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Content

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Monogenean Parasite Infections in Asian Seabass, *Lates calcarifer* (Bloch, 1790) during Crop Production in Earthen Pond Culture at Surat Thani, Thailand

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Abstract

This study aimed to identify monogenean parasites in Asian seabass (*Lates calcarifer*) and to assess infection rates in the host fish in an earthen pond culture during crop production in Surat Thani province, Thailand. One hundred fish were examined for parasite infections from July to November, 2017. Two species of monogeneans, *Laticola paralatesi* and *Laticola seabassi,* were detected on gill filaments. The highest 75% prevalence was observed in September. The highest mean intensity of *L. paralatesi* was recorded in September (5.8 parasites per fish). *L. seabassi* was detected at the highest mean intensity in November (2.6 parasites per fish), which was significantly different among the five months ($p < 0.05$). Moreover, the fish size was not significantly correlated with *L. paralatesi*, but there was a significant positive correlation between the weight of fish and the number of *L. seabassi* ($r = 0.928$; $p < 0.05$). This study corroborates that *Laticola* spp. is commonly found in Asian seabass, with parasite transmission from fish to fish, and the larval stage from wild infected fish live in natural water sources and is spread to fish ponds. The present work reveals monthly profiles of infestations by monogenean parasites in earthen pond culture and the results can be applied in fish health management. This is the first report on monogenean parasite infections in Asian seabass, in earthen pond cultures in southern Thailand.

Introduction

Lates calcarifer is a catadromous fish that is found in freshwater, brackish water and marine environments, including streams, lakes, billabongs, estuaries and coastal waters (Khrukhayan et al., 2016). They are born in seawater, then living in freshwater, then brackish water and adults again migrate to the sea to breed. This species is widely distributed throughout Southeast Asia including Thailand and in some other Pacific countries (northern Australia and Indo-West Pacific) (Mohamed-Jawad et al., 2012). The Asian seabass is a commercially important fish species grown in aquaculture. The culture systems are either pond or cage cultures. This species is tolerant of a wide range of salinities from freshwater to seawater, with the latter type of cultures used in fresh,

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brackish and marine water ecosystems (Thirunavukkarasu et al., 2009). These fish have been cultivated in brackish water as well as in freshwater ponds and marine cages in many Southeast Asian countries (Philipose et al., 2010). Also, earthen ponds are practiced on most farms because of the low production cost and the fast growth due to various kinds of natural food in the pond environment. Ponds are prepared traditionally and are also used for shrimp cultures (Jerry, 2013). In Thailand, Asian seabass cultures are found in eastern, central, and southern parts. In the southern part of Thailand, Songkhla and Nakhonsithammarat provinces are important to Asian seabass production. Asian seabass is an economically important fish species in Thailand and other countries of Asia and Pacific regions. Thailand has been the top producer of both fingerlings and marketable fish. The production has increased from 9,700 tons to 16,900 tons in 2014 (Department of Fisheries, 2015). The global production of Asian seabass had increased to 71,581 tons in 2014 (FAO, 2017; Joerakate et al., 2018). Moreover, Surat Thani province of Thailand also produces a substantial amount of Asian seabass along its coastal areas. Interestingly, Thai fishery organizations are promoting fish production in earthen ponds and have successfully initiated culture programs with the aid of a group of fish farmers at Klong Chanak sub-district, in Muang district, Surat Thani province.

The fish production is affected by diseases, especially parasites, viz., protozoans, monogeneans, digeneans, cestodes, nematodes, acanthocephalans and crustaceans (Yang et al., 2006; Ruckert et al., 2008; Petchsupa & Nilrat, 2008; Reed et al., 2009; Jerry, 2013; Mama et al., 2014). Parasite infections are an important consideration in animal health management, especially in aquaculture (Hutson et al., 2012). Many species of monogenean parasites in Asian seabass have been reported from Southeast Asia and other Pacific countries. The four species *Laticola lingaoensis, L. latesi, L. paralatesi* and *Diplectanum penangi* are reportedly found in the gills of fish from the South China Sea (Yang et al., 2006). Also, parasites in Asian seabass have been reported in Indonesia, in the context of severe parasite and disease outbreaks, when the fish were examined from Lampung Bay, South Sumatra. These fish were infected with monogeneans, such as *Pseudorhabdosynochus epinepheli, P. lantauensis, Benedenia epinepheli* and *Neobenedenia melleni* (Ruckert et al., 2008). Additionally, the ectoparasitic monogenean *Neobenedenia* sp. attaches to the body surface of its host using attachment organs. Histopathological changes in the fish associated with *Neobenedenia* sp. include epidermal damage. Cutaneous mucus secreted by mucous cells is an important component of teleost immune responses. *Neobenedenia* sp. infected fish had changes in epidermal thickness and decreased numbers of mucous cells (Trujillo-Gonzalez et al., 2015).

Parasite infections in Asian seabass have been reported in eastern and southern Thailand. In Pattani bay, fish rearing cages are affecting both wild and cultured fish species. Two species of monogenean parasites were identified, i.e. *Diplectanum latesi* and *D. papaverensis*. Histopathological changes in the fish included hemorrhage, edema, hyperplasia, inflammation, degeneration and necrosis (Petchsupa & Nilrat, 2008). In cage cultures in Nathap canal (Songkhla) and Saiburi canal (Pattani) 7 species of parasites were identified, namely *Trichodina japonica, Laticola latesi, L. lingaoensis, L. paralatesi, Lernanthropus latis, Caligus epidermicus* and *C. rotundigenitalis*. Histopathological changes in gill, liver, kidney and spleen included degeneration, edema, hyperplasia, necrosis and melanomacrophagy (Mama, 2015). Moreover, three species of monogeneans, *Laticola lingaoensis, L. paralatesi* and *Diplectanum penangi,* were isolated from cage cultures in Bangpakong River, Chachoengsao province (Khrukhayan et al., 2016). Parasite infections can affect animal health in aquaculture and require management responses. Therefore, parasites in fish have been considered an indicator of environmental health and important for the development of aquaculture (De Carvalho-Souza et al., 2009). Regarding pond cultures, knowledge of parasites affecting Asian seabass is poor. The present study aimed to identify parasitic monogeneans during crop production and to assess the infection rates in an earthen pond culture at Klong Chanak sub-district, Muang district, in Surat Thani province of Thailand.

Materials and methods

The study was conducted in the earthen pond culture during crop production from July to November 2017 at Klong Chanak sub-district, Muang district, Surat Thani province, in southern peninsular Thailand (9°10´56´´N, 99°20´16´´E) (Fig. 1). The fish pond is rectangular in shape with 6,400 square meter size and 150-200 centimeter water depth. A total of 15,000 fish

were raised in the earthen pond with a stocking density of 2.34 fish per square meter. Pond bottom is flat and slopes towards the drainage gate. The Asian seabass fry with size grading at proximately (n=20) 9.22±0.61 centimeter (8.0-10.0) and 9.14±1.56 gram (6.84-11.57) were obtained from a hatchery in Chachoengsao province, central Thailand. The worm-free fish were raised in an earthen pond and fed with chopped trash fish. After one month, the fish were sampled randomly by using a dip net and hook. Twenty specimens were examined once a month for 5 months. The live fish were transported to the laboratory in the Prince of Songkla University, Surat Thani campus, for examination of parasite infections. The fish were killed with 200 ppm clove oil (modified from Mladenio et. al, 2013). The total length and weight of fish were measured.

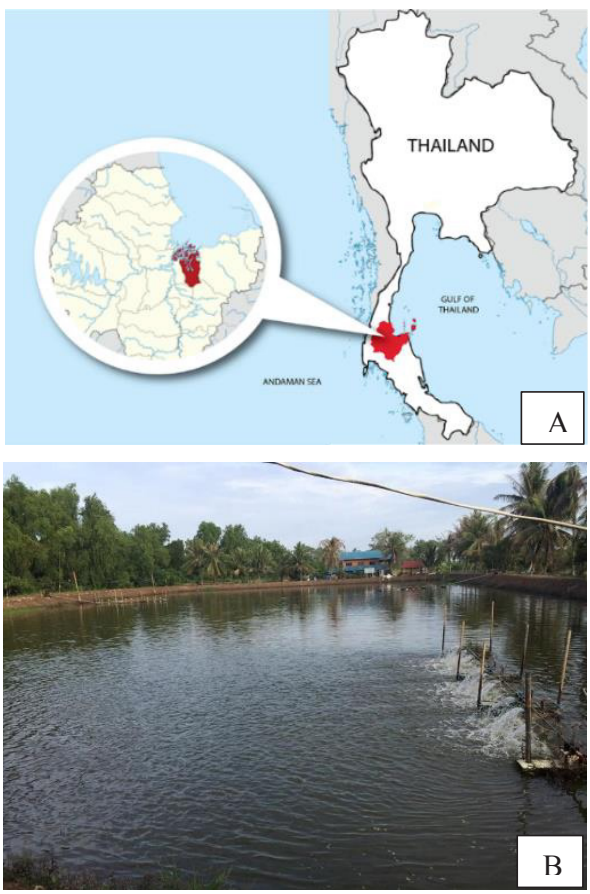

Fig. 1 Location of study site (A) Map of Klong Chanak sub-district, Muang district, Surat Thani province, southern Thailand; and (B) the earthen pond culture of Asian seabass

1. Water quality in fish pond

This study monitored the key water quality parameters, namely temperature, salinity, and pH. Water samples were measured for temperature using a thermometer; Hydrogen ion concentration using a pH meter with platinum electrode, calibrated with standard buffer solution prior to use; and salinity using a hand refractometer. The temperature varied in the range 30.6-31°C. The pH varied in the range 6.86-7.43, during the observed five months (July-November 2017). The salinity varied within 2-6 ppt, and the lowest and highest values in the pond culture were observed in September and July 2017 (Table 1).

Table1 Water quality parameters in earthen fish pond during crop production

Month (2017)	Water quality parameters		
	Temperature (°C)	Salinity (ppt)	pH
July	31±0	6±0	6.86±0.03
August	0.6±0.57	3±0	7.94±0.1
September	31±0	2±0	7.55±0.22
October	31 ±0	4±0	7.24±0.25
November	31 ±0	4±0	7.43±0.29

2. Parasite examination

Monogeneans were observed in Asian seabass reared in an earthen pond culture at Klong Chanak sub-district, Muang district, Surat Thani province. Gills were removed and each gill arch was cut out and gill filaments were separated; dorsal fin, pectoral fin, pelvic fin, anal fin and caudal fin were separated; scales were removed from both sides; and each part of the body was placed in a Petri dish containing 0.85% saline solution. The body surface of fish was scraped from anterior to posterior part using a cover slip. By examining under a stereomicroscope and a compound microscope, the counts of parasites were recorded (modified from Ruckert et al., 2008; Sonthi et al., 2016). In addition, internal organs including the stomach, intestine, liver, and gall bladder were observed from fish samples. No endoparasites were infecting these fish. Monogenean specimens were removed from the gill filaments using fine forceps and needles or pipettes under a dissecting microscope. Specimens were transferred to glass slides, covered with cover slips, fixed in ammonium picrate glycerine and sealed with nail varnish (modified from Moreira et al., 2015). To prepare permanent slides, some specimens were fixed in 4% formalin under a cover slip, then stained with Semichon's acetic-carmine. The stained slides were dehydrated through a graded ethanol series,

cleared in xylene, and mounted in permount (modified from Ruckert et al., 2008). Fresh specimens and permanent slides were observed under a compound microscope. Line drawings were done on fresh and stained materials with a drawing tube (UDA60) attached to a compound microscope. Monogenean parasites were identified based on genus *Laticola* spp. in Asian seabass (Yang et al., 2006; Chotnipat et al., 2015). Measurements are reported in micrometers with the means followed by ranges in parenthesis.

3. Data analysis

The prevalence and mean intensity were compared between the observed 5 months, following Bush et al. (1997). Prevalence was calculated as the number of infected hosts divided by the total number of examined hosts (expressed as a percentage):

$$\text{Prevalence} = \frac{\text{No. of infected hosts} \times 100}{\text{No. of examined hosts}}$$

Mean Intensity is the total number count of a particular parasite divided by the number of infected hosts:

$$\text{Mean Intensity} = \frac{\text{Total no. of a particular parasite}}{\text{No. of infected hosts}}$$

The data were subjected to correlation analysis conducted using statistical analysis programs. The mean intensity of each parasite in each month were compared using analysis of variance (ANOVA) then, by LSD post hoc test for multiple comparisons. Pearson's correlation was assessed between weight, length of fish and number of parasite infections. Statistical analysis used the significance threshold level of 0.05 ($p < 0.05$).

Results and discussion

A total of one-hundred live specimens were collected during crop production (July to November 2017) from the earthen pond culture. Fish total length ranged from 14.74 to 41.01 centimeters (28.9±3.07) and weight was from 44.66 to 1,013.81 grams (464.74±133.17) (Table 2). Thirty-nine of the collected fish were infected with 2 species of monogeneans on the gill filament (Fig. 2), *Laticola paralatesi* and *L. seabassi*, seen in the illustrations in Fig. 3 and 4. The study describes the total length-weight relationship with the two species monogeneans in the earthen pond culture. A comparison between fish size and monogenean infections were analyzed. A positive correlation was not statistically significant between the total length ($r = 0.626$; $p > 0.05$), weight ($r = 0.518$; $p > 0.05$) of fish and the number of *L. paralatesi*. In addition, fish infections with *L. seabassi* were not significantly correlated with total length ($r = 0.857$; $p > 0.05$). A significant positive existed between the weight of fish and the number of *L. seabassi* ($r = 0.928$; $p < 0.05$).

Table 2 Measurements of Asian seabass from earthen pond in Surat Thani province: mean length and mean weight (range in parentheses)

Month (2017)	Fish size (Mean±SD)	
	Length (cm)	Weight (g)
July	14.74 ± 1.75 (11-17.5)	44.66 ± 15.56 (17.39-72.69)
August	24.02 ± 2.35 (18-28)	208.55 ± 55.68 (81.53-316.93)
September	29.32 ± 3.65 (21.5-33.7)	399.59 ± 144.81 (120.78-521.8)
October	35.4 ± 3.38 (27.5-41.5)	657.06 ± 180.12 (287.72-939.56)
November	41.01 ± 4.24 (30-47)	1013.81 ± 269.67 (402.32-1458.08)

1. Description of *Laticola* spp.

Laticola paralatesi (n=20) (synonyms *Diplectanum paralatesi* Nagibina, 1976; *Pseudorhabdosynochus yangjiangensis* Wu & Li, 2005). Body 466.5 µm (300-600) long; greatest width 151.5 µm (140-170). *Laticola seabassi* (n=10) (synonyms *Pseudorhabdosynochus seabassi* Wu et al., 2005; *L. lingaoensis* Yang et al., 2006). Body 671 µm (500-880) long, with short peduncle; greatest width 179 µm (120-270) at level of gonads. Body surface was covered with tegumental scales which extend from peduncle to level of copulatory organ. Prohaptor moderately developed; consists of cephalic lobes; each head organ comprises 2-3 groupings of terminations of cephalic-gland ducts. Two pairs of eyespots, the anterior pair is smaller than the posterior pair. Pharynx oval shaped. Intestinal caeca terminate posterior to gonads near peduncle. Testis crescent shaped. Copulatory organ spoon shaped, distal tubular portion elongate. Oval shaped ovary. *L. paralatesi* presents a vase shaped sclerotized vaginal valve, with proximal duct extending from the vaginal valve to a seminal receptacle. *L. seabassi* presents vaginal valve shaped as blossoming flower of thistle with 2 central distally directed lobes. Vitellarium dense. Vitellarium extends posteriorly from the level of posterior pair of eye-spots to terminations of intestinal caeca. Opisthaptor, armed with dorsal and ventral pairs of anchors. Ventral squamodisc horseshoe shaped with

rows of rodlets; rodlets delicate, dumbbell shaped. Haptor consists of 14 marginal hooklets (Table 3, Fig. 3-4).

Table 3 Characteristics measured for two monogenean parasites (*Laticola* spp.)

Characteristic	Measurement (µm)	
	***L. paralatesi* (n=20)**	***L. seabassi* (n=10)**
Body length	466.5 (300-600)	671 (500-800)
Body width	151.5 (140-170)	179 (120-270)
Pharynx length	38.5 (25-50)	44.25 (35-62.5)
Pharynx width	29.12 (22.5-40.0)	41.75 (27.5-50)
Testis length	29.25 (25-32.5)	39.25 (25-50)
Testis width	36 (32.5-37.5)	45.75 (32.5-62.5)
Ovary length	82.5 (62.5-112.5)	79.75 (50-125)
Ovary width	37.25 (25-45)	42.75 (30-62.5)
Copulatory organ length	82.12 (75-85)	89.5 (75-112.5)
Haptor length	87.75 (75-105)	107.5 (80-137.5)
Haptor width	176.12 (155-200)	175.25 (137.5-212.5)
Number of rodlet rows	11-13	11-13
Ventral squamodisc length	76 (52.5-100)	84 (70-125)
Ventral squamodisc width	103.5 (80-125)	99 (87.5-112.5)
Dorsal anchor length	41.62 (37.5-50)	31.5 (22.5-45)
Ventral anchor length	47.75 (40-52.5)	35.25 (25-42.5)
Dorsal bar length	50.62 (50-52.5)	45.5 (32.5-55)
Ventral bar length	105 (95-125)	115 (100-137.5)
Number of marginal hooklets	14	14
Marginal hooklet length	10 (10-10)	10 (10-10)

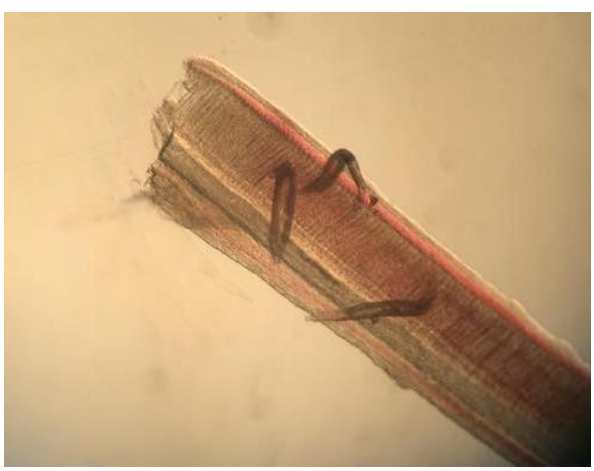

Fig. 2 *Laticola* spp. infecting gill filament of Asian seabass

The prevalence of parasite infections from July to November 2017 is shown in Fig. 5. The highest prevalence of *L. paralatesi* was observed in September with 75% (15/20) infection rate. *L.seabassi* was found in November with 40% (8/20) infection rate. The mean intensity of *L. paralatesi* was higher than of *L. seabassi* species, as seen in Table 3. The highest mean intensity of *L. paralatesi* were recorded in September with

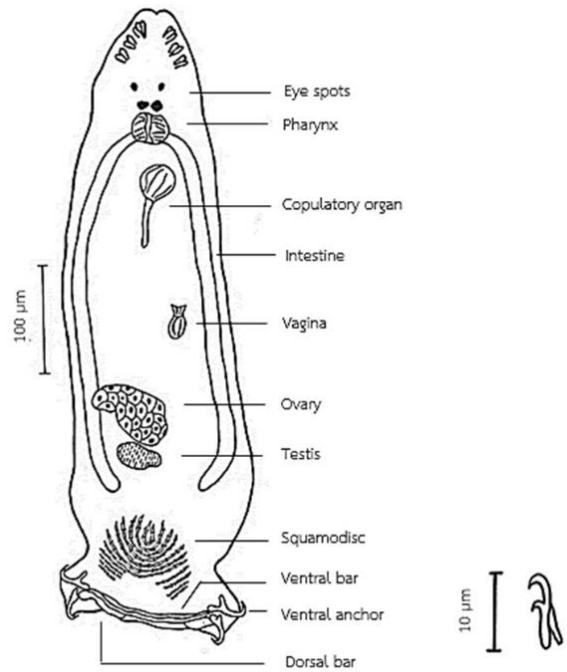

Fig. 3 Line drawing of *Laticola paralatesi* (ventral, composite) and marginal hooklet

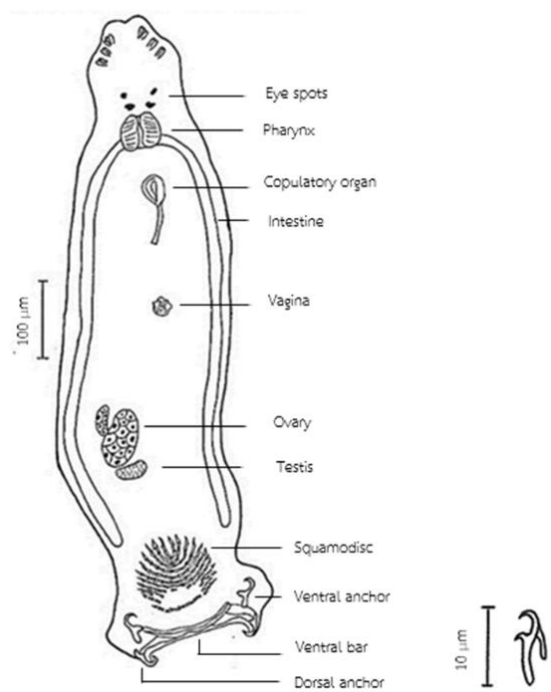

Fig. 4 Line drawing of *Laticola seabassi* (ventral, composite) and marginal hooklet

respective infection rates of 5.8 (87/15) parasites per fish, which was statistically significantly different from others ($p<0.05$). Meanwhile, *L. seabassi* had a high level in November, when the infection rate was 2.6 (21/8) parasites per fish (Table 3).

Table 3 Mean intensities of parasite infections by *L. paralatesi* and *L. seabassi*

Month (2017)	Infected fish/ Examined fish	Mean intensity (±SD)	
		L. paralatesi	*L. seabassi*
July	0/20	0^{a}	0^{a}
August	0/20	0^{a}	0^{a}
September	15/20	5.8 ± 3.16 (3-10)^{b}	0^{a}
October	12/20	4.45 ± 2.94 (1-8)^{b}	2 ± 0.69 (1-3)^{b}
November	12/20	2.9 ± 1.69 (1-5)^{bc}	2.6 ± 1.57 (1-5)^{b}

Remark: a,b,c Different superscripts indicate significant differences according to LSD post hoc test for multiple comparisons with $\alpha = 0.05$

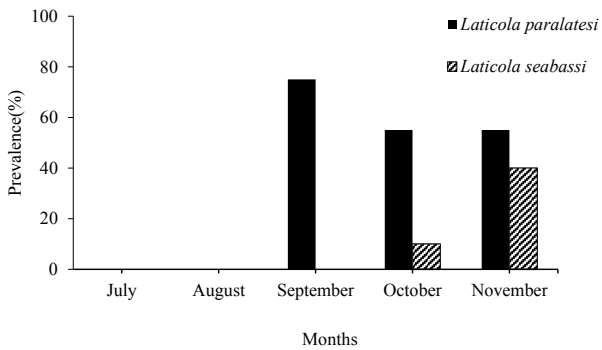

Fig. 5 Prevalence of monogenean infections in Asian seabass during crop production

Two monogenean species, *Laticola paralatesi* and *L. seabassi* were found in the gill filaments of infected fish. These two species belong to the genus *Laticola* and are members of the family Diplectanidae. The results showed that the prevalence and mean intensity of parasites per fish were highest in September 2017. In Thailand, two genera of monogenean parasites, *Laticola* spp. and *Dipletanum* spp., have been reported in cage cultures of Asian seabass. Parasite infestations in southern and eastern Thailand were found by *Dipletanum* sp., *D. papaverensis, D. latesi, D. penangi, L. latesi, L. lingaoensis* and *L. paralatesi* (Petchsupa & Nilrat, 2008; Mama, 2015; Sonthi et al., 2016; Khrukhayan et al., 2016). Similarly, Yang et al. (2006) reported 4 species of monogeneans in Asian seabass viz. *L. lingaoensis, L. latesi, L. paralatesi* and *D. penangi* infecting fish gills in the South China Sea. Also, four species of parasites were found, namely *Pseudorhabdosynochus epinepheli, P. lantauensis, Benedenia epinepheli* and *Neobenedenia melleni,* in the gills and on body surfaces of fish in Indonesia (Ruckert et al., 2008). This study was performed on fish samples from an earthen pond culture. Two species of diplectanid monogeneans were examined. However, monogeneans are mostly ectoparasites with a direct life cycle (Saraiva et al., 2015). This study indicated that *Laticola* spp. infected wild fish in this area, and the free-living larval stage (oncomiracidium) can contaminate water and enter a fish pond. This parasite managed to maintain transmission successfully in the culture system. Interestingly, free-swimming oncomiracidium of diplectanid monogenean can swim continuously for 4-8 hr to find definitive hosts under laboratory conditions (Erazo-Pagador & Cruz-Lacierda, 2010). This suggests that diplectanids enter farming systems through intake of natural water resources containing eggs and oncomiracidia or infected wild broodstock (Whittington et al., 1999). These parasites can cause gill damage. The gills are among the most delicate structures of a fish, and exposure outside the body makes them liable to damage by any irritant materials (Saraiva et al., 2015). It is well-known that these parasites are considered a threat to fish cultures and cause health status reduction and mortality in many species of farmed fish (Saraiva et al., 2015). Diplectanid parasite infections may cause damage to respiratory organs and histopathological changes in the gills, inducing darkened body, lethargy, loss of appetite, excess mucus production, lamellar fusion, hemorrhage, inflammation, degeneration, necrosis, hyperplasia, edema, swollenness and paleness (Petchsupa & Nilrat, 2008; Reed et al., 2009; Jerry, 2013; Mama et al., 2014; Mama, 2015). A comparison between fish size and number of parasite infections indicated that the larger fish tend to be more infected by monogeneans than the smaller fish. No parasites infected fish during the two first months of their culture, but parasites were observed in fish in the earthen pond after two months. While worm-free fingerlings were received from the hatchery, the parasite was initially observed in fish two months post-transfer into the earthen pond. This suggests that disease in farmed fish emerge from interactions between host, environment and pathogens. Generally, diseases are often caused by widespread pathogens that are commonly found in the culture environment (Gibson-Kuch, 2012). Some organisms, including eggs or larval stage monogenean parasites, contaminated the water source of the fish pond. On the other hand, the salinity was lowest in September 2017 matching the highest infection rate.

The effect of salinity on ectoparasite distribution of catadromous fish is such that the fish lose their ectoparasites after they migrate to marine water (Esch & Fernandez, 2013). However, parasites tend to increase in numbers in the larger fish, according to Ravi & Yahaya, (2016). *Neobenedenia melleni* monogenean species were analyzed for associations between fish length and parasitic intensity in infected fish, at Jerejak Island, Penang, Malaysia. A positive relationship between fish length and parasite diversity was found, because the larger fish show a larger infection surface area for parasitic colonization. Monogenean parasites affecting gills and body surface of fish can cause gill fluke disease and skin fluke disease. In contrast, there was no relationship between parasite infection and size of fish in another prior study (Olurin & Somorin, 2006; Amaechi, 2015). The problem of parasites infecting fish could be best handled through proper management procedures that eliminate the conditions favoring parasite infestation (Amaechi, 2015). The treatment of monogenean parasites has been investigated by Hutson et al. (2012), who reported that aqueous extracts from tropical seaweeds were evaluated for effects on the life cycle of an ectoparasite, *Neobenedenia* sp., infecting farmed *L. calcarifer.* The extracts of two seaweeds, *Ulva* sp. and *Asparagopsis taxiformis,* delayed embryonic development and inhibited egg hatching. The established treatment against monogeneans involves recurrent acute bathing of infected stock primarily in either formalin or in freshwater solutions (Jerry, 2013). Therefore, the rates of parasite infections are important to fish health management. Pond culture is more convenient for the control of parasite infections than a cage culture. However, parasite infections are important factors affecting fish health. Management begins with the prevention of disease rather than treatment. Prevention of fish disease is accomplished through water quality management, nutrition and sanitation. Hence, aquaculture system should consider using re-circulated water and fine filtration to reduce parasite transmission (Whittington et al., 1999). However, *Laticola* spp. are commonly found in Asian seabass in both pond culture and cage culture. Therefore, good management practices are required to promote the health status and fish production of earthen pond cultures in Thailand.

Conclusion

In this study, diplectanid monogenean parasites infecting Asian seabass in an earthen pond culture in Klong Chanak sub-district, Muang district, Surat Thani province, were assessed from July to November, 2017. Two monogenean species, *Laticola paralatesi* and *L. seabassi* were found from the gill filaments. The overall prevalence of parasites was dominant in September among the five months observed. The mean intensity of *L. paralatesi* was highest in September, while *L. seabassi* was highest in November. While there were no parasite infections in fingerlings from the hatchery, infected fish were found after two months in an earthen pond. This information supports developing methodologies to produce an integrated health management system of Asian seabass in earthen pond cultures.

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Pollen Resources for the Honey Bee, *Apis mellifera* in Roi-Et Province, Thailand

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Abstract

The objective of this research was to investigated pollen resources for the honey bee, *Apis mellifera* based on pollen morphology analysis. The samples of bee pollen loads and pollen of flowering plants were collected during September – November 2014 in Pathumrat district, Roi-Et province. Acetolysis technique was used for pollen preparation. Pollen morphology analysis was investigated under light microscope (LM). The results showed 22 species of blooming flowers and 14 plant species found in bee pollen loads. There were 8 plant families found among 14 plant species from bee pollen loads, including (1) Arecaceae (2) Asteraceae (3) Bignoniaceae (4) Caricaceae (5) Fabaceae (6) Iridaceae (7) Moringaceae and (8) Rubiaceae. Five plant species can be identified after compared to pollen morphology of blooming flowers, including (1) *Carica papaya* (2) *Helianthus annuus* (3) *Ixora coccinea* (4) *Sesbania grandiflora* (5) *Sphagneticola trilobata* and 9 unknown species. The information from this research will benefit both beekeepers and in crop pollination. There were 2 economic plant species (*C. papaya* and *H. annuus*) found in bee pollen loads, which indicated that *A. mellifera* could be a good pollinator for these crop pollination.

Introduction

The honey bee, *Apis mellifera* plays an important role in the pollination of many crops. They show different preferences for flora surrounding the colony. The bee forages for nectar and pollen by visiting thousands of flowers on their way. During foraging, the bees participate in pollination of crops and do so in large numbers (Bhalchandra et al., 2014; Crane & Walker, 1984; Delaplane & Daniel, 2000; Dukku, 2013). Honey bees consume nectar for energy, whereas consume pollen for proteins, lipids, minerals and vitamins (Herbert & Shimanuki, 1978).

A. mellifera is an introduced species and has been imported to Thailand for research and the beekeeping industry since more than 70 years (Suppasat et al., 2007; Wongsiri et al., 2000). The beekeeping is distributed throughout Thailand, but mostly located in the northern part. This species is used for pollination of many economic crops such as longan, litchi, durian, rambutan and other crops (Suwannapong et al., 2011). The beekeepers need to build up strong bee colonies before honey harvesting season (February-April). The bees need a lot more pollen during build up colony. Food plants of the bees has been observed based on different methods (Hepburn & Radloff, 1998), including direct observation

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of foraging bees (Ayansola & Davies, 2012; Makong, 2016), analysis of pollen loads removed from returning foragers (Köppler et al., 2007; Sopaladawan & Phinyo, 2018; Sopaladawan & Sonyoha, 2018; Sopaladawan et al., 2019), analysis of pollen stores in nests or hives (Ramanujam & Kalpana, 1992), palynological analysis of honey (Adekanmbi & Ogundipe, 2009) and pollen DNA barcoding (Bell et al., 2016).

This research investigated pollen resources for the bees, *A. mellifera* during colony build up period using morphological analysis on pollen from pollen loads of returning foragers. The results could provide information for beekeepers in choosing suitable area for beekeeping, and also benefit on potential economic crop pollination by bees.

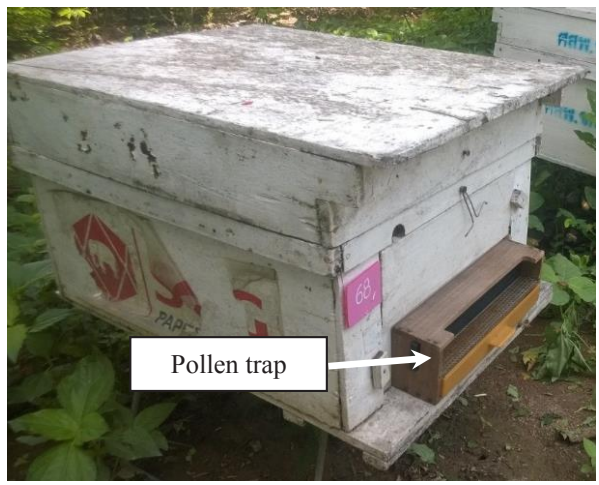

Fig. 1 Bee pollen loads collection using pollen trap

Materials and methods

1. Pollen collection

Pollen samples were collected from 2 different sources; (1) bee pollen loads and (2) pollen from blooming flowers. Both types of pollen were sampled once a month during colony built-up time in September-November 2014. Bee pollen were collected from 3 colonies of *A. mellifera* located at a local area of Pathumrat district, Roi-Et province (15º39´57.9´´ N, 103º25´45.9´´ E). A pollen trap was placed at the hive entrances of each colony (Fig. 1) at 7.00-11.00 am (Bhalchandra et al., 2014). Pollen loads from the bees were kept separately in plastic tubes at 4ºC for pollen identification. According to identify plant species from bee pollen, pollen from blooming flowers at the same area were also collected. This type of pollen was used as references of plant species from bee pollen loads.

2. Pollen preparation for pollen morphology analysis

Acetolysis method (Erdtman, 1966) was used for pollen morphology analysis, both from the bees and flowers. Firstly, pollen from bee pollen loads were separated by color and kept in 1.5 ml microcentrifuge tubes. Then, 10% KOH was added into the tube until it covered the pollen sample, the purpose of this step was to remove debris from the surface of pollen grains. The suspension was boiled in a water bath for 2-3 min. and filtered through a thin cloth. After filtration, pollen was transferred to a new tube and centrifuged at 3,000 rpm for 1 min., discarded the supernatant. Distilled water was subsequently added for pollen pellet washing, followed by being centrifuged at 3,000 rpm for 1 min. The supernatant was discarded and the washing step was repeated 2-3 times. Next, added glacial acetic acid to remove access water in pollen sample prior to add acetolysis mixture (Jones, 2014), centrifuged for 1 min and decanted the supernatant. Acetolysis mixture (9:1 acetic anhydride: conc. sulfuric acid) was then added to the tube and placed in warm water for 1 min, centrifuged, discarded the supernatant. Distilled water was added to wash the pollen sample, centrifuged and discarded the supernatant. Repeated the washing step for 2-3 times, followed by dehydrating the sample using 95% and 100% ethanol, respectively. For the final washing step, benzene was added to the sample, centrifuged for 1 min and discarded the supernatant. 2-3 drops of silicone oil were added to the pollen sample and it was kept until needed (Jones, 2014). The same method was used for preparation of pollen from blooming flowers.

3. Data analysis and plant species identification based on pollen morphology

Acetolyzed pollen samples were mounted on the slides for light microscopic examination at 40X magnification to perform morphology analysis of pollen. Pictures of 30 pollen grains per plant species were taken (Saensouk, & Saensouk, 2011) under the light microscope for size measurement using program AxioVision AC Rel. 4.1. Pollen symmetry, pollen aperture, and pollen shape were examined. Mean ± standard diviation (SD) of polar axis (P) and equatorial axis (E) of pollen grains from each plant species were calculated to use for comparison between bee pollen and pollen from flowers (Delaplane et al., 2013).

Results and discussion

The study showed totally 22 plant species of blooming flowers found in the study site during September-November 2014 (Table 1). Most of them were planted as human food and for home decorations. The 20, 22 and 22 species had bloomed in September, October and November, respectively. Whereas totally 14 species of plants were observed from bee pollen loads based on pollen morphology analysis (Fig. 2). The 7, 7 and 4 species were found in pollen loads of the bees in September, October and November, respectively (Fig. 3 and Table 2).

Table 1 List of 22 flowering plant species were found during September – November 2014 in Pathumrat district, Roi- Et province

Family name	Scientific name	Common name
Apocynaceae	*Cascabela thevetia*	Yellow oleander
Asteraceae	*Helianthus annuus*	Sunflower
	Sphaneticola trilobata	Singapore daisy/ Trailing daisy
Capparaceae	*Cleome viscosa*	Tickweed/ Asian spiderflower
Caricaceae	*Carica papaya*	Papaya
Convolvulaceae	*Argyreia nervosa*	Baby woodrose
	Ipomoea aquatic	Water morning glory
Cucurbitaceae	*Coccinia grandis*	Ivy gourd
	Cucurbita pepo	Pumpkin
	Luffa cylindrica	Sponge gourd
Fabaceae	*Cassia surattensis*	Scrambled Egg Tree/ Singapore Shower/ Sunshine Tree
	Lablab purpureus	Hyacinth bean
	Neptunia oleracea	Water mimosa
	Senna siamea	Siamese cassia
	Sesbania grandiflora	Corkwood tree
Labiatae	*Ocimum basilicum*	Sweet basil
Malvaceae	*Sida rhombifolia*	Bloom weed
Nyctaginaceae	*Bougainvillea sp.*	Paperflower
Portulacaceae	*Portulaca oleracea*	Flowering purslane
Rubiaceae	*Ixora coccinea*	Jungle geranium
Solanaceae	*Solanum melongena*	Eggplant
Zingiberaceae	*Alpinia galangal*	Siamese ginger

Eight families were found among 14 plant species from bee pollen loads, including (1) Arecaceae (2) Asteraceae (3) Bignoniaceae (4) Caricaceae (5) Fabaceae (6) Iridaceae (7) Moringaceae and (8) Rubiaceae. Five species of them could be identified after being compared to the pollen morphology of blooming flowers, including *Carica papaya, Helianthus annuus, Ixora coccinea, Sesbania grandiflora and Sphagneticola trilobata* and 9 unknown species (Fig. 2 and Table 2, 3). Amongst the 9 unknown species, 6 families were identified according to Adekanmbi (2009), Crompton & Wojtas (1993), Harley & Dransfield (2003), Perveen & Qaiser (2009), Rajukar et al. (2018), Rakarcha et al. (2018), Sawani et al. (2014), Sopaladawan & Phinyo (2018), Sopaladawan & Sonyoha (2018) and Sopaladawan et al. (2019) (Table 2, 3). Proportions of food plant species of *A. mellifera* to flowering plant species in September, October, November and in total were 0.35, 0.32, 0.18 and 0.64, respectively (Fig. 3). This suggested that number of food plant species of *A. mellifera* did not correlate to flowering plant species in each month. Whereas, high proportion of food plant species to flowering plant species found in total of 3 months. Earlier report by Sopaladawan et al. (2019) showed lower proportion (0.44) of food plant species to flowering plant species found in Nong Khai Province during the same time of this study, there were 43 flowering plant species, but only 15 species were pollen resources for *A. mellifera.* The results of this study and Sopaladawan et al. (2019) showed the same 7 flowering plant species, including *Cassia siamea, Coccinia grandis, Cucurbita moschata, Ixora coccinea, Ocimum basilicum, Portulaca oleracea* and *Sida rhombifolia.* Interestingly, none of them was found in bee pollen loads, which indicated that these 7 plant species are not good resources of pollen for *A. mellifera* bees.

Table 2 Pollen spectrum of 14 plant species in pollen loads of honey bee (*Apis melllifera*) in Pathumrat district, Roi-Et province during September-November 2014

Family name	Scientific name	September 2014	October 2014	November 2014
Arecaceae	Unknown 1	/	-	-
	Unknown 2	-	-	/
	Unknown 3	-	/	-
	Unknown 4	-	/	-
Asteraceae	*Helianthus annuus*	-	-	/
	Sphagneticola trilobata	/	-	-
	Unknown 5	/	/	-
Bignoniaceae	Unknown 6	-	/	-
Caricaceae	*Carica papaya*	/	-	-
Fabaceae	*Sesbania grandiflora*	-	/	/
	Unknown 7	/	/	/
Iridaceae	Unknown 8	-	/	-
Moringaceae	Unknown 9	/	-	-
Rubiaceae	*Ixora coccinea*	/	-	-

According to 5 identified plant species that were found in the bee pollen loads, 4 species of them had bloomed for the whole 3 months, including *C. papaya, I. coccinea, S. grandiflora* and *S. trilobata.* Whereas another species, *H. annuus* had bloomed only in November. Among 14 species that found in pollen loads of honey bees, only the Unknown 7 was found over 3 months (Table 2). There were 2 species of economic plants found in this study, which were papaya (*C. papaya*) and sunflower (*H. annuus*).

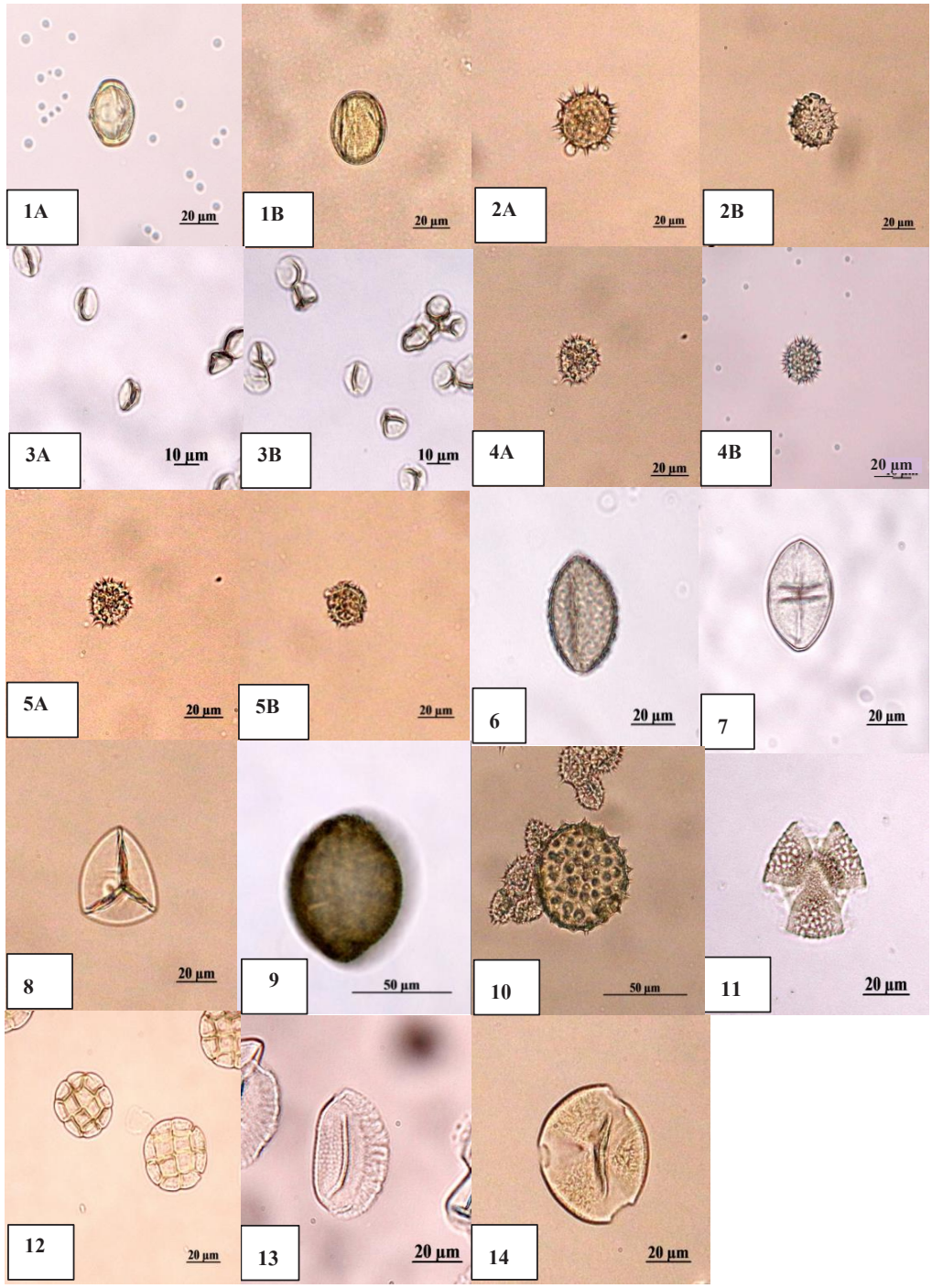

Fig. 2 Pollen grain morphology of food plant species for honey bee (*Apis mellifera*);
A = pollen from flowers, B = pollen from pollen loads
1= *Carica papaya,* 2 = *Helianthus annuus,* 3 = *Ixora coccinea,*
4 = *Sesbania grandiflora,* 5 = *Sphagneticola trilobata,* 6 = Unknown 1, 7 = Unknown 2
8 = Unknown 3, 9 = Unknown 4, 10 = Unknown 5, 11 = Unknown 6, 12 = Unknown 7, 13 = Unknown 8, 14 = Unknown 9

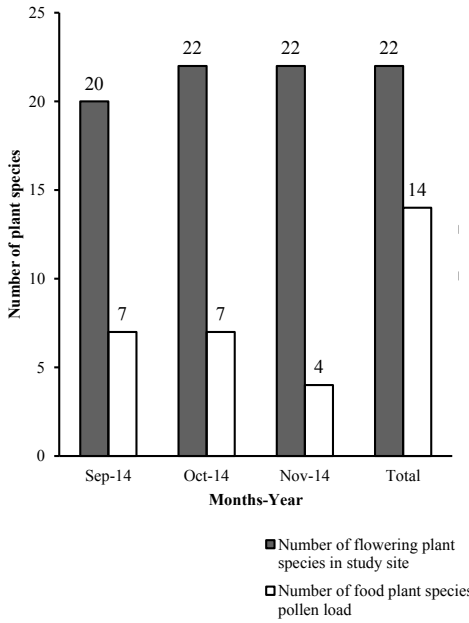

Fig. 3 Proportions of flowering plant species and food plant species in pollen loads of honey bee (*Apis mellifera*) were found during September – November 2014 in Pathumrat district, Roi-Et province

Papaya (*C. papaya*) is an important economic fruit crop in the tropical and subtropical countries. In 2008, papaya production in 20 countries over the world was over 9.1 million metric tons. India was the first ranked in papaya production, while Thailand was the 8th ranked. Annual papaya production in Thailand was about 215,000 tons in 2013, the largest papaya planting area in Thailand is in the eastern part. Thai people consume papaya both unripe and ripe forms (Janthasri & Chaiayaboon, 2016; Somsri, 2014). However, to increase papaya production, insect pollinators such as the hawk moth, stingless bees and honey bees had been considered to use for papaya pollination in Australia (Garrett, 1995; McGregor, 1976). There was no report on insect pollinators in papaya in Thailand. Another economic plant that could be identified from bee pollen loads was sunflower (*H. annuus*), which some beekeepers normally keep the bees in sunflowers field to collect honey (Andrada et al., 2004). Honey bee pollination activity is very important in seed production of hybrid sunflower (DeGrandi-Hoffman & Watkins, 2000; Sawatthum, 2020). So, this research could provide information on potential papaya and sunflower pollination services by *A. mellifera.*

Moreover, the study showed that honey bees also feed on non-crop flowers. The information of which plants are the bees foraged could be benefit on having those plants surround the fields or orchards to keep the pollinators close to the cropping system (Jones, 2014).

Conclusion

In summary, the study on pollen resources of the honey bee, *A. mellifera* in September-November 2014 at Pathumrat District, Roi-Et Province using morphology analysis on pollen from pollen loads of the bees showed 22 flowering plant species. Fourteen plant species were found in bee pollen loads. Five species of them can be identified based on pollen morphology, whereas 9 unknown species were observed. The results could be of benefit to beekeepers in which they get more information on pollen resources for the bees during the time of colony build up. Moreover, the results also provided more information on potential pollinators for economic crop pollination in Thailand.

Table 3 Pollen characteristics from pollen loads of honey bee (*Apis mellifera*)

Family name	Scientific name	Symmetry	Pollen aperture	Pollen shape (P/E)	P (µm) ± S.D.	E (µm)± S.D.
Arecaceae	Unknown 1	Bilateral	Monocolporate	Prolate	43.70±3.89	27.02±3.70
	Unknown 2	Bilateral	Monocolporate	Prolate	46.75±2.81	27.02±2.88
	Unknown 3	Radial	Tricolporate	Prolate spheroidal	39.61±2.86	35.43±2.82
	Unknown 4	Bilateral	Monocolporate	Prolate spheroidal	69.28±3.65	58.70±3.98
Asteraceae	*Helianthus annuus*	Radial	Periporate	Prolate spheroidal	29.79±1.31	28.12±1.23
	Sphagneticola trilobata	Radial	Periporate	Prolate spheroidal	20.09±0.95	18.55±0.88
	Unknown 5	Radial	Periporate	Prolate spheroidal	50.79±3.29	48.22±2.94
Bignoniaceae	Unknow 6	Radial	Tricolporate	Oblate spheroidal	35.83±1.64	34.07±1.77
Caricaceae	*Carica papaya*	Radial	Tricolpate	Subprolate	27.66±1.71	20.86±1.53
Fabaceae	*Sesbania grandiflora*	Radial	Periporate	Prolate spheroidal	19.62±0.73	18.99±0.86
	Unknown 7	Radial	Periporate	Prolate spheroidal	39.92±1.84	37.57±1.88
Iridaceae	Unknown 8	Bilateral	Monocolporate	Prolate	40.80±1.94	22.89±1.31
Moringaceae	Unknown 9	Radial	Tricolporate	Prolate spheroidal	41.13±2.31	39.08±2.14
Rubiaceae	*Ixora coccinea*	Radial	Tricolporate	Prolate spheroidal	12.79±0.87	10.23±0.93

Remark: P = Polar axis, E = Equatorial axis

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Aquatic Insect and Factors Influencing their Abundance in Temporary Habitats

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Abstract

Temporary water habitats are usually inhabited by a diverse fauna of aquatic organisms such as aquatic and semiaquatic species and may include rare and endangered species. In October and November 2016, aquatic insects were sampled in selected four temporary sampling sites in Kasetsart University, central Thailand. Aquatic D-hand net was used to capture the aquatic insects. Water variables in each habitat were simultaneously measured. A total of 4,820 aquatic insect belonging to 5 orders–Hemiptera (45.119%), Coleoptera (22.51%), Diptera (13.54%), Order Ephemeroptera (10.35%) and Odonata (8.42%) were collected. Eight families were recorded within the Order Hemiptera, with members of Family Notonectidae and the species *Anisops bouvieri* dominating. Five families were registered within Coleoptera, dominated by family Hydrophilidae, while order Odonata had 2 families dominated by family Libellulidae. Order Diptera was dominated by family Chironomidae. Order Ephemeroptera was dominated by family Baetidae. The values of the Shannon-Weiner index of diversity ranged from 2.118 to 2.487. Evenness values ranged from 0.643 to 0.795. The values of the Simpson index ranged from 0.7943 to 0.8900. Data of water variables and aquatic insects were analyzed with Principal Component Analysis (PCA). The correlations were found between aquatic insects and the water quality parameters of orthophosphate, nitrate-nitrogen, ammonia-nitrogen, temperature, alkalinity, electrical conductivity and total dissolved solids, were influenced the aquatic insect species.

Introduction

Temporary water habitats are diverse in form and geography. They are characterized by diverse physical and chemical conditions, regardless of their type and origin (Williams, 1996). The temporary habitats are include any habitat that intermittently has standing water and that, once inundated, holds water long enough for some species to complete the aquatic phases of their life cycle (Blaustein & Schwartz, 2001). This definition includes water body that might be classified elsewhere as temporary lakes, temporary ponds, rice fields or phytotelmata. Rain pools are small temporary ponds of variable duration formed in depressions where the rain

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is accumulated (Williams, 2006). Temporary ponds experience recurrent drought periods that may differ in duration, and are further characterized by the particular fauna that inhabits them and by the size of the populations that they can sustain (Williams, 1997). Temporary environments impose strict conditions on the fauna that inhabits them and require the development of different morphological, physiological and behavioral characteristics and adaptations to survive periods of drought, migration and changes in the life history (Wiggins et al., 1980; Wellborn et al., 1996; Williams, 1996). Temporary water habitats are usually inhabited by a diverse fauna of aquatic organisms, semiaquatic and terrestrial species. The adults of Hemiptera (aquatic bugs), Coleoptera (beetles), Odonata (dragonflies and damselflies), Diptera (true flies) and Ephemeroptera (mayflies) fly into the area and colonize the temporary habitats (Leitao et al., 2007; Pires et al., 2015); while other species spend their larval phase in the moist mud, growing rapidly in the aquatic medium and emerging as adults (Leitao et al., 2007; Pires et al., 2015). Temporary habitat condition was regulated the abundance and diversity of these organisms (Hayasaka et al., 2012; Mogi 2007), and therefore, temporary areas are colonized by organisms with short life cycles that are well adapted to the temporary habitats (Heiss et al., 1986).

Aquatic insect communities in temporary habitats are shaped by both abiotic and biotic factors. Hydroperiod, pattern of the water level in a temporary habitat, is the most important abiotic factor. The onset and duration of the hydroperiod will affect both invertebrate species richness and community composition, and the hydroperiod is at the same time the key factor to maintain distinct communities in temporary habitats (Spencer et al., 1999). Many aquatic insect taxa coexist in the water during floods and interactions between these insects are important for the species community structure. Predation is the most important of these biotic factors. Predatory insects of several taxa have a great impact on community structure in aquatic systems (Blaustein, 1998). Research on aquatic insect fauna in temporary habitat in Thailand is limited. With the aim to increase knowledge of temporary habitat fauna in the university campus, the objectives of this work were (1) to analyze some attributes of aquatic communities in temporary environments (2) to describe the abundance and diversity of aquatic insects in temporary habitats and (3) to relate the different temporary environments and aquatic insects.

Materials and methods

1. Sample collection and identification

Four temporary habitats as indicated with KU_KPS1, KU_KPS2, KU_KPS3 and KU_KPS4 (Fig. 1) were selected to sampling aquatic insects. Samples were collected once in October and November 2016 (where accumulation of water was registered). For the collection of aquatic insects, aquatic D-hand net (dimension of 30 × 30 cm frame, 250 μm mesh, 50 cm length) was dragged around the vegetation. At each sampling site, a stretch of approximately 1 m drag was chosen for collection of samples. Three such drags constituted one sample in each site. Collected insects were immediately sorted and preserved in 80% ethyl alcohol and taken back to the laboratory for identification. In the laboratory, aquatic insects were sorted in a Petri dish and identified to the lowest level using taxonomic keys by several authors (Dudgeon, 1999; Wiggins, 1996; Yule & Yong, 2004). Large aquatic insects were sorted by the naked eye whereas the sorting of the smaller ones was done under a dissecting microscope. All the sorted samples were kept in properly-labelled vials containing 80% ethanol.

2. Physicochemical water quality parameters

At the same collected aquatic insect site, selected physicochemical water quality parameters were recorded directly at the sampling site and included pH (measured by a pH-meter Waterproof Model Testr30), water temperature (WT) and air temperature (AT) (measured by a hand-held thermometer), and dissolved oxygen (DO, measured by a HACH® Model sensION 6 DO meter), total dissolved solid (TDS) and electrical conductivity (EC) (measured by a EURECH CyberScan CON110 conductivity/TDS meter). Water samples from each collecting period were stored in polyethylene bottles (500 mL). Ammonia- nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), orthophosphate (PO_4^{3-}), and turbidity (TUB) were determined in accordance with standard procedures [American Public Health Association (APHA) 1992]. Alkalinity (ALK) was measured by titration (APHA, 1992).

3. Data Analysis

The mean and standard deviation for each physicochemical variable was calculated per station. One-way ANOVA in combination with Tukey's (HSD) *post hoc* test was used to test for physicochemical parameters among sampling occasions and among the sampling sites using SPSS Version 20.0. The four community indices included: richness, evenness,

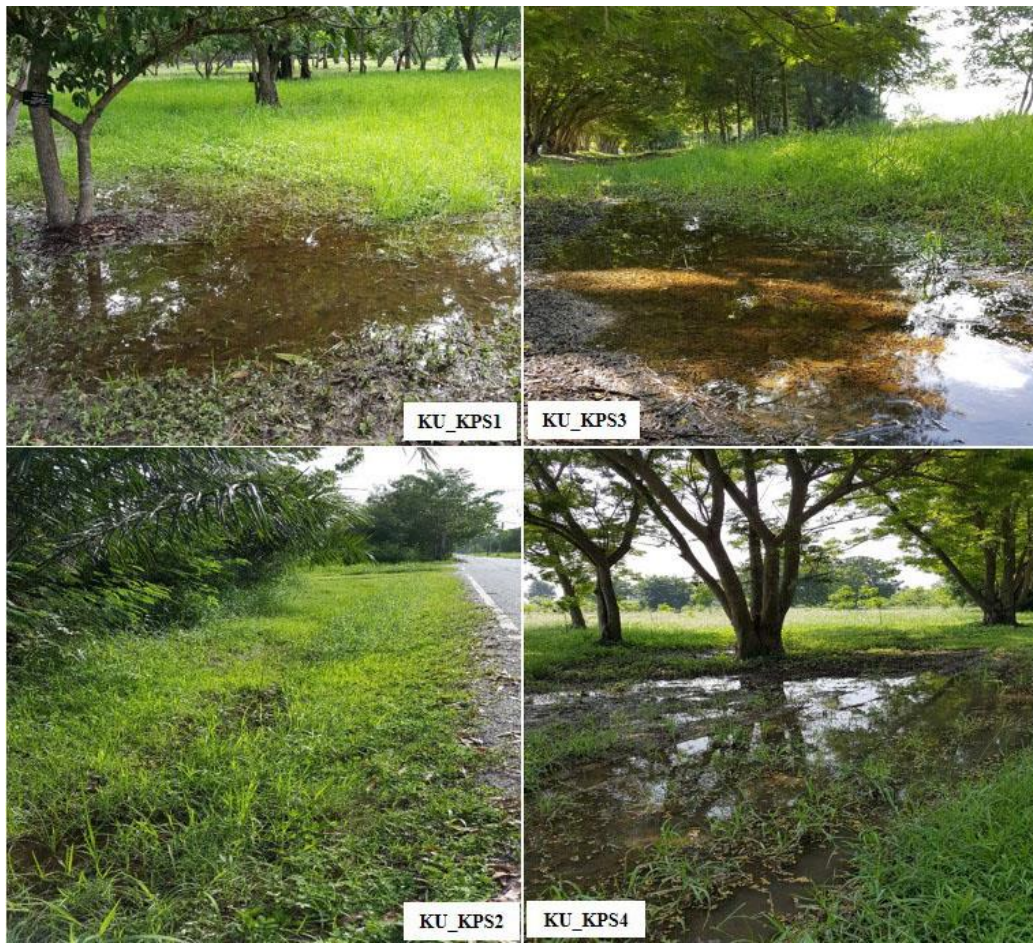


Fig. 1 Pictures of the selected of four temporary habitats sampled in Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province, Thailand (14° 0' 50.7204" N, 99° 58' 30.1002" E)

Shannon-Weiner diversity, and Simpson diversity were calculated using PC-ORD version 5.1 (McCune & Mefford, 2006). The Principal Component Analysis (PCA) was used to evaluate relationships between aquatic insects and environmental variables with PC-ORD version 5.10. Cluster analysis and non-metric multidimensional scaling (NMDS) were used to classify the sampling sites based on the aquatic insects using Ward's linkage method with Euclidean distance measure using PC-ORD software.

Results and discussion

1. Environmental variables in temporary habitat

All environmental variables (temperature, total dissolved solids, electrical conductivity, pH, alkalinity, ammonia-nitrogen, nitrate-nitrogen, orthophosphate and turbidity) showed significant differences ($p < 0.05$) across the temporary habitats (Table 1).

The average air temperature was 31.11ºC. The site of KU_KPS2 had the maximum temperature, and KU_KPS3 site had the minimum temperature. Mean air temperature was significantly high at site KU_KPS2 compared with sites KU_KPS1, KU_KPS3 and KU_KPS4 with significantly higher values ($p<0.05$). The average water temperature was 30.93ºC. The changes of water temperature are influenced by many variables including time of sampling and condition of the habitat. In the temporary habitats, the highest mean temperature was recorded in the KU_KPS4 (32.77±0.38°C) and KU_KPS3 site was recorded the minimum temperature (29.13±0.06°C). Aquatic insects preferred temperatures ranging 27.70 to 32.77°C.

Table 1 Environmental variables of water at four temporary habitats

Parameter/sites	KU_KPS1	KU_KPS2	KU_KPS3	KU_KPS4	*p*-value
Air temperature (°C)	31.17±0.61[a]	32.90±0.01[b]	29.70±0.10[a]	30.70±0.95[a]	0.001
Water temperature (°C)	32.33±0.46[c]	27.70±0.20[a]	29.13±0.06[b]	32.77±0.38[c]	0.000
Electrical conductivity (µS/cm)	209.67±3.06[a]	319.67±2.89[b]	190.13±56.21[a]	229.00±7.55[a]	0.002
Total dissolved solids (mg/L)	103.67±1.16[a]	160.00±2.65[b]	94.53±27.28[a]	114.67±3.22[a]	0.002
Dissolved oxygen (mg/L)	5.48±0.87[c]	2.87±0.98[a]	4.77±0.23[bc]	3.31±0.17[ab]	0.004
pH	7.33±0.06[a]	7.53±0.0.6[ab]	7.70±0.10[b]	7.50±0.10[ab]	0.004
Turbidity (NTU)	6.33±2.08[a]	14.33±1.53[b]	7.00±1.00[a]	38.00±2.65[c]	0.000
NH_3-N (mg/L)	1.20±0.06[c]	0.52±0.07[a]	0.83±0.11[b]	1.06±0.05[c]	0.000
NO_3-N (mg/L)	2.47±0.12[c]	1.50±0.17[a]	1.87±0.15[b]	2.60±0.10[c]	0.000
PO_4^{3-} (mg/L)	3.68±0.03[c]	1.72±0.04[a]	3.95±0.02[d]	2.55±0.17[b]	0.000
Alkalinity (mg/L)	79.33±1.15[a]	196.67±11.02[b]	85.33±2.31[a]	82.67±1.16[a]	0.000

Remark: a-c = the relationship of environmental factors is sinificant differences in the sampling sites

The levels of electrical conductivity at different sites showed wide variation, ranging between a mean of 190.13±56.21 µS/cm for the KU_KPS3 and 319.67±2.89 µS/cm for the KU_KPS2. KU_KPS1 and KU_KPS4 sites also recorded relatively high mean electrical conductivity levels of between 209.67±3.06 µS/cm and 229.00±7.55 µS/cm, respectively. Elevated levels of turbidity, total dissolved solids and electrical conductivity were recorded in KU_KPS2 and KU_KPS4, probably due accumulation of dissolved particles (Dida et al., 2015).

The dissolved oxygen varied considerably among the temporary sites in which the aquatic insects were caught, with the highest DO recorded in the KU_PKS1 (5.48±0.87 mg/L), followed by KU_KPS3 (4.77±0.23 mg/L) and KU_KPS4 (3.31±0.17 mg/L). The lowest (2.87±0.98 mg/L) was recorded in KU_KPS2. It was established that aquatic insects in the temporary sites were appeared in samples with DO values ranging between 2.87 mg/L to 5.48 mg/L.

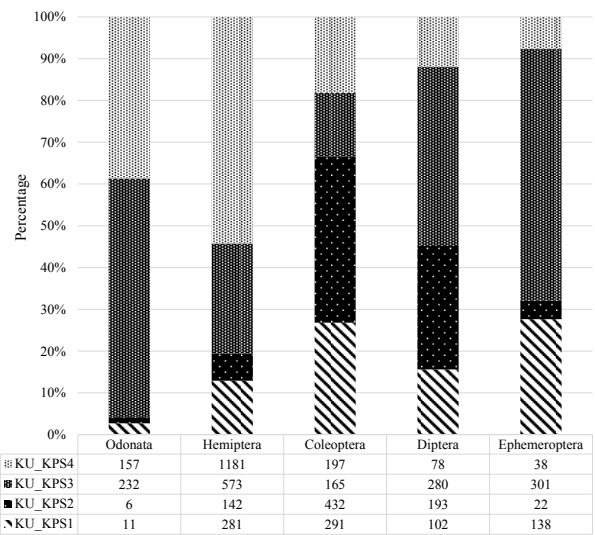
The measurement of water pH was varied markedly between different habitats, ranging between 7.33 to 7.70. The highest mean value (8.2 ± 0.5) was recorded in open puddle habitat KU_KPS3, while the lowest (7.33±0.06) was recorded in KU_KPS1.

As presented in Table 3, the highest mean alkalinity (196.67±11.02 mg/L) was recorded in the KU_KPS2 while the lowest were recorded in KU_KPS1 (79.33±1.15 mg/L). Mean water alkalinity values differed significantly between habitat types. The requirement of alkalinity for aquatic insects in the shared habitats varied, with values ranging between 79.33 mg/L, and 196.67 mg/L.

The orthophosphate concentration was highest (3.95±0.02 mg/L) in KU_KPS3 and lowest (1.72±0.04 mg/L) in KU_KPS2. The high concentration of phosphate in the temporary habitats may be due to land use management practices. Other important sources of phosphorus to freshwater are atmospheric precipitation, geochemical condition, and ground water (Lawniczak et al., 2016). Concentration of nitrate-nitrogen and ammonia-nitrogen ranged from 1.50±0.17 to 2.60±0.10 mg/L and 0.52±0.07 to 1.20±0.06 mg/L, respectively. In natural aerobic water, most nitrogen occurs as nitrates in varying amount depending upon the nature of water shed, seasons, degree of pollution and the abundance of plankton (Maitland, 1978).

2. Species diversity of aquatic insect

A total of 4,820 individuals comprising five aquatic insect orders, 22 families and 34 genera were identified (Fig. 2, Table 2).



	Odonata	Hemiptera	Coleoptera	Diptera	Ephemeroptera
KU_KPS4	157	1181	197	78	38
KU_KPS3	232	573	165	280	301
KU_KPS2	6	142	432	193	22
KU_KPS1	11	281	291	102	138

Fig. 2 The proportion of aquatic insects sampled in each temporary sampling habitats

Hemiptera was the dominant order and highest species richness belonging to eight family, together accounting 45.11% of the total number of aquatic insects collected. Family Notonectidae and the species Anisops bouvieri were highest abundance in all of Hemipteran family and species, respectively. In general, the genus Anisops was dominant and were found at all sampling stations and throughout the sampling time. They found the highest density up to 460 individuals in KU_KPS4. Overall, they also represented the highest abundance in all sites

surveyed with 1,102 individuals comprising 22.87% of the total insects collected. The relatively larger size of Notonectidae often makes these insect top predators in systems lacking vertebrates (Runck & Blinn, 1990). It is very common in temporary pools and permanent water bodies. This followed by Belostomatidae (476 individuals) and Gerridae (460 individuals) from orders of Hemiptera.

High abundance of hemipteran families especially Notonectidae, Gerridae and Belostomatidae were most related to the environmental conditions, as indicated by the PCA results. The Hemipterans are regarded as effective predators of freshwater snails and mosquito larvae in the aquatic ecosystems (Saha et al., 2007). It is also well known that notonectids are voracious predators of mosquito larvae (Saha et al., 2007). Gilbert & Burns

Table 2 Order, family, genus and species number for all aquatic insects sampled in four temporary habitats in October and November 2016

Oder	Family	Genus/Species	KU_KPS1		KU_KPS2		KU_KPS3		KU_KPS4	
			Oct	Nov	Oct	Nov	Oct	Nov	Oct	Nov
Odonata	Coenagrionidae	*Enallagma* sp.		5		3	3	2	28	31
	Libellulidae	*Hydrobasileus* sp.		4	2		28	3	39	
		Cratilla sp.		2		1	108	88	15	44
Hemiptera	Belostomatidae	*Diplonychus nitidus*				32	22	9	40	20
		Diplonychus rusticus		2	14	27	45	13	137	114
		Lethocerus indicus						1		
	Gerridae	*Limnogonus nitidus*	23	87	9	2	45	21	147	21
		Limnogonus sp.		38		5		57	3	2
	Helotrephidae	*Hydrotrephes yangae*				1				14
	Hydrometridae	*Hydrometra annamana*		1			1		3	3
		Hydrometra cracens					1			
		Hydrometra greeni		1				1	1	2
	Mesoveliidae	*Mesovelia horvathai*							1	
		Mesovelia vittegera	16	10	15	10		1		9
	Micronectidae	*Micronecta quadristrigata*	1		6	3			7	
	Notonectidae	*Anisops bouvieri*			9		201		435	25
		Anisops breddini	7						9	144
		Anisops lansvuryi		5			2	8		
		Anisops tahitiensis	2	65		2	59	39	2	
		Anisops sp.					37	10	2	39
	Veliidae	*Mircrovelia dauglasi*	13	1						
		Microvelia leveillei	6							
		Microvelia sp.		3	4				1	
Coleoptera	Dytiscidae	*Rhantus* sp.	10	12	29	25	13	3	22	5
		Hyphydrus sp.		61		1			4	
		Laccophilus sp.	3		6			2	2	2
		Neptosternus sp.	2	2	26	4	5		1	
		Copelatus sp.	2		6	32	30	22		
		Hydrovatus sp.	3		2					3
	Hydrophilidae	*Laccobius* sp.	8	19	12	26	2		2	
		Berosus sp.	20	14	12	5	5		17	
		Hydrophilus sp.	40	79	95	110	36	46	85	34
	Noteridae	*Canthydrus* sp.		1	3	1				2
	Scirtidae	*Hydrocyphon* sp.	3	4	5	13				
	Spercheidae	*Spercheus* sp.	4	4	13	6	1		14	4
Diptera	Ceratopogonidae	*Leptoconops* sp.				3				
	Chironomidae	*Chironomus* sp.	71	17	84	90	204	55	43	24
		Clinotanypus sp.			2					
		Parametriocnemus sp.					1			8
	Culicidae	*Aedes* sp.	4	2	2	2	2	7		1
		Culex sp.	2		4	1	6	2		1
	Tipulidae	*Limnophila* sp.		2				3		
	Stratiomyidae	*Odontomyia* sp.	2	1	3	5			1	
	Syrphidae	*Eristalis* sp.	1							
Ephemeroptera	Baetidae	*Cloeon* sp.	92	46	7	15	255	46	30	8

(1999) concluded that notonectid predators have the potential to alter mosquito communities via direct or indirect effects. Direct evidence of notonectid predation on mosquito larvae was later noted and this further confirmed their predominant role in mosquito larvae control (Chesson, 1984).

The second higher abundance of aquatic insect was order Coleoptera found in this study. Five families were registered within Coleoptera, accounted for 22.51%, dominated by family Hydrophilidae and Dytiscidae. Aquatic Coleoptera can be found in all types of freshwater (Fairchild et al., 2003) and they include a wide range of different feeding behaviours, represented by different families (e.g. many Dytiscidae are predators, many Hydrophilidae are algivores and detrivores) (Fairchild et al., 2000). Dytiscidae have three larval instars which pass their development in water, all adults are aquatic but may leave the water during migration or for over-wintering on land (Nilsson, 1996). Both larval and adult Dytiscids are generalist predators in aquatic habitats and feed on many different prey (Lundkvist et al., 2003). The dytiscid larvae are strictly predatory while the adults are partly scavengers, and larval prey choice is largely correlated with body size (Nilsson, 1996). Apart from predation on other invertebrates, large dytiscid larvae may also feed on small vertebrates. For example, increasing densities of Dytiscus larvae resulted in higher predation pressure on tadpoles (Pearman, 1995). This dominance of diversity and abundance of the Hydrophilidae among the Coleoptera is a common phenomenon in permanent and temporary ponds (Torres et al., 2012; Macchia et al., 2015). Ribera et al. (2003) considered both families typical of temporary environments. According to Ribera & Vogler (2000) the presence of Hydrophilidae and Dytiscidae in temporary ponds is due to their exceptional capacity to disperse.

Diptera with six accounted for 13.54% of the total number aquatic insects, dominated by family Chironomidae. Chironomidae are generally the most successful aquatic insect taxa and they inhabit all freshwater bodies, including polluted and eutrophic waters (Mackie, 2001). One of the main reasons for the great abundance of Chironomidae is that they exhibit all types of feeding behaviour and food preference (Nilsson, 1997). The larval abundance of the Culicidae, *Aedes* sp. and *Culex* sp. was low in number in all sites, because of high abundance of mosquito larvae predator were presented in all sites. Kweka et al. (2012) point out that the higher grass cover reduces sunlight penetration to the habitat which affects the algae biomass photosynthesis efficiency and other aquatic forms which are other sources of food to mosquito larvae. Grass cover influences oviposition site selection by mosquitoes hence directly effect on larvae abundance as observed by other researchers (Mala et al., 2011; Bashar, 2016).

Order Ephemeroptera were less presented with the family Baetidae and comprised 10.35% of the aquatic insect in temporary habitats. The family Baetidae can be found in all temporary habitats in this study which this families are very common in any kind of freshwater. They are mainly diversified in unpolluted running water, especially in the tropics. Although they are less diversified in standing waters, with genera like *Cloeon,* the Baetidae constitute an important part of the insect biomass in ponds. Most species of Baetidae are collector-gatherers, feeding mainly on detritus (Gattolliata & Nieto, 2009).

Odonata with two family accounted for 8.42% of the total number aquatic insects, dominated by family Libellulidae and genus *Cratilla.* Anisoptera was abundant in most of the water bodies sampled. This might be due to their high dispersal ability (Lawler, 2001; Kadoya et al., 2004) and their adaptability to wide range of habitats (Suhling et al., 2004; 2005). Less abundance of damselflies was probably due to their limited dispersal ability, undulating environment afforded by the temporary water bodies (Kadoya et al., 2004) and partial or absence of shade cover (Clark & Samways, 1996). The abundance of damselflies temporary habitat could be attributed to the presence of shade over the habitat from the trees present around the water bodies and to the presence of aquatic vegetation. This is in confirmation with the findings of Subramanian (2005) who revealed that shade and aquatic vegetation could favour Zygoptera more than Anisoptera. The abundance of Libellulidae (Anisoptera) and Coenagrionidae (Zygoptera) in the present study might be due to their shorter life cycle and widespread distribution (Norma-Rashid et al., 2001) and tolerant to wide range of habitats (Samways, 1989).

Table 3 showed the species diversity indices. The highest Shannon-Weiner index of diversity of 2.487 was recorded in KU_KPS1_Nov and the lowest (2.118) was in KU_KPS4_Nov, indicating the presence of a quite high diversity of aquatic insects in temporary ecosystems. Normally, the Shannon index in real ecological units ranges between 1.5 and 3.5 (Magurran, 2004). The value of diversity index can indicate the level of diversity in temporary habitats. Higher value of H′ indicates that the

species diversity in the location is high. The diversity of insects in aquatic ecosystems tends to increase with increased nutrients and these optimum environmental conditions favour their abundance in this habitat. Their abundance has been associated with the presence of high food quality and better water quality conditions prevailing in the habitats (Hepp et al., 2013).

Table 3 Number of individual, taxon richness, Shannon-Weiner diversity index, Simpson`s diversity index and Evenness index of the four sampling stations

Sites/month	Total individual	Taxon richness (S)	Evenness index (E)	Shannon-Weiner index (H')	Simpson dominance (D`)
KU_KPS1_ Oct	335	23	0.742	2.328	0.8504
KU_KPS1_ Nov	488	27	0.755	2.487	0.8886
KU_KPS2_ Oct	370	24	0.777	2.469	0.8620
KU_KPS2_ Nov	425	26	0.739	2.406	0.8619
KU_KPS3_ Oct	1112	24	0.722	2.296	0.8614
KU_KPS3_ Nov	439	22	0.795	2.457	0.8900
KU_KPS4_ Oct	1091	27	0.643	2.118	0.7943
KU_KPS4_ Nov	560	24	0.762	2.421	0.8666

Evenness values ranged from 0.643 in KU_KPS4_Oct to 0.795 in KU_KPS3_Nov. The evenness value in the present study was recorded as high in almost all the sites, indicating a relatively even distribution of taxa in the habitats. The high species diversity and evenness in almost all the sites are an indication of good water quality (Abhijna et al., 2013). The values of the Simpson index ranged from 0.7943 in KU_KPS4_Oct to 0.8900 in KU_KPS4_Oct. The high scores of diversity indices, such as those of the Shannon-Wiener index and Simpson's index, indicate that clean or unpolluted water support more diverse taxa, thus making them useful for detecting organic pollution (Maneechan & Prommi, 2015). Higher numbers of taxa (family) collected from a habitat imply a richer community that usually lives in a healthier environment. Based on the scores, all temporary sites supported relatively rich aquatic insect fauna.

Cluster analysis based on Bray-Curtis similarity distance (Fig. 3) showed that sites Oct_2 and Nov_2 showing high similarity followed by sites Oct_3 and Nov_3. Sites Oct_4 and Nov_4 had the most distinctive aquatic insect composition comparing to other sites. This was expected as this site had the high taxa richness (27 and 23 genera) and taxa abundance (range from one to 435). This station also recorded the low to slightly high diversity (2.118 and 2.421) as illustrated by Shannon-Weiner index.

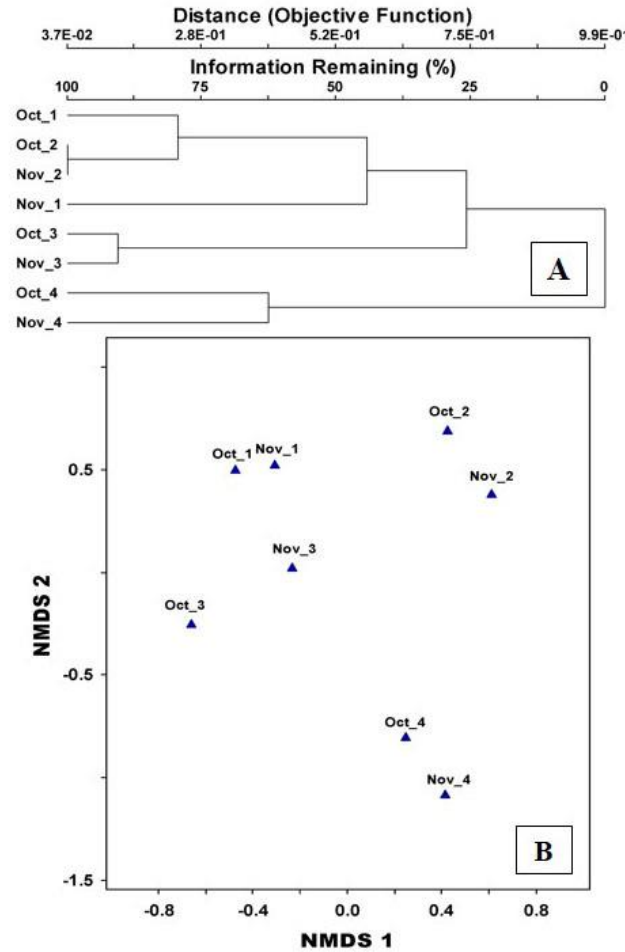


Fig. 3 Cluster analysis of aquatic insects collected during October and November 2016 in four temporary habitats (A) and Non-metric Multidimensional (NMDS) Scaling (B) of sampling sites based on aquatic insect data

3. Aquatic Insects and Environmental Parameters.

In order to determine the trend of the relationship between the physicochemical parameters with the aquatic insects in each site, a principal component analysis (PCA) was performed.

PCA ordination for data of aquatic insects can be separated into three groups (Fig. 4). The first group was located in the Oct_1, Nov_1, Oct_2 and Nov_2. The second group was located in the Oct_3 and Nov_3. The third group was located in the Oct_4 and Nov_4. PCA analysis revealed a correlation between the aquatic insect taxa and physicochemical variables (Fig. 4).

Aquatic insects, *Hydrocyphon* sp., *Laccobius* sp., *Odontomyia* sp., *Neptosternus* sp., *Clinotanypus* sp.,

Laccophilus sp., *Eristalis* sp., *Leptoconops* sp., *Berosus* sp., *Canthydrus* sp., *Spercheus* sp., *Hydrophilus* sp., *Rhantus* sp., *Helochares* sp., *Mesovelia* sp., *M. vittegera, M. leveillei* and *M. dauglasi* were negatively related to temperature, alkalinity, total dissolved solids and electrical conductivity in the first group.

Aquatic insects, *Limnophila* sp., *Copelatus* sp., *Aedes* sp., *Limnogonus* sp. *Chironomus* sp., *Culex* sp., *Cratilla* sp., *Cloeon* sp., *Hyphydrus* sp., *Copelatus* sp., *A. lansvuryi, A. tahitiensis, H. cracens* and *L. indicus* were positively related to ammonia-nitrogen, orthophosphate and dissolved oxygen in the second group.

Water quality variables such as turbidity and nitrate-nitrogen were positively related to aquatic insect *Hydrobasileus* sp., *Hydrovatus* sp., *Amphiops* sp., *Enallagma* sp., *Anisops* sp., *Parametriocnemus* sp., *A. breddini, H. yangae, H. greeni, D. indicus, D. rusticus, H. annamana* and *M. horvathai* in the third group.

In this study temporary habitats were sampled during flood, which were the periods of greatest rain fall in the study area. It is likely that the abundance and richness of aquatic insects in this flood period is associated with heavy precipitation that favored the formation of temporary habitats and individuals dispersal flights. Various research work in other countries (Schneider & Frost, 1996; Wellborn et al., 1996; Spencer et al., 1999; Boix et al., 2001) postulated the presence of various factors that influence the structure of the communities living in temporary environments, emphasizing the importance of hydroperiods. An association between species richness and water permanence has already been reported in the literature (Schneider & Frost, 1996; Spencer et al., 1999; Fontanarrosa et al., 2009), this association was also found in several temporary ponds during this study. At longer hydroperiods, more species will be able to complete their development and maintain viable populations. According

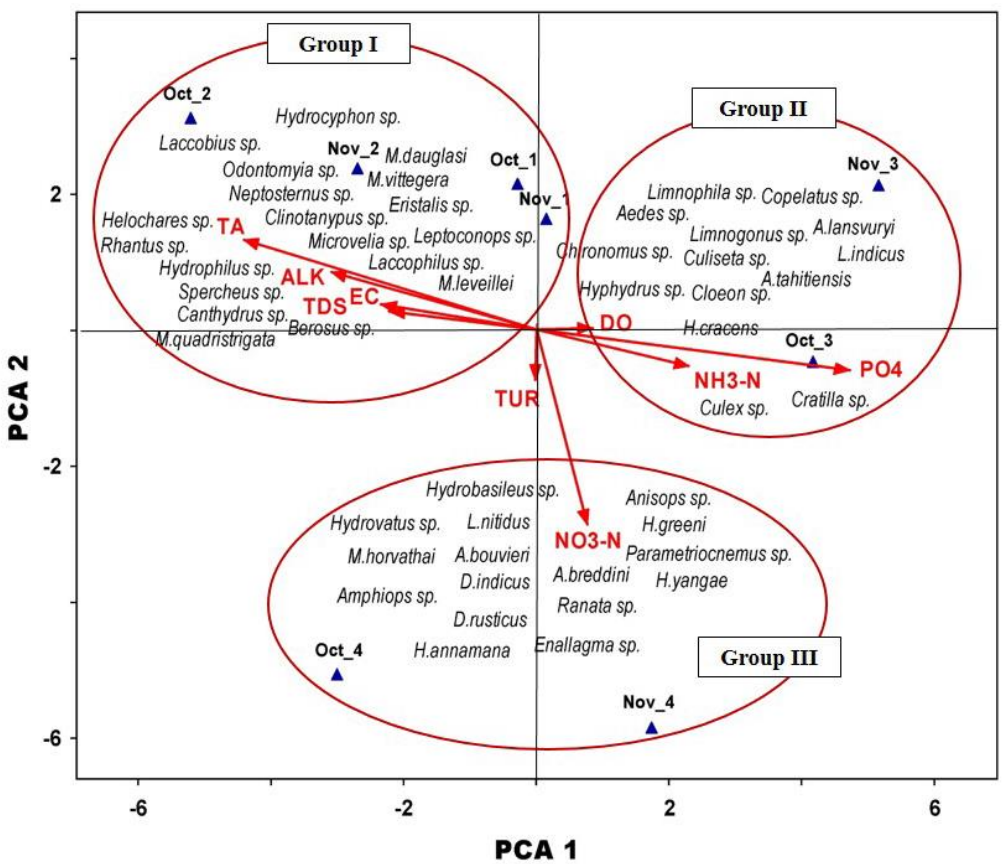


Fig. 4 Principal component analysis (PCA) on aquatic insects, environmental variables and sampling sites. The first and second PC axes explain 23.91% (eigenvalue: 10.75) and 20.93% (eigenvalue: 9.41), respectively, of the variation in the data set

to Spencer et al. (1999) the longer permanence of ponds also implies a longer time available for colonization. In this contribution, the temporary ponds that remained for a maximum of 10 days showed very few significant correlations between the analyzed variables, perhaps due to the rapid drying.

Conclusion

This is the first of a series of contributions which intend to study and evaluate the dynamics of aquatic insects in temporary environments. Based on the results, the aquatic insect communities inhabiting temporary habitats in the University in central Thailand are diverse, and include several species, such as *Diplonychus rusticus, Limnogonus nitidus, Rhantus* sp., *Hydrophilus* sp., *Chironomus* sp., *Aedes* sp., *Culex* sp. and *Cloeon* sp., that frequently inhabit these environments due to their biological adaptations. Also, less frequent and abundant species, such as *Hydrometra cracens, Mesovelia horvathai* and *Eristalis* sp. were registered in once time. Finally, the environmental variables are the factors that mainly determine the composition of these environments. We are aware that there are numerous open questions and unresolved issues that need to be addressed in future investigations. However, these data contribute to the knowledge about aquatic insects, as well as the ecology of the species that inhabit these temporary environments, which is currently very limited.

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Cytotoxicity Activity of Crude Extracts of Leum Phua Khaow-Mak (*Oryza sativa* L. variety Leum Phua) against Fibroblast Cell

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Abstract

Leum Phua Khaow-Mak is a fermented food, which provides high amounts of nutrients and antioxidant bioactive compounds such as phenolic compounds. Leum Phua Khaow-Mak extract compounds also have potential for applications in cosmetics and function food formulations. The objective of this research was to investigate cytotoxic activity of crude extracts from Leum Phua Khaow-Mak to human dermal fibroblast cells using sulforhodamine B (SRB) colorimetric assay method. Leum Phua glutinous rice (*Oryza sativa* L. variety Leum Phua) was fermented with a starter (Look Pang) at room temperature for 5 days. Fermented rice samples were extracted with 95% ethanol for 24 hours. The solvent was evaporated at 45°C to obtain dried crude extracts. The antioxidant capacity of crude extracts was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. Butylated hydroxytoluene (BHT) was used as a standard antioxidant. The EC_{50} value of the extract was 0.367 mg/ml, as similar to that of BHT (EC_{50} = 0.239 mg/ml). A dried sample was dissolved with 10% (v/v) dimethyl sulfoxide (DMSO) in the cell culture media. Cytotoxic activity of crude extracts to human dermal fibroblast cell was evaluated. The cell cultures were fixed in ice-cold 40% trichloroacetic acid (TCA) and were dyed using 0.05% sulforhodamine B for 30 min. Sodium lauryl sulfate was used as the positive control. The optical density was measured at 510 nm in a microplate reader. The percentage of viability of human dermal fibroblast cell were calculated and compared with sodium lauryl sulfate. The results showed that the concentrations of crude extracts from Leum Phua Khaow-Mak at 0.0001-1.0 mg/ml have the percentage of viability at 105.17-102.29 respectively. The percentage of viability of sodium lauryl sulfate at 0.0001-1.0 mg/ml have the percentage viability at 99.43-4.91 respectively. The percentage of viability at the concentrations of 0.1 and 1.0 mg/ml were low. These obtained results indicated that the concentrations of crude extracts from Leum Phua Khaow-Mak in the range between 0.0001 and 1 mg/ml were non-toxic to human dermal fibroblast cells.

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Introduction

Leum Phua glutinous rice is a colored rice known to be used for over 100 years by the Hmong people. It is a local variety found in the mountains of the North. Khao Rai Leum Pua, is still planted in Phop Phra District, Tak Province, in Thailand. Main pigment in both seed coat and whole grain of Leum Phua glutinous rice is purple black color containing phenolic compounds e.g. anthocyanin (Suttajit et al., 2006). These bioactive compounds show high nutritive values, such as Omega-3, 6 & 9, anthocyanin, gamma oryzanol, vitamin E and good minerals for health. Leum Phua rice has been identified as a source of natural antioxidants and reported to have antioxidant activity higher than other black rice varietals (Srisuwan et al., 2013). The antioxidant was contributed to inhibit the oxidation reaction and also to terminate the free radical chain reaction. The strength skin cell wall was reduced and collagen of cells was degraded leading to the decreasing of moisture and skin elasticity. Then, the wrinkles of skin cells were generated. By the addition of antioxidant compounds, the skin damage and wrinkle were protected and may slow-down the cell degeneration (Fereidoon & Chi-Tang, 2005). Moreover, The effect of high concentration of antioxidants in Luem Phua rice could improve learning and memory in mice (Srisuwan et al., 2013).

Khaow-Mak is known as one of the famous traditional Thai foods. It is produced from the fermentation process of rice using microorganisms. The starter or Look Pang is a semi-circular starch ball that incorporates various fermentative biological species such as molds and yeast. In the production process of Look Pang, Thai herbs such as pepper, garlic and galangal are added as antibacterial agents (Taechavasonyoo et al., 2013). Khaow-Mak has been documented as a rich source of probiotics and bioactive compounds, offering various food properties that influence human health. Several studies showed that the fermentation can increases bioactive compounds, such as minerals, vitamins, phenolic compounds and proteins digestibility. The degradation of antinutritional compounds as phytic acid, fermentation can lead to improved nutritional quality of food (Zhai et al., 2015; Plaitho et al., 2013 ; Michela et al., 2019). In addition, previous studies have examined the amount of phenolic compounds, flavonoid and antioxidant activities of Khaow-Mak crude extracts from colored rice varieties, such as Red Brown Jasmine Rice, Mun Poo Brown Rice, Sangyod Brown Rice, Black Jasmine Rice (Hom-nin), Rice Berry, Luem Pua Glutinous Rice, Muser Purple Rice and Black Glutinous Rice. It was found that the extract from Luem Pua Glutinous Rice obtained highest amount of phenolic content, flavonoids content and antioxidant efficiency (EC_{50}). According to previous results (Wattanuruk et al., 2018; Semsang et al., 2016), the rice varieties at after fermentation had the antioxidant activity higher than before fermentation. Therefore, there is a potential to further develop the extracts as an active ingredient in cosmetic and food for health and beauty products.

Fibroblast cells is a component of connective tissue in the skin. In recent years, various studies have been conducted to verify the capacity of fibroblasts to regenerate the skin structure. Collagen is the most common form of extracellular envelope, which forms fibers in an extracellular environment; therefore, it determines the shape of tissues. Collagen is stored as a precursor in fibroblastic cells and is secreted to the extracellular medium. This extracellular matrix and collagen form the structural framework of tissues in animals and plays an important role in tissue repair. These connective tissue are very important to the skin and can also combine with the water in the skin cells to maintain the skin moisture. In addition, elastin proteins are typically spindle-shaped can arrange adjacent collagen. Main function of elastin proteins is to anchor the skin cells to increase both strength and elasticity. If the fibroblast cells are damaged, causing from an ultraviolet radiation (UVR) and environmental insults, the skin can be broken down. This results in the loses of skin elasticity, reduction of the number of dermal fibroblasts, loss of skin thickness and can be wrinkles and hyperpigmentation in the skin (Nilforoushzadeh et al., 2017). Several studies have reported that the phenolic compounds of rice extracts showed immune and inflammatory cell function as well as antioxidant activity, so compounds with antioxidant properties will be useful against oxidative damage of connective tissue (Havsteen, 2002; Palungwachira et al., 2019).

The objective of this research was to check the antioxidant activity of Leum Phua Khaow-Mak by measuring DPPH free radical scavenging assay. The cytotoxicity studies carried out by using sulforhodamine B (SRB) colorimetric assay of crude extracts from Leum Phua Khaow-Mak againg the fibroblast cell. The method is most widely used for in vitro cytotoxicity screening of natural compounds on cultured cells (Skehan et al., 1990). The results will be further used in the production

of skin cosmetic to create new value of fermented rice.

Materials and methods

1. Preparation of crude extracts from Leum Phua Khaow-Mak

Leum Phua glutinous rice (Tak province) was washed by water for 5 min. The rice was soaked in water for 6 hours before mixing with distilled water (1:3 w/v) and cooked with the ordinary rice cooker. Cooked rice was cooled at room temperature and fermented with 0.5% Look-Pang (a mixed culture of yeasts and molds) at room temperature for 5 days in a glass container (0.5g/100 g of raw rice). The fermented rice was dried in an oven at 60°C for 24 hours. Dried rice samples were extracted with 95% ethanol under stirring condition using an orbital shaker at 120 rpm for 24 hours. The ethanol extracts were separated using the centrifugation at 6,000 rpm for 10 min and were filtered through a filter paper (Whatman No.1, Whatman International, England). The remaining soluble extracts were reprocessed by the same method and the extracts were combined and transferred to a flat-bottomed flask. The solvents were evaporated by a rotary evaporator at 45°C until dry. The sample was kept at -10°C until used (Plaitho, 2016).

2. DPPH radical scavenging activity

DPPH free radical scavenging activity was carried out according to (Fukumoto & Mazza, 2000) with some modifications. 0.02 g of Khaow-Mak extracts was diluted with 40 ml of 99.99% ethanol. A series of concentrations of the extract sample at 31.25, 62.50, 125, 250 and 500 mg/ml was prepared. BHT (butylated hydroxytoluene) was used as standard (positive-control) (Prabnok et al., 2016). Briefly, 1 ml of each extract was allowed to react with 2 ml of 0.1 mmol/l DPPH solution for 30 min in the dark before the absorbance was read at 517 nm. The radical scavenging activity was calculated as

$$\% \text{ Inhibition} = [(AB - AA)/AB] \times 100$$

where AA was the absorption of tested extract solution and AB was the absorption of blank sample.

The sample concentration providing 50% effective concentration (EC_{50}) was calculated from the graph ploting inhibition percentage againt sample concentration.

3. Cultivation of human dermal fibroblast cell

Human dermal fibroblast cell lines (passage 50: ATCC®PCS-201-012, USA) were cultivated in a culture medium containing Dulbecco' modified Eagle medium (DMEM) and supplemented with 10% Fetal bovine serum (FBS) (v/v) and 1% Penicillin-Streptomycin antibiotics (v/v) (Gibco, USA). The cells were mixed to obtain a homogeneous cell suspension and transferred to a sterilized tissue-culture bottle and incubated at 37°C in a humidified incubator with 5% CO_2 for 24 hours. Cell concentration was adjusted with a growth medium to obtain a seeding density of 10^5 cells/ml by hematocytometer chamber under a microscope (Vichai & Kirtikara, 2006).

4. Cytotoxicity of crude extracts to fibroblast cell by SRB

Dried sample of crude extract from Leum Phua Khaow-Mak was weighed and dissolved in 10% (v/v) dimethyl sulfoxide (DMSO) (Labscan, Thailand) and culture media. The samples were then sterilized using a membrane filter (0.2 µ) and were diluted to obtain five concentrations of (mg/ml) 0.0001, 0.001, 0.01, 0.1 and 1 in 10% (v/v) DMSO in sterilized cultured media. Sodium lauryl sulfate were prepared in a sterilize cultured media to obtain the concentration of 0.0001, 0.001, 0.01, 0.1 and 1 mg/ml. Ten microliters of the sample was added into each well of a 96 well tissue culture plate. Negative control was performed by adding 10 µl of 10% (v/v) DMSO into a well of test sample. Sodium lauryl sulfate (10 µl) was added into a well for a positive control. Added 10 µl of sterilized cultured media to negative control well of sodium lauryl sulfate. Added 100 µl cells suspension to each well already containing compounds. Set aside a plate contain only cells suspension for a no-growth (day 0). The cell culture was incubated at 37°C in a humidified incubator with 5% CO_2 for 72 hours.

Cytotoxic activity of crude extracts to human dermal fibroblast cell was evaluated. Sulforhodamine B (SRB) is used for cell density determination, which performed to assess growth inhibition by a colorimetric assay by staining total cellular protein with the dye SRB. This assay cell cultures were fixed with 100 µl of ice-cold 40% (w/v) Trichloroacetic acid (99.0% TCA: Merck, Germany) per well, incubated at 4°C for 1 hour. The plates were washed with distilled water to wash non-viable cells. Then, and dried at room temperature. SRB solution (Sigma-Aldrich, USA) in 50 µl (0.05% w/v in 1% glacial acetic acid, Sigma, USA) was added to each well and left at room temperature for 30 min. The plates

were quickly rinsed with 1% (v/v) acetic acid to remove an unbound dye. Then, the plates were dried at room temperature for 30 min before added in 200 µl of 10 mM Tris base solution (Sigma, USA) pH 10.5 [Tris (hydroxyl methyl) aminomethane]. The plate was then shaken to solubilize the protein-bound dye. The optical density (OD) was measured at 510 nm in a microplate reader. Percentage of viability of fibroblast cell was calculated and compared with sodium lauryl sulfate using the equation below (Skehan et al., 1990 ; Vichai & Kirtikara, 2006). Morphology of fibroblast cell was studied by compound light microscope (100x).

$$\% \text{ of control cell growth} = \frac{\text{mean ODsample - mean ODday 0 x 100}}{\text{mean ODneg control - mean ODday 0}}$$

5. Statistical analysis

All values were expressed as means of each treatment group. Student's unpaired t-tests were used to compare between the two groups. $P<0.01$ was considered statistically significant.

Results and discussion

DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized (Asgarpanah et al., 2011), which can be quantitatively measured from the changes in absorbance. In this experiment, the EC_{50} value of the Leum Phua Khaow-Mak extracts was 0.367 mg/ml, as similar to that of BHT (EC_{50} = 0.239 mg/ml). BHT was used as standard antioxidant in the performed experiments. It also known as dibutylhydroxytoluene, is a lipophilic organic compound, chemically a derivative of phenol, that is useful for its antioxidant properties. These obtained results indicated that the crude extract was able to scavenge DPPH radicals. Leum Phua is one of the aromatic and indigenous purple sticky rice, enriched with flavonoids, especially anthocyanins, and have total antioxidant higher than other varieties black rices of Thai rice namely, Black Rose, Hawm Nil, and Klam (Suwannalert & Rattanachitthawat, 2011). In addition, our previous studies have examined the amount of phenolic compounds, flavonoids, antrocyanins and antioxidant activities of Khaow-Mak crude extracts from colored rice varieties around Thailand. The results showed that Leum Phua glutinous rice showed the highest antioxidant activity of DPPH and it contains high amount of phenolic compounds. The results indicated crude extracts from Leum Phua Khaow-Mak might be used as a natural antioxidant (Wattanuruk et al., 2020). Moreover, Rice Research and Development Division (2019) (Rice Department, Ministry of Agriculture and Cooperatives of Thailand) also reported that Leum Pua rice has higher antioxidant than other colored rice. Boonsit et al., (2010) reported that Leum Phua glutinous rice contains higher antioxidants and bioactive compounds such as anthocyanins, γ-oryzanol and phenolic compounds than white rice. In other research, Leum Phua cultivar exhibits high antioxidant content and activity of bran extracts from Thai rice cultivars. The bran extract obtained from the black rice cultivar Leum Phua exhibited a potent scavenging effect towards DPPH radical, correlated with its high content in phenolic compounds (Peanparkdee et al., 2019; Pansiri et al., 2019).

The SRB assays performed similarly, exhibiting moderate to excellent correlation in the evaluation of the cytotoxicity of chemicals on cultured cells.The assays are used for cell density determinations, based on the measurement of cellular protein content (Vajrabhaya & Korsuwannawong, 2018). The method was used to test the toxicity of compounds for adherent cell by dyeing. The assay relies on the ability of SRB for the binding of protein components with the cells and has been fixed with a tissue culture plate. SRB is a bright-pink aminoxanthene dye with two sulfonic groups to bind with the basic amino acid residues under mild acidic conditions for the survive cellular protein contents. Strong intensities of SRB staining indicates number of cell viabilities (Thongdeeying et al., 2007). Cell viability was expressed as a percentage of the control values.

The crude extracts from Leum Phua Khaow-Mak at concentration between 0.0001and 1 mg/ml were focused. The results show the human dermal fibroblast cells with stained SRB are similar as the control group. The standards, sodium lauryl sulfate were prepared. When the concentration of sodium lauryl sulfate increases, the stains of SRB dyed were reduced (Fig. 1). The concentrations of Leum Phua Khaow-Mak extracts were prepared at the concentrations of 0.0001, 0.001, 0.01, 0.1, and 1 mg/ml and their percentages of viabilities were 105.17, 105.82, 103.54, 103.49, and 102.29, respectively.

Whereas, the concentrations of sodium lauryl sulfate standards were 0.0001, 0.001, 0.01, 0.1, and 1 mg/ml cell and their percentages of viabilities were 99.43, 96.06, 94.32, 9.85, and 4.91, respectively. Then, the concentrations of 0.1 and 1.0 mg/ml had the lower survival cell percentage (Table 1). In addition, the study of cells appearance under the microscope method was found that the testing cells with Leum Phua Khaow-Mak crude extracts displayed the similar appearance with group of control cells, with no alteration of the typical spindle-shape cell morphology. On the other hands, the exposure cells with sodium lauryl sulfate were exhibited the different appearance from the group of control cells. Because of the cell division, the irregular shapes and the tissue were indistinguishable from the control (Table 2). Many researcher groups reported that sodium lauryl sulfate that are known to produce irritation when applied topically to skin. The contact irritants were cytotoxic for keratinocytes and fibroblasts and suppressed growth at lower concentrations than the contact sensitizers. The contact irritants also produced histological changes (hyperplasia, incomplete keratinization, loss of the granular layer, acantholysis and necrosis) in organ-cultured skin (James et al., 2007; Corinne et al., 1998).

The results showed that the crude extracts from Leum Phua Khaow-Mak with all concentrations do not affect the toxicity of the cell components. Due to the extracts containing with polyphenol, flavonoid and anthocyanin, these bioactive compounds in the outer membrane of rice as seed coat or rice bran with either black or red with high amounts of antioxidant (Lee et al., 2007 ; Shen et al., 2009). Based on literature (Chen et al., 2018), no obvious cytotoxic effect was found with the increase of the concentration of the alcohol free rice bran fermentation solution. Moreover, the fermented alcohol-free rice bran did not cause irritation. However, fermented alcohol-free rice bran had also antiaging, melanin prohibition, whitening and moisturizer. Moreover, the fermented purple plain sap samples showed high antioxidative, the high amounts of tyrosinase inhibition, the high MMP-2 inhibition activities with low cytotoxicity to normal human skin fibroblast by SRB assay in comparing to other rice sap samples (Aranya et al., 2011). On the other hand, it is well accepted that several plants are the richest source of antioxidants. In other studied, the rice cell extracts (at 25–100 µg/ml) were not cytotoxic to the human dermal fibroblasts and keratinocytes. Furthermore, significantly enhanced the migratory ability of the two cell types (Kim et al., 2016). The hydroglycolic crude extract of Thai red Hom–Kularb–Drice rice bran on UVB induced photoaging of human skin fibroblast. The extract showed a no cytotoxic response was showed in human skin fibroblast (Yakaew et al., 2020). Black Rice Extract (BRE) treatment did not affect cell morphology and viability of HaCaT and human dermal fibroblasts. These findings suggest that BRE contained antioxidative flavonoid components such as cyanidin 3-O-β-D glycoside and taxifolin 7-O -glucoside (Han et al., 2018).

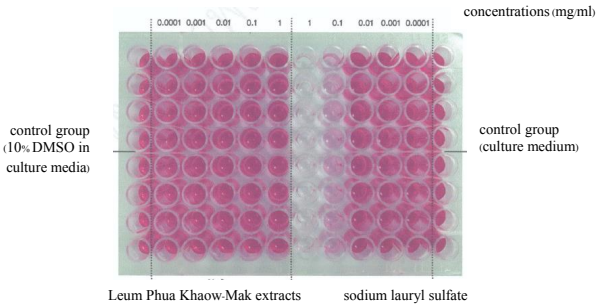


Fig. 1 The strong intensity of SRB staining is show that number of cell viability

Table 1 The percentage of viability of human dermal fibroblast cell

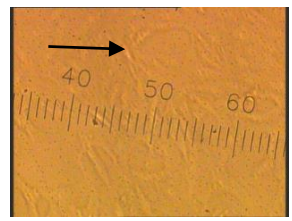
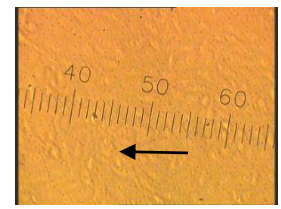
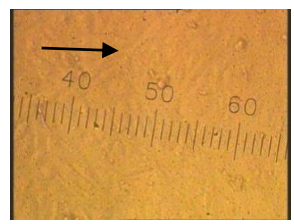
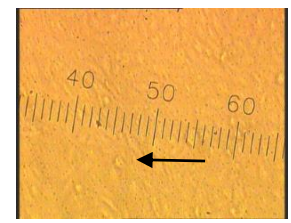
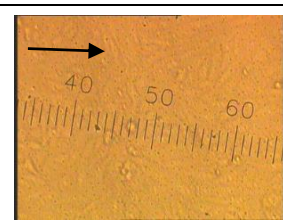
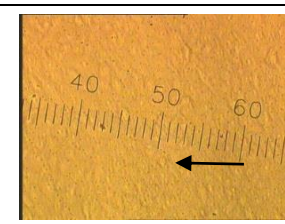
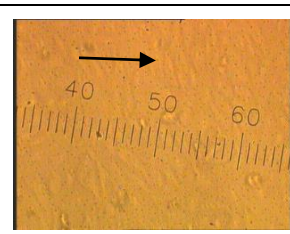
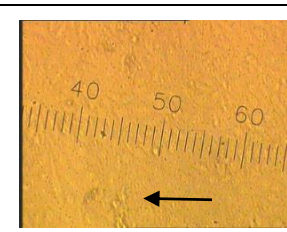
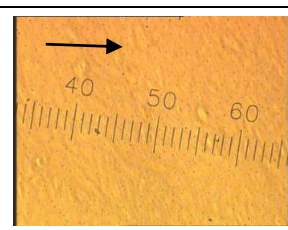
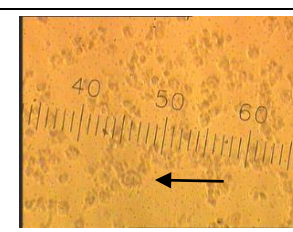
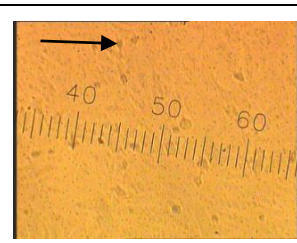
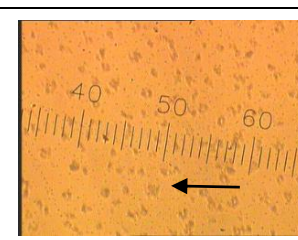
Test sample	Viability of human dermal fibroblast cell (%)				
	0.0001 (mg/ml)	0.001 (mg/ml)	0.01 (mg/ml)	0.1 (mg/ml)	1 (mg/ml)
Leum Phua Khaow-Mak extracts	105.17±3.93	105.82±2.69	103.54±2.69	103.49±2.76	102.29±4.42
Sodium lauryl sulfate	99.43±2.42	96.06±1.07	94.32±0.89	9.85*±1.94	4.91*±0.37

Remark: Each value are expressed as four replicate (mean±S.D.), (*p < 0.01)

Conclusion

In conclusion, the extract of Leum Phua Khaow-Mak exhibits good antioxidant and cytotoxic activities. The results can be summarized that EC_{50} value of the extract was 0.367 mg/ml, as similar to that of BHT (EC_{50} = 0.239 mg/ml). The concentrations of crude extracts from Leum Phua Khaow-Mak at 0.0001-1.0 mg/ml have the percentage of viability at 105.17-102.29 respectively. The percentage of viability of sodium lauryl sulfate at 0.0001- 1.0 mg/ml have the percentage viability at 99.43-4.91 respectively. The percentage of viability at the concentrations of 0.1 and 1.0 mg/ml were low. These obtained results indicated that the concentrations of crude extracts from Leum Phua Khaow-Mak in the range between 0.0001 and 1 mg/ml were non-toxic to human dermal fibroblast cells. The pure compounds of this strain might be further applied

Table 2 Characteristic of human dermal fibroblast cell under compound light microscope (100x) after was tested with Leum Phua Khaow-Mak extracts and sodium lauryl sulfate

concentrations (mg/ml)	Leum Phua Khaow-Mak extracts	Sodium lauryl sulfate
0 (control group)		
0.0001		
0.001		
0.01		
0.1		
1		

as antioxidant or anti-aging ingredients in cosmetic, food and other industries, including prevention of cellular oxidative damage.

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Antioxidant Activity and Allelopathic Potential of *Lippia nodiflora* (L.) Michx on Germination and Growth of *Neptunia javanica* Miq. seeds

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Abstract

The research objective was to evaluate the antioxidant activity, the allelopathic potential on germination growth of *Neptunia javanica* Miq. seeds and the total phenolic content (TPC) of crude extracts and fractions from *Lippia nodiflora* (L.) Michx. In this research, the ethanol crude extracts of leaves (LE), stems (SE) and roots (RE) from *L. nodiflora* were subjected to study the antioxidant activity by using DPPH free radical scavenging assay and the allelopathic potential on germination seeds of *N. javanica.* The RE extract had the highest antioxidant activity with IC_{50} value of 136.87 ppm. The allelopathic effect of LE extract at a concentration of 20 mg/mL completely inhibited the seed germination of *N. javanica.* The LE fractions were separated by liquid-liquid partition with hexane and ethyl acetate system to afford the leaf hexane fraction (LH), leaf ethyl acetate fraction (LEtOAc) and leaf hydroethanolic fraction (LW). The antioxidant, TPC and allelopathic activities of all fractions were investigated. The antioxidant activity of LW (158.21) and LEtOAc (175.45) fractions were better than those of LE (296.87) extracts, while LH fraction showed the lowest antioxidant activity at 722.08 ppm. The active allelopathic compounds in LEtOAc at a concentration of 10 mg/mL showed complete inhibition of radicle and hypocotyl on germination seed growth and 50% of seed growth inhibition (IC_{50}) was 3.14 mg/mL. Crude extracts and fractions showed more inhibited radicle growth than hypocotyl growth of *N. javanica* seeds. The TPC of leaves crude extracts and fractions were the highest. Interestingly, the total phenolic content of LEtOAc fraction, was the highest (0.072 mg GAE/g) that was related to the good allelopathic properties, compared with other fractions and crude extracts. Our results indicate that the ethanolic crude extracts and an ethyl acetate fraction of *L. nodiflora* leaves can be applied to weed control, especially for *N. javanica.*

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Introduction

Nowadays, human beings have more awareness of health and the environment. The chemical residue is one of the factors affecting health and the environment. Due to synthetic chemicals used for weeding, some will remain in the soil which affects the environment and human health. There are many ways to reduce chemical pollution; one of them is the natural plant extracts replacement. The effect chemicals from some plants have been released into the environment affected to another plant by the positive or negative is called allelochemical or allelopathy (Rice, 1984; Al-Samarai et al., 2018). Allelochemicals was found in many parts of plant such as leaf, stem, flower, pollen, root and seed, might be useful in agriculture systems (Iqbal et al., 2019). Some important allelochemicals may be flavonoids, alkaloids, terpenoids, steroids, tannins and phenolic compounds (Anwesa & Sanjib, 2015). These compounds have a various biological activity, including antioxidant, antimicrobial and allelopathic activities (Fonseca et al., 2017; Abd El-Gawad et al., 2015; Dacoreggio et al., 2019). The previous researches, the allelochemical from the plant could be extracted and fractioned by using various polarities of a solvent such as water and organic solvent (methanol, ethanol, ethyl acetate, dichloromethane and hexane). Fonseca et al. (2017) research -the ethanol extract and fraction (hexane, dichloromethane, ethyl acetate and hydroethanol) from dry powder leaves *Smilax brasiliensis* Sprengel on the antioxidant and allelopathic activities- showed higher antioxidant activity than BHT (2,6-di-tert-butyl-4-methylphenol) and the allelopathic effect on the growth of onion showed inhibition of hypocotyl and radicle at the lower concentration tested, *S. brasiliensis* Sprengel could be used as a natural antioxidant and herbicide. In another report, Dacoreggio et al. (2019) found that the leaves aqueous extracts of *Psidium cattleianum* Sabine, showed antibacterial, antioxidant and against on germination *Lactuca sativa* seed. The all tested extract show considerable on allelopathic activity, with growth inhibition on seed more than 50%. Teixeira de Oliveira et al. (2019) studied the allelopathic effect of the ethanol extract and dichloromethane fractions of the aerial parts of *Lippia alba*. The dichloromethane fraction extract from the aerial part of *L. alba* showed inhibiting the growth of *L. sativa* (lettuce) and *Allium cepa* (onion) seeds.

Lippia nodiflora (L.) Michx. synonym *Phyla nodiflora* (L.) Greene (Verbenaceae family) has a few common names such as lippie, frog fruit, bukkan, jaipapli (Hindi), busbusi, chachahan (Philippines) and vernacular names in Thai also known as ya kelt pla. (Sharma & Singh, 2013; Stuartxchange, 2018) or yaa riet pla (Al-Snafi, 2019). *L. nodiflora* is a small creeping perennial medicinal herb, small white to pink flowers, roots sprout from some stem nodes with growing rapidly to the ground covering on sandy loams, sand, gravel, clay loams soil in floodplains or wetlands together with capable on fixating sand and conserving earth and water (Fu et al., 2013). The deep rooting system of this plant is better to hold soil than grass. *L. nodiflora* is well known for applying to prevent sand or soil surface from the riverbank erosion. This plant is a fast-growing and resistant to sunlight so we can see it throughout India, Sri Lanka, Tropical Africa, Ceylon, Baluchistan, South and Central America, Taiwan and The Philippines (Elakovich & Stevens, 1985; Sharma & Singh, 2013; Sharma, 2018). In Thailand, *L. nodiflora* can be found in many provinces such as Nakhon Ratchasima, Kanchanaburi, Lampang, Mae Hong Son, Khon Kaen, Kanchanaburi, Nakhon Sawan and Phetchaburi (BGO Plant Databases, 2013a). *L. nodiflora* is a traditional medicinal plant boiled dried stems in water for a cough remedy, and when the patient is coughing up blood. A bandage using a poultice of stems is able to treat chronic ulcers and cure blister (Medthai, n.d.). Some researchers have described chemical constituents, antimicrobial, antioxidant activities, anticancer properties, including allelopathic activities of *P. nodiflora* L. or *L. nodiflora* L. The aerial part of *L. nodiflora* contains many bioactive components e.g. antioxidant (Ashokkumar et al., 2008). Lin et al. (2014) reported that the HPLC-fingerprint and antioxidant constituents of flavone pure compounds were isolated from dried aerial part *P. nodiflora* methanol extract. The pure compounds such as onopordin, cirsiliol and eupafolin displayed a strong antioxidant activity with DPPH scavenging assay. The aerial part of *P. nodiflora* was extracted with hexane, chloroform, ethyl acetate, and methanol extract by using a soxhlet extractor. Preliminary screening of phytochemicals was implemented for different extracts of *P. nodiflora.* The ethyl acetate extract from aerial parts showed dominant antimicrobial and antifungal activity (Priya & Ravindhran, 2015). Liau et al. (2017) studied on antioxidant and anticancer properties of solvent partitioned extracts from leaves and stems of *P. nodiflora* L. The extracts were obtained using methanol, ethyl acetate and water fractions contained

higher amount of TPC (total phenolic content) than hexane and chloroform fractions. The DPPH radical scavenging activity of this plant increased in a concentration level depends on leaves and stems extracts. The EC_{50} value, leaves methanol extract showed higher antioxidant activity compared to stems methanol extract. The allelopathic effectiveness of *L. nodiflora*, from some researches on various seeds inhibited germination. Fu et al. (2013) reported that the investigation on the allelopathic effect on three crops as wheat, radish and rape with 4% and 1% aqueous extract of fresh aerial part of *P. nodiflora*. The result of this research showed allelopathic effect of inactive aqueous extract on the three crops, so this plant will not be harmful on wheat, radish and rape growth. Ali et al. (2019) reported that allelopathy of leaves leachates *L. nodiflora* 10 mg in sandwich method showed non effect on growth of *Lactuca sativa* L. (lettuce) seed.

From the researches mentioned above, the antioxidant and allelopathy activities of *L. nodiflora* have not been reported. Thus, the main objective of this study is to determine the DPPH radical scavenging, total phenolic content and allelopathy capacity of crude extracts and fractions of *L. nodiflora*. This will be the potential way of allelopathy on weed *Neptunia javanica* Miq. seed.

Materials and methods

1. Chemical reagents

All chemical reagents used in this study are analytical grade (AR). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Gallic acid monohydrate of Sigma-Aldrich, Folin-Ciocalteu's RS reagent, L-ascorbic acid (Vitamin C) of CARLO ERBA, Hexane, Ethyl acetate, Ethanol of RCI Labscan, Sodium Carbonate (Na_2CO_3) of KEMAUS.

2. Instruments

The determination of DPPH activity and total phenolic content were measured by a Jasco V-650 series UV-Visible spectrophotometer (JASCO international Co., Ltd., Thailand). The preparation of crude extracts was removed solvent to dryness with vacuum rotary evaporator (Rotavapor R-210, Heating bath B491, Vacuum pump V-700, CTL911) of BÜCHI, Thailand.

3. Plant materials

Lippia nodiflora (L.) Michx. and seed of *Neptunia javanica* Miq. were collected from Bang Bo District in Samutprakarn Province. The *L. nodiflora* (L.) and *N. javanica* Miq. were identified by comparing with herbarium database of Botanical Garden Organization (BGO) Plant Database, The Botanical Garden Organization, Chiang Mai, Thailand. *L. nodiflora* (L.) Michx. in the other name *P. nodiflora* (L.) Greene is a herbarium database of QBG No. 62171 and *N. javanica* Miq. is QBG No. 112212 (BGO Plant Databases, 2013b). Voucher specimen of *L. nodiflora* (L.) Michx. (WM/LP 1/2019) and *N. javanica* Miq. (WM/NJ 1/2019) were kept at the Division of Chemistry, Rajamangala University of Technology Krungthep, Bangkok, Thailand.

4. Preparation of plant materials

Plant material *L. nodifloa* (L.) Michx. (Fig. 1) was cleaned and dried under shade at room temperature and then separated into three parts of the plant as leaves, stems and roots. The stems and roots were made into small pieces approximately 2 cm each. Each part of plant was dried in a hot air oven at 50°C for 8 hrs. and ground by house blender.

Fig. 1 The morphological feature of *Lippia nodiflora* (L.) Michx

5. Extraction of plant

The fine leaves, stems and roots of *L. nodiflora* were soaked in ethanol at a ratio of dried plant per solvent (1:5 g/mL) at ambient temperature for 72 hrs. The ethanol extracts were filtrated with Whatman No.1 filter paper, the residue was repeatedly extracted for 4 times. The filtrates were combined and evaporated by using a vacuum rotary evaporator to afford sticky crude extracts of leaves ethanol (LE), stems ethanol (SE) and roots ethanol (RE) crude extracts. These crude extracts were reserved in a refrigerator at 4°C until further use.

6. Antioxidant activity of ethanol crude extracts

DPPH (2,2'-Diphenyl-1-picrylhydrazyl) radical scavenging assay: the DPPH radical scavenging activities of leaves ethanol crude extract (LE), stems ethanol crude extract (SE) and roots ethanol crude extract (RE) were tested according to Sudha & Srinivasan (2014) briefly 1 mL of each different concentration sample (62.5-1000

ppm) added to 3 mL of 0.1 mM of freshly prepared DPPH solution. The reaction mixtures were mixed and incubated in dark condition for 30 minutes at room temperature. The decrease of absorbance at 517 nm was determined by using a UV-Vis spectrophotometer (Jasco V-650 spectrophotometer). The IC_{50} (the concentration of specimen required to reduce the absorbance of DPPH by 50%) was calculated graphically. The percentage inhibition of the DPPH radical was calculated as following:

$$\text{DPPH radical scavenging activity (\%)} = 1 - \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

7. Allelopathic activity of ethanol crude extracts

The *Neptunia javanica* Miq. (Phak Kased Kok) seeds (Fig. 2) preparation for seed germination assay was prepared accordingly to the method of Yongvanich et al. (1997). In brief, the seeds were cleaned with distilled water and soaked in warm water (50-60°C) for 4 minutes, replaced the warm water with distilled water and kept at the room temperature for 24 hrs. The swollen seeds were selected for allelopathic assay.

Fig. 2 The botanical characteristic and seeds of *Neptunia javanica* Miq

This allelopathic activity of ethanolic extracts from leaves (LE), stems (SE), and roots (RE) were tested as described by Kalegari et al. (2012). The various concentrations of crude extracts (2.5, 5, 10 and 20 mg/mL) were dissolved in ethanol. The filter paper (Whatman No.1) was placed in the Petri dish (9 cm diameter, 2 cm height) and added 5 mL of crude extracts and fractions in each plate. After that, the solvent was let to evaporate within 24 hrs. at room temperature and each dish was added 5 mL of distilled water. The swollen *N. javanica* seeds were used for each experiment consisting of 10 germination seeds placed on filter paper in a Petri dish then kept in the dark place, triplicated for each treatment. Moreover, a control treatment was designed with distilled water and ethanol AR. The anti-germination activity could be calculated by using the following formula (Fonseca et al., 2017).

$$G\% = \frac{(M_a - M_c)}{M_C} \times 100$$

G%= Growth percentage, M_a = mean value of seeds, M_c = mean value of the control

$$R\% = \frac{(R_c - R_a)}{R_c} \times 100$$

R% = Radicle inhibition percentage, R_c= mean radicle length value of control, R_a= mean radicle length value of sample

$$H\% = \frac{(H_c - H_a)}{H_c} \times 100$$

H% = Hypocotyl inhibition percentage, H_c= mean hypocotyl length value of control, H_a = mean hypocotyl length value of sample

8. Liquid-liquid partition of leaves ethanol extract and bioactivity

In this study, we focused on leaves crude extract. Due to the effect of LE showed the completely inhibition of seed germination of *N. javanica*. The leaves crude extract for a liquid-liquid partition of ethanolic extract was performed according to Liau et al. (2017) method with different solvent modification. The dissolved dry crude extract 16.43 g in a mixture of ethanol:water (1:1) was added hexane (50 mL) 4 times to separate hexane extract and then added ethyl acetate (50 mL) 4 times to separate ethyl acetate extract. Each part of the solvent layer was removed by vacuum rotary evaporator to obtain hexane fraction extract (LH), ethyl acetate fraction extract (LEtOAc) and hydroethanolic fraction extract (LW), respectively. All fractions were tested antioxidant (62.5-1000 ppm) and allelochemicals (2.5, 5 and 10 mg/mL) potential germination on *N. javanica* seeds.

9. Total phenolic content of crude extracts and leaves fractions

The determination of total phenolic compounds in the samples was performed by using the Folin-Ciocalteu method according to the methodology described by Liau et al. (2017). Briefly, 100 µl (1 mg/mL) each of leaves, stems and roots crude extracts and leaves fractions or standard gallic acid solution was mixed with 4.5 mL of distilled water and 100 µl of Folin-Ciocalteu reagent. After 3 minutes, 300 µl of sodium carbonate solution

(2%w/v in water) was added and mixed. The mixture was kept for 2 hrs. in the dark at room temperature. The absorbance of each sample was measured by the UV-Vis spectrophotometer (Jasco V750) at 760 nm. Total phenolic content (TPC) was expressed as milligram gallic acid equivalents (GAE) per gram extract (mg GAE/g extract) and calculated by a standard curve of gallic acid solution (0, 15.625, 31.25, 62.5, 125, 250, 500, 1000 µg/mL). Each sample was analyzed 3 times.

10. Statistical analysis

The data were analyzed using One-way Analysis of Variance (ANOVA – SPSS Version 17) with Tukey's test and $p<0.05$ was utilized to evaluate significance between samples.

Results and Discussion

1. Extraction of plant

The fine leaves, stems and roots of *L. nodiflora* were soaked in ethanol and repeatedly extracted 4 times to obtain crude extracts as shown in Table 1. The dried powder of leaves (90.00 g), stems (100.00 g) and roots (18.40 g) were extracted by maceration (ratio of dried plant: ethanol; 1:5) for 3 days and repeated 4 times. After the ethanol extractions of three parts were filtered and evaporated under reduced pressure, the extract yields of leaf (LE), stem (SE) and root (RE) were 11.97, 12.22 and 7.02%w/w, sticky black-brown, respectively, as shown in Table 1.

Table 1 Percentage yield of leaves, stems and roots ethanolic crude extracts *L. nodiflora*

Extracts	Percentage yield (%w/w)	Color of crude extract
LE	11.97	Semisolid black green
SE	12.22	Semisolid black green
RE	7.02	Sticky black-brown

2. Antioxidant activity of ethanolic crude extracts

The antioxidant activity of leaves, stems and roots ethanol crude extracts (62.5-1000 ppm) were determined by DPPH radical scavenging activities. The data activity showed in Table 2, the vitamin C (3.125-25 ppm) was represented as a standard. The linear regression standard equation of vitamin C was y=3.8707x-3.9422 with $r^2 = 0.9963$. The IC_{50} of vitamin C was 13.94 ppm. The effect of ethanol solvent extracted from different parts of *L. nodiflora* showed significance ($p<0.05$) on DPPH radical scavenging activity. The IC_{50} value defined as the concentration of an antioxidant that caused a 50% decrease in the DPPH absorbance. The antioxidant activity of RE extracts was higher than those of LE and SE extracts as shown in Table 2. To compare with vitamin C, the antioxidant activity of RE extracts was lower than vitamin C to 9.82 times. The LE extracts had slightly higher antioxidant activity than SE extracts which was correlated to the report of Liau et al. (2017) who studied DPPH scavenging activities from methanol extracts of leaves and stems, and found that antioxidant activity of leaf extracts was higher, compared to stem extracts.

Table 2 DPPH radical scavenging activity in the percentage of ethanolic crude extracts *L. nodiflora*

Conc. (ppm)	DPPH radical scavenging activity (%)		
	Ethanolic crude extracts		
	LE	SE	RE
62.5	11.71±2.83^{e}	11.16±2.83^{e}	27.40±1.97^{d}
125	22.66±0.75^{d}	22.69±1.49^{d}	47.28±2.81^{c}
250	45.52±2.41^{c}	41.75±0.70^{c}	83.30±0.23^{b}
500	80.78±0.43^{b}	78.23±0.60^{b}	86.91±0.20^{ab}
1000	84.91±0.54^{a}	90.60±0.08^{a}	90.81±0.27^{a}
IC_{50} (ppm)	**296.87**	**310.41**	**136.87**

Remark: Values with different letters superscripts are in each column indicate values with significant variation at $P<0.05$ and obtained after round off to two decimal points

3. Allelopathic activity of ethanolic crude extracts

The allelopathic effect of LE, SE and RE extracts on germination of *N. javanica* seeds comparing to the water control for 7 days. The allelochemical activity of LE, SE and RE extracts tested on the growth of *N. javanica* seeds as shown in Table 3. The LE extracts at a concentration of 20 mg/mL showed no growth of seeds (Fig. 3c), while SE and RE extracts showed 80.00% (Fig. 3d) and 33.33% (Fig. 3e) of seed germination, compared to the water and ethanol as controls (Fig. 3a and 3b). The increasing concentrations of crude extracts could significantly more inhibit on seed germination ($p<0.05$). LE, SE and RE extracts also reduced the length of radicle and hypocotyl (Table 3). The radicle inhibition of LE, SE and RE extracts were 50-100, 28-61 and 76-90%, respectively, while hypocotyl inhibition of LE, SE and RE extracts were 13-100, 15-42 and 34-61%, respectively. The LE, SE and RE extracts had more effective on radicle than hypocotyl length growth.

Table 3 The effect of allelopathic activity of ethanolic crude extracts *L. nodiflora* on germination and growth of *N. javanica* seed in day 7

Sample concentration (mg/mL)	Percentage (%) of seed germination (G%)	Radicle length (mm)	Hypocotyl length (mm)	Percentage inhibition (%)*	
				Radicle inhibition (R%)	Hypocotyl inhibition (H%)
Water control	100.00±0.00^{a}	58.86±2.47^{a}	74.75±1.31^{a}	0.00±0.00^{f}	0.00±0.00^{h}
Ethanol control	100.00±0.00^{a}	57.36±2.07^{a}	72.88±1.64^{a}	2.54±3.52^{f}	2.50±2.05^{h}
Leaves extract					
2.5	100.00±0.00^{a}	27.38±2.00^{c}	64.71±1.53^{b}	53.48±3.40^{d}	13.43±2.05^{g}
5	80.00±0.00^{c}	23.80±1.45^{c}	56.45±1.60^{c}	59.56±2.47^{d}	24.48±2.14^{f}
10	73.33±5.77^{c}	22.83±1.80^{c}	50.22±2.92^{de}	61.21±3.06^{d}	32.81±3.91^{de}
20	0.00±0.00^{e}	0.00±0.00^{f}	0.00±0.00^{h}	100.00±0.00^{a}	100.00±0.00^{a}
Stems extract					
2.5	96.67±5.77^{a}	42.37±1.76^{b}	63.28±2.07^{b}	28.01±2.99^{e}	15.34±2.78^{g}
5	93.33±5.77^{ab}	40.10±2.22^{b}	55.81±1.28^{c}	31.87±3.78^{e}	25.34±1.72^{f}
10	83.33±5.77^{bc}	24.33±3.39^{c}	53.26±2.66^{cd}	58.66±5.76^{d}	28.75±3.56^{ef}
20	80.00±0.00^{c}	22.46±2.58^{c}	43.07±2.55^{f}	61.84±4.39^{d}	42.38±3.41^{c}
Roots extract					
2.5	100.00±0.00^{a}	14.09±1.33^{d}	48.74±1.14^{de}	76.06±2.26^{c}	34.79±1.53^{de}
5	100.00±0.00^{a}	11.97±1.65^{d}	47.78±2.27^{ef}	79.66±2.82^{c}	36.08±3.04^{cd}
10	100.00±0.00^{a}	10.93±1.42^{de}	46.93±1.43^{ef}	81.43±2.42^{bc}	37.22±1.91^{cd}
20	33.33±5.77^{d}	5.82±1.94^{ef}	28.52±0.96^{g}	90.11±3.30^{b}	61.85±1.29^{b}

Remark: *Comparison to water control. R%=radicle length percentage inhibition, H%=hypocotyl length percentage inhibition. Values represented mean ± S.D. and obtained after round off to two decimal points. Superscripts with different letters are in each column indicate values with significant variation at P<0.05

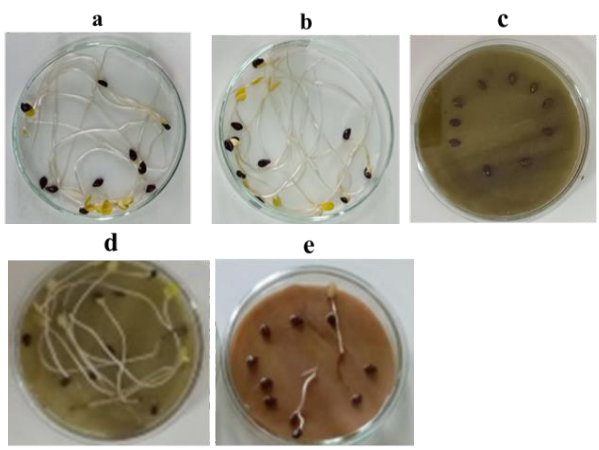

Fig. 3 Germination of *N. javanica* in control and crude extracts of *L. nodiflora* at 20 mg/mL, after 7 day. a=water control, b=ethanol control, c=Leaves ethanol extract (LE), d=Stems ethanol extract (SE), e=Roots ethanol extract (RE)

4. Liquid-liquid partition of leaves ethanol extract and bioactivities

In this study, the effect of LE showed completely inhibited seed germination of *N. javanica.* The further study, the crude extract of leaves (16.43 g) was separated by a liquid-liquid partition with hexane and ethyl acetate to obtain three fractions (hexane fraction; LH, ethyl acetate fraction; LEtOAc and hydroethanolic fraction; LW). The LW (69.22%w/w) had the highest percentage of yields, compared with LH (26.18%) and LEtOAc fractions (2.45%w/w). The physical property of hexane and ethyl acetate fractions were semisolid black green, while hydroethanolic fraction was sticky black-brown (Table 4).

Table 4 Percentage yield and physical property of leaves fractions *L. nodiflora*

Sample	Percentage yield (%w/w)	Physical property of fractions
Leaves fractions		
Hexane (LH)	26.18	Semisolid black green
Ethyl acetate (LEtOAc)	2.45	Semisolid black green
Hydroethanolic (LW)	69.22	Sticky black-brown

5. DPPH radical scavenging activity of leaves fractions

Three fractions (LH, LEtOAc and LW) tested antioxidant activity by DPPH scavenging assay and IC_{50} showed in Table 5. The results showed that the antioxidant activity of LW, LEtOAc and LH fractions (at concentration of 500 ppm) was 92.65, 83.13 and 40.75%, respectively. The effect of high polarity solvent was significantly increased antioxidant activity of extracts ($p<0.05$). The IC_{50} values of LW, LEtOAc and LH fractions were 158.21 ppm, 175.45 ppm and 722.08 ppm, respectively. The IC_{50} of LEtOAc and LW displayed lower value that means higher antioxidant activity than LH. The crude extracts and fractions of *L. nodiflora* had less antioxidant activity than vitamin C (IC_{50} = 13.94 ppm). The Fig. 4 showed IC_{50} DPPH scavenging activity of crude extracts (LE, SE, RE) and leaves fractions (LH, LEtOAc and LW) in comparison with vitamin C as a standard. The degrees of DPPH scavenging activity from highest to lowest were vitamin C>RE> LW>LEtOAc>LE>SE>LH. The LEtOAc (175.45 ppm) fractions showed antioxidant activity more than LE (296.87 ppm), which is similar to the report of Sudha & Srinivasan (2014) who studied DPPH scavenging activities of ethyl acetate fraction (26.06 µg/mL) and found higher antioxidant activity of LEtOAc than methanol extracts of aerial part (24.66 µg/mL) of *L. nodiflora.*

6. Allelopathic activity of leaves fractions

From the 100% of anti-germination of LE (20 mg/mL of concentration) as shown in Fig. 3, LE was fractionated to obtain hexane fraction (LH), ethyl acetate fraction (LEtOAc) and hydroethanolic fraction (LW). Three fractions of LH, LEtOAc and LW at various concentrations

Table 5 DPPH radical scavenging activity in percentage of leaves fractions *L. nodiflora*

Conc. (ppm)	DPPH radical scavenging activity (%)		
	Fraction extracts of leaves		
	LH	LEtOAc	LW
62.5	3.12±0.27^{e}	22.07±0.65^{d}	24.84±1.58^{d}
125	12.02±0.76^{d}	39.62±0.78^{c}	42.72±0.82^{c}
250	22.29±0.53^{c}	67.17±2.10^{b}	72.99±0.53^{b}
500	40.75±1.03^{b}	83.13±1.35^{a}	92.65±0.29^{a}
1000	65.01±0.26^{a}	-	-
IC_{50} (ppm)	**722.08**	**175.45**	**158.21**

Remark: Values with different letters superscripts are in each column indicate values with significant variation at P<0.05 and obtained after round off to two decimal points

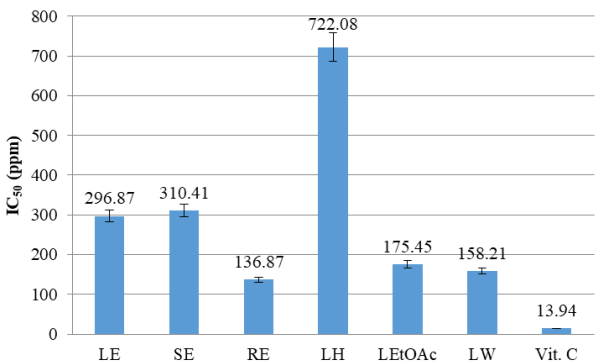

Fig. 4 IC_{50} values (ppm) of the DPPH radical scavenging activity of crude extracts, leaves fractions *L. nodiflora* and the vitamin C (standard)

of 2.5, 5.0 and 10 mg/mL were tested germination on *N. javanica* seeds for 7 days. The results showed the different level of inhibitory activity to growth of *N. javanica* radicle and hypocotyl (Fig. 5). The LH and LW fractions did not effect seed germination, while 10, 5 and 2.5 mg/mL of LEtOAc could significantly reduce seed germination to 0%, 33.33% and 60%, respectively (Fig. 6B), compared to the water control ($p<0.05$). The inhibiting effect of LH, LEtOAc and LW fraction concentration display in Table 6 on length of radicle and hypocotyl growth in percentage. The LH fraction presented 69-73% of radicle inhibition (R%) and 36-40% of hypocotyl inhibition (H%), R% and H% activities of LW fraction were 75-78 and 27-30%, respectively, in comparison to the water control (Table 6). All concentrations of leaf fractions showed inhibitory activity on the growth of radicle more than those of hypocotyl. The inhibition activity on seed germination, length of radicle and hypocotyl growth of LH, LEtOAc and LW fractions at 10 mg/mL concentration were compared to water control is shown in Table 6 and Fig. 5. From the assumption, the LEtOAc may have alkaloid, flavonoid, terpenoids and glycosides in leaves of *L. nodiflora* (Tamilselvi et al., 2018) and these compounds had allelopathic activity. From this research, LEtOAc significantly inhibited *N. javanica* weed. The LEtOAc showed completely inhibited seed germination at lower concentration (10 mg/mL showed Fig. 6B) than LE (20 mg/mL showed Fig. 6A). The LEtOAc displayed inhibitory activities on radicle length (1.63 times showed in Fig. 7A) and hypocotyl growth (3.05 times showed in Fig. 7B) than LE at 10 mg/mL. The active ingredient of leaves ethanol crude extract increased allelopathic activity after partition with ethyl acetate.

Table 6 The effect of allelochemical activity of hexane, ethyl acetate and hydroethanolic leaves fractions *L. nodiflora* on germination and growth of *N. javanica* seed in the day 7

Sample concentration (mg/mL)	Percentage (%) of seed germination	Radicle length (mm)	Hypocotyl length (mm)	Percentage (%) inhibition*	
				Radicle inhibition (R%)	Hypocotyl inhibition (H%)
Water control	100.00±0.00^{a}	58.86±2.47^{a}	74.75±1.31^{a}	0.00±0.00^{e}	0.00±0.00^{g}
Ethanol control	100.00±0.00^{a}	57.36±2.07^{a}	72.88±1.64^{a}	2.54±3.52^{e}	2.50±2.20^{g}
LH					
2.5	100.00±0.00^{a}	17.94±0.00^{b}	47.57±0.91^{cd}	69.52±0.07^{d}	36.37±1.23^{de}
5.0	100.00±0.00^{a}	16.55±2.11^{bc}	44.45±1.68^{de}	71.88±3.59^{cd}	40.54±2.25^{cd}
10.0	96.67±5.77^{a}	15.49±1.87^{bc}	44.20±1.50^{de}	73.68±3.19^{cd}	40.86±2.01^{cd}
LEtOAc					
2.5	60.00±0.00^{b}	7.22±2.21^{d}	39.10±2.84^{e}	87.73±3.7^{b}	47.69±3.79^{c}
5.0	33.33±5.77^{c}	1.39±0.46^{e}	14.29±2.46^{f}	97.64±0.79^{a}	80.88±3.29^{b}
10.0	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{g}	100.00±0.00^{a}	100.00±0.00^{a}
LW					
2.5	100.00±0.00^{a}	16.76±2.54^{bc}	54.30±2.80^{b}	71.58±4.32^{cd}	27.36±3.75^{f}
5.0	100.00±0.00^{a}	13.39±0.56^{bc}	52.55±2.38^{bc}	77.23±0.96^{c}	29.69±3.19^{ef}
10.0	100.00±0.00^{a}	12.80±2.08^{c}	51.96±1.47^{bc}	78.25±3.54^{c}	30.49±1.97^{ef}

Remark: *Comparison to water control. LH=Leaves hexane fraction, LEtOAc=Leaves ethyl acetate fraction, LW=Leaves hydroethanolic fraction, R%= radicle length percentage, H%= hypocotyl length percentage. Values represented mean ± S.D. and obtained after round off to two decimal points. Superscripts with different letters are in each column indicate values with significant variation at P<0.05

7. Total phenolic content of crude extracts and leaves fractions

The total phenolic contents (TPC) of extracts (leaves, stems, roots) and leaves fractions (hexane, ethyl acetate and hydroethanolic) from the *L. nodiflora* expressed as mg gallic acid equivalents per gram dry extract (mg GAE/g extract) and showed various significant ($p<0.05$) in different extracts and fractions, and ranged from 0.011±0.000 to 0.072 mg GAE/g. The regression equation of standard curve of gallic acid was $y = 1.0045x - 0.0015$ with $r^2 = 0.9971$. The results of TPC of extracts and fractions were showed in Table 7. In the present study,

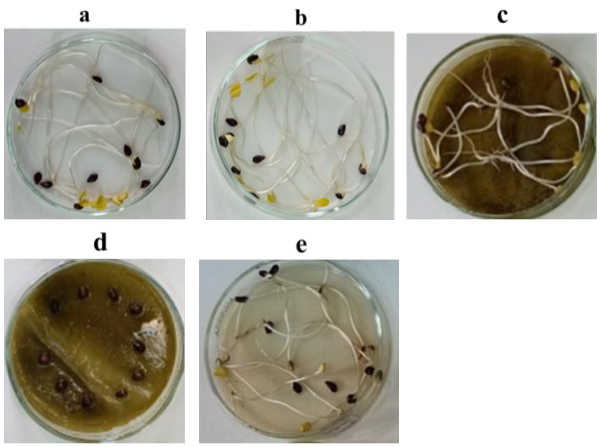

Fig. 5 Germination of *N. javanica* seeds in control and fractions of *L. nodiflora* at 10 mg/mL, a=water control, b=ethanol control, c=Leaves hexane fraction (LH), d=Leaves ethyl acetate fraction (LEtOAc), e=Leaves hydroethanolic fraction (LW)

the ethyl acetate fraction (LEtOAc) and hexane fraction (LH) had levels of TPC at 0.072 and 0.011 mg GAE/g, respectively (Table 7), which similar to the report of Liau et al. (2017) who studied TPC. The ethyl acetate fraction and hexane fraction had the total phenolic contents at 0.081 and 0.020 mg GAE/g, respectively.

Conclusion

In this study, *Lippia nodiflora* collected from Bang Bo in Samutprakarn Province showed biological activity on antioxidant, allelopathy and total phenolic content (TPC). The roots extract (RE) showed higher antioxidant and TPC than leaf (LE) and stem extracts (SE). The RE and SE had no effect on germination of *N. javanica* seeds, while LE showed lower antioxidant activity and TPC than those of RE. However, LE exhibited allelopathic activity on germination and seed growth. The leaves ethyl acetate fraction (LEtOAc) of

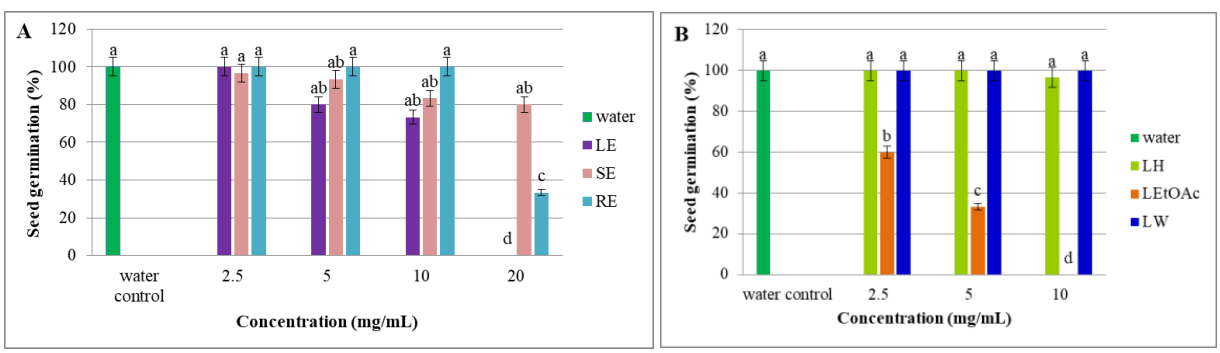

Fig. 6 Percentage of seeds germination on *N. javanica* of crude extracts and leaves fractions of *L. nodiflora.* (A) = Seed germination percentage of crude extracts, (B) Seed germination percentage of fraction extracts. Different letters indicate significant difference (Tukey's test, $p<0.05$)

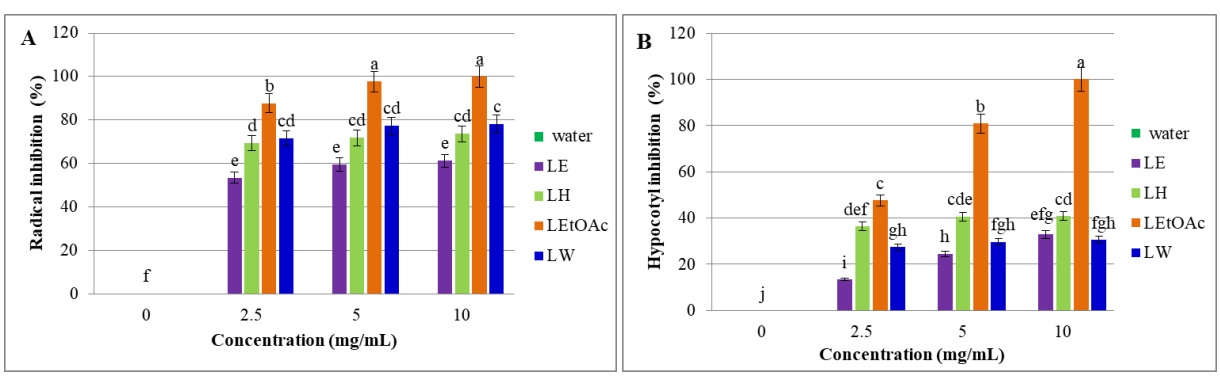

Fig. 7 Effect of four concentration of *L. nodiflora* leaves crude extract and fractions on germination of *N. javanica* seeds. (A) = Radicle growth inhibition percentage, (B) Hypocotyl growth inhibition percentage. Different letters indicate significant difference (Tukey's test, $p<0.05$)

Table 7 Total phenolic content of ethanolic extract from leaves, stems, roots and fractions from leaves consisting of hexane, ethyl acetate and hydroethanolic

Sample	TPC (mg GAE/g)
Leaves ethanol crude extract (LE)	0.032±0.001[d]
Leaves hexane fraction (LH)	0.011±0.000[f]
Leaves ethyl acetate fraction (LEtOAc)	0.072±0.001[a]
Leaves hydroethanolic fraction (LW)	0.046±0.001[c]
Stems ethanol crude extract (SE)	0.022±0.000[e]
Roots ethanol crude extract (RE)	0.053±0.000[b]

Remark: Values with different letters superscripts are in each column indicate values with significant variation at P<0.05 and obtained after round off to three decimal points

L. nodiflora had the highest total phenolic contents and antioxidant and allelopathic activities, compared with other fractions and crude extracts. The leaves crude extract (LE) at least 20 mg/mL and leaves ethyl acetate fraction (LEtOAc) at least 10 mg/mL will be recommended to reduce seed germination and inhibit radicle and hypocotyl growth of *Neptunia javanica* weed. Both LE and LEtOAc should be an optional application in control of *N. javanica* seed and other weeds. To further study, LEtOAc of *L. nodiflora* will be isolated and characterized allelochemicals to specify allelophatic activities on weeds.

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Development of Horse Mango (*Mangifera foetida*) Jam and Consumer Acceptance

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Abstract

The purposes of this research were to develop horse mango (*Mangifera foetida* Lour.) jam, to investigate the quality of the developed jam and consumer acceptance of horse mango jam. The methodology of research was conducted by studying three concentration levels (35%,45% and 55%) of ripe horse mango flesh suitable to be processed into horse mango jam and evaluating the jam properties: physical, chemical and microbiological quality and consumer acceptance of the jam. The experimental results indicated that ripe horse mango flesh could be suitably processed as jam, and that horse mango also had a unique smell. Furthermore, the quality of horse mango jam was investigated by measuring the quality of jam according to the Thai Community Product Standard (TCPS) criteria for coloring (L*a* b*) and spreadability used to deform the jam, it was found that 3 horse mango jams are concentrated in terms of color, odor and flavor, according to TCPS criteria: L*, a* decreased and b* increased when the concentration of horse mango jam increased. The texture of the jam decreased when the concentration of horse mango increased. Horse mango jam selection by sensory evaluation from 50 panelists found that horse mango jam 55% received the most favorable rating in terms of color, odor, flavor and overall liking and there was a significant difference with 35%, 45% jam ($p < 0.05$). When using 55% horse mango jam for chemical and microbiological quality, it was found that jam contains the amount of β-carotene at 1.04 g, moisture at 28.5 g, ash 0.56 g protein 0.45 g, carbohydrate and fat 0.73 g per 100 grams of horse mango jam. The amount of microbes detected was not up to the standard of TCPS. For the acceptance of 100 consumers, consumers accepted 97% of jam and decided to buy horse mango jam at 76%.

Introduction

Horse mango (*Mangifera foetida*) is in genus *Mangifera* that belongs to Anacardiaceae family, which is in the same genus as mango. It is an oval shaped and shell is green when ripe and also is orange-yellow with a sweet, sour taste, has a strong specific smell and a very rough texture (Wong & Ong,1993). Horse mango is a native plant in Indonesia, Malaysia, Myanmar, Singapore, Vietnam and Thailand. Horse mango is a native fruit of

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southern Thailand it can be found in many provinces of the southern region for instance, Phang Nga, Phuket, Trang, Krabi, Phatthalung Nakhon Si Thammarat, etc. (Kostermans & Bompard, 1993). It has names for other dialects such as mamud, som mut, malmut, muangmod, limus, bachang, machang etc., horse mango is cultivated widely and often mixed with other plants and will produce a lot during January to April.

Horse mango is a fruit that has high nutritional value, such as carbohydrates, dietary fiber, beta-carotene, vitamin C including other vitamins, including B vitamins and niacin (Lim, 2012), which have an antioxidant capacity 31.53 - 97.30% (Ikram & Khairul, 2009). The edible portion of horse mango represents 56% of fruit weight. For every 100 g edible portion of flesh, it contains 78.5 g water, 0.8 g protein, 17.9 g carbohydrates, 16 mg calcium, 19 mg phosphorus, 0.09 mg thiamine, 255 µg carotenes, and 47.4 mg vitamin C. There are the antioxidant capacity and antioxidant components in fresh, powder and fiber products prepared from horse mango (*Mangifera foetida*) fruit for example, reducing, flavonoid, carotenoid, and ascorbic acid contents were in the fresh, fiber and powder, whereas based on β-carotene bleaching method, order of antioxidant activity was fiber, powder, fresh in order. (Tyug et al., 2010).

Due to the unique characteristics of horse mango, when ripe has a strong smell and a coarse texture, the ripe horse mango is eaten or used relatively little, resulting in relatively low prices for fresh fruit. Farmers therefore prefer to use horse mango for cooking. However, there are still a lot of horse mangoes that cannot be sold. Mostly, horse mango is consumed as of soft fruit, and is popularly consumed as a savory dish like yellow curry. The researchers found that, there are only few products apply horse mango as an ingredient. There are studies have concentrated on the antioxidant properties of commercial mango (*Mangifera indica*), no research has been published on horse mango (*Mangifera foetida*). Thus, there is a need to explore the health promoting properties of this underutilized fruit. Horse mango can be produced into different food ingredients and products for example powder and fiber. Therefore, processing horse mango into a product adds value to the product.

Horse mango creates diversity for consumers and helps to conserve local plants in the southern region because if the products are not used in the future, farmers may turn to grow economic crops such as natural rubber, palm oil, which may cause local plants to disappear. Therefore, there is an idea to bring the ripe horse mango to develop into horse mango jam products. Because jam is one of the preservation methods that can be stored for a long time and has a simple method, while also helping to increase the value of raw materials from horse mango. In addition, there has never been a report about processing horse mango into jam products.

The best suitable technique to preserve perishable fruits is jam preparation which is an ancient way for preservation in several parts of the world. Jam is made of the pulp or the puree of a single or a mixed fruit by boiling fruits with sugar (sucrose), pectin, acid and other ingredients (preservative, coloring, limited amount of fruit peals and flavoring materials). Normally, jam should contain at least 40% of fruit component and the expected total soluble solid content should not less than 68%. Thus, jam should be in relatively stiff, solidity and set enough to carry the fruit tissues in position. Jams are full of sugar, energy, fiber, life-sustaining vitamins, minerals and amino acids. However, it does not contain any fat or cholesterol. In relation with the above reason, jam consumption is able to reduce the risk of cardiovascular disease (Bekele et al., 2020). In accordance with the announcement of the Ministry of Public Health in Thailand, jam is a product made from fruit ingredients. It can be whole fruit, piece of fruit, pulp or smoothie mixed with sugar, or mixed with fruit juice or concentrated fruit juice, after that make it suitable for consistency. It also includes vegetables suitable for jam, which is fresh, rotten, unhealthy or moldy by washing off dust, pesticides and other contaminated substances. The Ministry of Public Health has set the quality of the jam such as it must have the characteristic flavor of the jam, total soluble solid not less than 65%, pH between 2.8 to 3.5, no pathogenic microbes and there are no sweeteners other than sugar. It's consistent with the Codex Alimentarius specify that finished jam should contain more than 65% TSS (Codex Standard 79, 1981).

This research aims to increase the value of horse mango by developing into horse mango jam products along with studies in terms of physical quality and studies of consumer acceptance of horse mango jam products. Thai Community Product Standard is the quality specifications that are appropriate for community products to be trusted, accepted and guarantee for consumers of products by focusing on sustainable development for developing the quality of community products.

Materials and methods

1. Preparation of horse mango pulp

Horse mangoes were purchased from fresh markets and farm located at Nakhon Si Thammarat, Phatthalung and Trang provinces. Horse mango was used 8-10 cm. in size, with 20% yellow skin and above. Horse mangoes were washed, peeled and washed again because horse mango has quite a lot of rubber. The skins were carefully peeled with a kitchen knife and removed the seed from the flesh that yield of fruit is 60%. The yellow flesh is shown in Fig. 1 and 2. Then sliced horse mango into small pieces and blended with a blender, then filtered by a 60-mesh sieve to become pulp horse mango for making horse mango jam.

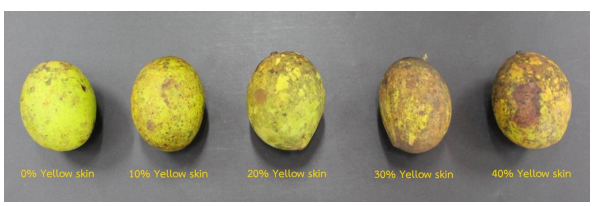

Fig. 1 The characteristics of horse mango

Fig. 2 The characteristics of horse mango's yellow skin with 20% yellowness

2. Preparation of horse mango jam

Horse mango jam products were developed by studying the suitable quantity of horse mango pulp at 3 different levels of concentration 35%, 45% and 55%, as shown in Table 1. Then, horse mango pulp mixture was heated at a temperature of about 80ºC, after that added sugar, pectin and stirred the mixture to be dissolved and added citric acid while measuring the amount of total soluble solid content should not less than 65% according to the standards was announced by the Ministry of Public Health, Thailand. Jam was filled in to sterilized container while was heat and was analyzed for physical, chemical and microbiological quality characteristic and sensory evaluation.

Table 1 Formula of horse mango pulp at different levels of concentration

Ingredients (%)	Horse mango pulp (% w/w)		
	35	**45**	**55**
Horse mango pulp	35.0	45.0	55.0
Water	21.0	11.0	1.0
Sugar	43.4	43.4	43.4
Pectin	0.2	0.2	0.2
Citric acid	0.4	0.4	0.4
Total	**100**	**100**	**100**

3. Quality of horse mango jam and selection of horse mango jam formula

3.1 Physical quality

3.1.1 Quality inspection in terms of appearance, color and flavor of all 3 levels of horse mango jam was conducted by 5 experts from the Culinary Technology and Service Program to inspect horse jam. From the analysis, sniffing and tasting according to the criteria of the Thai Community Product Standard No.342 in the topic of jam, which has set the criteria as follows: 3 points means good, 2 points means moderate and 1 point means abnormal.

3.1.2 Determination of color values of all 3 levels of horse mango jam, measure the color values with a color meter (Minolta colorimeter CR–410, Japan) and report the results as L* or brightness (0 = black, 100 = white), a* (+a = red, -a = green) and b* (+b = yellow, -b = blue)

3.1.3 Texture characteristics determination by applying all 3 horse mango jam levels to measure the spreadability with a texture analyzer (Stable Micro System TA.XT Plus). It uses a 45° conical probe Perspex (P/45C), pre-test speed 1 mm/s, distance 20 mm and trigger force 5 g to read the area under the graph as the spreadability of g.sec. (Sompongse et al., 2016)

3.2 Selection of horse mango jam formula

Horse mango jam formula was selected by sensory evaluation of all 3 levels of horse mango jam by liking the 9 point hedonic scaling test (9 = extreme like, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = extreme dislike) (Lawless & Heyman, 2010). The 50 untrained panelists was selected randomly from the students, the staffs and the lecturers of Suan Dusit University, Trang Center considered the different parameters like color, odor, flavor, texture, spreadability and overall acceptability, then analyzed for statistical results, then horse mango jam formula that received the most

acceptance scores and will be tested for microbiological quality and consumer acceptance testing.

3.3 Chemical quality

Horse mango jam with the most acceptable scores from sensory evaluation were investigated for chemical quality by measuring

3.3.1 Total soluble solid (TSS) by Hand Refractometer (ATAGO MASTER-M, China) (AOAC, 2000).

3.3.2 Beta-carotene (Britton et al., 2004)

2 g of finely ground horse mango samples were weighed. After that, saponification was done with ethanolic. KOH, soaked in ice and added hexanes to be used as extractors, rinse the Hexanes with the water for 2-3 more times to remove all the hexanes solution, then dried by rotary evaporator in the bath and analyzed by high-performance chromatography-diode array detection (HPLC-DAD). In the analysis, the solution must be prepared according to the standard concentration at 0, 0.5, 1.0, 2.0 3.0 and 4.0 g, then measured the absorbance of beta-carotene, calculate beta-carotene content from the area under the peak which is compared with the standard solution.

3.3.3 The content of the main compound in food is moisture, ash, protein, carbohydrate and fat (AOAC, 2016)

3.3.4 The pH value of horse mango jam was determined by using digital pH meter (SevenCompact, Mettler Toledo, Switzerland)

3.4 Microbiological quality

The analysis of microbiological quality in horse mango jam with the most accepted scores was conducted by total plate count, *Samonella* spp., *Staphylococcus aureus,* coliform by MPN, yeast and mold by FDA, BAM (Maturin & Peeler 2001) according to Thai Community Product Standard; TCPS (ICS 67.080.01)

4. Consumer acceptance test

The most highly-rated horse mango jam products from the sensory evaluation were tested for consumer acceptance using the 9 point hedonic scaling test (Lawless & Heyman, 2010). The test site is the Central Location Test (CLT) and 100 consumers (50 males and 50 females, 15-50 years old) at Huai Yot district, Trang province in terms of appearance, color, odor, flavor and overall acceptance.

5. Statistical Analysis

The physical quality and chemical analysis are done by planning a Complete Randomized Design (CRD). The selection of horse mango jam formula and consumer testing was done by planning a Randomized Complete Block Design (RCBD), then analyzing the variance and comparing the mean value with DMRT at 95% confidence level and interpret statistical results by using SPSS software.

Results and discussion

1. Physical quality

The results of the quality analysis of all 3 levels of horse mango jam showed that horse mango jam was yellow in color according to horse mango's characteristics. All 3 levels of jams were rated in terms of appearance, color and flavor (complies with the TCPS standard) and there was no significant difference $p > 0.05$ (Table 2).

Table 2 The acceptance's score of color, odor and flavor according TCPS standard

Characteristics	Horse mango puree (% w/w)		
	35	45	55
Appearance^{ns}	2.2 ± 0.45	2.2 ± 0.45	2.4 ± 0.55
Color^{ns}	2.6 ± 0.55	2.6 ± 0.55	2.6 ± 0.55
Flavor^{ns}	2.2 ± 0.45	2.2 ± 0.45	2.4 ± 0.54

Remark: ns means non significant differences ($p \geq 0.05$)

The results of color measurement of all 3 levels of horse mango jam showed that, when the concentration of horse mango jam increased; L* and b* values decreased, increased a* and were significantly different at $p < 0.05$. This is due to the characteristic of flesh horse mango says, when the ripeness level increases, the flesh becomes dark yellow until it becomes darker. Therefore, when the quantity of horse mango pulp increases, it results in less brightness (Table 3). In the past study, changing of color values as the results of thermal degradation during heat treatment, enzymatic browning, millard reaction and ascorbic acid degradation. (Ma et al., 2008)

The results showed that the texture characteristics of all 3 levels of horse mango jam by measuring the spreadability that indicates the force used to deform the jam, it was found that the spreadability tends to decrease as the concentration of horse mango increases. Horse mango at 45% and 55% concentration will have no significant difference on spreadability ($p > 0.05$) (Table 3). It therefore showed that when the amount of horse mango puree increases, the force used to deform the jam or decrease the force of the bread slice. Therefore, it

could be said that the viscosity of the jam decreases. This was due to an increase in the volume of horse mango puree while the sugar content remains the same, resulting in reduced gel set. Thus, the force used to deform the jam is reduced.

Table 3 Quality of coloring, texture of difference level for horse mango

Horse mango jam	Quality of coloring			Quality of texture
	L*	a*	b*	spreadability (g.sec)
Horse mango 35%	39.25 ± 0.18^{a}	4.46 ± 0.16^{b}	19.74 ± 0.38^{a}	557.62 ± 69.48^{a}
Horse mango 45%	36.47 ± 0.14^{b}	5.03 ± 0.19^{b}	15.01 ± 1.34^{b}	335.36 ± 18.14^{b}
Horse mango 55%	30.09 ± 0.22^{c}	5.96 ± 0.39^{a}	6.54 ± 0.21^{c}	337.15 ± 19.29^{b}

Remark: Each value is presented as mean ± standard deviation (n=3), different superscripts in the same column indicate significant differences ($p < 0.05$)

2. Selection of horse mango jam formula

The results of the selection of horse mango jam formula by testing the preference of all 3 levels of horse mango jam tester showed that, the testers rated the highest liking in terms of color, odor, flavor, spreadability and overall liking to horse mango jam at 55% concentration and were significantly different from horse mango jam at 35 and 45% but the texture did not different significantly from other concentrations ($p \geq 0.05$) (Table 4).

Table 4 Acceptance scoring of horse mango puree

Characteristics	Horse mango puree (% w/w)		
	35	45	55
Color	6.84 ± 0.99^{b}	6.64 ± 1.37^{b}	7.42 ± 1.26^{a}
Odor	6.38 ± 1.46^{b}	6.32 ± 1.15^{b}	7.24 ± 1.31^{a}
Flavor	6.40 ± 1.64^{b}	6.48 ± 1.50^{b}	7.80 ± 1.26^{a}
Texture^{ns}	6.88 ± 1.57	6.74 ± 1.41	6.94 ± 1.15
Spreadability	5.90 ± 1.54^{b}	7.38 ± 1.37^{a}	7.60 ± 1.05^{a}
Total acceptance	6.80 ± 0.93^{b}	6.88 ± 1.32^{b}	7.74 ± 1.03^{a}

Remark: Each value is presented as mean ± standard deviation (n=3), different superscripts in the same column indicate significant differences ($p < 0.05$), ns shown that there was no statistically significant difference ($p > 0.05$)

3. Chemical quality

The most favorite 55% horse mango jam products were analyzed for chemical quality by measuring total soluble solids, beta-carotene, moisture, ash, protein, carbohydrate and fat content. The pH value of horse mango jam found in 3.0 in according to Ministry of Public Health in Thailand and Codex. Moreover, there was the reported that pH value of the fruit is an important factor in jam processing because it is related with gel formation. The pH value of jam production should be between 3 and 3.5 (Bekele et al., 2020). However, the optimal pH for pectin gelatin is between pH 2.8 and 3.5. There were few other factors involved in reaching the perfect set and getting pectin to gel properly, but pH is absolutely key factor.

Table 5 Content of chemical quality of 55% horse mango jam

Chemical quality	Quantity (% w/w)
Beta-carotene	1.04
Moisture	28.5
Ash	0.56
Protein	0.45
Carbohydrate	70.4
Fat	0.73

4. Microbiological quality

Microbiological quality inspection for horse mango jam at a concentration of 55% in accordance with the Thai Community Product Standard (TCPS) 342/2018 found that horse mango jam has a standardized microbial value (Table 6). The production of horse mango jam was packed with the sterilized process, for the reason that the resulting in the amount of microbes is not exceeding the standard.

Table 6 Microbiological value

Microbiological	Microbiological value	
	TCPS standard 342/2561	Mamud jam 55%
Total plate count	Less than $1x10^{4}$ cfu/g	Less than 10 cfu/g
Salmonella spp.	Not detected	Not detected
Staphylococcus aureus	Less than 10 cfu/g	Less than 3 cfu/g
Coliform	Less than 3 MPN/g	Less than 3 MPN/g
Yeast and mold	Less than $1x10^{2}$ cfu/g	Less than 10 cfu/g

5. Consumer acceptance test

The consumer test results for 55% horse mango jam products selected for 100 consumers were as follows:

5.1 General information of consumers

General information of 100 consumers who tested acceptance of horse mango jam products, there were respondents consisting of equal numbers of males and females (50% of each gender), between the ages of 20-30 years (33%), most of them had education levels below bachelor degree (46%), most of them are students (43%) and an average monthly income of less than or equal to 10,000 baht (53%).

5.2 Jam consumption behavior

Consumer behavior data revealed that, most consumers choose to buy jam products by using flavor criteria (87%), frequency of eating jam 1-2 times a week (48%), the places to buy jam products are department stores (53%).

5.3 Consumer acceptance of horse mango jam products

Consumer test results on horse mango jam products by allowing the testers to taste horse mango jams and give scores on color odor, taste, texture, spreadability and overall liking on bread and overall liking. The results of their liking for the various features of horse mango jam products are as follows: the preference for color was at the level of 4.26, which was high; the odor was at the level of 3.87, which was moderate; the taste was 3.89, which was moderate; the texture is at a rating of 3.99, which was high; the spreadability on the bread was at the level of 4.03, which was high and overall liking was at the 4.00, which was high. Consumers acceptance 97% of horse mango jam products and decided to buy horse mango jam products, 76%. From the consumer acceptance test scores, horse mango can be used to make horse mango jam products. Although horse mango had a strong smell, when used as jam, the smell of horse mango had no effect on consumer acceptance.

Conclusion

The development of jam from horse mango fruits indicated that ripe horse mango flesh could be suitably processed as jam and applied on bread, Horse mango also had a unique smell. Furthermore, the quality of horse mango jam was investigated by measuring the quality of jam according to the Thai Community Product Standard (TCPS) criteria for coloring measurement (L* a* b*) and spreadability measurement used to deform the jam, it was found that all 3 horse mango jams are concentrated in terms of color, smell and flavor, according to TCPS criteria: L*, a* decreased and b* increased when the concentration of horse mango jam increased. The texture of the jam decreased when the concentration of horse mango increased. Horse mango jam selection by sensory evaluation from 50 panelists found that horse mango jam 55% received the most favorite rating in terms of color, odor, flavor and overall liking and there was a significant difference with 35% and 45% horse mango jam ($p < 0.05$). When using 55% horse mango jam for chemical and microbiological quality, it was found that Jam contains beta carotene at 1.04 g and moisture at 28.5g per 100 grams of horse mango jam. The amount of microbes detected was not up to the standard criteria of TCPS. For the acceptance of 100 consumers per horse mango jam product, consumers accepted 97% of horse mango jam products and decided to buy horse mango jam products at 76%.

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Reliability of the Modified O'Sullivan Functional Balance Test in Person with Spinal Cord Injury

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Abstract

The modified O'Sullivan functional balance test is a short and easy scale that is commonly used in clinical practice, but this test lacks of standardized instructions that may affect its reliability. This study aimed to determine the reliability of the modified O'Sullivan functional balance (mOFB) test in persons with spinal cord injury. Various test instructions were given and VDO recorded in twelve chronic spinal cord injuries (lesion level C5-L5). Inter-rater and intra-rater reliability were determined by 5 physical therapists who have clinical experience ranging from 1 to 10 years. All raters scored the patient's performance from from observing the video twice, 7 days apart. Inter-rater and intra-rater were calculated by interclass correlation coefficient (ICC). The mOFB test showed excellent intrarater reliability (ICCs range from 0.93 (0.86-0.98) to 0.96 (0.92-0.99)), whereas interrater reliability ranged from poor to moderate (ICC range from 0.38 (0.12-0.69) to 0.53 (0.26-0.80). The different test instructions including posture alignment adjustment before testing, amount of resistance, and amount and direction of reaching led to decreased intra-rater reliability to poor and moderate. This study confirmed that a lack of a clear testing instructions and grading criteria decreased the reliability of the modified O'Sullivan functional balance test.

Introduction

For patients with spinal cord injury, wheelchair is the most important and most frequently used as their primary means of mobility (Post et al., 1997; Bergstrom & Samuelsson, 2006). Balance control in upright sitting on wheelchair is a necessary component in engaging the use of upper limbs in functional activities such as feeding, dressing, transferring (Gao et al., 2015), as well as preventing falls during wheelchair navigation over obstacles and up or down inclines (Sisto et al., 2009). Balance training in sitting, therefore, is very important to maximize opportunities for independent mobility, functional activities and preventing falls in patients with spinal cord injury. The successful of this training requires balance measurement tool that is reliable, valid and feasible to use in a clinical setting. Two main approaches of balance assessment consist of laboratory and functional assessments. The laboratory instrument such as force plate transducers and accelerometer

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provides objective and quantitative measurement without tester's bias but it is expensive and requires extensive training and testing. Clinical balance scale was developed to be used in clinical setting that does not require expensive equipment and is easy to use. The clinical balance scale assesses balance ability and its changes over time though ordinal scale that assesses a set of functional task which requires balance control during maintaining sitting posture or during doing activity (Horak, 1997).

A survey was conducted to gather information regarding types of balance measurement used amongst physical therapists working in the neurological area in Thailand. 130 respondents (86.09%) replied that they always or almost always measured balance by using clinical balance test (Chinsongkram et al., 2018). Among those, 16.56% used the standardized balance measures such as Berg Balance Scale (BBS), Time Up and Go (TUG) and Balance Evaluation Systems Test (BESTest), whereas 69.53% used the test that resembles O'Sullivan functional balance scale. The O'Sullivan functional balance (OFB) scale can be used to define both static and dynamic control in sitting and standing in elderly and patients with neurological disorders (O'Sullivan & Schmitz, 2007). The popularity of the OFB scale in Thailand may be due to it being short and easy to use; this test can be completed within 5 minutes (O'Sullivan & Schmitz, 2007). The OFB scale has been modified further by adding external resistance for disturbing static balance and weight shifting for disturbing dynamic balance and adjusted the grading criteria in accordance with the modified test. However, the modified O'Sullivan functional balance (mOFB) scale does not provide cleartest instructions, i.e., no clear test instruction of starting position, test command, amount of resistance and distance of weight shifting. When using this test without test instructions, a variety of testing procedures such as test commands or starting position may affect the reliability of this test. From literature review, psychometric properties of the mOFB scale had never been investigated in the population with spinal cord injury before. Therefore, it is necessary to examine the reliability that is the first step of psychometric properties study in the use of the mOFB scale in patients with spinal cord injury. The aim of this study was to determine the reliability in persons with spinal cord injury and determine the reliability of this test when use with different test instructions.

Participants and methods

Twelve participants with spinal cord injury were recruited from Pathum Thani of Thailand during October, 2017 to March, 2018. The sample size calculation by G*power version 3.1.9.2 was based on a power of 0.80 and alpha level of 0.05. An expect intraclass correlation coefficient of this study was 0.90. An intraclass correlation coefficient for null hypothesis was at 0.5 which represents poor reliability. The minimum sample size required was 12 therefore that is in line with the recommendation from guideline to determination of sample size requirements for estimating the value of intraclass correlation coefficient (Bujang & Baharum, 2017). Individuals were included in this study following criteria: diagnosis of spinal cord injury at 4th cervical spinal cord or below with stable medical conditions, age between 18-70 years, independent sitting and able to follow instructions to complete the assessment. Individuals were excluded from the study if they presented with other problem that is sufficient to disturb balance such as respiratory problem, bed ridden, postural hypotension, fracture and stroke. Interrater and intrarater reliability were determined by 5 physical therapists. Raters consisted of 1 lecturer in neurological physical therapy and 1 physical therapist with more than 5 years of neurological rehabilitation experience and 3 physical therapists with less than 5 years of neurological rehabilitation experience.

Outcome measurement in this study was the modified O'Sullivan functional balance (mOFB) scale. It consists of functional balance items in sitting and standing that focus on the ability to maintain a position and postural adjustments to voluntary movements such as head/trunk turning, picking up object off floor and weight shifting. This test has 4 items including; static balance in sitting, dynamic balance in sitting, static balance in standing and dynamic balance in standing. The static balance items are sequenced according to the level of difficulty from sitting or standing supported to unsupported and against external resistances in all directions. Likewise, the dynamic balance items progress from sitting or standing unsupported to minimal weight shifting and full range weight shifting in all directions. Due to lack of standardized test instructions, a starting position varies based on patients' performance such as ring sitting or long sitting in patients with quadriplegia and high sitting in patients with paraplegia. Testing time in each item ranges from 30 seconds to 120 seconds.

Amount of resistance in static balance test ranges from minimum to trigger isometric contraction of trunk muscle, to maximum of triggering trunk movement. The direction of apply external resistances or weight shifting was varied including anterior, posterior, left side, right side and add up and down direction for weight shifting test. (Chinsongkram et al., 2018) The distance of weight shifting in dynamic balance test ranges from reaching within arm range to reaching over arm range. Influence of the above-mentioned testing procedures in the mOFB scale (i.e., starting position, testing time, amount of resistance, direction of resistance and direction of weight shift) on the testing reliability were examined further in this study. Grading criteria of the mOFB test is scored on a 5-level ordinal scale from zero (unable to maintain balance) to normal (normal balance performance) as shown in Table 1. This test requires minimal testing equipment and short time (less than 5 minutes) for administering.

Table 1 Grading definition of the modified O'Sullivan functional balance test

Grade	Descriptors
	Static balance
Normal	Patient able to maintain steady balance without handhold support and maintain steady balance against the external resistance for disturb balance in all directions.
Good	Patient able to maintain steady balance without handhold support and maintain steady balance against the external resistance for disturb balance in some directions.
Fair	Patient able to maintain steady balance without handhold support but cannot maintain steady balance against the external resistance for disturb balance.
Poor	Patient requires handhold support and moderate to maximal assistance to maintain position.
Zero	Patient unable to maintain balance.
	Dynamic balance
Normal	Patient accepts maximal challenge and can shift weight easily within full range in all directions.
Good	Patient accepts moderate challenge and can shift weight within range in some directions.
Fair	Patient accepts minimal challenge; cannot maintain steady balance when weight shifting
Poor	Patient unable to accept challenge or move without loss of balance.
Zero	Patient unable to maintain balance.

This study was approved by the Human Research Protection Committee at Rangsit University, Thailand (number RSEC 33/2560). After signing the consent forms, participants completed demographic and clinical information including; age, weight, height, and level of spinal cord injury lesion, time since spinal cord injury, cognitive impairment and postural hypotension screening. The evaluation with the mOFB test was performed in a same setting and videotape recording was performed in the same view in all participants. Each participant performed the test in ring and high sitting with various testing instructions and testing procedures to examine the effect of varied instruction and procedure on the test reliability. The test instruction conditions including starting position in sitting, adjust postural alignment before test, testing time in static balance test, amount of resistance in static balance test, distance of weight shifting in dynamic balance test, and directions of apply external resistance or weight shifting. All participants received the same verbal instruction and were allowed to rest as needed during the test.

Video clip of each test condition were edited and randomized for patient's sequence in each test instruction and procedure before sent out to raters to prevent rater from remembering scores from previous test condition in the same patient. All raters scored the patient's balance grade from each video clip on 2 separate occasions. The second occasion was performed 7 days after the first occasion (Portney & Watkins, 2007; Shultz et al., 2013; Schlager et al., 2018). Intrarater reliability was assessed by comparing the score of occasion 1 and score of occasion 2 in each rater. Interrater reliability was determined by comparing the score from occasion 1 between 5 raters. Each rater scored video clip separately on the separate scoring sheets for each occasion and did not discuss scoring among participants and occasions.

Descriptive statistical analysis of demographic and baseline clinical characteristics of participants were conducted. For score distribution, the floor and ceiling effects were calculated as the percentage of sample scoring the minimum or maximum possible grade, respectively. Ceiling and floor effects of 20% or greater are considered significant. Interrater and intrarater were calculated by interclass correlation coefficient (ICC). ICC model 2, k was used for interrater reliability and model 3, k was used for intrarater reliability. ICC value of 0.80 and above indicates excellent reliability, 0.5-0.79 indicates moderate reliability and less than 0.5 indicates poor reliability (Portney & Watkins, 2007).

Results and discussion

Twelve chronic patients with spinal cord injury; 9 females and 3 males with the average age of 36.08 ±

7.46 years, participated in this study. The average time since onset spinal cord injury was 11.83 ± 9.40 years and level of spinal cord injury was ranged between C5 to L5 with above T1 level in 3 persons and below T1 level in 9 persons. The average of body mass index was 20.22 ± 1.51. Maximum functional independent in all participants were independent wheelchair activity.

The distribution of participants' score of the modified O'Sullivan functional balance scale in all testing conditions of static and dynamic balance test is displayed in Fig. 1. It can be seen that the distribution of scores from static sitting balance test covered the whole grade. The static sitting balance ability of most patients is in the grade fair (40.2%), good (36.5%) and poor (13%), respectively. In dynamic sitting balance, the balance ability of most patients is in the grade poor (40.4%), fair (31.4%) and good (15.7%), respectively. The analysis of the floor effect and ceiling effect demonstrated that there were 0-0.1% of participants receiving the lowest possible score (grade zero) and 2.2-2.8% of participants receiving the highest possible score (grade normal), suggesting there were no floor and ceiling effect of the modified O'Sullivan functional balance test in patients with chronic spinal cord injury.

The intrarater and interrater reliability of the the mOFB scale from 5 raters scoring, in appropriate test instructions are presented in Table 2. The appropriate test instructions including adjust postural alignment before test, testing time in static balance test is 60 seconds, minimum amount of resistance to trigger isometric contraction of trunk muscle, distance of weight shifting is reaching over arm range, and apply external resistance or weight shifting in all directions. The interrater reliability of the static sitting balance test was poor both in ring sitting and high sitting position. The ICCs of interrater reliability test in dynamic sitting balance was indicating moderate reliability in both ring sitting and high sitting position. In contrast, the intrarater reliability of the static sitting balance test and dynamic sitting balance test were excellent. Comparable reliability between ring sitting and high sitting condition suggested that sitting position had no effect on the testing reliability.

Table 2 Intraclass correlation coefficient and 95% confident interval of interrater reliability and intrarater reliability of the modified O'Sullivan functional balance test during proper conditions in patients with spinal cord injury

Compare	Interrater reliability ICC (95%CI)		Intrarater reliability ICC (95%CI)	
	Ring sitting	High sitting	Ring sitting	High sitting
Static balance	0.38 (0.12-0.69)*	0.40 (0.14-0.71)*	0.94 (0.88-0.98)*	0.93 (0.86-0.98)*
Dynamic balance	0.52 (0.19-0.80)*	0.53 (0.26-0.80)*	0.96 (0.91-0.99)*	0.94 (0.88-0.99)*

Remark: * Significant level of ICC at $p < 0.001$

The intrarater reliability of the mOFB scale in each rater, when scoring in different test instructions is shown in Table 3. Intrarater reliability of all raters decreased from excellent during the appropriate test instruction (Table 2) to poor and moderate when using varied instruction and procedures (Table 3). All test instruction factors led to deteriorating effect on intrarater reliability except the testing time factor.

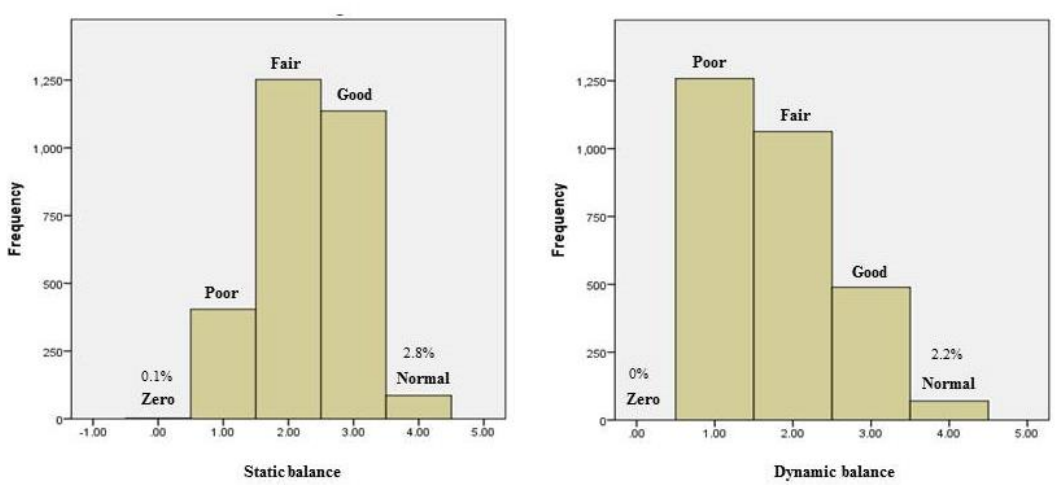

Fig. 1 Score distribution of the modified O'Sullivan functional balance scale in chronic patients with spinal cord injury during static balance test (left panel) and dynamic balance test (right panel)

Table 3 Intra-rater reliability of the modified O'Sullivan functional balance test in different testing instruction and procedures

Compare	Intrarater reliability; ICC (95%CI)			
	Posture alignment adjustment	Testing time	Amount of resistance and distance of weight shifting	Direction of resistance and weight shifting
Static balance	0.53 (0.45-0.61)*	0.95 (0.94-0.97)*	0.48 (0.44-0.56)*	0.55 (0.51-0.60)*
Dynamic balance	0.61 (0.53-0.68)*	0.95 (0.95-0.97)*	0.64 (0.60-0.69)*	0.63 (0.59-0.68)*

Remark: * Significant level of ICC at $p < 0.001$

This is the first study to determine whether the modified O'Sullivan functional balance (mOFB) scale is reliable for assessing balance impairments in patients with spinal cord injury. Although this study demonstrated that the mOFB scale lack of floor and ceiling effects, this scale may not be suitable for assessing balance in patients with chronic spinal cord injury with lesion between C5 to L5 due to its low rater reliability under certain testing conditions.

When using with clear and appropriate testing instruction and testing procedures, the mOFB scale showed excellent intrarater reliability but poor interrater reliability. The possible explanation of poor interrater reliability may be from diverse understanding of grading criteria. Interrater reliability in dynamic balance test tended to be higher than static balance due to the fact that dynamic balance test is more difficult and require higher balance control than static balance. Therefore, the sway that indicates balance impairment may be clearly noticeable, thus, most raters could reach agreement in scoring. From our results, it can be seen that the mOFB scale may not be preferable when being used by several assessors such as re-evaluation or case referral.

On the other hand, the intrarater reliability decreased significantly when the instruction and procedures were not controlled and varied. Several factors listed in this study (adjustment of posture, amount and direction of external resistance, distance of weight shifting) influenced the intrarater reliability of the mOFB scale, but not the testing duration which ranged from 30 seconds to 120 seconds, suggesting that varying of instructions and procedures could possibly affect the amount of observed sway. Therefore, the scores were different when graded by the same rater using the same grading definition. These findings emphasized the importance of having test instruction and procedures for testers for administering the mOFB scale with the same method. Our results are in line with the previous survey study that showed no consensus of test instruction and grading definition of the mOFB test in Thailand (Chinsongkram et al., 2018). Another study indicated that clear and consensus grades definitions and test instruction of the scale would improve reliability of this test (Iansek & Morris, 2013). An appropriate testing instructions and procedures that we used in this study could be used for developing clear and consensus test instruction of the mOFB scale including; (1) the assessor needs to adjust the postural alignment before starting the test to ensure that the patient sits up straight, (2) the proper testing time for static balance test is 60 seconds, (3) the amount of external resistance given to the patient should be minimum just enough to trigger isometric contraction of trunk muscle, (4) the distance of weight shifting needs to be farther by reaching over arm range, and (5) assessor should apply external resistance or instruct weight shifting in all directions. However, the reliability and validity of adjusted test must be studied for confirmation.

Conclusion

The mOFB scale is reliable for measuring balance in persons with chronic spinal cord injury when being used by same rater with an appropriate testing instructions and procedures. Both interrater and intrarater reliability of this scale reduce to moderate and poor when measuring without a clear testing instruction and grading criteria. The future studies are needed to verify if the criteria suggested in this study could improve rater reliability of the mOFB scale.

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Promoting Physical Activity among Buddhist Monks: The Role of Nurse

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Abstract

Buddhist monks suffer from chronic illnesses that could be prevented or delayed if they engaged in proper physical activity. Because a monk's lifestyle differs from a normal Buddhist, physical activity maybe neglected. Physical activity is usage of energy by using muscles in various parts of the body. If the body does not completely metabolize excess fat, it will result in accumulation of fat. Nurses have an important role in promoting physical activity among Buddhist monks, which will result in better health for monks, prevention of complications of diseases and reduce risk of noncommunicable diseases (NCDs) such as diabetes, hypertension, chronic kidney disease, hyperlipidemia, obesity, heart disease, cancer and osteoporosis, which is usually caused by monks' lack of physical activity. Buddhist monks have religious disciplines and duties that make their life style differ from normal citizens. This article shows the role of nurses in promoting physical activity among Buddhist monks that doesn't go against monks' discipline; consisting of evaluation of physical activity using Global physical activity questionnaire, International physical activity questionnaire, calculation of energy usage (Metabolic Equivalent: MET), different ways for monks to exercise such as fast walking, Taichi-Qigong, Yoga and concentration activity, including the spread of awareness to monks and nurses to support and design physical activity program that is adequate and suitable for monks. If nurses become more active and emphasize this topic more, there will be more projects and programs that promotes physical activities among monks to improve monks' physical well-being.

Introduction

Monks are missionaries of Buddhism. They practice Buddhism strictly and their roles are to spread the religion, improve people's virtue and morality and be a good role model for Buddhists,. The way that monks take care of themselves and the environment in temples can affect community health. The way monks live is different than normal Buddhists; they have to obey the 227 precepts of Buddhism, be composed physically, verbally and mentally. You'll find that 95 percent of Thais practice Buddhism, and use the principles in their daily life to the point that Buddhism is the foundation of Thai's tradition and culture (Health Assembly, 2012).

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From an academic survey regarding monks' health, most suffer from chronic diseases, most common ones such as diabetes, hypertension, ischemic heart diseases, dyslipidemia, obesity, chronic obstructive pulmonary disease and arthithis. There are many factors that contribute to these illnesses such as genetic, behaviour, belief, medical care and environment. One important causes is the food given to monks by people who lack the knowledge, understanding and acknowledgement of the negative consequences of some of the food, for example, food with high fat or salt content (Angkatavanich et al., 2014). Moreover, monks have little to medium physical activity, most activities are ones that they perform on a daily basis such as walking or cleaning, older monks have less physical activity, most are idle like sleeping after breakfast and lunch (Tongterm & Kaewma, 2019). These reason causes monks to suffer from non-infective chronic diseases. A health query of monks throughout Thailand, totaling 122,680 monks, in 2016 showed that the most common disease are diabetes and hypertension, and a health query of 6,375 monks in Bangkok in 2016 showed a reduction of healthy monks from 60.3% to 28.5%. The 5 most common diseases that asked for treatment were as follow; hyperlipidemia, hypertension, diabetes, chronic kidney disease and osteoarthritis where the risk behaviors are smoking, food and lack of exercise. (Priest hospital, 2017) Osteoarthritis is more common in older and overweight monks. (Tangtrakulwanich et al., 2006) Also, a number of monks that suffer from diabetes still have inappropriate health behavior and use inappropriate drugs. (Yensabai et al., 2016) Even though Dharma practice and meditation can reduce blood pressure and stress for monks (Moceri & Cox, 2019), there should be more promotion for physical activity for monks. This article will emphasize physical activity that will help reduce risk factor of diseases among monks, answering Thailand's statement in the 71st World Health Assembly, where many countries support the promotion of physical activity. (World Health Organization, 2018) As for Thailand, we have programs promoting physical activity but are unable to reach some of the population, especially monks.

Monks have limitation in their movement, because as a missionary, they have to be composed physically due to the precepts of Buddhism. Some of the activities that monks perform on a daily basis are asking for alms and cleaning temples which are appropriate according to Buddhist precept, but are inadequate to prevent and recover from chronic non-infective diseases. Thus, the role of health care providers plays an important part in promoting physical activity that will improve monks' lives while being able to carry out the duty of missionary, reducing risk factors of chronic non-infectious diseases.

The importance of physical activities

Physical activity helps strengthen the circulatory system and respiratory system. For monks, it plays a very important role in keeping their circulation, heart and gas exchange healthy to prevent diseases that may be caused by lack of exercise. If monks care more about their health, it will keep their blood vessels, heart and metabolism healthy.

Definition and evaluation of physical activity

Physical activity means movements of body in daily life that induces metabolism including working, travelling and recreational activities. We can rank the levels of physical activity by using the combination of risk of disease and WHO classification that uses Metabolic Equivalent of Task (MET). MET is the objective measure of the ratio of the rate at which a person expends energy, relative to the mass of that person, while performing some specific physical activity compared to a reference where 1 MET = 1 kcal/kg/hr which is roughly equal to the rate of energy produce by a person seated at rest. Therefore, the level of physical activity in monks can be divided into 3 levels (Department of Health, 2018), as follows

Low physical activities mean movements that use little energy, movements that occur normally in daily life, using less energy than 600 MET- minutes/week such as low distance walking, carrying light items, meditation, praying etc.

Moderate physical activities mean activities that cause moderately tiredness, but still able to talk while performing the activity, continuously for more than 10 minutes each time, using energy between 600 MET-minutes/week and 1,500 MET-minutes/week such as fast walking, sweeping, washing clothes etc. Moderate physical activity uses 4 times more energy than sitting still.

Severe physical activities mean activities that cause severe tiredness, make the participant unable to hold a conversation while performing the activity, induce faster breath and heartbeat, continues for more than 10 minutes each time, using more energy than 1,500 MET -minutes/week such as sprinting, heavy lifting,

construction work etc. Severe physical activity uses 8 times more energy than sitting still.

The comparison between the MET value during activity and resting metabolic rate will results in a value called METs, multiply that by the amount of minute of the activity duration to get the MET-minutes value. Calculate MET-min/week of severe, moderate and walking activities using a reference standard values (Ainsworth et al., 2011) results in 1) An average MET value for walking = 3.3 METs 2) moderate-intensity activities = 4.0 METs and 3) vigorous-intensity activities = 8.0 METs. Calculating the values for each activity (per week) results in 1) Walking MET-minutes/week = 3.3*walking minutes*walking days 2) Moderate MET-minutes/week = 4.0*moderate-intensity activity minutes*moderate days and 3) Vigorous MET-minutes/week = 8.0*vigorous-intensity activity minutes *vigorous-intensity days. Total Physical Activity MET-minutes/week = sum of Walking + Moderate+ Vigorous MET minutes/week scores.

Thai Health Promotion Foundation promoted "Klai Rok Thai Buddhist Monk Project" program to promote healthiness among monks and show that monks can exercise within the rules of the 10 routines of monks' activities, because lack of physical activity is a significant factor that contributes to manifestation of chronic diseases and 5.1 percent of Thais death, and illustrate poster showing energy usage of various monk activity per 40 minutes in calories unit, shown in Table 1.

Table 1 Energy usage in 40 minutes (Klai Rok Thai Buddhist Monk Project, 2017)

Monks activities	Monks younger than 50 years old	Monks older than 50 years old
Alms round	210	190
Walking around	100	80
Sweeping	140	120
Arm swings	220	200
Clothes washing	110	95
Temple works	240	210

Monks are present in temple and other religious sites all over Thailand. Study on monks suffering from non-communicable diseases show that they have moderate physical activities (Laochai & Preechawong, 2020), so health care staffs should promote physical activities among monks and be able to evaluate the level of physical activity of each monk to suggest the appropriate physical activity to prevent diseases. Common surveys for evaluation used globally are as follow:

Global physical activity questionnaire (GAPQ) from WHO (2002) is a survey for physical activity and sedentary behavior. It has a total of 16 questions divided into 4 parts including physical activity during work, physical activity during travelling, physical activity during recreational activity and sedentary behavior. Each question is a Yes/No question that also ask for the duration of activity. Gathering data on level of activity and duration allows the calculation of the MET-min/week value mentioned above.

International Physical Activity Questionnaire (IPAQ) - Short and Long Forms Contents (IPAQ Research Committee, 2005) is a commonly used survey that has been translated to many languages. The short form has only 4 questions while the long form has 27 questions composed of 5 parts including; part (1) job-related physical activity which has 7 questions, part (2) transportation physical activity which has 6 questions, part (3) housework, house maintenance, and caring for family which has 6 questions, part (4) recreation, sport, and leisure-time physical activity which has 6 questions, and part (5) time spent sitting which has 2 questions. This survey gathers data to evaluate the level of physical activity of the past 7 days.

The surveys mentioned above will let the health care staffs know about monks' level of physical activity, and also let the monks know about their level of physical activity. The uses of these surveys will allow for more concrete data which can be used to promote physical activity among monks in the future.

Promotion of physical activity in monks

Nurses that are taking care of monks should have a role in promoting physical activity among monks. They must be knowledgeable about monks' rules and limitations and understand how to treat them, for example, in Thai language; the words people used with monks are different from the words used with normal people. The desired effect from exercise of monks differs from other people. Normally people exercise to gain worldly benefit, strength or looks, but exercising for monks is limited to just keeping the body from illnesses. So, for monks, no matter what type of exercise, consider it as Dharma practice too. So, the meaning of exercise for monks is movements based on 4 natural body gestures, which are standing, walking, sitting, and sleeping. Be conscious of the movement to strengthen both the mind and the body. The promotion of physical activity among

monks should follow the following:

1. Activity must be composed and be accepted according to Buddhist teaching.

2. The activity should be held in a private place such as the monks' room or temple grounds etc. The place should also have good air flow, have smooth ground, and be dry.

3. Regarding clothes worn during activity, the Sorong might be worn without the yellow robe for more flexible movements. Shoes should be worn to prevent injuries, especially for monks who suffer from diabetes.

4. Activity should be appropriate for the monk's age and health condition.

5. There should always be warmups and cooldowns before and after the activity.

6. Activity should be held when the monk isn't either hungry or shortly after eating a meal.

7. Activity should not be held when it's too hot.

8. If there are any abnormalities during the activity such as chest pain, shortness of breath, palpation, headache etc. the activity should be halted, and if the condition does not get better, a doctor from a nearby hospital should be consulted.

activities for monks that are allowed by monk discipline are these 4 exercises that stretch body muscles and resemble monk's normal activities (Priest hospital, 2010)

The first exercise is a breath training and muscle relaxing exercise. It strengthens shoulder and arm muscles. First, the monk sit cross-legged, rest both hands face up or face down on his knees in a relaxed position. Then, inhale and rotate both hands toward the body and contract both shoulder until they move up into a sit up straight position. Then, exhale and move both hands back to the first position as shown in Fig.1. Repeat at least 10 times. This exercise is good for monks that want to do physical activity while meditating.

The second exercise strengthens hands, elbows and shoulders muscles. First, the monk sits up straight cross-legged, exhale and rotates both hands to face the body at chest level. Then, push both hands forward until the elbows straighten with both arms parallel to the floor. Then, inhale and rotate both hands and pull both arms back into the first position as shown in Fig. 2. Repeat at least 10 times.

Fig. 1 Breath training and muscle relaxing exercise; strengthen shoulders and arms muscles (Priest hospital, 2010)

Monks can perform physical activities to strengthen their circulation system by consistently moving such as when they take alms, sweeping etc. The activity should be moderate level and the duration should be around 30 minutes 3-5 times per week.

Exercise for monks

Exercise for monks can reduce or prevent illnesses that are caused by lack of physical activity, which can be perform in various ways such as fast walking, arm swings, Taichi-Qigong, yoga etc. Examples of physical

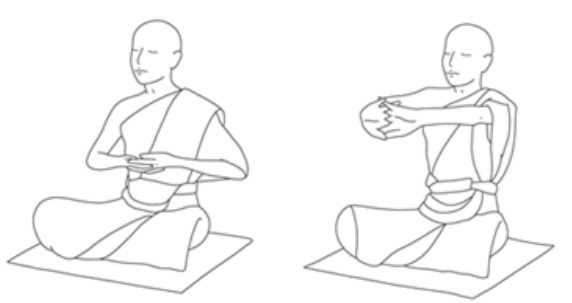

Fig. 2 Hands, arms, shoulders stretching exercise (Priest hospital, 2010)

The third exercise uses the lower body muscles and hips. The monk sits up straight and faces both feet toward each other, grip the feet with his hands and pull them toward the body while inhaling keeping the knee from moving up. Then, exhale, lean forward, bends both elbows while gripping the feet with hands until he feels the stretch at the back, stay in this position for a moment. Then inhale and lean back into the first position as shown in Fig. 3. Repeat at least 10 times.

Fig. 3 Exercise that uses lower body muscles and hips (Priest hospital, 2010)

The fourth exercise stretches knees muscles and lower back muscles. This exercise is good for monks who have muscles and knee problems. First, the monk sit stretching both legs forward, strait back, both hands placed on the knees. Then, exhale, lean forward and push both hands toward the feet until he feels the stretch at the back. Stay in this position for 5 seconds then slowly inhale, lean back, pull both hands back into the first position as shown in Fig. 4. Repeat at least 10 times (The 50th Anniversary Mahavajiralongkorn hospital foundation, 2016). All these 4 exercises can be performed during both warm up and cool down.

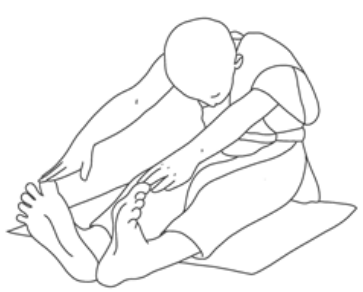

Fig. 4 Exercise that stretches knees and lower back.

There are also other easy ways to do physical activities for monks; they can be performed during normal monk activities to strengthen the circulatory and respiratory system to reduce risk of illnesses, for example:

1. Physical activities that helps strengthen the circulatory and respiratory system

Fast walking, more than 10,000 steps per day can help reduce blood pressure, and if it is done every day, it will help control the sugar level and increase the amount of beneficial lipid in the body. 12,000 to 15,000 steps per day will help lose weight, improve metabolism of the body, and strengthen bones and muscles (Kupnirattisayakul, 2012).

The process of this activity should be as follow; each step should not be too big, it should be small but frequent and along with arm swings. The proper speed is different for each person, an easy way to tell is that you should feel tired with a bit of sweat but can still talk and not be gasping for breath; you should feel that you breathe faster and your heart beat faster. The duration is 20-30 minutes, but beginner may split it into 2 rounds of 15 minutes with a 2-3 minutes break in between.

Arm swings should be performed in a spacious place with good air flow such as in a garden. Doing it continuously for 10 minutes will benefit your circulation and makes you feel happier. For better results, do it more frequently and longer in duration, this will help reduce accumulation of fat, reduce blood pressure, reduce stress, make you feel refreshed and relaxed.

The process of this activity should be as follows; stand up straight, align boot feet with shoulders, relax your arms so they fall by gravity, the fingers shouldn't split, face your hands backward, calm your mind. Swing both arms forward to about 30 degrees in angle, breathe in, and swing them backward to about 60 degrees in angle, breathe out. Do this for at least 10 minutes each time, and at least 30 minutes per day, for 5 days per week (Fig. 5).

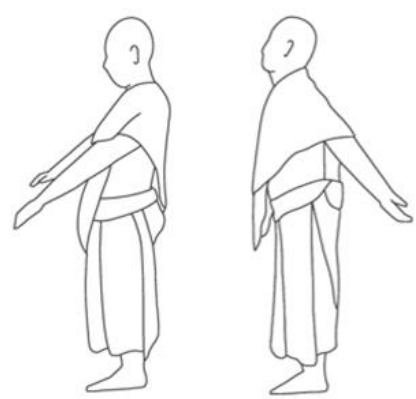

Fig. 5 Arm swings

2. Physical activities that strengthen the muscles of monks

Monks that have muscle weakness (Muscle strength grade II-III) should perform Yoga, Qigong, and stretching exercise. For weak monks, these exercises will help stimulate nerves and muscles to help strengthen them (Fig. 6).

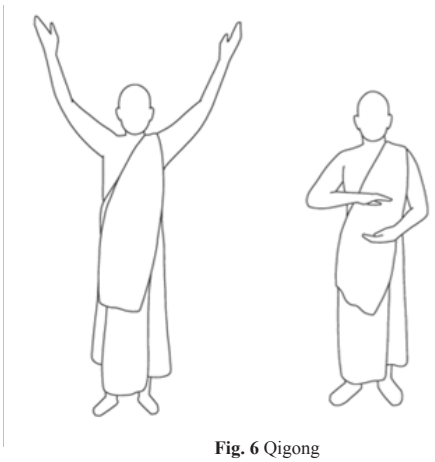

Fig. 6 Qigong

3. Physical activities that improve muscle flexibility for monks

Slowly stretching muscles can reduce the risk of injuries. Stretching should only be done to the point where the performer feels the stretch but not pain, alternate with relaxing them, do movements repeatedly to improve flexibility. Monks can increase the duration of stretch to 1 minute, but if there is potential for injuries, they should keep it at no more than 20 seconds (Fig. 7).

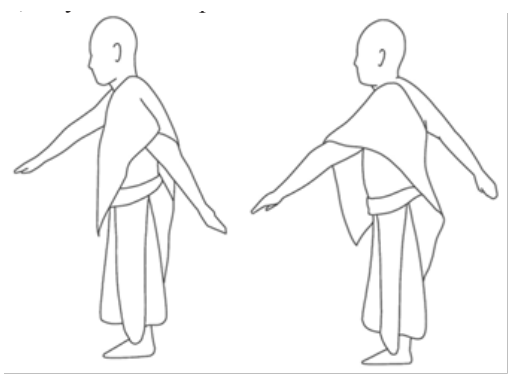

Fig. 7 Stretch exercise

Benefits of physical activity

1. Heart and lungs works well, which allows for longer duration of physical activity. Reduce resistance of blood vessels, which reduce risk of illness from hypertension. Reduce harmful lipid (Triglyceride, LDL) and increase beneficial lipid (HDL), which reduce risk of vessels diseases (Hiranrat, 2003).

2. Musculoskeletal system; strengthen muscles, more fluid joints; reduce risk of injuries from slipping and falling accidents.

3. Nervous system; stimulate nerves, better reflexes, improved memory and concentration. Reduce risk of brain conditions, stress and improve mental health.

4. Gastrointestinal; Complete digestion of food, allow for consistent excretion.

5. Better immunity, more resistant to diseases. Increase agility, improve personality and self-confidence.

6. Endocrine; help use up excess energy, control body weight, reduce risk of obesity, increase insulin sensitivity.

Physical activity can benefit monks in many ways like mentioned. There are still many more ways to exercise that aren't mentioned here. If we can design an activity that goes along with religious rules, monks will be more familiar to exercising. This is a great opportunity for monks to become stronger while keeping their normal life style and duties.

Roles of nurses in promoting physical activity for monks

Nurses have to take different approaches when promoting physical activity for monks than for normal people. Tanakronpaisal (2011) said that monks are a group of people that have very little contact with healthcare services; they have less opportunity to receive primary, secondary and tertiary medical services, they also have to do religious activities which give them less time to take care of their health. Additionally, the lack of health knowledge and lack of physical activity leads to health problems. Primary healthcare nurses in Sweden succeeded in promoting health for citizens, because they gave importance to promoting health and have good organization such as giving professional growth to nurses that performed well in promoting health. (Maijala et al., 2016) A health-promoting organizational culture (e.g., the respectful management of health promotion) and nurses' health orientation and development (e.g., nurses' professional growth and work well-being) were found as the main elements required for the success of the health promotion practices in primary health care.

Factors that promote physical activity are promotion of self-confident, belief that physical activities are beneficial, individual evaluation of physical activity and physical activity promotion training (Crisford et al., 2018) [Self-efficacy in physical activity (PA) promotion, positive beliefs in the benefits of PA, assessing patients' PA, and PA promotion training were the main factors associated with engaging in PA promotion.]. So, nurses should teach monks about physical activity, its health benefits, variants that do not go against religious rules, give proper evaluation and adjustment to their physical activity to reduce risk of chronic diseases. A community-based intervention program, based on self-efficacy theory, improves older adults' self-care behaviors as well as health outcomes related to hypertension and dyslipidemia. randomized controlled trial Body mass index, blood pressure, hyperglycemia, and high-density lipoprotein cholesterol in the intervention group improved significantly from baseline (Wu et al., 2019). So, if nurses can utilize intervention program based on self-efficacy theory to promote physical activity for monks, it will benefit monks and reduce risk of hypertension and dyslipidemia.

We can summarize the roles of nurses as:

1. Health educator role: health promotion and health prevention especially diet and physical activities. Nurses should provide information on proper nutrition such as low salt, low fat food and how to exercise to burn excess fat. They should also promote people to choose proper food to give to monks. Moreover, nurses should promote monks to be more considerate about their health by having proper nutrition and exercise. They should teach monks about exercises that do not go against religious rules, so monks can perform those exercise properly by themselves.

2. Health care provider: health problem screening, nursing activity for the monks who got the NCDs. Nurses and health care team should provide annual checkup for monks such as examination of blood pressure, blood sugar, and BMI, to diagnose illnesses and provide treatment. Furthermore, they should also inform monks on how to perform self-basic diagnosis like measuring blood pressure, BMI, or feet examination.

3. Advocator and campaign for health promotion project: nurses should hold events promoting physical activity for monks where people can participate, and campaign for promotion of monks' health which has monks as leader or a good role model.

What could the nurses do to promote physical activities for Buddhist monks?

Begin by setting a mindset that monks daily activities such as alms round, cleaning, or other chores can be performed to increase physical activity. Then provide information on physical activity that do not go against religious rules to monks such as the ones mentioned in this article, yoga, resistance exercise using dumbbells or other weights to increase muscle strength. Moreover, nurses can develop integrated health promoting programs that emphasize physical activity, proper nutrition and stress management and suggest them to the temple leader or an affiliate organization.

Conclusion

Nurses' role to promote physical activity among monks is considered to be an active role and is very important to prevent chronic non-infectious diseases that are caused by lack of physical activity. So, Nurses should be knowledgeable about physical activity promotion and proper treatment for monks. They should use the proper words in conversations, be able to convey the right information about exercising and not go against Buddhism discipline. They should also be able to evaluate physical activity levels of monks, design proper physical activity programs for each monk and make people understand about the negative effects of lack of physical activity. To make monks become more knowledgeable about physical activity and be able to perform physical activity, and to make common citizen understand and support physical activity among monks. For sustainability of adequate physical activity, resulting in monks being able to complete their duties and enjoy better quality of life.

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Book Review

Suwanna Pichaiyongvongdee

Book name:	Innovations in Food Technology
Author:	Mishra, P., Mishra, R.R., & Adetunji, C.O.
Published:	Springer Singapore, 2020
Paperback:	522 pages
Language:	English
ISBN:	978-981-15-6121-4

"Innovations in Food Technology" gathers a collection of essays that describe recent innovations in food technology including food processing, packaging, food safety. and novel ingredients for in the future. The book has four 4 section:

Section 1: Food processing and food microbiology
Section 2: Nutritional security
Section 3: New and enhanced food materials, as well as processing innovations to extend shelf life
Section 4: Reduction toxic effects

In addition, this book also addresses the health potential of various nutraceuticals, bio-absorption of metals and their positive impacts on living systems, as well as methods for reducing food wastage, preventing the loss of nutritive value, qand preserving or enhancing palatability.

Given the above content, this book will be highly interesting for food scientists, both in academia and the food industry. It will also benefit advanced graduate students and senior researchers.

Reviewer

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

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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.
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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.

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Original Articles

Review article

Book Review

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