

Phytochemical Characterization of *Alternanthera sessilis* and Assessment of its Growth Promoting Potential on the Freshwater Prawn *Macrobrachium rosenbergii*

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Abstract: This study was aimed to understand the primary and secondary phytochemical profiles of *Alternanthera sessilis* and its influence on growth promotion of *Macrobrachium rosenbergii*. Complete extraction of *A. sessilis* leaf using petroleum ether (non-polar), followed by acetone (middle polar) and ethanol (polar) was performed first. Then the primary and secondary phytochemical profiles were studied. *A. sessilis* contains alkaloids, terpenoids, flavonoids, tannins, saponins, polyphenols, cardiac glycosides and quinones. GC-MS spectrum of *A. sessilis* revealed the presence of 17 secondary phytochemicals, totally. Among these, 5 compounds have possessed bioactive properties. Artificial feeds were prepared with incorporation of three different concentrations (0.1, 0.5 and 1%) of ethanolic extract of *A. sessilis* and fed to *M. rosenbergii* PL for 90 days. Among the three levels, 1.0% of ethanolic extract of *A. sessilis* incorporated feed produced the best growth performance, by showing the highest survival rate, weight gain and contents of total protein, amino acid, carbohydrate, lipid and ash, when compared with control, which was confirmed by the lowest food conversion rate. Thus, the present work recommends incorporation of *A. sessilis* as feed additive to attain sustainable culture of *M. rosenbergii*.

Keywords: Herbal extraction, Active principles, Prawn culture.

1. INTRODUCTION

India is making rapid strides with its blue revolutions and holds second position in the world in aquaculture production, producing 7.76% in 2013, yet far below from China, which holds number one position with almost 61.35% production. Crustacean, which includes crabs, lobsters, crayfish, prawns, shrimps etc. consisted of 50,000 to 67,000 species and has become one of the fastest growing animal production sectors worldwide. Among these about 4048 species of shrimps and prawns are known to the world (Radhakrishnan *et al.*, 2012).

In India, the freshwater prawn fishery of the rivers Krishna, Godavari, Parkas, Ganges, Mahanadi, Hooghly and Cauvery indicate that the major commercial species are *Macrobrachium rosenbergii* and *Macrobrachium malcolmsonii*. Their aquacultures have become more popular due to nutritious delicacy for mankind, and earn valuable foreign exchange because of good demand in the international markets. *M. rosenbergii* has shorter life history than that of marine shrimps, and it is a fast growing species, reaches considerable size within short period under intensive culture. The nutritive value of any crustacean depends up on its protein, amino acid, lipid, fatty acid, carbohydrate, vitamins and minerals (Bhavan *et al.*, 2010). Other Palemonid prawns, such as *Macrobrachium nobilii*, *Macrobrachium lamarrei*, *Macrobrachium scabriculum*, *Macrobrachium birmanicumchoprai*, *Macrobrachium mirabile*, *Macrobrachium rude*, *Macrobrachium hendersonii*, *Macrobrachium villosimanus* and *Macrobrachium hendersonii* also support local fisheries (Chandrasekaran and Sharma, 1997; Mariappan *et al.*, 2003).

Feed is the major operational cost for most aquaculture enterprises (D'Abramo and Sheen, 1994). The formulation of well-balanced diets and their adequate feeding are the most important for successful aquaculture. The formulation of feeds by using locally available low cost agricultural and animal husbandry byproducts (such as soy bean meal, rice brawn, wheat brawn and ground nut oilcake,

fishmeal, poultry waste, blood meal and prawn meal etc.) have crucial role in aquaculture industry (Mitra *et al.*, 2005; Langer *et al.*, 2011).

Currently, researchers have focused on herbal plants, greens, algae, vegetable and fruit wastes as low cost food supplements for better growth and survival of *Macrobrachium* (Rebecca and Bhavan, 2011; Shanthi *et al.*, 2012; Bhavan and Radhakrishnan, 2012; Radhakrishnan *et al.*, 2013; Aarumugam *et al.*, 2013; Bhavan *et al.*, 2011, 2012, 2013a,b, and 2014a,b; Dhanalakshmi *et al.*, 2016; Rajkumar *et al.*, 2017). In this line, *Alternanthera sessilis* a common pan-tropical weed of shady, damp soils in cultivable and waste land has been chosen for screening of its phytochemicals, and whether can it be used as a feed additive for *Macrobrachium* culture. *A. sessilis* is a popular leafy vegetable, and is used as a folk medicine in Southeast Asia. For example, the juice of this plant is an ingredient in medicinal hair oils (Gupta, 2014). It serves as good fodder as well. The genus, *Alternanthera* has possessed anti-inflammatory, antimicrobial, antiviral, antitumor, anti-malarial, and anti-ulcer properties (Guerra *et al.*, 2003; Hilou *et al.*, 2006). In this study, *A. sessilis* leaf was subjected to extraction with three different solvents, such as petroleum ether, acetone and ethanol for determining its phytochemicals. The ethanolic extract of *A. sessilis* was incorporated with artificial feed formulated, and its growth promoting potential was assessed on *M. rosenbergii* PL.

2. MATERIALS AND METHODS

2.1. Preparation of *A. sessilis* Leaf Extract

The medicinal herb, *A. sessilis* was collected from Bharathiar University campus, Coimbatore, Tamil Nadu, India. The herb was thoroughly washed with freshwater, blotted and spread out and dried for two weeks at room temperature. Shade dried herb was ground to fine powder. *A. sessilis* powder (50 g) was packed with Whatmann No. 1 filter paper and put into Soxhlet apparatus, successively and sequentially extracted with 300 ml (1:6 w/v) of individual solvent (petroleum ether, acetone and ethanol) for 6-9 h each (30 to 36 cycles) based on the polarity (non-polar to polar) until a clear colorless solution was obtained. The extract obtained was filtered by using double layer muslin cloth, and concentrated at 40-50°C using rotary vacuum evaporator (ROTAVAP) with ultra-cryostat and dried at 40°C under hot air oven. The dark green, gummy solid obtained was used for further investigation.

2.2. Qualitative Analysis of Phytochemicals

The presence of primary phytochemical compounds, such as presence of alkaloids, terpenoids, flavonoids, tannins, polyphenols, saponins, cardiac glycosides and quinones was screened by adopting the standard qualitative procedures (Trease and Evans, 1989).

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Each extract of *A. sessilis* was subjected to GC-MS (The Trace GC Ultra and DSQII model MS with inbuilt pre-filter to reduce the neutral particles, Thermo Fisher Scientific Company Pvt. Ltd.) analysis for identification of different secondary phytochemical compounds with following working conditions [Injector port temperature: 250 °C; Interface temperature: 250 °C, and source was maintained at 200 °C; The oven temperature: programmed as variable, 70 °C for 2 mins, 150 °C @ 8 °C /min, up to 260 °C @ 10 °C /min; injector used was splitless mode; Column: The DB-35 MS Nonpolar (Agilent Co., USA) with dimensions of 0.25 mm OD x 0.25 µm ID x 30 metres length; Carrier gas: Helium was used at 1 mL/min; Scan: 50-650 Da; Motor vacuum pressure: <40; Ionization energy: -70eV].

Peaks resolved with relative abundance of 0-100 were considered as major compounds. To show the minor peaks, the chromatogram was magnified. Identification of various components present in each extract was done by comparison of retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. National Institute Standard and Technology (NIST4) and WILEY9 (Vandendool and Kratz, 1963) on-line library source was also used for matching the identified components.

2.4. Preparation of Feeds

The following branded feed basal ingredients (BI) were used to formulate the experimental feed. For protein source fish meal (25%), groundnut oilcake (25%) and soybean meal (25%) were taken. For carbohydrate source wheat bran (10%) was taken. For lipid source Sunflower oil (2%) was taken.

Tapioca flour (5%) and egg albumin (7%) were used as binding agents. The powdered basal ingredients such as fish meal, groundnut oilcake, soy bean meal, wheat bran were thoroughly mixed. Then sterilized water was used to prepare the dough, which was steam cooked and cooled at room temperature. Then Sunflower oil was added with the dough and mixed well. The ethanolic extract of *A. sessilis* was prepared freshly and incorporated with the dough at three different concentrations 0.1%, 0.5% and 1.0%. Then tapioca flour and egg albumin were added and mixed well. Finally, 1% of vitamin B-complex forte with vitamin C (BECOSULES® CAPSULES, Pfizer Ltd., Navi Mumbai, India) and a pinch of salt (Approximately, 100mg) were also added and thoroughly mixed. Double distilled water (ddH₂O) was adequately added for maintaining the mixer in moist and paste form. This was pelletized in a manual pelletizer fixed with 3 mm diameter mesh. The pellets were immediately dried in a thermostatic oven at 37-40 °C for one hour to quickly reduce the moisture in order to keep them intact, and then shade dried until they reached constant weight. To maintain its brittleness and prevent fungal attack they were kept in air tight jars, stored at -20 °C and used afresh. The proximate composition of organic matters present in the basal diet formulated was determined by adopting the methodology of Castell and Tiews (1980) as given in AOAC (1995) manual, which contains 40.50% crude protein, 5.60% crude fat, 3.40% crude fibre, 9.00% total ash, 8.60 % moisture, 32.90% carbohydrate (total nitrogen free extract) and gross energy, 4281 kcal/kg.

2.5. Feeding Trial

The post larvae (PL-30) of the freshwater prawn, *Macrobrachium rosenbergii* were procured from nursery pond at Singanallur (Lat.10.99°N; Lon. 77.02°E), Coimbatore, Tamil Nadu, India. They were transported to the laboratory in polythene bags filled with oxygenated water. They were acclimatized to ambient laboratory conditions for 2 weeks (at that time it reached PL-45) in cement tank (6 × 3 × 3 feet) with ground water (temperature, 27±1.0 °C; pH, 7±0.14; total dissolved solids, 0.9±0.006 g/l; dissolved oxygen, 7.2±0.55 mg/l; BOD 30.0±1.28 mg/l; COD, 125.0±3.1 mg/l; ammonia, 0.028±0.005 mg/l). During acclimatization the prawns were fed with boiled egg albumin and artificially formulate feed of our own. More than 50% of tank water was routinely renewed every day in order to maintain a healthy environment and adequate aeration was also provided. This was to ensure sufficient oxygen supply to the prawns and an environment devoid of accumulated metabolic wastes. The unfed feeds, faeces, moult and dead prawns if any were removed by siphoning without disturbing the prawns.

Four groups of *M. rosenbergii* PL-45 (2.63 ± 0.11 cm in length and 0.15 ± 0.02 g in weight) each with 30 individuals were maintained in 30 L plastic tanks in a triplicate experimental set-up. They were starved for 24 h before beginning of the feeding trial. One group served as control and fed with feed formulated by using BI only, and the other three groups were fed with experimental feeds prepared by incorporation of ethanolic extract of *A. sessilis* (0.1%, 0.5% and 1.0%). The feed was allocated to the prawns for two times a day (8:00 and 20:00 hrs) at 10% of body weight. The experiment was extended for a period of 90 days, at that time the prawns reached juvenile stage. The unfed feed, feces and moult if any, were collected on daily basis by siphoning with minimum disturbance to the prawns while renewing the aquarium water. For morphometric and nutritional analysis 10 prawns from each group were randomly measured and the mean was considered as a single value (mean of 10 individual measurements = one observation), and three such observations were made to fulfill the triplicate analysis.

2.6. Evaluations of Nutritional Indices

After 90 days of feeding trial, the growth parameters, such as survival rate (SR), length gain (LG), weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER) and food conversion ratio (FCR) were determined by following equations of Tekinay and Davies (2001). Survival rate, SR (%) = Total No. of live prawn / Total No. of prawns introduced initially × 100. Length gain, LG (cm) = Final length (cm) – Initial length (cm). Weight gain, WG (g) = Final weight (g) – Initial weight (g). Specific growth rate, SGR (%) = $\log w_2 - \log w_1 / t \times 100$ (where, w₁ & w₂ represents initial and final weight (g) respectively, and, 't' is the total number of experimental days). Protein efficiency ratio, PER (g) = Weight gain (g) / Protein intake. Feed conversion ratio, FCR (g) = Total quantity of feed intake (g) / Weight gain of the prawn (g).

2.7. Estimations of Biochemical Constituents

On the final day of the experiment, the contents of basic biochemical constituents such as total protein (Lowry *et al.*, 1951), total carbohydrate (Roe, 1955), total lipid [extracted by using chloroform-methanol mixture (Folch *et al.*, 1957) and estimated by following the method of Barnes and Blackstock (1973)], ash and moisture (APHA 2005) of individual diet fed prawns were estimated.

2.8. Statistical Analysis

The data were expressed as mean \pm SD, and analyzed by one-way analysis of variance (ANOVA) using SPSS (v20), and subsequent post hoc multiple comparison, Duncan's multiple range test (DMRT) to compare the significant differences among treatments at $p < 0.05$.

3. RESULTS

3.1. Primary Phytochemicals of *A. sessilis*

The petroleum etheric leaf extract of *A. sessilis* showed presence of 6 primary compounds, such as alkaloids, terpenoids, flavonoids, tannins, polyphenols and quinones. Of which alkaloids, terpenoids and polyphenols were moderately present. Flavonoids, tannins and quinones were poorly present (Table 1). In acetonic extract of *A. sessilis* showed presence of 7 compounds. Of which polyphenols and cardiac glycosides were luxuriantly present. Tannins and saponins were moderately present. The other compound, such as terpenoids, flavonoids and quinones were poorly present (Table 1). Similarly, ethanolic extract of *A. sessilis* contained 6 primary compounds, such as, flavonoids tannins, polyphenols, saponins, cardiac glycosides and quinones. Of which tannins, polyphenols and saponins were luxuriantly present. Quinones was moderately present. The other compounds, such as flavonoids and cardiac glycosides were poorly present (Table 1).

Table 1. Presence of primary phytochemicals in *A. sessilis* leaf extracts

Phytochemicals	Solvents		
	Petroleum ether (non-polar)	Acetone (middle-polar)	Ethanol (Polar)
Alkaloids	++	--	--
Terpenoids	++	+	--
Flavonoids	+	+	+
Tannins	+	++	+++
Polyphenols	++	+++	+++
Saponins	--	++	+++
Cardiac glycosides	--	+++	+
Quinones	+	+	++

+, Poor presence; ++, Moderate presence; +++, Luxuriant presence; --, Absence

3.2. Secondary Phytoconstituents of *A. sessilis*

Overall the GC-MS analyses of *A. sessilis* leaf extracts contains of 17 different secondary compounds, they are depicted in table 2. The petroleum etheric extract of *A. sessilis* revealed the presence of 7 different secondary compounds {N-3-Oxopropylcrotonylamide; Dibenz[a,h]anthracene, 5,6,12,13-tetrahydro; 6-Formyl-2,2'-bis[1,3-ithiolo[4,5-b][1,4]dithiin-2,2'-diylidene]; 1-phenyl-1-hydro-1-(diethoxy-oxo-phosphinyl)-phosphine - (pentacarbonyl) tungsten complex; 2-Pentadecanone, 6,10,14-trimethyl; 9,12-Octadecadienoic acid (Z,Z); 3-Cyanobenzaldehyde dimethyl acetyl}. Of which, 2 compounds {2-Pentadecanone, 6,10,14-trimethyl; 9,12-Octadecadienoic acid (Z,Z)} are possessed bioactive properties (Tables 2 and 3; Figs. 1 and 1a).

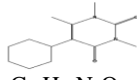
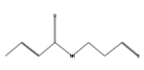
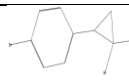

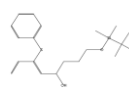
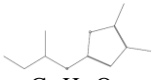


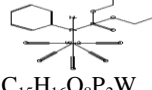
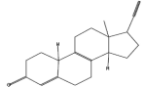
The acetonic extract of *A. sessilis* showed presence of 5 different secondary compounds {5-Cyclohexyl-1,3,6-trimethyluracil; 2-sec-Butoxy-4,5-dimethylfuran; 19-norpregn-4,18-dien-20-yn-3-one; 9,12-Octadecadienoic acid (Z,Z); (13E,16S,17R,18R)-17,18-Epoxy-16,18-dimethyl-10-phenyl[11]cytochalasa-6(7),13-dien-1,21-dione}. Of which, 2 compounds {5-Cyclohexyl-1,3,6-trimethyluracil; 9,12-Octadecadienoic acid (Z,Z)} are possessed biological properties (Tables 2 and 4; Figs. 2 and 2a).

In the case of ethanolic extract of *A. sessilis*, 6 different secondary compounds {1-(p-bromophenyl)-2,2-dibromocyclopropane; (Z)-1-[(tert-Butyldimethylsilyl)oxy]-6-(phenylthio)-5,7-oc; (2R,3S,4R)-

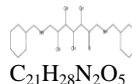

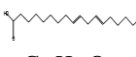

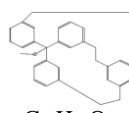

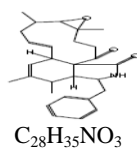
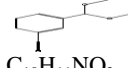
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1,2:3,4-Di-O-Isopropylidene-1,2,3,4-hexadeca netetraol; 1,7-dideoxy-d-mannoheptulose 1,7-bisbenzylamine; 17-Methoxy [2.2.2] (1,3,5) benzene (3,3',3'') triphenylmethanophane; Methyl 5-trimethylsilyloxy) -bis [O (18) eicosanoate} were detected. Of which, 2 compounds {(2R,3S,4R)-1,2:3,4-Di-O-Isopropylidene-1,2,3,4-hexadeca netetraol; 1,7-dideoxy-d-mannoheptulose 1,7-bisbenzylamine} are possessed bioactive properties (Tables 2 and 5; Fig. 3).

Table 2. Presence of secondary phytochemical compounds in *A. sessilis* leaf extracts

Solvents						
Petroleum ether (non-polar)			Acetone (middle-polar)		Ethanol (Polar)	
Peak RT	Name of the compounds identified	Chemical structure and molecular formula	Name of the compounds identified	Chemical structure and molecular formula	Name of the compounds identified	Chemical structure and molecular formula
5.08	--	--	5-Cyclohexyl-1,3,6-trimethyluracil	 C ₁₃ H ₂₀ N ₂ O ₂	--	--
5.53	N-3-Oxopropylcrotonylamide	 C ₇ H ₁₁ NO ₂	--	--	--	--
5.85	--	--	--	--	1-(p-bromophenyl)-2,2-dibromocyclopropane	 C ₉ H ₇ Br ₃
8.58	Dibenz[a,h]anthracene, 5,6,12,13-tetrahydro	 C ₂₂ H ₁₈	--	--	--	--
9.57	--	--	--	--	(Z)-1-[(tert-Butyldimethylsilyloxy]-6-(phenylthio)-5,7-oc tadien-4-ol	 C ₂₀ H ₃₂ O ₂ SS i
10.05	--	--	2-sec-Butoxy-4,5-dimethylfuran	 C ₁₀ H ₁₆ O ₂	--	--
12.09	NV	NV	--	--	--	--
13.88	--	--	NV	NV	--	--
14.15	--	--	--	--	(2R,3S,4R)-1,2:3,4-Di-O-Isopropylidene-1,2,3,4-hexadeca netetraol	 C ₂₂ H ₄₂ O ₄
16.32	6-Formyl-2,2'-bis[1,3-ithiolo[4,5-b][1,4]dithiin-2,2'-diylidene]	 C ₁₁ H ₆ OS ₈	--	--	--	--
17.85	--	--	NV	NV	--	--
19.91	1-phenyl-1-hydro-1-(diethoxy-oxo-phosphinyl)-phosphine - (pentacarbonyl) tungsten complex	 C ₁₅ H ₁₆ O ₈ P ₂ W	--	--	--	--
22.29	--	--	19-norpregn-4,18-dien-20-yn-3-one	 C ₂₀ H ₂₄ O	--	--

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22.73	--	--	--	--	1,7-dideoxy-d-mannoheptulose 1,7-bisbenzylamine	 C ₂₁ H ₂₈ N ₂ O ₅
25.72	2-Pentadecanone, 6,10,14-trimethyl	 C ₁₈ H ₃₆ O	--	--	--	--
31.02	9,12-Octadecadienoic acid (Z,Z)	 C ₁₈ H ₃₂ O ₂	--	--	--	--
31.27	--	--	9,12-Octadecadienoic acid (Z,Z)	 C ₁₈ H ₃₂ O ₂	--	--
32.14	--	--	--	--	17-Methoxy [2.2.2] (1,3,5)benzene (3,3',3'') triphenylmethanophane	 C ₃₂ H ₃₀ O
36.15	--	--	--	--	Methyl 5-trimethylsilyloxy - bis [O (18) eicosanoate	 C ₂₄ H ₅₀ O ₃ Si
36.38	--	--	(13E,16S,17R,18 R)-17,18-Epoxy-16,18-dimethyl-10-phenyl[11]cytochalas-6(7),13-dien-1,21-dione	 C ₂₈ H ₃₅ NO ₃	--	--
38.20	3-Cyanobenzaldehyde dimethyl acetyl	 C ₁₀ H ₁₁ NO ₂	--	--	--	--

RT, Retention time; NV, Not validated

Table 3. GC-MS profiles of secondary phytochemical compounds in petroleum etheric extract of *A. sessilis* leaf

RT	Name of the compound	P	MF	MW	Area (%)	SI	RSI	Biological properties by literature only
5.53	N-3-Oxopropylcrotonylamide	80.59	C ₇ H ₁₁ NO ₂	141	71.58	853	875	--
8.58	Dibenz[a,h]anthracene, 5,6,12,13-tetrahydro	10.08	C ₂₂ H ₁₈	282	0.00	365	727	--
12.09	NV	NV	NV	NV	NV	NV	NV	NV
16.32	6-Formyl-2,2'-bis[1,3-ithiolo[4,5-b][1,4]dithiin-2,2'-diylidene]	46.22	C ₁₁ H ₆ OS ₈	410	0.00	432	751	--
19.91	1-phenyl-1-hydro-1-(diethoxy-oxo-phosphinyl)-phosphine - (pentacarbonyl) tungsten complex	60.06	C ₁₅ H ₁₆ O ₈ P ₂ W	570	0.02	433	846	--
25.72	2-Pentadecanone, 6,10,14-trimethyl	60.34	C ₁₈ H ₃₆ O	268	0.02	712	837	Skin creams, lotion, cosmetic products (Hussain <i>et al.</i> , 2004)
31.02	9,12-Octadecadienoic acid (Z,Z)	29.96	C ₁₈ H ₃₂ O ₂	280	0.49	764	792	Anti-inflammatory, acne reductive, and moisture retentive (Diezel <i>et al.</i> , 1993; Letawe <i>et al.</i> , 1998; Darmstadt <i>et al.</i> , 2002)
38.20	3-Cyanobenzaldehyde dimethyl acetyl	31.83	C ₁₀ H ₁₁ NO ₂	177	0.28	490	837	--

RT, Retention time; NV, Not validated; P, Probability; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar index

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Table 4. GC-MS profiles of secondary phytochemical compounds in acetonic extract of *A. sessilis* leaf

RT	Name of the compound	P	MF	MW	Area (%)	SI	RSI	Biological properties by literature only
5.08	5-Cyclohexyl-1,3,6-trimethyluracil	18.28	C ₁₃ H ₂₀ N ₂ O ₂	236	53.96	839	839	Cough suppressant (De Blasio <i>et al.</i> , 2012)
10.05	2-sec-Butoxy-4,5-dimethylfuran	5.99	C ₁₀ H ₁₆ O ₂	168	0.01	344	799	--
13.88	NV	NV	NV	NV	NV	NV	NV	NV
17.85	NV	NV	NV	NV	NV	NV	NV	NV
22.29	19-norpregn-4,18-dien-20-yn-3-one	4.98	C ₂₀ H ₂₄ O	280	0.03	364	477	--
31.27	9,12-Octadecadienoic acid (Z,Z)	31.63	C ₁₈ H ₃₂ O ₂	280	2.75	729	789	Anti-inflammatory, acne reductive, and moisture retentive (Diezel <i>et al.</i> , 1993; Letawe <i>et al.</i> , 1998; Darmstadt <i>et al.</i> , 2002)
36.38	(13E,16S,17R,18R)-17,18-Epoxy-16,18-dimethyl-10-phenyl[11]cytochalasa-6(7),13-dien-1,21-dione	64.26	C ₂₈ H ₃₅ NO ₃	433	1.03	390	896	--

RT, Retention time; NV, Not validated; P, Probability; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar index

Table 5. GC-MS profiles of secondary phytochemical compounds of *A. sessilis* extracted with ethanol

RT	Name of the compound	P	MF	MW	Area (%)	SI	RSI	Biological properties by literature only
5.85	1-(p-bromophenyl)-2,2-dibromocyclopropane	7.17	C ₉ H ₇ Br ₃	352	38.87	288	519	--
9.57	(Z)-1-[(tert-Butyldimethylsilyloxy]-6-(phenylthio)-5,7-oc tadien-4-ol	16.10	C ₂₀ H ₃₂ O ₂ SSi	364	0.46	395	662	--
14.15	(2R,3S,4R)-1,2:3,4-Di-O-Isopropylidene-1,2,3,4-hexadeca netetraol	42.30	C ₂₂ H ₄₂ O ₄	370	0.53	459	824	Detergents and cleaning agents (OECD, (1994)
22.73	1,7-dideoxy-d-mannoheptulose 1,7-bisbenzylamine	9.02	C ₂₁ H ₂₈ N ₂ O ₅	388	1.73	400	497	Allergy, hay fever, cold (WebMD, 2016)
32.14	17-Methoxy [2.2.2] (1,3,5)benzene (3,3',3'') triphenylmethanophane	91.90	C ₃₂ H ₃₀ O	430	14.36	558	670	--
36.15	Methyl 5-trimethylsilyloxy)-bis [O (18) eicosanoate	7.55	C ₂₄ H ₅₀ O ₃ Si	414	5.09	292	546	--

RT, Retention time; NV, Not validated; P, Probability; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar index

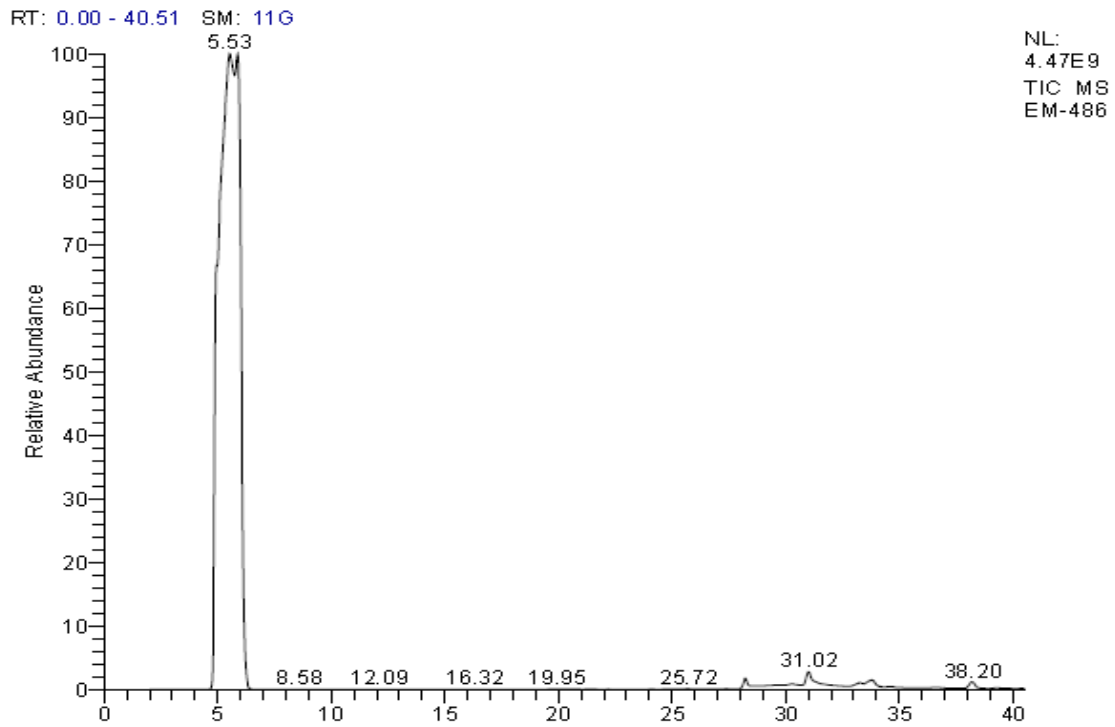


Fig. 1. GC-MS chromatogram of petroleum etheric extracted *A. sessilis* (Relative abundance up to 100)

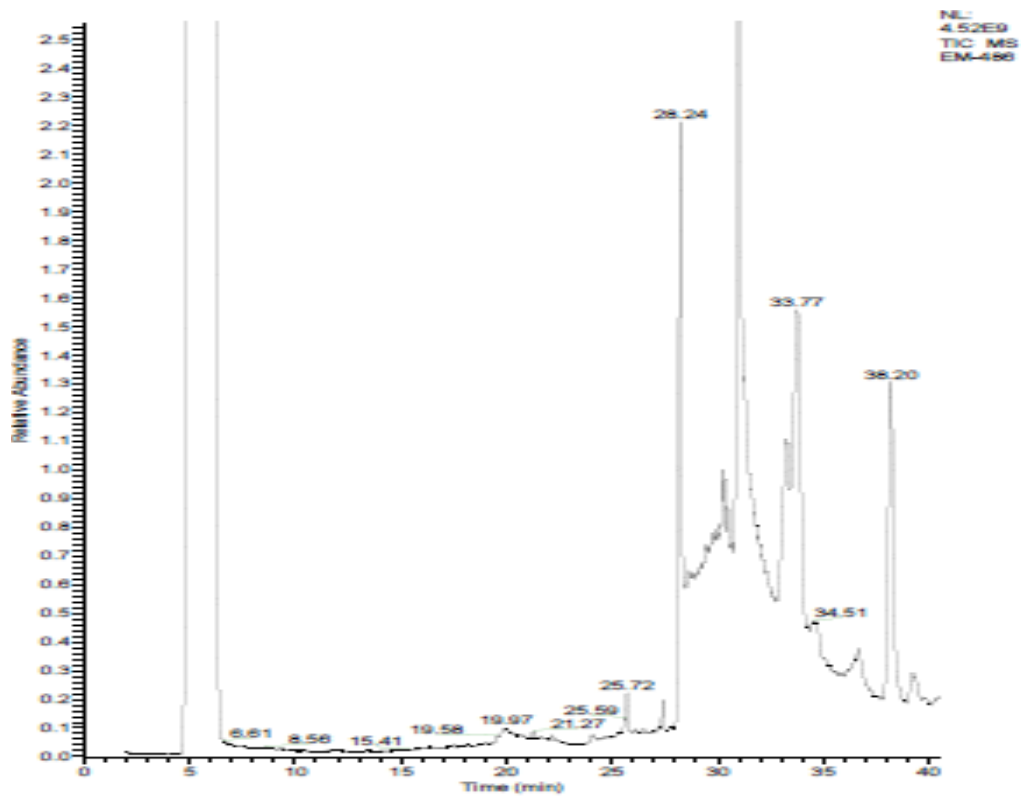


Fig. 1a. GC-MS chromatogram of petroleum etheric extracted *A. sessilis* (Relative abundance up to 2.5)

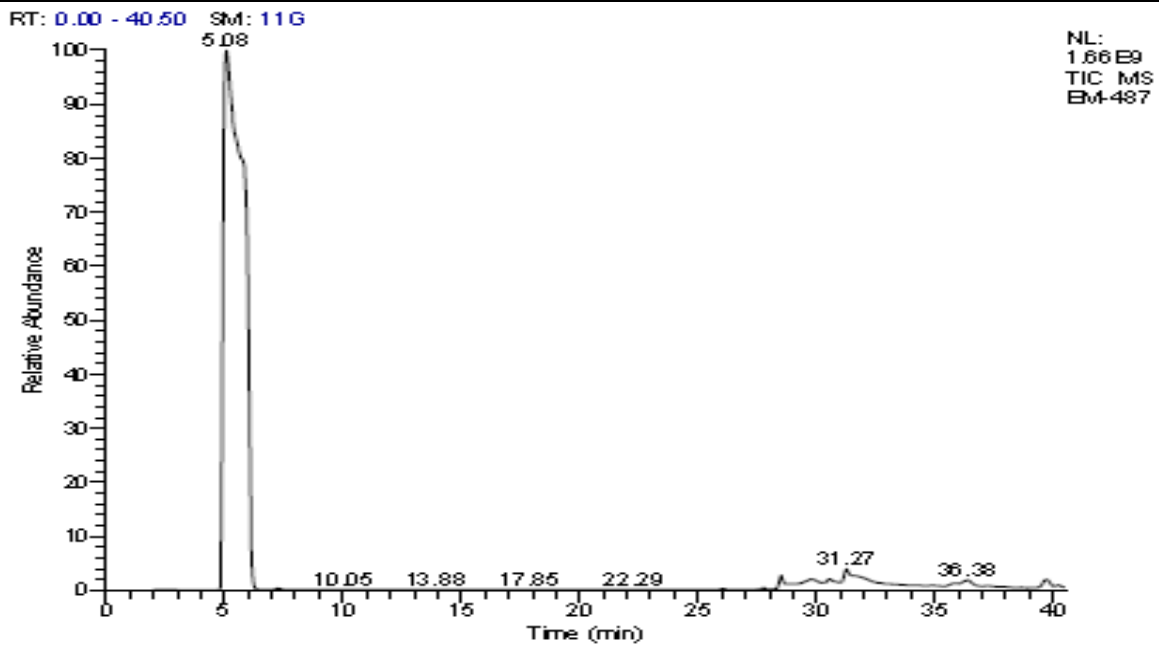


Fig. 2. GC-MS peak level in the chromatogram graph of acetonitrile extract of *A. sessilis* (Relative abundance up to 100)

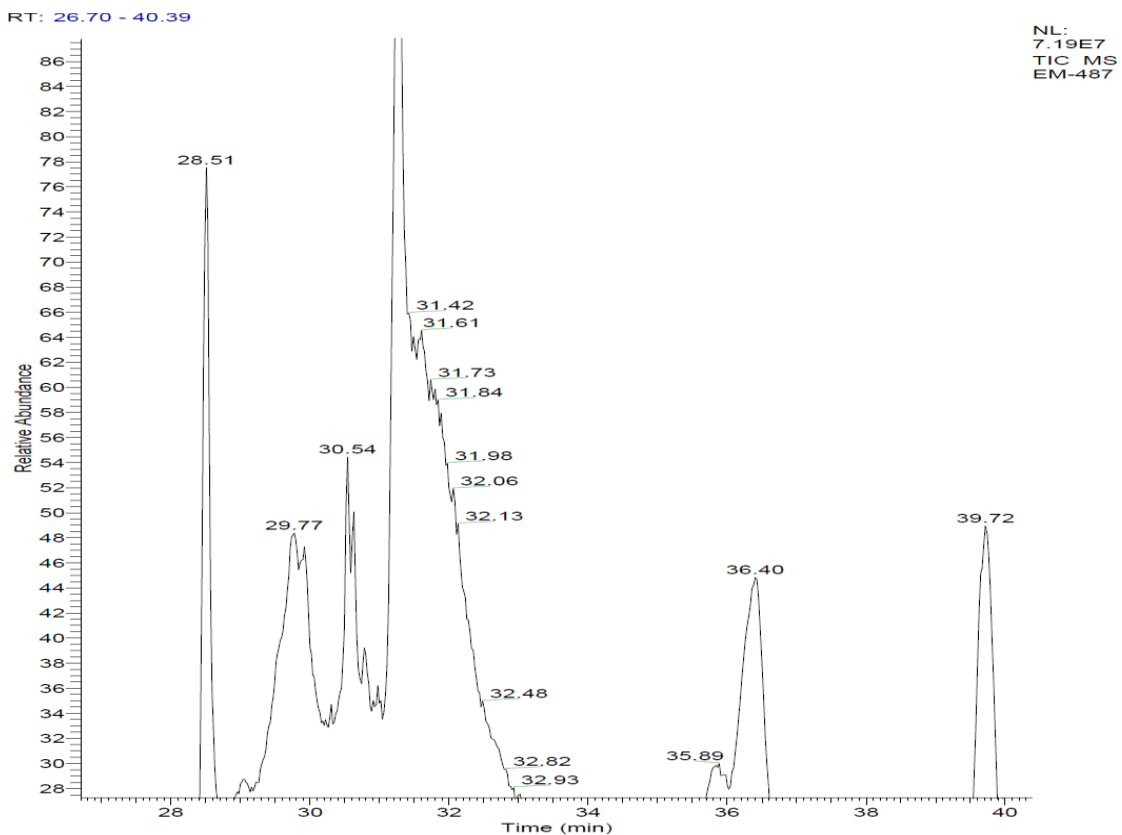


Fig. 2a. GC-MS peak level in the magnified chromatogram graph of acetonitrile extract of *A. sessilis* (Relative abundance up to 56)

3.3. Nutritional Indices

The morphometric analyses revealed that the nutritional indices such as SR, WG, SGR and PER were found to be significantly increased in 1% ethanolic extract incorporated feed fed prawns ($p < 0.05$) followed by 0.5% and 0.1% when compared with control (Table 6). In the case of FCR, the data naturally appeared in the decreasing trend that the lowest FCR was recorded in 1% of ethanolic extract incorporated feed fed prawns, which reflects the best quality of feed.

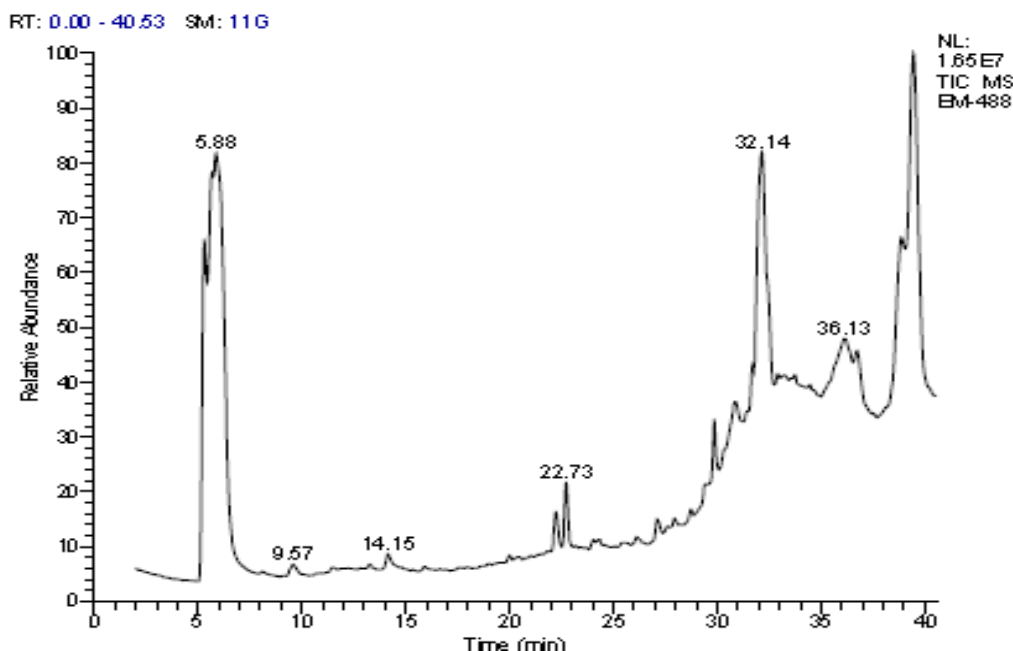


Fig. 3. GC-MS peak level in the chromatogram graph of ethanolic extract of *A. sessilis* (Relative abundance up to 100)

Table 6. Nutritional indices of *M. rosenbergii* fed with ethanolic extract of *A. sessilis* leaf incorporated feeds

Parameter	Control	Ethanolic extract		
	BI	BI+0.1%	BI+0.5%	BI+1%
SR (%)	66.66±6.66 ^d	81.11±5.09 ^c	85.55±6.33 ^b	88.66±3.33 ^a
Length (cm)	4.09±0.22 ^d	5.15±0.18 ^{bc}	5.25±0.15 ^b	5.40±0.18 ^a
Weight (g)	0.59±0.06 ^d	1.14±0.14 ^{bc}	1.20±0.21 ^b	1.41±0.22 ^a
WG (g)	0.44±0.01 ^d	0.99±0.11 ^{bc}	1.05±0.23 ^b	1.25±0.24 ^a
SGR (%)	0.68±0.02 ^d	0.96±0.02 ^{bc}	0.98±0.13 ^b	1.09±0.09 ^a
FCR (g)	3.05±0.12 ^a	1.63±0.10 ^b	1.53±0.05 ^{bc}	1.29±0.09 ^d
PER (g)	0.77±0.03 ^d	1.89±0.07 ^c	2.07±0.05 ^b	2.24±0.11 ^a

Each value is mean ± standard deviation of three individual observations.

Initial length and weight were 2.63 ± 0.11 cm and 0.15 ± 0.02 respectively

Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at $p < 0.05$ (one-way ANOVA and subsequent post hoc multiple comparison with DMRT).

BI, basal ingredients; SR, survival rate; WG, weight gain, SGR, specific growth rate; FCR, food conversion ratio; PER, protein efficiency ratio

3.4. Biochemical constituents

The basic biochemical constituents, such as total protein, total carbohydrate, total lipid and ash were found to be significantly increased in 1% ethanolic extract incorporated feed fed prawns ($p < 0.05$) followed by 0.5% and 0.1% when compared with control (Table 7). In the case of moisture content, the most decrease was found to be in 1% ethanolic extract incorporated feed fed prawns ($p < 0.05$) followed by 0.5% and 0.1% when compared with control (Table 7).

Table 7. Concentration of biochemical constituents (mg/g wet wt.) in *M. rosenbergii* fed with ethanolic extract of *A. sessilis* leaf incorporated feeds

Parameters	Initial	Control	Ethanolic extract		
		BI	BI+0.1%	BI+0.5%	BI+1%
Total protein	43.91±2.74	82.78±4.47 ^d	115.93±3.44 ^c	120.74±4.57 ^b	133.5±3.77 ^a
Total carbohydrate	21.06±1.98	30.35±2.59 ^d	45.69±3.13 ^{bc}	46.94±2.27 ^b	49.01±2.51 ^a
Total lipid	9.32±2.05	18.21±2.54 ^d	25.89±2.54 ^{bc}	26.91±2.04 ^{ab}	27.94±1.65 ^a
Moisture (%)	76.66±4.32	65.33±2.08 ^a	53.33±3.64 ^b	52.66±2.45 ^{bc}	50.33±2.58 ^d
Ash (%)	10.65±1.02	13.18±1.95 ^c	17.91±1.57 ^{bc}	18.72±1.83 ^{ab}	19.24±1.31 ^a

Each value is mean ± standard deviation of three individual observations.

Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at $p < 0.05$ (one-way ANOVA and subsequent post hoc multiple comparison with DMRT)

BI, basal ingredients

4. DISCUSSION

Similar to that of the present study, the presence of alkaloids, flavonoids, phenols, steroids, terpenoids, saponins and glycoside have been reported in ethanolic extract of *A. sessilis* (Sivakumar and Sunmathi, 2016). The presence of other phyto-constituents such as proteins, amino acids, carbohydrates, fixed oil and sterols have also been reported in *A. sessilis* by several workers (Debnath *et al.*, 2014; Sivakumar and Sunmathi, 2016). The phytochemicals, such as flavonoids, anthrax quinines and terpenes stimulate glucose uptake in cells (Latha and Pari, 2003). Certain flavonoids exhibited hypoglycemic activity (Ahmad *et al.*, 2000) and also beta cell regeneration in pancreas (Latha and Pari, 2003). The primary phytochemicals serve as health tonic in aquaculture nutrition (Citarasu, 2010; Pourmoghim *et al.*, 2015).

The detected hexadecanoic acid, octadecanoic acid and propanoic acid in the present study have also been reported to be present in the hydro-alcoholic extraction of *A. sessilis* (Shabi *et al.*, 2010).

The detected hypocholesterolemic compounds such as 9, 12-Octadecadienoic acid (Z,Z)- and Octadecanoic acid have also been reported in *Justicia wynaadensis* (Ponnamma and Manjunath, 2012). The other compounds reported in *J. wynaadensis* are gamma-Tocopherol, Vitamin E, Ergost-5-en-3.Beta.-ol and Stigmasta-5, 22-dien-3.beta.-ol (Ponnamma and Manjunath, 2012).

Vinodh and Senthil Kumar (2014) have reported that the aqueous extract of *A. sessilis* leaf contains the following secondary phytochemicals: 1-vinylclohexa-1,4-diene; 1-isopropylcyclohexa-1,4-dienein; 5-butyl-4-methylnaphalen-1(4H)-one; 4a,8-dimethyl-1-methylene-1,4,4a,4b,5,8,8a,9,10,10 adecahydrophenanthrene; 1-(1-hydroxyethyl)-1-(methoxymethyl)-7a-methyloctahydro-3H-cyclopropa[a]naphthalene-2(7bH)-one; 10,13,17- trimethyl-3-methylylene 2,3,4,5,6,10,12,13,14,15, 16,17-dodecahydro-1Hcyclopenta [a] phenanthrene; 1,1-bis(1-methoxyvinyl)-9b-methyl-4,5,6,7,8,9, 9a,9b,9cdecahydro-1H-cyclopropa [c]phenanthrene; 1,1-bis(1-methoxyvinyl)-9b-methyl-2-methylenetetradecahydro-1H-cyclopropa [c] phenanthrene; Dimethyl 17-ethyl-9b-methyl-2-oxo-1a,2-dihydro-7 Hcyclopropa [c] phenanthrene-1,1(9aH,9bH,9cH) - dicarboxylate and 7-ethyl-1,1-bis(1-methoxyvinyl)-2,7,9b-trimethyl-1a,2,7,8,9a,9b,9c-octahydro-1Hcyclopropa [c] phenanthrene. None of the compound was detected in the present study.

The phytocomponents derived from various plants have been used in traditional medicine for the treatment of several diseases. Recent studies showed that the incorporation of medicinal plants (raw material, extracts and phytocomponents) as a source of fish/prawn feed, which stimulate the growth and immune system (Chakrabarti *et al.*, 2012; Bhavan *et al.*, 2014a,b; Pourmoghim *et al.*, 2015; Dhanalakshmi *et al.*, 2016).

Similar to that of the elevated SR, WG, SGR and PER, and elevated total protein, carbohydrate and lipid recorded in the present study in ethanolic extract of *A. sessilis* incorporated feed fed prawns, it has also been reported in Nutripro-aqua, herbal based diet fed *M. rosenbergii* (Kesavanth *et al.*, 2003). The increased nutritional indices has also been reported in freshwater prawns fed with *Alteranthera sessilis*, *Eclipta alba*, *Cissus quadrangularis*, *Allium sativum*, *Andrographis paniculata*, *Coriandrum sativum*, *Curcuma longa*, *Menthe arvensis*, *Murraya koenigii*, *Ocimum sanctum*, *Trigonella foenum-graecum*, *Withania somnifera*, *Zingiber officinale*, *Cynodon doctylon*, *Syzygium cumini*, *Phyllanthus emblica*, *Azadirachta indica*, *Ricinus communis*, *Papaver somniferum*, *Elettaria cardamomum*, *Foeniculum vulgare*, *Syzygium aromaticum*, *Mentha arvensis*, and *Trigonella foenum-graecum* incorporated feeds (Bhavan *et al.*, 2011; Bhavan *et al.*, 2012; Shanthi *et al.*, 2012; Poongodi *et al.*, 2012; Radhakrishanan *et al.*, 2013; Bhavan *et al.*, 2013a,b; Bhavan *et al.*, 2014a,b; Dhanalakshmi *et al.*, 2016).

Generally herbs are good appetizer, they contain bioactive compounds which stimulate the feeding rate and food consumption ratio, and thus improved biochemical constituents in prawns have been reported (Sambhu and Jayaprakash, 2001; Bhavan *et al.*, 2012, 2013a,b; 2014a,b). Herbal growth promoters helped to induce the transcription, leading to increased RNA, which coupled with increased amino acid and finally enhanced protein synthesis in *Penaeus monodon* (Citarasu, 2010). It has been

reported that, *W. somnifera* supplemented spawners showed higher protein values in the haemolymph as well as positively regulated the larval quality in *P. monodon* (Babu *et al.*, 2008).

5. CONCLUSION

In the present study, the primary phytochemicals and secondary bioactive compounds present in ethanolic extract of *A. sessilis* have the potency to enhance the growth, survival and nutritional quality of *M. rosenbergii*. Hence, *A. sessilis* leaf can be taken as a feed additive in on-farming feed preparation, and thus, sustainable aquaculture of freshwater prawns can be promoted.

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