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Malaysian herbs as feeding attractants and enhancers for the giant freshwater prawn (*Macrobrachium rosenbergii*) and the whiteleg shrimp (*Litopenaeus vannamei*)

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Abstract

Feeding attractiveness of 21 fresh herbs was examined for the giant freshwater prawn (*Macrobrachium rosenbergii*) and the whiteleg shrimp (*Litopenaeus vannamei*) at different growth stages in tanks. The feeding attractant and enhancement were assessed by behavioural observations of the test animals in response to pelleted feeds incorporated with the herbal extracts in different concentrations. For *M. rosenbergii*, peppermint (*Mentha piperita*) and dokudami (*Houttuynia cordata*) were the most attractive herbs at all stages, and garlic (*Allium sativum*) strongly attracting to the juveniles but only weekly to the adults, postlarvae and larvae. Peppermint significantly enhanced feeding of *M. rosenbergii* at all stages except for the larvae. Garlic was a significant feeding enhancer for *M. rosenbergii* juveniles and postlarvae. The inclusion of the herbal extract at high concentrations had a negative effect on the feeding. As far as *L. vannamei* is concerned, the red chilli (*Capsicum annuum*) was the best feeding attractant and ginger (*Zingiber officinale*) and peppermint were moderate attractants for adults and juveniles. While galangal (*Alpinia galangal*), ginger and yellow onion (*Allium cepa* sp.) enhanced feeding in *L. vannamei* adults, but red chilli, garlic and peppermint did not enhance feeding. Evidently, the strong feeding attractants were not necessarily effective feeding enhancers. The incorporation of several herb extracts at higher concentrations caused a negative effect on the feeding of the test animals. This could be due to the presence of feeding deterrents in the herb such as saponins which are known to lower food palatability in insect, decapod crustaceans and fishes. More long-term work is warranted to determine if inclusion of feeding enhancing herbs in artificial feed promotes growth performance of the shrimp.

Keywords: Herbs, Peppermint, Ginger, Feeding enhancer, Feeding attractant

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Introduction

Interest in research and medicinal properties of plants for potential use in aquaculture is growing steadily. Hormones, antibiotics, vitamins and several other chemicals are no longer considered attracting for several reasons, mainly the resistance development, high cost and concerns about their environmental impact and consumer health (Rebecca and Bhavan, 2014). Natural plant products have been reported to promote various activities such as stress mitigation, growth, appetite and immunostimulation in aquaculture animals (review in Citarasu, 2010). Recently, many authors reviewed herbal applied in aquaculture. Harikrishnan et al. (2011) reviewed 242 articles and summarized the medicinal plant extracts and their products that modulate the immune response in fish and shellfish. Cristea et al. (2012), Olusola et al. (2013), Vaseeharan and Thaya (2014) reviewed and evaluated medicinal the effectiveness of many plant products. Rebecca and Bhavan (2014) summarized findings on the medicinal effects of several plant products on fishes in India. Bulfon et al. (2015) reviewed the effects of products of more than 70 plant species on fish growth, haematological profiles, immune responses and resistance to infectious diseases.

In general, feeding behaviour of prawns and shrimps progresses as follow: 1) detecting and locating food by vision and olfaction, 2) approaching and touching it, 3) holding or grabbing it, 4) conveying it to the mouth after manipulation by walking legs or maxillipeds and 5) ingestion. If the food is not palatable, it will be rejected after the manipulation (Kawamura et al., 2017, 2018). Several plant extracts are reported to stimulate appetite and promote weight gain when they are administered to cultured fishes (reviewed in Reverter et al., 2014). Herbal supplements are also reported to enhance food intake in decapod crustaceans. Rebecca and Bhavan (2014) showed that the dietary supplementation of ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) and garlic (*Allium sativum*) in different concentrations enhanced the feed intake in the larvae of *Macrobrachium rosenbergii*.

Venkatramalingam et al. (2007) showed that *Penaeus monodon* postlarvae consumed significantly higher number of ginger-enriched *Artemia* compared to those fed unenriched *Artemia*. Similarly, Citarasu et al. (2002) reported that postlarvae and pre-adults of *P. monodon* fed *Artemia* nauplii enriched with five Indian medicinal plants showed significantly increased food consumption compared to those of unenriched *Artemia* group.

This study aimed was aimed at determining the effectiveness of herbs locally available in Malaysia as feeding attractants and feeding enhancers in the giant freshwater prawn (*Macrobrachium rosenbergii*) (Family Palaemonidae) and the marine whiteleg shrimp (*Litopenaeus vannamei*) (Family Penaeidae) at different growth stages. *M. rosenbergii* is native to Malaysia and is a popular species for aquaculture production worldwide (Yong et al., 2018). This species was introduced into coastal Asian countries in the early 2000 and became one of the most cultured species worldwide including Malaysia (Sanudin et al., 2015). This makes the studies on feeding attractants particularly more commercially important for growth of whiteleg shrimp aquaculture.

Materials and Methods

Experiments were done in the shrimp hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah. The trials were carried out in rearing tanks placed inside the roofed hatchery to avoid the effect of "beaker stress" on the feeding behaviour of the test animals. All the experimental trials were conducted in accordance with the 'Researcher's guidelines on code of practice for the care and use of animals for scientific purposes, Universiti Malaysia Sabah'.

Test animals and husbandry

M. rosenbergii zoea larvae (X), postlarvae (PL), juveniles and adults were obtained from the hatchery. Figure 1 shows the frequency distribution of the initial body length of the PL, juveniles and adults. The experiments were conducted for about five months.

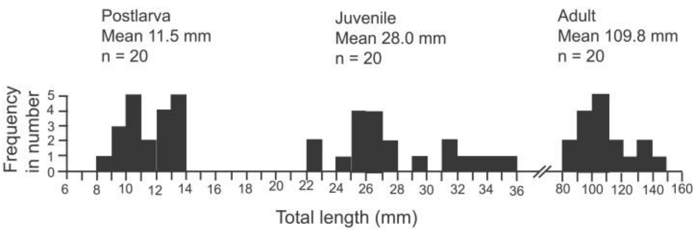

Figure 1. Frequency distribution of total length of *Macrobrachium rosenbergii* used in the experiments.

Three different low- density polyvinyl (LDPV) tanks were used to stock the prawn depending on their stage of life (Table 1). Prawns were fed twice a day (08:00 am and 04:00 pm) with formulated powderd feed (Gold Coin, Malaysia) which contained 37% crude protein and 4–6% crude lipid for larvae. Pelleted feed (Sheng Long- Royal Dragon, DT313, Vietnam) containing 40% crude protein and 5–7% crude lipid were offered to the for PL, juveniles and adults

A total of 150 juveniles and 50 adults of *L. vannamei* were obtained from a shrimp farm in Tuaran, Sabah. The shrimp specimens were acclimatized to the captive conditions in different LDPV rearing tanks (diameter 135 cm top and 120 cm bottom, height 80 cm, water depth 50 cm) equipped with a water flow- through system was placed in the roofed hatchery for a few days.

Table 1. Size of tanks and stocking density of *Macrobrachium rosenbergii* used in the experiment.

	Shrimp stage			
	Larva	*Postlarva*	*Juvenile*	*Adult*
Tank volume (L)	360	500	1000	1000
Tank diameter (cm)	100	135	153	
Water depth (cm)	40	40	50	
Stocking density	4,500–4,600	1500–1,600	400–500	15

The shrimp were fed with the similar commercial pelleted feed (Sheng Long- Royal Dragon, DT313, Vietnam) about 15% of body weight, twice a day at 07:30 am and 04:30 pm. The initial total length of the juveniles ranged from 11.1 cm to 13.3 cm (mean, 12.3 cm) (Figure 2), and body weight from 8.6 g to 13.3 g (mean, 10.7 g). The experiments were conducted for three months.

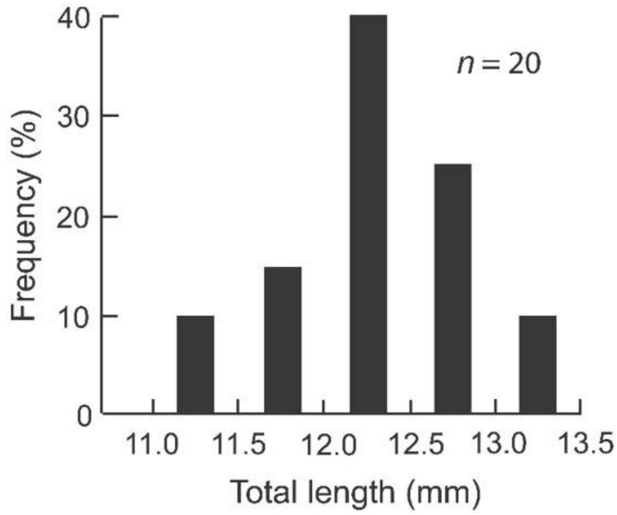

Figure 2. Frequency distribution of total length of *Litopenaeus vannamei* used in the experiments.

Herbs used

Sixteen fresh herbs were procured from a local market and botanical garden for the experiments on *M. rosenbergii* and ten herbs for *L. vannamei* (Table 2). Peppermint and dokudami were propagated in a small-scale aquaponics in the shrimp hatchery.

Procedure for attractiveness test

The behaviour of the test animals in response to herbs was categorized as approach, touch, bite, non-detach and jump-back (Table 3) and number of test animals exhibited each behaviour was visually and video recorded for 15-min with a digital camera (OLYMPUS Tough, Olympus Corporation, Tokyo, Japan). Three replicates of attractiveness test were carried out for each herb. After each test, water renewal of 20% was carried out to remove the smell of herbs in the tank.

Table 2. List of 21 herbs used.
*Denotes herbs used only for *Litopenaeus vannamei*.

Portion used	Common name	Scientific name
Leaf	Basil	*Ocimum basilicum*
	Betel	*Piper betel*
	Belalai gajah*	*Clinacanthus nutans*
	Coriander	*Coriandrum sativum*
	Curry leaf	*Murraya koenigii*
	Dokudami	*Houttuynia cordata*
	Flat-leaf parsley	*Petroselinum crispum*
	Galangal*	*Alpinia galanga*
	Lemongrass*	*Cymbopogon* sp.
	Peppermint	*Mentha piperita*
	Pokok pecah beling	*Strobilanthes crispus*
	Sabah snake grass	*Clinacanthus nutans*
	Wild strawberry	*Fragaria* sp.
	Willow-leaved justicia	*Justicia gendarussa*
Underground rhizome or bulb	Blue ginger	*Alpinia galanga*
	Garlic	*Allium sativum*
	Ginger	*Zingiber officinale*
	Red onion	*Allium cepa* sp.
	Turmeric*	*Curcuma longa*
	Yellow onion	*Allium cepa* sp.
Fruit	Red chilli*	*Capsicum annuum*

Table 3. Description of behavioural pattern of test animals in response to herbs for *Macrobrachium rosenbergii* and *Litopenaeus vannamei*.

Behaviour	Description
Approach	Animals moved toward the herb but did not touch it.
Touch	Animals approached and touched a herb with feeding appendages.
Bite	Biting herb with mouthparts
Non-detach	Animals continued clinging to a herb and was not detached when the herb was slightly shaken vertically.
Jump-back	Animals quickly jumped backward after touching a herb.

For *M. rosenbergii* larvae, PL and juveniles, herbs of 16 different species were paired and displayed to the prawns in different combinations. The herbs were clipped on a red pin and tethered by transparent monofilament (0.25 mm thick, 50 cm long) to a plastic rod at an interval of 50 cm (Figure 3A). Since the larvae were strongly attracted to a silvery clip rather than herbs in a preliminary observation, the silvery clip was replaced with a red clip which did not attract the larvae at all. The position of two herbs was interchanged.

Adults of *M. rrosenbergii* stopped eating and withdrew back into their shelter in response to disturbance caused by movement of people around the tank.. In order to observe and record their behaviour, the herbs were placed in feeding trays (circular, 15 cm diameter, 1 cm depth) and the prawn behaviour was video recorded with the digital camera mounted on a tripod, and no human activity was carried out near the tank during the recording period (Figure 3B). The two trays were placed simultaneously on the tank bottom, each with a different type of herb. The evaluation was based on five different prawn behaviours shown towards the herbs; the approach to the herb, touch, bite, no-detachment when herbs were shaken, and quick jump back from the herb after touching it. The attractiveness was evaluated for the larvae, PL and juveniles in a similar way.

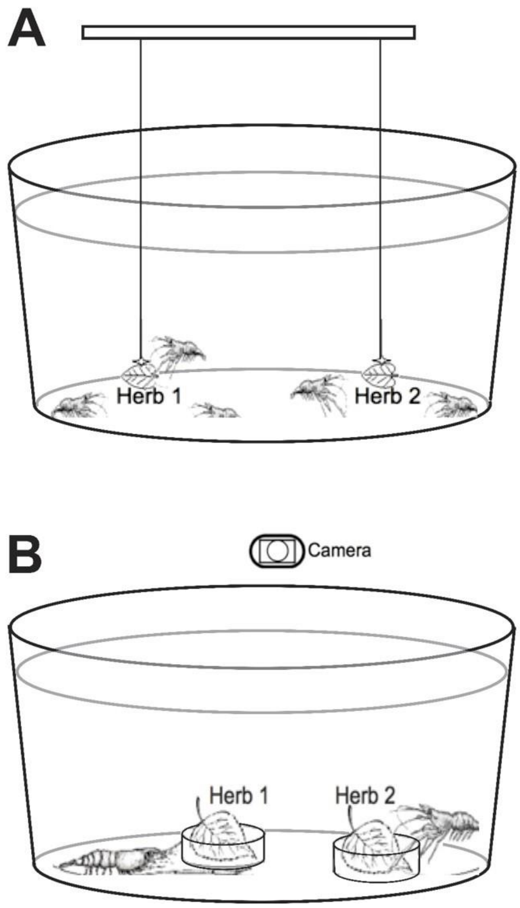

Figure 3. Diagrammatic representation of experimental tank with herbs tethered for *Macrobrachium rosenbergii* larvae, PL and juveniles (A) and adults (B) in feeding attractiveness test.

The attractiveness was evaluated based on the behavioural response of *M. rosenbergii* to the herbs. The relative magnitude of each behavioural response was recorded as positive '*' or '**' when the response was made by more than five individuals and negative '–' (when no specimen exhibited it).

The experiment with *L. vannamei* was conducted in the 1000 L LDPV rearing tank. The tank contained 50 juveniles or adults. A piece of sliced herb was clipped on a red pin and tethered in the tank by a transparent monofilament (0.25 mm thick, 50 cm long) from a plastic rod. The behavioural response of the shrimp to the herb was visually observed and recorded.

The feeding attractiveness of the herbs was accessed by the number of frequencies of each behaviour exhibited by the test animals: approach, touch and jump-back, in response to test herbs. And the behavioural responses were scored and magnitude of attractiveness (MA) calculated for each herb.

MA was defined by a following formula:

$$MA = N_{\mathrm{approach}} \times 1 + N_{\mathrm{touch}} \times 3 + N_{\mathrm{jumpback}} \times -1$$

where, N is number of shrimps which exhibited respective behavioural response. Mean MA and its 95% confidence interval were calculated for each herb and statistically analyzed (*t*-test).

Procedure for feeding enhancement test

The test animals were the same with those used in the feeding attractiveness test. For *M. rosenbergii,* six most attractive herbs from the above trials were selected for the feeding enhancement test: *M. piperita*, *Houttuynia cordata*, *Allium sativum*, *A. cepa* sp., *Coriandrum sativum*, and *Piper betel*. For *L. vannamei,* eight herbs were used: *H. cordata*, *M. piperita*, *Curcuma longa*, *Zingiber officinale*, *Alpinia galanga*, *A. sativum*, *A. cepa* sp., and *Capsicum annuum*. Each kind of herb was weighed, hand-grinded in a mortar, added with distilled water, manually stirred well, and filtered out using a filter paper (Whatman No. 1). For adults, juveniles and PL of *M. rosenbergii* and *L. vannamei the* pelleted feed was used. For larvae of *M. rosenbergii*, egg custard was used as the feed because larvae were unable to consume pelleted feeds. Egg custard was made followed Yong et al. (2018). The pelleted feeds or egg custard were soaked in the herb extract of different nominal concentrations for 5 min then the feeds were filtered out from the extract with a feeding mesh tray of pore size 0.5 mm and served as test feeds. The pelleted feeds or egg custard without herb extract served as control feeds.

Concentration of herb extract

The nominal concentration (Cn) was defined as:

$$Cn = [\text{Amount of herb (g)} / \text{Volume of water (mL)}] \times 100$$

Pelleted feed (Gold Coin, Malaysia for *M. rosenbergii*; Sheng Long- Royal Dragon, DT313, Vietnam for *L. vannamei*) were soaked in herb water extracts of different concentrations (from 1% to 75%) and given to the test animals in the respective rearing tanks. After the pelleted feed were soaked in the extract, mean weight of the pelleted feed increased 40.2% (mean of five replications). Therefore, real concentration of the extracts in the pelleted feed (Cr) was calculated as:

$$Cr = Wp\,(0.402 \times Cn) / Wp$$
$$= (0.402 \times Cn)\ (\%)$$

where Wp is weight of pelleted feed. The values of Cr ranged from 0.4% to 30.2%.

Recording of feeding behaviour and assessment of feeding enhancement

For *M. rosenbergii* adults, juveniles and PL, video recording was started immediately after simultaneous placing two feeding trays containing test or control pelleted feeds on the tank bottom (Figure 4A). For the larvae, a piece of egg custard with or without herb extract was selected and tethered using a fishing line in the experimental tank for observation of behavioural response of the larvae (Figure 4B). Feeding behaviour of the test animals was video recorded for 15 min. The position of test and control feeds was interchanged. This procedure was repeated three to seven times for each herb extract concentration.

Feeding enhancement was assessed by the number of test animals which exhibited each behaviour in response to test and control pelleted feeds; approach, touch, bite or ingestion, and jump-back. The number of animals in the three replicates was summed and compared between test and control pelleted feeds by the χ^2-test at $\alpha = 0.05$.

L. vannamei is more aggressive than *M. rosenbergii* in feeding. *L. vannamei* uses its third walking legs when it has to fight for a pellet with a competitor, violently waving it until the competitor withdraws. *L. vannamei* holds as many as eight pellets at once at the mouth (Kawamura et al., 2018). Therefore, feeding enhancement was assessed based on the number of pellets consumed in 10 min.

The feeding enhancement test with *L. vannamei* was conducted in the 1000 L LDPV rearing tank which contained 50 adults or 50 juveniles. Test and control pelleted feeds were delivered with a plastic netting tray (feeding tray) (20 cm diameter, 1 cm deep). The tray was partitioned into half and contained test pelleted feeds in a half side and control pelleted feeds in the other half side (paired delivery) (Figure 5). The feeding tray placed on the tank bottom was retrieved after 10 min and the number of pelleted feeds remained was counted. The number of pelleted feed consumed was calculated by subtracting the number of pelleted feed remained from the initial number of pelleted feed, summed for three replicates and the feeding enhancement was assessed by the χ^2-test at $\alpha = 0.05$.

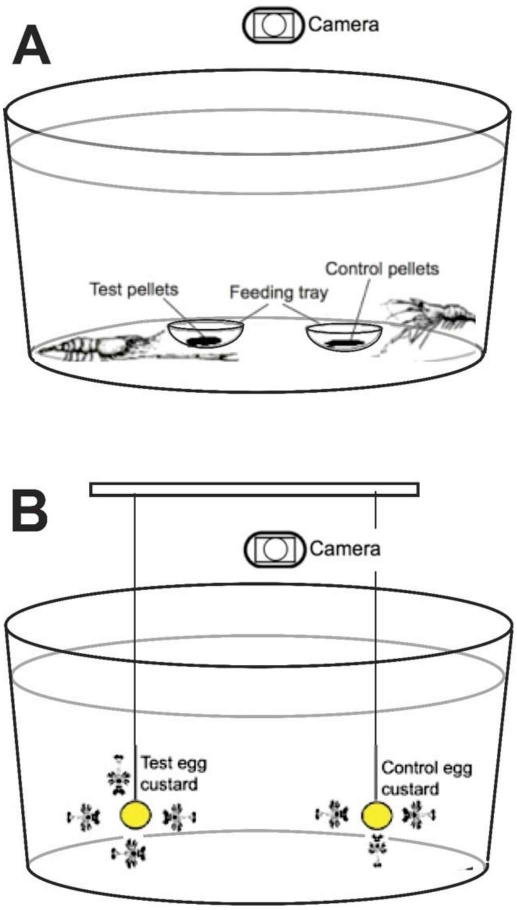

Figure 4. *Macrobrachium rosenbergii*: diagrammatic representation of experimental tank with control and test pelleted feeds in feeding trays for adults, juveniles and PL (A) and control and test egg custards tethered with monofilament lines for larvae (B).

Figure 5. *Litopenaeus vannamei*: Netting (feeding) tray containing test pelleted feed one side and control pelleted feed on the other side.

Results

Feeding attractiveness

M. rosenbergii larvae, PL and juveniles were attracted well to the herbs and continued to cling to and did not detach from peppermint and yellow onion even when the herbs were vertically shaken (Figure 6). Results of the attractiveness tests with *M. rosenbergii* are shown in Table 4 for the adults, juveniles, PL, and larvae. The adults showed strongest response to *M. piperita* and *H. cordata* followed by *A. sativum* and *P. betel*, and did not show any response to *A. galanga*, *Z. officinale*, *M. koenigii*, and *C. nutans* in one of the three trials. The juveniles were attracted to the herbs except for *C. sativum*, *M. koenigii*, and *Fragaria* sp.. The PL were attracted to all the herbs. The larvae were not attracted to *Fragaria* sp., *J. gendarussa*, and *S. crispus*.

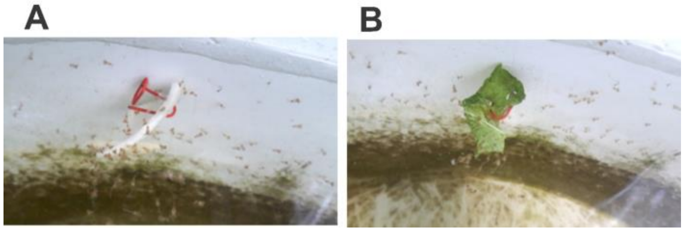

Figure 6. *Macrobrachium rosenbergii* larvae touching and biting yellow onion (A) and peppermint (B) tethered with monofilament line in rearing tank.

Results of the attractiveness tests with *L. vannamei* are shown in Figure 7 for juveniles and adults. All the herbs used attracted *L. vannamei* juveniles and adults, but the magnitude of attractiveness (MA) was herb species specific. In juveniles, *Z. officinale* and *C. annuum* had the highest MA followed by *M. piperita*. In adults, *C. annuum* showed highest MA followed by *Z. officinale* and *M. piperita*. The MA tended to be higher in juveniles than in adults.

Feeding enhancement

The PL and juveniles of *M. rosenbergii* approached the feeding trays, touched the test and control pelleted feed with pincers, and conveyed them to the mouth or held one or two pieces with pincers and left the trays. When one dominant specimen was eating the pelleted feed in the tray, other specimens did not come to the tray. Several specimens immediately jumped-back after touching the tray.

The results of feeding enhancement test with *M. rosenbergii* were selected as an example to show the process of statistical analysis (Table 5) and all the results were summarized in Table 6. The effect of inclusion of herb extract into pelleted feeds was very different depending on herb species regardless of the attractiveness of herbs. *A. sativum*, *M. piperita*, *S. crispus* enhanced feeding of the test animals at moderate extract concentrations (Table 6). *C. sativum*, *H. cordata*, *J. gendarussa*, and *A. cepa* sp. did not enhance feeding of *M. rosenbergii* at all (Table 6). Feeding enhancement differed depending on stage of test animals. Inclusion of *A. sativum* and *M. piperita* extracts produced negative effects on the feeding of the larvae in all the extract concentrations (Table 6).

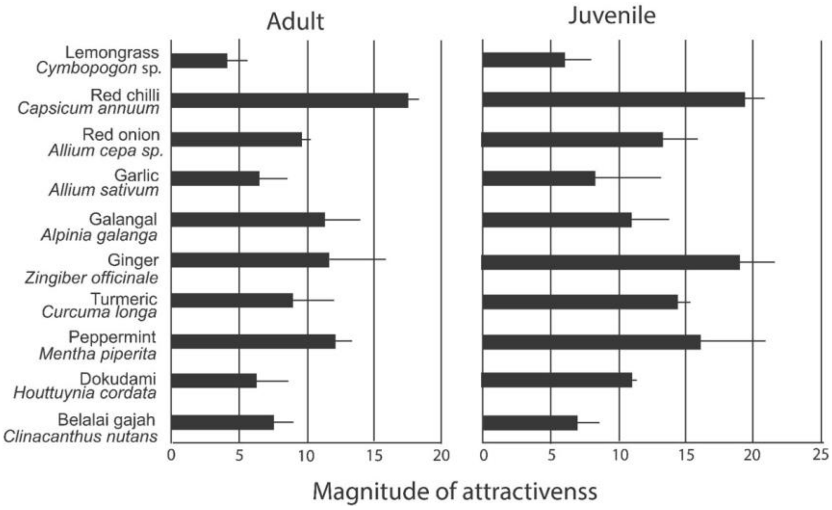

Figure 7. Results of attractiveness test for adults and juveniles of *Litopenaeus vannamei*.

Table 4. *Macrobrachium rosenbergii*: Results of attractiveness by herbs and stage. *, **, **-** denote “positive response”, “positive response in a mass and “no animal showed the behaviour” respectively.

Growth stage	Herb	Behavioural pattern				
		Approach	Touch	Bite	Non-detach	Jump-back
Adult	Basil *Ocimum basilicum*	–	–	–	–	–
	Betel *Piper betel*	*	*	*	*	–
	Blue ginger *Alpinia galanga*	*	*	–	–	–
	Coriander *Coriandrum sativum*	*	*	–	–	–
	Curry leaves *Murraya koenigii*	–	–	–	–	–
	Dokudami *Houttuynia cordata*	**	**	**	**	–
	Flat-leaf parsley *Petroselinum crispum*	*	*	–	–	–
	Garlic *Allium sativum*	*	*	*	*	–
	Ginger *Zingiber officinale*	*	–	–	–	–
	Peppermint *Mentha piperita*	**	**	**	**	–
	Pokok pecah *Strobilanthes crispus*	–	–	–	–	–
	Red onion *Allium cepa* sp.	*	*	–	–	–
	Sabah snake grass *Clinacanthus nutans*	–	–	–	–	–
	Wild strawberry *Fragaria* sp.	–	–	–	–	–
	Willow-leaved justicia *Justicia gendarussa*	–	–	–	–	–
	Yellow onion *Allium cepa* sp.	*	*	–	–	–
Juvenile	Basil	*	*	*	*	–
	Betel	**	**	**	**	–
	Blue ginger	*	*	*	*	–
	Coriander	*	*	–	–	*
	Curry leaves	*	*	–	–	–
	Dokudami	**	**	**	**	–
	Garlic	**	**	**	**	*
	Ginger	*	*	*	*	*
	Flat-leaf parsley	*	*	*	*	*
	Peppermint	**	**	**	**	–
	Pokok pecah	**	**	*	*	–
	Red onion	*	*	*	*	–
	Sabah snake grass	**	**	*	*	*
	Wild strawberry	*	*	*	*	–
	Willow-leaved justicia	**	*	*	*	–
	Yellow onion	**	**	**	*	*
Postlarva	Basil	**	**	**	**	–
	Betel	**	**	**	**	–
	Blue ginger	**	**	**	**	*
	Coriander	**	**	**	**	–
	Curry leaves	**	*	*	*	–
	Dokudami	**	**	*	*	–
	Garlic	*	*	*	*	*
	Ginger	**	**	*	*	–
	Flat-leaf parsley	**	**	**	**	–
	Peppermint	**	**	**	**	–
	Pokok pecah	**	**	**	**	–
	Red onion	**	**	**	**	–
	Sabah snake grass	*	*	*	*	*
	Wild strawberry	**	**	**	**	–
	Willow-leaved justicia	**	**	**	**	–
	Yellow onion	**	**	**	**	*
Larva	Basil	**	**	*	*	–
	Betel	**	**	*	*	–
	Blue ginger	**	**	–	–	*
	Coriander	*	*	*	*	*
	Curry leaves	*	*	*	*	–
	Dokudami	**	**	*	*	–
	Flat-leaf parsley	**	**	**	*	–
	Garlic	**	*	*	*	*
	Ginger	**	**	*	*	*
	Peppermint	**	**	**	**	–
	Pokok pecah	–	–	–	–	–
	Red onion	*	*	*	*	–
	Sabah snake grass	*	*	*	*	–
	Wild strawberry	–	–	–	–	–
	Willow-leaved justicia	–	–	–	–	–
	Yellow onion	**	**	**	**	–

Table 5. *Macrobrachium rosenbergii*: Results of enhancement test showing the number of animals which exhibited four behavioural patterns in response to pellet feeds with peppermint (*Mentha piperita*) extract at different concentrations and statistical analysis. TP, test pellet; CP, control pellet.

Nominal extract concentration (%)	No. of trials		Behavioural pattern							
			Approach		Touch		Bite		Jump-back	
			TP	CP	TP	CP	TP	CP	TP	CP
50	4	Total no.	93	94	86	78	69	74	12	5
		$\chi 2$	0.005		0.390		0.113			
		Probability	0.10 < P < 0.95 (no effect)							
30	7	Total no.	213	134	189	117	188	114	11	12
		$\chi 2$	19.986		16.941		18.132			
		Probability	P < 0.005 (significant positive effect)							
25	5	Total	39	15	29	12	26	9	2	0
		$\chi 2$	10.667		7.049		8.257			
		Probability	P < 0.005 (significant positive effect)							
20	3	Total	33	24	21	14	16	11	5	5
		$\chi 2$	1.421		1.400		0.926			
		Probability	0.10 < P < 0.90 (no effect)							
15	3	Total	17	18	14	15	14	12	1	1
		$\chi 2$	0.029		0.034		0.154			
		Probability	0.10 < P < 0.90 (no effect)							

Table 6. *Macrrorachium rosenbergii*: Summarized results of the feeding enhancement test by herb, stage of prawn and herb extract concentration. (++), (−−), (+) and (−) denote significant positive or negative effect (P < 0.01), insignificant positive or negative effect (0.05 < P < 0.10) respectively. No effect, P > 0.10.

Herb	Stage	Nomimal extract concentration (%)	No. of trials	Effectiveness
Betel *Piper betel*	Adult	5	3	(−)
		10	3	No effect
		30	3	No effect
		50	3	(−−)
	Juvenile	5	3	No effect
		10	4	(++)
		30	3	No effect
		50	3	(−)
	Postlarva	5	3	(+)
		10	3	(++)
		30	3	(+)
		50	3	No effect
Coriander *Coriandrum sativum*	Adult	10	3	No effect
		30	3	No effect
		50	3	(−−)
	Juvenile	10	5	(+)
		25	4	(+)
		50	3	No effect
	Postlarva	10	7	(+)
		20	3	(+)
		25	3	(+)
		30	3	No effect
		50	3	No effect
Dokudami *Houttuynia cordata*	Adult	1	3	No effect
		5	3	No effect
		10	3	No effect
		20	3	No effect
		30	3	No effect
	Junenile	1	3	No effect
		5	5	No effeect
		10	5	(+)
		20	5	No effect
		30	3	(−−)
		50	2	(−)
	Postlarva	1	3	No effect
		5	3	(+)
		10	3	(+)
		20	3	No effect
		30	3	No effect

Herb	Stage	Nominal extract concentration (%)	No. of trials	Effectiveness
Garlic *Allium sativum*	Adult	10	4	No effect
		30	3	(−−)
		50	4	No effect
		75	3	(−−)
	Juvenile	10	3	No effect
		25	3	(++)
		30	4	(++)
		50	3	No effect
		75	3	No effect
	Postlarva	10	3	(++)
		20	3	(++)
		30	3	(++)
		50	3	No effect
		75	3	(−)
	Larva	10	3	No effect
		30	3	(−−)
		50	3	(−−)
		75	3	(−−)
Peppermint *Mentha piperita*	Adult	10	3	No effect
		15	3	(+)
		25	4	(++)
		30	3	(++)
		50	3	No effect
		75	3	(−)
		95	3	(−−)
	Juvenile	15	4	(−)
		20	3	No effect
		25	3	(++)
		30	7	(++)
		50	3	No effect
	Postlarva	15	3	No effect
		20	3	(++)
		25	3	(++)
		30	3	(++)
		50	3	No effect

Herb	Stage	Nominal extract concentration (%)	No. of trials	Effectiveness
Pokok pecah beling *Strobilanthes crispus*	Adult	5	3	(−)
		10	3	No effect
		30	3	No effect
		50	3	(−−)
	Juvenile	5	3	No effect
		10	3	(++)
		30	3	No effect
		50	3	(−)
	Postlarva	5	3	(+)
		10	3	(++)
		30	3	(+)
		50	3	No effect
Willow-leaved justica *Justicia gendarussa*	Adult	5	3	No effect
		10	3	No effect
		20	3	No effect
		30	3	No effect
	Juvenile	5	3	No effect
		10	3	(+)
		20	5	No effect
		30	3	(−−)
		10	3	(+)
		20	3	No effect
		30	3	No effect
Yellow onion *Allium cepa* sp.	Adult	10	3	No effect
		25	3	No effect
		50	3	(−−)
	Juvenile	10	3	No effect
		25	3	(−)
		50	3	No effect
		75	3	(−)
		95	5	No effect
	Postlarva	5	3	No effect
		10	3	(+)
		20	3	(+)
		30	3	No effect
		50	3	No effect

Table 7. *Litopenaeus vannamei:* Summarized results of feeding enhancement test for adults and juveniles. (++), (−−), (+), and (−) denote significant positive or negative effect ($P < 0.01$), insignificant positive or negative effect ($0.05 < P < 0.10$) respectively. No effect, $P > 0.10$.

Stage	Herb	Nominal extract concentration (%)	Total no. of pellet taken		χ2 test		Effectiveness
			TP	CP	χ^2 value	Probability	
Adult	Red chilli *Capsicum annuum*	5	65	54	1.017	0.25 < P < 0.50	No effect
		10	32	50	3.951	0.025 < *P* < 0.05	(−−)
	Dokudami *Houttuynia cordata*	5	84	93	0.458	0.25 < *P* < 0.50	No effect
		10	30	45	3.000	0.05 < *P* <0.10	(−)
	Galangal *Alpinia galanga*	10	49	47	0.042	0.25 < *P* < 0.50	No effect
		30	115	59	18.023	*P* < 0.005	(++)
	Garlic *Allium sativum*	10	72	60	1.091	0.25 < *P* < 0.50	No effect
		20	67	75	0.451	0.25 < *P* < 050	No effect
		30	39	74	10.841	*P* < 0.005	(−−)
	Ginger *Zingiber officinale*	10	64	77	1.200	0.25 < *P* < 0.50	No effect
		20	73	66	0.354	0.25 < *P* < 050	No effect
		30	84	42	14.000	*P* < 0.005	(++)
	Peppermint *Petroselinum crispum*	5	76	77	0.065	0.25 < *P* < 0.50	No effect
		10	66	63	0.070	0.25 < *P* < 0.50	No effect
		30	60	79	2.597	0.10 < *P* < 0.25	No effect
	Yellow onion *Allium cepa* sp.	10	123	124	0.004	0.90 < *P* <0.95	No effect
		30	82	44	11.460	*P* < 0.005	(++)
Juvenile	Red chilli	5	46	50	0.167	0.25 < P < 0.50	No effect
		10	146	134	0.514	0.25 < *P* < 0.50	No effect
	Dokudami	5	142	136	0.130	0.25 < *P* < 0.50	No effect
		10	52	146	44.626	*P* < 0.005	(−−)
	Galangal	10	57	90	7.408	0.01 < *P* < 0.05	(−)
		30	72	126	14.727	*P* < 0.005	(−−)
	Garlic	10	56	101	12.898	*P* < 0.005	(−−)
		20	92	73	2.188	0.10 < *P* < 0.25	No effect
		30	95	109	0.961	0.25 <*P*< 0.50	No effect
	Ginger	10	67	124	17.011	*P* < 0.005	(−−)
		20	117	103	0.891	0.25 < P < 0.50	No effect
		30	109	115	0.723	0.25 < P < 0.50	No effect
	Peppermint	5	102	131	3.609	0.05 < *P* < 0.1	(−)
		10	109	146	5.369	0.01 < *P* < 0.025	(−−)
		20	99	114	1.056	0.25 < *P* < 0.50	No effect
		30	113	116	0.039	0.50 < *P* < 0.75	No effect
	Turmeric	10	92	116	2.769	0.05 < *P* < 0.1	(−)
		30	77	113	6.821	0.01 < *P* < 0.05	(−−)
	Yellow onion	10	92	136	8.491	*P* < 0.005	(−−)
		20	94	140	9.043	*P* < 0.005	(−−)

When the feeding tray was placed on the tank bottom, most of the juvenile *L. vannamei* approached to it right away and quickly tasted the feed. While most of the adults slowly approached the tray and stayed there for a few minutes. The results of the feeding enhancement test with *L. vannamei* are summarized in Table 7. Inclusion of extracts of *A. galanga*, *Z. officinale*, and *A. cepa* sp. at 30% concentration significantly enhanced the feeding of the adults but not of the juveniles. In the other herbs, the inclusion of extract either showed no response or produced negative effect on the feeding of the adults and juveniles. The most attractive *C. annuum* did not enhance feeding or gave negative effects on the feeding of adult at extract concentration of 5% and 10% respectively.

Discussion

Response of *M. rosenbergii* and *L. vannamei* to the herbs altered with the stage of growth. In *M. rosenbergii*, while the post larvae were strongly attracted to 11 of 16 herbs, the adults were strongly attracted to only two herbs; *H. cordata* and *M. piperita*. In *L. vannamei*, the *C. annuum* and *Z. officinale* were the strongest attractants to the juveniles but the adults were strongly attracted only to *C. annuum*. While the inclusion of *A. sativum* and *M. piperita* significantly enhanced the feeding of *M. rosenbegii,* these herbs had no effect on the feeding of *L. vannamei*. This different response with growth stages and between *M. rosenbergii* and *L. vannamei* indicates difference in the nature of their chemosensory system at different growth stages, which resulted in herb species specific effect on the feeding of the test animals.

Unexpectedly, a strong feeding attractant was not necessarily an effective feeding enhancer. In *M. rosenbergii*, the strongly attractive *H. cordata* and *A. cepa* sp. did not enhance feeding of all the stages. *M. piperita* enhanced feeding of the adults, juveniles and PL but negatively affected the feeding of the larvae of *M. rosenbergii*. Similar negative effect on the feeding of the *M. rosenbergii* larvae was also seen with the *A. sativum*. In *L. vannamei*, strong feeding attraction with *C. annuum*, and moderate feeding attraction towards *H. cordata. M. piperita* did not enhance feeding at all. In our preliminary observations, the adult and juvenile of *L. vannamei* fed well the garlic fragments scattered on the rearing tank bottom. When the garlic fragments were placed among pelleted feeds, *L. vannamei* selectively grabbed garlic fragments rather than the pelleted feeds. This result of the preliminary observations differs with the feeding enhancement tests done with inclusion of herb extracts into the pelleted feeds.

This discrepancy can be explained by the olfactory and gustatory mixture perception. Attraction is mediated by olfaction whereas feeding enhancement is mediated by gustation. Gustatory binary mixture perception can lead to several perceptual qualitative interactions that include suppression (partial masking, whereby one quality is suppressed by the other or synergy, whereby one quality is enhanced by the other) (Duchamp-Viret et al., 2003). In insects, crustaceans, and teleost fishes, although peripheral mixture interactions may occur at the receptor level, the so-called 'global' inhibition could also take place within the olfactory lobe of the brain (Desig et al., 2006). The inhibitory interneurons can synapse back onto the pre-synaptic terminals of receptor neurons and such an inhibitory system effectively inhibits the responses of receptors. The global inhibition is considered as a gain control preventing saturation of taste-evoked responses, thus allowing encoding taste over a wide dynamic range (Wachowiak et al., 1999). Obviously, the herb extracts might negatively affect the food intake of *M. rosenbergii* and *L. vannamei* to the pelleted feeds.

The administration of several herb extracts at higher concentration caused a negative effect on the feeding of *M. rosenbergii* and *L. vannamei* in this study. This could be due to some feeding deterrents contained in the herb extracts such as saponins. Saponins are bitter off-taste in humans (Günther-Jordanland et al., 2016). Saponins occur in a great number of plant species, both wild plants and cultivated crops and marine echinoderms (Puglisi et al., 2014). All the herbs used in this study contain saponins (Corea et al., 2005; Juntachote et al., 2006; Wei et al., 2009; Srividya et al., 2010; Khairul-Bariyah et al., 2012; Ahamefula et al., 2014; Kavitha et al., 2014; Saputra et al., 2016; Salomi et al., 2016; Hozayen et al., 2016; Shafique et al., 2018; Mainasara et al., 2018; Sang et al., 2019). Saponins are non-volatile compound (Lanzotti, 2006) and taste substances (Kawamura et al., 2001; Günther-Jordanland et al., 2016). The most observed effect is lowered food intake in insects (Shinoda et al., 2002; Geyter et al., 2007; Golawska, 2007; Saha et al., 2009), decapod crustaceans (Chen et al., 1996; Nagesh et al., 1999; Yeh et al., 2006) and fishes (McClintock and Baker, 1997; Bureau, et al., 1998). Saponins are feeding deterrents in the kuruma prawn (*Penaeus japonicus*) (Chen et al., 1996), and cause shell softening in the tiger shrimp (*P. monodon*) (Cruz-Lacierda, 1993; Parado-Estepa, 1995).

The feeding responses of insects are regulated by feeding stimulants and feeding deterrents. In the presence of deterrents, food ingestion does not occur despite the presence of feeding stimulants. Feeding stimulants are indispensable as behavioural releasers for insect feeding (Hisao, 1969). But the effect of herb extract on the feeding was concentration dependent in this study. In moderate inclusion levels the betel, garlic, and peppermint significantly enhanced the feeding of *M. rosenbergii* adults, juveniles and postlarvae, but at higher concentrations there was either no effect or negative response.

Soybean meal is the most widely used alternative ingredient to replace fishmeal because of its lower cost per unit protein and palatability. It is practically feasible to produce it in bulk quantities. However, commercial soy protein typically contains high levels of saponins (Oakenfull, 2001; Lucas et al., 2001). Floreto et al. (2000) examined the feasibility of soy-based diets for the pound culture of the American lobster (*Homarus americanus*) and reported that low palatability of diets containing ≥ 50% soybean meal as dietary protein was observed. Juveniles fed these diets ignored, fled or even transported the pellet out of their shelters at each feeding time. This behaviour was reported also for the juvenile marble goby (*Oxyeleotris marmorata*) where some of the juveniles expelled out the feed containing 20% soybean meal soon after ingestion (Yong et al., 2013). However, this behaviour was not observed in *L. vannamei* when test diets contained the essential amino acid (arginine, leucine, methionine) supplements, which are known to be attractants in crustaceans (Lim and Dominy, 1990). Floreto et al. (2000) noted that the fleeing behaviour exhibited by the juvenile American lobster suggests that soybean meal itself may contain deterrents to feeding. De Santis et al. (2016) reported significant variation for the saponin content among the evaluated genotypes of plants. Saponin contents also vary depending on the plant species of its origin (Jiang et al., 2018). The genotype and origin of soybean should be selected for the higher replacement of soybean meal with fishmeal.

In conclusion, following herbs are recommended as feeding attractants: *H. cordata* and *M. piperita* for *M. rosenbergii* at all stages, and *C. annuum*, *Z. officinale*, and *M. piperita* for *L. vannamei*. As feeding enhancers, following herbs are recommended: *P. betel*, *Allium sativum*, and *M. piperita* for *M. rosenbergii*, and *A. galanga*, *Z. officinale*, and *A. cepa* sp. for *L. vannamei*. Effective feeding attractants can be used in culture ponds. More long-term work is warranted to determine if inclusion of feeding enhancing herbs in artificial feed promotes growth performance of the prawn and shrimp.

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References

Ahamefula, I., Onwuka, G.I. & Chibuzo, N. (2014). Nutritional composition of turmeric (*Curcuma longa*) and its antimicrobial properties. **International Journal of Scientific & Engineering Research** 5, 1085–1089.

Bulfon, C., Volpatti, D. & Galeotti, M. (2015). Current research on the use of plant-derived products in farmed fish. **Aquaculture Research** 46, 513–551.

Bureau, D.P., Harris, A.M. & Cho, C.Y. (1998). The effects of purified alcohol extracts from soy products on feed intake and growth of Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). **Aquaculture** 161, 27–43.

Chen, J.C., Chen, K.W. & Chen, J.M. (1996). Effect of saponin on survival, growth, molting, and feeding of *Penaeus japonicus* juveniles. **Aquaculture** 144, 165–175.

Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. **Aquaculture International** 18, 403–414.

Citarasu, T., Sekar, R.R., Babu, M.M., et al. (2002). Developing *Artemia* enriched herbal diet for producing quality larvae in *Penaeus monodon*. **Asian Fisheries Science** 15, 21–32.

Corea, G., Fattorusso, E., Lanzotti, V., et al. (2005). Antispasmodic saponins from bulbs of red onion, *Allium cepa* L.Var. Tropea. **Journal of Agricultural and Food Chemistry** 53, 935–940.

Cristea, V., Antache, A., Grecu, I., et al. (2012). The use of phytobiotics in aquaculture. **Lucrări Ştiinţifica - Seria Zootehnie** 57, 250–255.

Cruz-Lacierda, E.R. (1993). Effect of rotenone and saponin on the shell quality of juvenile tiger shrimp, *Penaeus monodon*. **The Israeli Journal of Aquaculture-Bamidgeh** 25, 126–130.

De Santis, G., Maddaluno, C., D'Ambrosio, T., et al. (2016). Characterisation of quinoa (*Chenopodium quinoa* Willd.) accession for the saponin content in Mediterranean environment. **Italian Journal of Agronomy** 11, 277–281.

Desig, N., Giurfa, M., Lachnit, H., et al. (2006). Neural representation of olfactory mixtures in the honeybee antennal lobe. **European Journal of Neuroscience** 24, 1161–1174.

Duchamp-Viret, P., Duchamp, A. & Chaput, M.A. (2003). Single olfactory sensory neurons simultaneously integrate the compound of an odour mixture. **European Journal of Neuroscience** 18, 2690–2691.

Floreto, E.A.T., Bayer, R.C. & Brown, P.B. (2000). The effect of soy-based diet, with and without amino acid supplementation, on growth and biochemical composition of juvenile America lobster, *Homarus americanus*. **Aquaculture** 189, 211–238.

Geyter, E.D., Lambert, F., Greelen, D., et al. (2007). Novel advances with plant saponin as natural insecticides to control pest insects. **Pest Technology** 1, 97–105.

Golawska, S. (2007). Deterrence and toxicity of plant saponins for the pea aphid *Acyrthosiphon pisum* Harris. **Journal of Chemical Ecology** 33, 1598–1606.

Günter-Jordanland, K., Dawidi, C., Dietz, M., et al. (2016). Key phytochemicals contributing to the bitter off-taste of oat (*Avena sativa* L.). **Journal of Agriculture and Food Chemistry** 64, 9639–9652.

Harikrishnnan, R., Balasundaram, C. & Heo, M.S. (2011). Impact of plant products on innate and adaptive immune system of culture finfish and shellfish. **Aquaculture** 317, 1–15.

Hisao, T.H. (1969). Chemical basis of host selection and plant resistance in oligophagous insects. **Entomologia Experimentalis et Applicata** 12, 777–788.

Hozayen, W.G., El-Desouky, M.A., Soliman H.A., et al. (2016). Antiosteoporotic effect of *Petroselinum crispum*, *Ocimum basilicum* and *Cichorium intybus* L. in glucocorticoid-induced osteoporosis in rats. **BMC Complementary and Alternative Medicine** 16, 165–176.

Jiang, X., Hansen, H.C.B., Strobel, B.W., et al. (2018). What is the aquatic toxicity of saponin-rich plant extracts used as biopesticides? **Environmental Pollution** 236, 416–424.

Juntachote, T., Berghofer, E., Bauer, F., et al. (2006). The application of response surface methodology to the production of phenolic extracts of lemon grass, galangal, holy basil and rosemary. **International Journal of Food Science and Technology** 41, 121–133.

Kavitha, K., Sangeetha, K.S.S., Sujatha, K., et al. (2014). Phytochemical and Pharmacological profile of *Justica gendarussa* Burm f.–review. **Journal of Pharmacy Research** 8, 990–997.

Kawamura, G., Bagarinao, U.B., Senimann, N.S., et al. (2018). Comparative morphology and function of feeding appendages in food intake behaviour of the whiteleg shrimp, *Litopenaeus vannamei*, and the giant freshwater prawn, *Macrobrachium rosenbergii*. **Borneo Journal of Marine Science and Aquaculture** 2, 26–39.

Kawamura, G., Bagarinao, U.B. & Yong, A.S.K. (2017). Sensory systems and feeding behaviour of the giant freshwater prawn, *Macrobrachium rosenbergii*, and the marine whiteleg shrimp, *Litopenaeus vannamei*. **Borneo Journal of Marine Science and Aquaculture** 1, 80–91.

Kawamura, G. Yacoob, S.Y. & Anraku, K. (2001). The gustatory response of the Nile tilapia (*Oreochromis niloticus*) to saponin. **UPV Journal of Natural Sciences** 6, 106–110.

Khairul-Bariyah, S., Ahmed, D. & Ikram, M. (2012). *Ocimum basilicum*: A review on phytochemical and pharmacological studies. **Pakistan Journal of Chemistry** 2, 78–85.

Lanzotti, V. (2006). The analysis of onion and garlic. **Journal of Chromatography A** 1112, 3–22.

Lim, C. & Dominy, W. (1990). Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp (*Penaeus vannamei*). **Aquaculture** 87, 53–63.

Lucas, E.A., Khalil, D.A., Daggy, B.P., et al. (2001). Ethanol-extracted soy protein isolate does not modulate serum cholesterol in golden Syrian hamsters: a model of postmenopausal. **Journal of Nutrition** 131, 211–214.

Mainasara, M.M., Bakar, M.F.A., Waziri, A.H., et al. (2018). Comparison of phytochemical, proximate and mineral composition of fresh and dried peppermint (*Mentha piperita*) leaves. **Journal of Science and Technology** 10, 85–91.

McClintock, J.B. & Baker, B.J. (1997). Palatability and chemical defense of eggs, embryos and larvae of shallow-water Antarctic marine invertebrates. **Marine Ecology Progress Series** 154, 121–131.

Nagesh, T.S., Jayabalan, N., Mohan, C.V., et al. (1999). Survival and histological alterations in the juvenile tiger shrimp *Penaeus monodon* exposed to saponin. **Aquaculture International** 7, 159–167.

Oakenfull, D. (2001). Soy protein, saponins and plasma cholesterol. **Journal of Nutrition** 131, 2971–2972.

Olusola, S.E., Emikpe, B.O. & Olaifa, F.E. (2013). The potentials of medicinal plant extracts as bio-antimicrobials in aquaculture. **International Journal of Medicinal Aromatic Plants** 3, 404–412.

Parado-Estepa, F.D. (1995). Research on crustaceans. In: **Towards Sustainable Aquaculture in Southeast Asia and Japan.** (T.U. Bagarinao & E.E.C. Flores, eds.), pp 187–198. SEAFDEC Aquaculture Department, Iloilo, Philippines.

Puglisi, M.P., Sneed, J.M., Sharp, K.H., et al. (2014). Marine chemical ecology in benthic environmentws. **Natural Product Reports** 31, 1510–1553.

Rebecca, A.A. & Bhavan, P.S. (2014). Growth promotion and survival enhancement of fish freshwater prawn *Macrobrachium rosenbergii* post larvae fed with *Allium sativum*, *Zingiber officinale* and *Curcuma longa*. **International Journal of Pure and Applied Zoology** 2, 138–149.

Reverter, M., Bontemps, N., Lecchini, D., et al. (2014). Use of plant extract in fish aquaculture as an alternative to chemotherapy; current status and future perspectives. **Aquaculture** 433, 50–61.

Saha, S., Walia, S., Kumar, J., et al. (2009). Screening for feeding deterrent and insect growth regulatory activity of triterpenic saponins from *Diploknema butyracea* and *Sapindus mukorossi.* **Journal of Agricultural and Food Chemistry** 58, 434–440.

Salomi, M.V. & Manimekalal, R. (2016). Phytochemical analysis and antimicrobial activity of four different extracts from the leaves of *Murraya koenigii*. **International Journal of Current Microbiology and Applied Sciences** 5, 875–882.

Sang, J., Rimal, S. & Lee, Y. (2019). Gustatory receptor 28b is necessary for avoiding saponin in *Drosophila melanogaster*. **EMBO Reports** 20, e47328.

Sanudin, N., Tuzan, A.D., Kawwamura, G., et al. (2015). Effect of different lighting condition on feeding activity and eye adaptation of post larvae *Penaeus vannamei*. **Journal Teknologi** 77, 33, 1–6.

Saputra, A., Andayani, S. & Nursyam, H. (2016). Total Quantity of phenol and isolation methanol tannin extract of red betel leaf (*Piper crocatum*). **International Journal of PharmTech Research** 9, 146–153.

Shafique, S., Shafique, S. & Ahmad, A. (2018). Biochemical and molecular screening of varieties of chili plants that are resistant against Fusarium wilt infection. **European Journal of Microbiology and Immunology** 8, 12–19.

Shinoda, T., Nagao, T., Nakayama, M., et al. (2002). Identification of a triterpenoid saponin from a crucifer, *Barbarea vulgaris*, as a feeding deterrent to the diamondback moth, *Plutella xylostella*. **Journal of Chemical Ecology** 28, 587–599.

Srividya, A.R., Dhanabal, S.P., Misra, V.K., et al. (2010). Antioxidant and antimicrobial activity of *Alpinia officinarum*. **Indian Journal of Pharmaceutical Sciences** 72, 145–148.

Vaseeharan, B. & Thaya, R. (2014). Medicinal plant derivatives as immunostimulants: an alternative to chemotherapeutics and antibiotics in aquaculture. **Aquaculture International** 22, 1079–1091.

Venkatramalingam, K., Christopher, J.G. & Citarasu, T. (2007). *Zingiber officinalis* and herbal appetizer in the tiger shrimp *Penaeus monodon* (Fabricius) larviculture. **Aquaculture Nutrition** 13, 439–443.

Wachowiak, M. & Cohen, L.B. (1999) Presynaptic inhibition of primary olfactory afferents, C.H. mediated by different mechanisms in lobster and turtle. **Journal of Neuroscience** 19, 8808–8817.

Wei, O., Yu, L.J. & MengRan, L. (2009). Chemical constituents from *Houttuynia cordata*. **Chinese Journal of Natural Medicines** 7, 425–427.

Yeh, S.P., Liu, C.H. Sung, T.G., et al. (2006). Effect of saponin on hematological and immunological parameters of the giant freshwater prawn, *Macrobrachium rosenbergii*. **Aquaculture** 261, 1432–1439.

Yong, A.S.K., Kawamura, G., Lim, L.S., et al. (2018). Growth performance and survival of giant freshwater prawn *Macrobrachium rosenbergii* larvae fed coloured feed. **Aquaculture Research** 49, 2815–2821.

Yong, A.S.K., Ooi, S.Y. & Shapawi, R. (2013). The utilization of soybean meal in formulated diet for marble goby, *Oxyeleotris marmoratus*. **Journal of Agriculture Science** 5, 139–149.