used to characterize the genetic diversity of diazotrophs using *16S rRNA* gene (Zehr et al., 2003). The significant factor that affects the nitrogenase enzyme complex activity is the high sensitivity to oxygen. The major problem of free-living nitrogen-fixing bacteria is inhibition of nitrogenase by oxygen, especially aerobic species such as cyanobacteria (blue-green algae) and the free-living aerobic bacteria,

such as *Azotobacter* and *Beijerinckia*. The aerobic bacteria have different methods to solve this problem. In *Azotobacter* species, the protection of nitrogenase is maintained a deficient concentration of oxygen in their cells by increased in respiration to decrease the oxygen concentration around nitrogenase. Another mechanism for nitrogenase protection is the production of extracellular polysaccharide and maintains water within the layer to limit oxygen diffusion into the cell (Bertsova et al., 2001). In the case of *Beijerinckia derxii,* it contains two lipoid structures consist of poly-β-hydroxybutyrate (PHB). It can produce polysaccharide slime which are exopolysaccharides that can protect the nitrogenase from oxygen, called protective O_2 barrier (Thuler et al., 2003). Most of the biological nitrogen fixation (BNF) is carried out by diazotrophs in symbiosis with legume plant. But in some conditions, free-living nitrogen fixer in soil may fix the massive amounts of nitrogen (up to 60 kg N ha^{-1} $year^{-1}$) (Burgmann et al., 2004). The studies of free-living nitrogen-fixing bacteria are focused on both aerobic and anaerobic bacteria such as *Azotobacter vinelandii, Klebsiella pneumoniae, Clostridium pasteurianum* and *Rhodobacter capsulatus*. The two main steps of the free-living nitrogen-fixing bacteria are ammonia formation and nitrification. The NH_3 is formed by reducing the atmospheric nitrogen. When the bacteria die, NH_3 from bacteria is released from cells into the soil or surrounding ecosystems and can be converted to nitrates by nitrifying bacteria. Nitrates can be absorbed and beneficial to plants.

Application of endophytes in agriculture

Nowadays, modern agriculture has used pesticides excessively which has caused changes in soil microbial populations (Pampulha & Oliveira, 2006). It may have a direct effect on microbial growth and microbial diversity due to the overall changes in ecological structure. Recently, many endophytic bacteria have been studied for application in the agricultural field. They have the ability to produce plant growth-promoting substances to influence the growth of plants directly such as IAA production (Lee et al., 2004), siderophore and ammonia production, increase phosphate solubilization and nitrogen fixation. The endophytic bacteria can control phytopathogens, insects and nematodes through the production of new compounds and antifungal metabolites (Berg et al., 2005; Hallmann et al.,1998; Azevedo et al., 2000). They stimulate the host plant growth by nitrogen fixation, enhance the solubility of minerals and phytohormones production (Audipudi et al., 2007). From the characters of the endophytic bacteria suggesting that they can be used as biofertilizers. Dhevendaran et al. (2013) reported that *Azotobacter chroococcum, Azotobacter beijerinckii* and *Azotobacter vinelandii* produced IAA, a growth-promoting hormone. The increasing pH stimulated the growth and synthesis of IAA lead to higher growth of the seedling of *Ocimum sanctum.* They suggest that the application of *Azotobacter* and *Azospirillum* species as biofertilizers is a testimony to the impact that IAA has on seed growth. From the IAA production of these bacteria dominates the application in agricultural in the next few years.

Pathogenic bacteria including pathogenic *Salmonella* sp., *Escherichia coli, Vibrio cholerae* and *Pseudomonas aeruginosa* were described as endophytic bacteria. They may be associated with the use of manures contaminated with fecal bacteria (Holden et al., 2009). The importance of agricultural practices for preserving the natural diversity of endophytic bacteria is emphasized that plants or crops may become the hosts for human pathogens and the source of foodborne illness (Brandl, 2006). The best methods for using endophytes in agriculture are not yet known. The most obvious way is adding bacteria into the soil or to soak the seeds in the culture solution. However, the addition of bacteria is often unsuccessful on the field level because of many factors in the environment (O'Callaghan, 2016). Many questions have not been answered about the use of endophytic bacteria as food supplements in the agricultural field. However, if properly managed, they can show the ability to pathogenic control and abiotic stress from climate change, osmotic stress, exposure to heavy metals and xenobiotic molecules (Howden et al., 2007; Johnson et al., 2013).

The endophytes show great potential in biotechnology. They produce substances that beneficial to plants and they can decompose contaminated molecules in the soil such as pesticides, makes them a promising tool for bioremediation. Moreover, the inoculation of endophytes in plants is economical because it increases productivity and utilizes low-cost farming techniques that have a little environmental impact. For the development of efficient endophytes in agricultural, the bacteria must be selected formulated concerning the agricultural field and environmental conditions.

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Book Review

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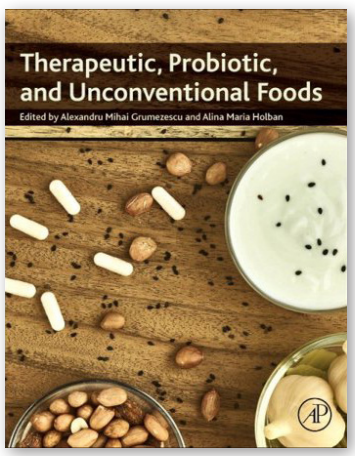

Book name: Therapeutic, Probiotic and Unconventional Foods (1st Edition)
Authors: Alexandru M. Grumezescu and Alina M. Holban
Published: Elsevier, Academic Press, USA, 2018
Paperback: 484 pages
Language: English
ISBN: 978-0-12-814625-5

Eating habits have a great impact on humans' health, environment, industry, and economy. Numerous diseases have arisen as endemic to modern society, such as obesity, osteoporosis, cancer, diabetes, allergies, and dental problems, which can occur at an early age and could be related to eating habits and preferences.

Alternative medical practices often include food-related products and currently there are numerous scientific proves to demonstrate the efficiency of some dietary components in preventing and even treating diseases. The food industry has also changed to fulfill consumers' requirements, and modern technologies allowed the production of differently processed foods, with improved aspects, such as flavor and lower costs.

Therapeutic, Probiotic and Unconventional Foods, first edition was aimed to bring together the most recent progress in the field of food dietary supplements and food products with therapeutic value, empathizing their bioactive components and trends in obtaining unconventional products. This volume was edited by Alexandru M. Grumezescu and Alina M. Holban. The book focuses on probiotic foods, addressing the benefits and challenges associated with probiotic and prebiotic use. This book has 3 sections with 21 chapters, and was written by researchers from around the world.

Section 1 : Probiotics and Prebiotics
Section 2 : Therapeutic Foods and Ingredients
Section 3 : Unconventional Foods and Food Ingredients

This book is recommended to scientists, food researchers, students, and industrial companies who seek scientific evidence on recent tools and perspectives in functional and unconventional foods. This book is a resourceful, interesting, and updated reference for any the reader interested in learning about trends and progress in Therapeutic, Probiotic, and Unconventional Foods.

Reviewer

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Guidelines for Writing and Submitting Original Manuscripts for Publication in Journal of Food Health and Bioenvironmental Science

Journal of Food Health and Bioenvironmental Science is an academic publication that aims to publish manuscripts such as original articles, review articles, and book reviews in the fields of food, health, biological and environmental disciplines and other related fields.

The journal is published thrice annually. All manuscripts accepted for publication are copyrighted by Suan Dusit University; reproduction, in whole or in part, requires written approval from Suan Dusit Unversity. Excluding errors incurred during the printing process, all content contained within articles is the author's responsibility. Due to self-supporting of Journal of Food Health and Bioenvironmental Science, authors are required to pay 500 Bath as a processing fee and 3,000 Bath for peer review process after the submission. The submission fee is non-refunable.

Publication Process

1. The journal accepts original manuscripts for consideration, from January to December. Due to self-supporting of Journal of Food Health and Bioenvironmental Science, authors are required to pay 500 Bath as a processing fee and 3,000 Bath for peer review process after the submission
2. The editorial board adjourns to consider the merits or submitted manuscripts and the scope of the journal. During this phase the integrity and accuracy of the manuscripts content is assessed.
3. An editorial letter is issued to the author for manuscripts that the editorial board deems inappropriate for publication. If the editorial board approves the manuscripts, an editorial letter will be sent to the author and the article will be subjected to peer review.
4. Articles that are deemed appropriate for publication are subjected to peer review by a panel of three experts in the appropriate field. In order to be deemed appropriate for publication, an article must be by recommended two of the three experts via the double-blinded review system.
5. The qualitative assessments of the expert panel returned by the manuscript’s author. The author is expected to make the appropriate alterations indicated by the experts’ feedback.
6. The author returns the edited document; the editorial staff examines the changes to make sure they are congruent with the experts' recommendations as well as the journal format.
7. The revised version is granted the University's recognition of "Accepted" for publication status with the Journal of Food Health and Bioenvironmental Science Stamp on every page. Information regarding publication status (Accepted) is located on the journal's website (http/ research dusit ac.th/new/e-Journal)
8. The editorial tearm conducts an accuracy check for all articles before sending the manuscripts to the printer to create a draft journal issue.
9. The editorial board conducts a review of the draft journal issue before publication on the journal's website (http://research.dusit.ac.th/new/e-Journal). Suan Dusit University will place their official seal of approval on each page of the manuscript and to verify before formal publication.
10. Upon approval by each author, the final version of the journal will be published as a physical journal and online publication, accessible on website (http://research.dusit.ac.th/new/e-Journal). Together with sending a physical journal to peer reviews, authors and involved sectors.

Publication Criteria

1. The original manuscript is concise and interesting to the academic community.
2. The content of the manuscript represents quality and theory of the discipline and also possesses knowledge with practical applications.
3. The manuscript's content is consistent with the aim and scope of the journal.

4. Manuscripts submitted to Journal of Food Health and Bioenvironmental Science must not have been published previously in or actively involved in the publication process of another journal.

5. All content within the manuscript must be the product of the author himself. Any use of intellectual property within must be appropriately credited to its original authors.

6. The author must comply with the writing style established by Journal of Food Health and Bioenvironmental Science.

7. There are four levels of assessments given to reviewed manuscripts:

7.1 Requires minor or no revisions prior to publication.

7.2 Requires moderate revisions prior to publication.

7.3 Requires intensive editing and revisions followed by a future evaluation. 7.4 Unsuitable for publication

In order to be assigned the "Accepted" status, an article must be assessed as “Requires minor or no modification prior to publication” by two of the three experts from the peer review process.

Formatting Guidelines

It is the author's responsibility to format manuscripts to the standards of Journal of Food Health and Bioenvironmental Science. The details of format style are contained herein,

1. Format

1.1 Single page printing on A4 paper with a width of 19 cm and height of 26.5 cm. The vertical and horizontal spacing from the margins must be 3.5 cm and 2.5 cm, respectively.

1.2 Typefaces and layout: English must be typed using Time New Roman using Microsoft word. Specific font format guidelines are as follows.

1.2.1 The header contains the page number, aligned on the right side, in 12 pt. font.

1.2.2 The title in English languages must be 12 pt. font, bolded, and center aligned. The title should not exceed two lines of text.

1.2.3 The author's name in English language must be typed 9.5 pt. font and centered below the title. Asterisks (*) should proceed the authors' names which is correspond to the appropriate author.

1.2.4 Affiliations should match each author with their appropriate affiliated institutions and organizations. In case of different affiliations, superscript numbers should follow the surname a and affiliation a.

1.2.5 A footnote must be placed on the first page of the article with the text “*Corresponding Author”, and the next line of text should contain “e-mail”.

1.2.6 “Abstract” in English must be 9.5 pt. font, bolded, left aligned, and placed below the Thai keywords section. Abstract text must be 9 pt. font, with 1 tab indentation from left and right margins.

1.2.7 “Keywords:” should appear in English language in 9.5 pt. font, placed beneath the English abstract text and be aligned with the left margin. English keywords must be 9 pt. font, and should not exceed four words. Each keyword should be separated by a comma (,) and space.

1.2.8 Regardless of language choice, the main text headings used throughout the paper must be 9.5 pt. font, bolded, and aligned with the left margin.

1.2.9 Bulleted items must appear as 9 pt. font, bolded, and be indented 1.5 tabs from the left margin.

1.2.10 Body text must appear as 9 pt. normal font, and be indented 1 tab from the left and right margins.

1.2.11 “References” must be 9.5 pt. font, bolded, and be aligned with the left margin. Individual entries must be 9 pt. font and should follow American Psychological Association (APA) formatting guidelines. Any lines of text for a single entry that exceed the first line should use a “hanging indent” of 1.5 tabs from the left margin.

1.3 An appropriate page length for publication in the Journal is approximately 15 pages.

2. Citing

Should follow American Psychological Association (APA) formatting guidelines. Click http://jfhb.dusit.ac.th/flie/Ref%20Guidelines. pdf to see the example.

3. Ordering of Titles in Journal of Food Health and Bioenvironmental Science

The written manuscript may contain only English. The content should be easy to understand and clear. If the author uses abbreviation, full word must appear before any abbreviation.

3.1 The title should be brief, the length should not exceed 100 characters.

3.2 The authors if there are more than six authors only the first author is listed, followed by "et al."

3.3 Affiliated entities associated with the author should appear in English languages.

3.4 The abstract must be written in English language. The abstract should briefly summarize the research and not exceed 250 words or 15 lines of text.

3.5 The "Keywords" section must contain no more than four keywords that allow for appropriate searching and selection based upon the article's topic.

3.6 The "Introduction" section should provide background information relevant to the research, provide information regarding the manuscript's content and state the objectives of the work.

3.7 The "Materials and methods" section delineates the procedures, how the research was conducted, sampling method (i.e. simple random samples) and population, and the creation and development of research tools used for data collection and analysis.

3.8 The "Results" section or "Results and Discussion" presents data obtained during the research and may be displayed as tables, graphs, illustrations, and accompanying explanations. Tables should be not have left and right borders and are normally black and white printed. No more than five tables should be present in the "Results" section. Pictures within the section should be clear and use simple black and white coloring with an accompanying caption, the author wishes to use colors for any item they may do so; however, the author will be responsible for the additional costs of color printing.

3.9 The "Discussion" section or "Result and Discussion" should explore the significance of the results of the work and address whether or not the data support the research hypothesis and compare research findings to other similar research works.

3.10 The "Conclusions" section should summary of the main topic covered or a re – statement of the research problem.

3.11 The "Acknowledgements" (if any) section should provide help during the research (e.g., providing materials, laboratory, equipment, etc.) and funding.

Sending Original manuscript

1. Compose the manuscript using the format of the Journal of Food Health and Bioenvironmental Science.

2. Send the manuscript via ScholarOne website https://mc03.manuscriptcentral.com/jfhb

Journal of Food Health and Bioenvironmental Science Publication Ethics

Editorial Regulations

- It is the duty of the Editors to consider the submitted manuscripts related to field of food, health, biological and environmental disciplines and other related fields. The consideration will be based solely on the content. The ethnicity, country of origin, gender, sexual orientation, political affiliation, or religious belief of authors does not influence the editor's decision.
- Throughout the submission, the editors must not share the information about the submissions to anyone except the authors, reviewers and JFHB staffs.
- Editors must make sure the manuscript has no substantial vested interests authors or affiliated organizations.
- The editorial staff have to assure that the manuscript has been peer-reviewed by at least two reviewers in the field of Food, Health, biological and environmental disciplines or other related field appropriate for each manuscript. The editorial staffs also have to be careful about the copyright Infringement, falsification of data, and plagiarisms. If there is an offense according to the said regulations, the editor must investigate and seek for evidence before consider reject the manuscript.
- In case that the Editors suspect that the manuscript might have been published elsewhere, the editor should investigate and consider reject.
- In case of unethical publishing practices that are later uncovered, the action will still be taken seriously.

Reviewer Regulations

- Reviewers are expect to give constructive and professional comments. Improper criticism must be avoided.
- If the manuscript given is lies beyond area of expertise, the reviewers should inform the staff immediately.
- Reviewers must keep the manuscript confidential. Do not share any information of the manuscript to anyone other than the editorial staff.
- In case that the reviewers find that the other works contained in the manuscript are not well credited, reviewers are required to inform the editorial staff.
- If there are conflicts of interests, reviewers should inform the editorial staff. Editors will decide whether the reviewer is appropriate for the manuscript or not.

Author Regulations

- The authors should write the manuscript related to the theme of Food, Health, biological and environmental disciplines. The research manuscript should contained relevant background information, efficient methodology, APA style citation, accurate results, and reasonable discussion.
- The authors should follow the journal guidelines strictly.
- Any opinion or perspective made in the manuscript must be explicitly highlighted as "opinion" or "perspective"
- The authors must be careful and aware that fraudulent information and omission of important information are unethical author behaviors.
- The authors must be able to provide research data if the Editor see needed.
- Authors must reference other works properly. Any work involved in the manuscript also must be well credited.
- The authors must make sure that the manuscript has not been published elsewhere before and is not currently in the publication process in other journals.

- The person must have made significant contributions to the manuscript, participate and give important efficient content during revisions and provide approval for publication in order to be listed as an author. Researchers who do not meet the above criteria should be listed in the Acknowledgements section.
- Author should identify any conflicts of interest that might have influenced the data and/or interpretations of data.
- To make the efficient revision, the authors should respond to all the given critiques and suggestions during the revision.
- If the authors find errors in their works that need to be correct, the author should inform the editors immediately.