ผลของสารสกัดหยาบจากลูกใต้ใบ (*Phyllanthus urinaria* Linn.) ต่อการเจริญเติบโต การใช้ประโยชน์จากอาหาร และอัตราการรอดตายของกุ้งขาวแวนนาไม (*Litopenaeus vannamei* Boone)

Effects of Crude Extract of *Phyllanthus urinaria* Linn. on Growth Performance, Feed Utilization and Survival Rate of Pacific White Shrimp (*Litopenaeus vannamei* Boone)

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Received: 10 May 2018, Revised: 19 October 2018, Accepted: 15 January 2019

บทคัดย่อ

ลูกใต้ใบชนิด *Phyllanthus urinaria* Linn. เป็นพืชสมุนไพรชนิดหนึ่งที่มีสรรพคุณทางเภสัชอย่างหลากหลาย ได้ถูกนำมาผสมกับอาหารกุ้งเพื่อศึกษาประสิทธิภาพที่มีต่อการเจริญเติบโต การใช้ประโยชน์จากอาหาร และอัตราการรอดตายของกุ้งขาวแวนนาไม (*Litopenaeus vannamei* Boone) โดยเริ่มการทดลองด้วยการให้กุ้งกินอาหารที่มีสารสกัดหยาบจากลูกใต้ใบในระดับความเข้มข้นที่แตกต่างกัน ได้แก่ 0, 1, 10, 100, 1,000 และ 10,000 ppm เป็นระยะเวลา 56 วัน จากนั้นพิจารณาผลของปัจจัยชี้วัดที่สนใจ ซึ่งหลังจากสิ้นสุดการทดลอง พบว่ากุ้งที่กินอาหารผสมสารสกัดหยาบจากลูกใต้ใบที่ความเข้มข้น 10,000 ppm แสดงค่าของปัจจัยชี้วัดทางด้านการเจริญเติบโตและประสิทธิภาพการใช้ประโยชน์จากอาหารมากกว่ากุ้งที่กินอาหารทดลองอื่นเล็กน้อย แต่มีความแตกต่างทางสถิติ ($p < 0.05$) เฉพาะกับชุดควบคุมเท่านั้น ในส่วนของอัตราการรอดตายของกุ้งที่กินอาหารในแต่ละชุดการทดลองพบว่าไม่มีความแตกต่างทางสถิติ ($p \geq 0.05$) จากผลการทดลองข้างต้น สามารถสรุปได้ว่าสารสกัดหยาบจากลูกใต้ใบชนิด *P. urinaria* มีผลในการเสริมการเจริญเติบโตได้เล็กน้อย แต่ไม่มีผลต่อการเพิ่มอัตราการรอดตายของกุ้งขาวแวนนาไม

คำสำคัญ: สารสกัดหยาบ, ลูกใต้ใบชนิด *Phyllanthus urinaria*, กุ้งขาวแวนนาไม, การเจริญเติบโต, อัตราการรอดตาย

คณะวิทยาศาสตร์และเทคโนโลยีการประมง มหาวิทยาลัยเทคโนโลยีราชมงคลศรีวิชัย อำเภอสิเกา จังหวัดตรัง 92150
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ABSTRACT

Phyllanthus urinaria Linn., one of the medicinal plants possessing various biomedical effects, had been applied in shrimp feed in order to study its efficacy on growth performance, feed utilization and survival rate of Pacific white shrimp (*Litopenaeus vannamei* Boone). Initially, the experiment was carried out by feeding shrimp for 56 days with diets containing *P. urinaria* crude extract at the levels of 0, 1, 10, 100, 1,000 and 10,000 ppm, and then the results of interested parameters were investigated. At the end of the feeding trial, the obtained results indicated that shrimp fed with the diet containing 10,000 ppm of *P. urinaria* crude extract slightly showed higher rate of growth performance and feed utilization than the others; however, the significant difference ($p < 0.05$) was only found on the controlled group. In case of survival rate of shrimp ingesting on each experimental diet, there were no significant differences ($p \geq 0.05$) among various treatments evaluated in this parameter. All in all, it can be concluded that *P. urinaria* crude extract has a slight effect on growth promotion, but it does not affect to increment in the survival rate of Pacific white shrimp.

Key words: crude extract, *Phyllanthus urinaria*, *Litopenaeus vannamei*, growth performance, survival rate

INTRODUCTION

Nowadays, Pacific white shrimp (*Litopenaeus vannamei* Boone) is one of economically penaeid shrimp usually cultured in several countries, especially in South America and Southeast Asia. Because of its economic value, this shrimp species has been cultured, overspreading the coastal area and mangrove swamp, bringing about environmental problems. Moreover, the intensified condition of this shrimp aquaculture causes crowding stress stimulation, leading to malfunction of immunity and subsequent occurrence of disease outbreak which is a major constraint of shrimp aquaculture, resulting in significant socio-economic losses in affected areas (Kumar *et al*., 2014).

To protect from infectious disease and promote shrimp growth, chemicals and drugs usage has proliferated among shrimp farms. This practice leads to diverse problems due to the use of drugs and chemicals results in generation of toxic residues causing risks to the environment, animals and consumers (Jones *et al*., 2004). Moreover, the over-usage of antibiotic drugs causes the appearance of antibiotic-resistant strains of bacteria, leading to subsequent diverse problems which are complicated to handle (Waldvogel, 2004). Besides, shrimp products may be rejected during export due to the accumulation of antibiotics is over-regulated levels (Alderman and Hasting, 1998).

To decrease aforementioned problems, the alternative natural ways for shrimp aquaculture should be actualized, for instance, the replacement of antibiotics with bioactive compounds derived from medicinal plants and algae for bacteriostatic and bactericidal purposes. In this case, some herbal plants possess the biological substances which can officiate as growth promoters, immunostimulants, antioxidants, antiviral agents and so on (Direkbusarakom, 1998; Citarasu, 2010; Chitmanat, 2013). Interestingly, one of the medicinal plants in the Phyllanthaceae family such as the *Phyllanthus* plants possess numerous bioactive phytochemicals including tannins, flavonoids and lignans. Tannins present in the form of gallotannin such as amariin, phyllanthusiin D, geraniin, corilagin and elecarpusin. Flavonoids present in the form of rutin and quercetin-3-0-glucoside. Lignans present in the form of phyllantin and hypophyllanthin (Gruenwald *et al*., 2000). Furthermore, the plants in this genus also contain alkaloids, saponins and glycosides (Akin-Osanaiye *et al*., 2011). The phytochemicals in *Phyllanthus* plants can present the properties of anti-hepatitis (Wang *et al*., 1995; Ram, 2001; Ahmed *et*

al., 2002), antioxidant (Raphael *et al*., 2002; Pinitsoontorn *et al*., 2012; Maity *et al*., 2013), anti-inflammation (Kiemer *et al*., 2003; Dang *et al*., 2011), and apoptosis in tumor cells (Huang *et al*., 2003).

Consequently, the purpose of this investigation was to evaluate the efficacy of *P. urinaria*, one of the *Phyllanthus* plants, on growth performance, feed utilization and survival rate of Pacific white shrimp (*L. vannamei*) in order to acquire a natural alternative approach for sustainable shrimp aquaculture in Thailand.

MATERIALS AND METHODS

1. Experimental animals

Healthy Pacific white shrimp (*L. vannamei*) in post larvae 15 stage were obtained from a commercial farm in Thailand, acclimated in 8,000-L concrete ponds (250 shrimp/m^3) with suitable condition of ambient environment, and fed with commercial controlled diet, 4 meals daily for 3 months in order to increase shrimp size to around 7-8 g used in this trial. When grown to the usable size, shrimp were graded and transferred to black plastic tanks containing 400-L of brackish water (15 ppt) for setting the experiment.

2. Plant material and extraction

P. urinaria was collected from the planting grounds in Rajamangala University of Technology Srivijaya, Sikao district, Trang province, Thailand. Then, whole plants were thoroughly washed with distilled water to remove dirt and contaminants, and then oven-dried at 50 ^oC. The dried plant was chopped into small pieces, finely ground with an electric blender, and then macerated in 99.9 % ethanol for 7 days. The derived crude extract was filtered and concentrated at 40 ^oC by using a rotary evaporator under low pressure, then stored at -20 ^oC until used (modified method of Poompachee and Chudapongse, 2012).

3. Experimental diet preparation

The commercially formulated feed was thoroughly mixed with *P. urinaria* crude extract in different evaluated levels by top-coating, then air-dried and re-coated with fish oil at 1.5 % of shrimp feed so as to protect dissolution of effective substance from pellets and to enhance shrimp palatability. The diets were air-dried once again and stored at -20 ^oC until used. All experimental diets were prepared weekly.

4. Experimental design for feeding trial

The experiment was conducted as complete randomized design (CRD) in which every treatment was randomly assigned to different plastic tanks. In this trial, six treatments with triplicate, divided through various levels of *P. urinaria* crude extract supplemented to commercial diet at the concentration levels of 0 ppm (control), 1 ppm (0.001 g/kg diet), 10 ppm (0.01 g/kg diet), 100 ppm (0.1 g/kg diet), 1,000 ppm (1 g/kg diet) and 10,000 ppm (10 g/kg diet). In each replicate, 50 shrimp of equal size were stocked in black plastic tanks containing 400 L of seawater and fed with experimental diets, 4 meals daily for 56 days. At first, daily feeding rate was 6% of total body weight, after that it was daily re-adjusted according to feed intake of shrimp in each tank. In regard to trial system management, the cultured water was monitored and adjusted to maintain an appropriate condition through an installation of aeration and water circulation system which was cleaned daily. At last, all data form feeding trials were recorded for assessment of growth performance, feed utilization and survival rate of experimental shrimp.

5. Parameters assessment from feeding trial

After 56-days feeding period, there are three parts of data collection for evaluating the efficacy of *P. urinaria* crude extract on shrimp growth performance, feed utilization and survival rate. The first part, individual weight of shrimp in each treatment was recorded at the beginning and the end of the feeding trial in order to determine growth parameters including average body weight (g), percent weight gain (%), specific growth rate (%/day), and average daily growth (g/individual/day). The second part, amount of diet used in each treatment was daily recorded for calculation of feed conversion ratio, feed efficiency

ratio and feed intake (g/individual). In the final part, the survival rate of shrimp was calculated through enumeration of remaining shrimp in the tank. In brief, all parameters were calculated using the equations as follows:

Average body weight (g)
= final biomass / final shrimp number

Specific growth rate (%/day)
= [natural log (final mean biomass) - natural log (initial mean biomass)] / duration of feeding (days) × 100

Weight gain (%)
= [(final mean biomass - initial mean biomass) / initial mean biomass] × 100

Average daily growth (g/individual/day)
= (final mean biomass - initial mean biomass)/ duration of feeding (days)

Feed conversion ratio
= total feed intake / shrimp weight gain

Feed efficiency ratio
= shrimp weight gain / total feed intake

Feed intake (g/individual)
= total feed intake / number of shrimp

Survival rate (%)
= (initial shrimp number / final shrimp number) × 100

6. Statistical analysis

All data obtained from this experiment were statistically analyzed using a one-way analysis of variance (ANOVA) in the statistical program (IBM SPSS Statistics 21), and Duncan's multiple range test (DMRT) was used to determine the significant differences between the means. The comparisons were done at a significant level of 0.05 (Duncan, 1995).

RESULTS AND DISCUSSION

To date, the research steps of medicinal plants applied in aquaculture comprise of *in vitro* studies focused on an investigation of plant biological activities and bioactive molecules, and *in vivo* studies focused on plant effect on aquatic animal physiology and potential treatment in aquaculture (Reverter *et al*., 2017). Many scientific studies have revealed that herbal plants containing various active compounds could be used in aquaculture because they produce several effects providing benefits for aquatic animals such as disease agents elimination, growth promotion, immunostimulation, and so on (Citarasu, 2010; Chakraborty and Hancz, 2011). In case of the effects on growth performance of aquatic animals, diverse medicinal plants have been studied, for instance, the study of Punitha *et al*. (2008) revealed that diet supplemented with a mixture of methanolic herb extracts including *Cynodon dactylon*, *Piper longum*, *Phyllanthus niruri*, *Tridax procumbens* and *Zingiber officinalis*, could increase the final weight result of grouper (*Epinephelus tauvina*) at 41% higher than controlled fish. Then, Prasad and Mukthiraj (2011) reported that the diet containing *Andrographis paniculata* extract could increase growth and immune functions of *Oreochromis mossambicus*. In addition, the report of Putra *et al*. (2013) specified that grouper (*Epinephelus coioides*) fed with a diet containing ethanolic extract of *Sauropus androgynous* exhibited the results on increment of growth and feed utilization. These aforementioned results may be caused by active compounds of medicinal plant extract which could improve digestibility and availability of nutrients, resulting in feed conversion increment, leading to more protein synthesis which is necessary for growth.

In this study, *P. urinaria*, one of beneficial medicinal plants, was used for studying its effects on growth performance, feed utilization and survival rate of Pacific white shrimp (*L. vannamei*), and the derived results revealed that the use of this medicinal plant could slightly promote growth of tested shrimp, but it did not affect to increment of shrimp survival rate.

As specified in Table 1, the obtained results revealed that shrimp fed with the experimental diet containing 10,000 ppm (10 g/kg diet) of *P. urinaria* crude extract exhibited the highest values of all growth parameters analyzed when compared to shrimp fed with the other experimental diets. In this case, the shrimp fed with this diet showed a significant difference ($p < 0.05$) in average body weight (g) when compared to shrimp fed with controlled diet and diets containing 1 and 100 ppm of *P. urinaria* crude extract. Moreover, it also showed significant differences ($p < 0.05$) in percent weight gain (%), specific growth rate (%/day) and average daily growth (g/individual/day) when compared to shrimp fed with controlled diet.

In terms of the parameters for estimation of feed utilization of shrimp fed with several experimental diets containing graded levels of *P. urinaria* crude extract, the derived results specified in Table 2 revealed that feed conversion ratio and feed efficiency ratio of all treatments evaluated had presented a similar trend with indication of significant differences ($p < 0.05$) when the results obtained from shrimp fed with experimental diet containing 10,000 ppm of *P. urinaria* crude extract compared to the results got from shrimp fed with controlled diet. In case of feed intake, there were no significant differences ($p \geq 0.05$) among controlled and treatment groups in this parameter estimated.

Moreover, shrimp fed with diet containing 10,000 ppm of *P. urinaria* crude extract had shown the survival rate higher than shrimp fed with controlled and the other experimental diets; however, the significant differences ($p \geq 0.05$) among various treatments evaluated did not present in this parameter (Table 2).

In shrimp, the growth performance depends on the fitness of heptopancreas because this organ contains fibriller cells (F-cells) and blister-like cells (B-cells) which produce and secrete the enzymes essential for digestion in shrimp foregut (Gibson and Barker, 1979; Icely and Nott, 1992; Sousa and Petriella, 2000; Sousa *et al*., 2005). Then, the nutrients obtained from digestion have been absorbed and stored as lipid droplet in the cytoplasm of resorption cell (R-cells) located in hepatopancreas (Loizzi, 1971; Vogt *et al*., 1989; Johnston *et al*., 1998). Therefore, this organ is a source of energy and nutrients which are necessary for the growth of shrimp through ecdysis. Moreover, this organ is responsible for detoxification and metabolic waste excretion carried out by F-cells (Vogt and Quinitio, 1994).

As above informations, shrimp fed with the diet containing 10,000 ppm of this herbal plant extract had shown the results of all parameters investigated slightly better than shrimp fed with the diet containing the other concentration levels tested and controlled. This is because *P. urinaria* crude extract possesses anti-hepatotoxic properties contributing to hepatocyte nourishment and hepatitis protection (Wang *et al*., 1995). It is beneficial for a function of hepatopancreas on production of enzymes used for supporting digestibility. Furthermore, the extract of this medicinal plant could protect shrimp heptopancreas from pathogenic infection, for example, previous research of Direkbusarakom *et al*. (1995) found that 1 mg/ml of *P. urinaria* crude extract possessed antiviral properties against Yellow head virus (YHV) causing necrosis of shrimp lymphoid organ. Moreover, 10 ppm of this medicinal extract showed the antibacterial activity against *Vibrio* spp. usually infected in shrimp (Direkbusarakom, 1998). Therefore, abovementioned properties may promote shrimp health and fitness, resulting in promotion of growth performance.

All in all, *P. urinaria* may be a potential source of natural growth promoter for sustainable shrimp aquaculture. Nevertheless, its phytochemicals concerning growth enhancement should be further studied and separated from crude extract so that it can be more effectively used with lower concentration which is more worthwhile.

Table 1 Effect of *P. urinaria* extract-supplemented diets on growth performance of Pacific white shrimp after 56-days feeding period (Mean ± SD).

Concentrations of *P. urinaria* extract	Average body weight (g)		Weight gain (%)	Specific growth rate (%/day)	Average daily growth (g/individual/day)
	Initial	Final			
0 ppm	7.56±0.65^{a}	23.75±2.41^{c}	213.96±3.17^{b}	2.04±0.02^{b}	0.29±0.01^{b}
1 ppm	7.57±0.66^{a}	24.05±2.40^{bc}	217.92±5.25^{ab}	2.07±0.03^{ab}	0.29±0.01^{ab}
10 ppm	7.56±0.63^{a}	24.22±2.30^{abc}	220.26±13.12^{ab}	2.08±0.07^{ab}	0.30±0.02^{ab}
100 ppm	7.57±0.66^{a}	24.17±2.43^{bc}	219.36±4.41^{ab}	2.07±0.02^{ab}	0.30±0.01^{ab}
1,000 ppm	7.56±0.66^{a}	24.44±2.41^{ab}	223.14±4.32^{ab}	2.09±0.02^{ab}	0.30±0.01^{a}
10,000 ppm	7.57±0.65^{a}	24.77±2.08^{a}	227.36±5.11^{a}	2.12±0.03^{a}	0.31±0.01^{a}

Note: Mean values within the same column sharing the different superscript were significantly different at $p < 0.05$.

Table 2 Effect of *P. urinaria* extract-supplemented diets on feed utilization and survival rate of Pacific white shrimp after 56-days feeding period (Mean ± SD).

Concentrations of *P. urinaria* extract	Feed conversion ratio	Feed efficiency ratio	Feed intake	Survival rate (%)
0 ppm	1.84±0.16^{b}	0.55±0.05^{b}	28.63±1.26^{a}	92.67±4.16^{a}
1 ppm	1.78±0.20^{ab}	0.57±0.06^{ab}	28.34±2.14^{a}	94.00±6.93^{a}
10 ppm	1.71±0.09^{ab}	0.58±0.03^{ab}	28.87±0.17^{a}	95.33±1.15^{a}
100 ppm	1.75±0.07^{ab}	0.57±0.03^{ab}	28.15±0.47^{a}	94.00±2.00^{a}
1,000 ppm	1.62±0.07^{ab}	0.62±0.03^{a}	27.13±0.68^{a}	98.00±2.00^{a}
10,000 ppm	1.59±0.06^{a}	0.63±0.03^{a}	27.05±0.66^{a}	98.00±2.00^{a}

Note: Mean values within the same column sharing the different superscript were significantly different at $p < 0.05$.

CONCLUSION

P. urinaria crude extract with the concentration level of 10,000 ppm can slightly promote growth performance of Pacific white shrimp, but it does not affect to increment of shrimp survival rate. This medicinal plant may be a promising source of bioactive compounds acting as growth promoters. The use of this plant extract may be an alternative way for sustainable shrimp aquaculture without chemical and drug usage leading to further negative effects.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Rajamangala University of Technology Srivijaya for supporting the research fund.

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