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Phytochemical Characterization of *Eichhornia crassipes* and *Sargassum cristaefolium*, and Their Effects on the Growth of the Prawn *Macrobrachium rosenbergii*

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Original Research Article

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Abstract: The main aim of this study was to see the primary and secondary phytochemicals of the common water hyacinth, *Eichhornia crassipes* and the marine brown alga, *Sargassum cristaefolium*, and evaluation of their effects on the growth of the freshwater prawn, *Macrobrachium rosenbergii* post-larvae (PL). The phytochemical properties of *E. crassipes* and *S. cristaefolium* were analyzed using petroleum etheric (non-polar solvent) and ethanolic (polar solvent) extracts. Presence of primary phytochemical components, such as alkaloids, terpenoids, flavonoids, tannins, polyphenols, saponins, cardiac glycoside and quinines, and significant amounts of total phenolic and total antioxidant contents were recorded in *E. crassipes*, and *S. cristaefolium*. *E. crassipes* showed the presence of totally 14 secondary metabolic components, of which 5 from petroleum etheric extract and 9 from ethanolic extract. Among these 3 compounds, Dodecanoic acid; 6,7-Dimethoxy-2-tetralone; and, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, and 5 compounds, Phenol, 2-methoxy-5-(1-propenyl)-, (E); Cycloheptane, 1,3,5-tris(methylene); 4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol; Tetradecanoic acid; and, Loliolide of the respective solvent extracts possessed bioactive properties. In *S. cristaefolium*, totally 13 secondary metabolic components were detected, of which 6 from petroleum etheric extract and 7 from methanolic extract. Among these, one compound in each extract, Docosane, and cis-2-[2-(hydroxymethyl)cyclopentyl] ethanol, respectively possessed bioactive properties. The basal diet was prepared using fish meal, groundnut oilcake, soy bean meal, wheat bran, sunflower oil tapioca flour, egg albumin and vitamin-B complex with vitamin-C and used as control diet. The fishmeal was replaced with raw powder of *E. crassipes*, and *S. cristaefolium*, independently at 5, 10 and 15%. Similarly, each solvent extract of *E. crassipes*, and *S. cristaefolium*, was independently incorporated with the basal diet at 0.5, 1.0, and 1.5%. These diets were fed to *M. rosenbergii* PL for 60 days. Significant improvements in survival rate, weight gain, food conversion ratio, content of total protein and ash were recorded in experimental PL when compared with control. This was found to be the best at 10% incorporation of raw powder of each weed, and 1.5% ethanolic extract, as well as pertroleum etheric extract of each weed. Among these two weeds, *E. crassipes* produced a little better performance than that of *S. cristaefolium*. Among the two solvents, ethanolic extract produced better result than that of petroleum etheric extract. This may be due to presence of more number of bioactive components in ethanolic extract, or due to the presence of a particular bioactive component. Therefore, the raw powders of *E. crassipes* and *S. cristaefolium* are recommended as ingredients, and their ethanolic extracts are recommended as feed additives in aqua feed formulations for sustainable culture of *M. rosenbergii*.

Keywords: Water hyacinth, Brown alga, Prawn, Survival, Growth.

INTRODUCTION

The fisheries and aquaculture sectors play a vital role in addressing various social issues like food and nutritional security, employment generation, livelihood support, upliftment of rural economy, and economy of the nation as well. The world is facing an increasing demand for quality food supply. Aquaculture provides ample opportunity for augmented protein rich food production to feed the growing human population. In India, aquaculture of fishes, prawns, shrimps, crabs, lobsters, crayfishes, molluscs etc., are practiced at extensive, semi-intensive and intensive levels. There are two freshwater prawn species of commercial importance due to their nutritious delicacies and export

values [1, 2]. They are *Macrobrachium rosenbergii* and *Macrobrachium malcolmsonii*. Among these two *M. rosenbergii* is more popular because of its little larger abdominal portion than that of *M. malcolmsonii*, which has little bigger cephalothorax instead. Therefore, *M. rosenbergii* is considered to be a species with increasing potential for aquaculture worldwide [3]. The freshwater prawn farming is simpler than marine prawn with lower cost since the ponds can be build-in small, medium and large sized ones, both on the cost and inland as well [4]**.** The average national production of finfish, crustacean and molluscs from still water ponds has increased from 2.2 tonnes/ha/year to as high as 8-12 tonnes/ha/year [5, 6]. The human being consumed nearly 10,000,000 tons of crustaceans in the year 2005 [7].

The growth depends upon the quality of feeds offered. In any farm, the feed management requires major operational cost, which is non-affordable to small framers. Fishmeal is the one of the major and important ingredients in any aqua feed. It is a depleting resource and therefore its demand is at high. Therefore, ideal alternatives and byproducts are required. At present about 20 million tonnes of manufactured aqua feed are being used in aquaculture sector, of which the majority is consumed in shrimp culture [8]. If the rapid growth of aquaculture persists, the feed requirement may increase many fold. Hence, more scientific understanding and interventions are required for sustainable aquaculture.

Recently, we have been used cereals, pulses, vegetable waste, fruits waste, greens, herbals, fish oil, vegetable oil, sunflower oil and Cod liver oil as feed supplements for better survival and growth of freshwater prawns [9-15]**.** The Herbal supplements have also been used to promote growth and survival of freshwater prawns [16-30]. The chicken waste meal has been tested as an alternative to fishmeal [31]. Most recently, we have checked the efficacy of certain algae as feeds to *M. rosenbergii* [32-37], and the fishmeal has also been replaced by *Chlorella vulgaris*, *Turbinaria ornata* and *Gracilaria corticata* [38, 39]**.**

In the present study, the primary and secondary phytochemicals of the common water hyacinth, *Eichhornia crassipes* and a marine brown alga, *Sargassum cristaefolium* were analyzed to understand their bioactive compounds. In addition, these weeds were used as ingredients for partial replacement of the fishmeal, and, their petroleum etheric (non-polar) and ethanolic (polar) extracts were used as feed additives to evaluate the growth, survival and concentrations of basic biochemical constituents of *M. rosenbergii* post-larvae (PL).

MATERIALS AND METHODS

Collection and identification

The water hyacinth, *Eichhornia crassipes* was collected from Muthannakulam Lake (Lat, 10° 59'27" N; Lon, 76° 56'42" E), Coimbatore, Tamil Nadu, India. The marine brown alga, *Sargassum cristaefolium* was procured from Mandapam Coast (Lat. 9° 17'N; Lon. 79° 19'E), Gulf of Mannar, South-east coast of Tamil Nadu, India. They were authenticated by Botanical Survey of India (BSI), Coimbatore, India. The proximate composition of *E. crassipes* and *S. cristaefolium* were estimated following the method of Castell and Tiews [40] as given in AOAC [41].

Preparation of *E. crassipes* and *S. cristaefolium* extracts

The collected weeds were thoroughly washed with freshwater, blotted and spread out and dried for 2 weeks at room temperature. They were shade dried individually and ground to fine powders. The powdered samples were stored in sterile containers for further use. Each powder (75 g) was packed in Whatman No. 1 filter paper separately and Soxhlet extractions were done with 450 ml (1:6 w/v) of petroleum ether and ethanol individually for 6-9 h each (30 to 36 cycle) until a clear colorless solution was obtained. Fresh powder was used for each solvent extraction [42]. These extracts were filtered using double layer muslin cloth, concentrated at 40-50 °C using rotary vacuum evaporator (ROTAVAP) and dried at 40 °C under hot air oven. The dark, semi solids obtained were used for further investigation.

Qualitative analysis of phytochemicals

Each solvent extract was subjected to primary phytochemical analysis for screening the presence of alkaloids, terpenoids, flavonoids, tannins, polyphenols, saponins, cardiac glycosides and quinones [43].

Gas chromatography-mass spectrum (GC-MS) analysis

Each extract 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 under the following working conditions [Injector port temperature: 250^{o}C; Interface temperature: 250 ^{o}C, and source was maintained at 200 ^{o}C; The oven temperature: programmed as variable, 70 ^{o}C for 2 mins, 150 ^{o}C @ 8 ^{o}C /min, up to 260 ^{o}C @ 10 ^{o}C /min; the 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].

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 [44] on-line library

sources were used for matching the identified components.

Feed formulation

The following branded basal ingredients (BI) were used to formulate the experimental diets. 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, and soybean meal and wheat bran were thoroughly mixed to prepare dough with sterilized water, steam cooked and cooled at room temperature. Then the Sunflower oil, 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 were added, and mixed well. Sterilized water 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 ºC 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 ºC to be used afresh. This was used as control diet, which devoid of inclusion of these weeds in any form. The proximate composition of organic matters present in the basal diet was determined by adopting the methodology of Castell and Tiews [40] as given in AOAC [41] manual. The fishmeal was replaced with raw powder of *E. crassipes*, and *S. cristaefolium*, independently at 5, 10 and 15% (1.25, 2.50, and 3.75 g, respectively). Similarly, each solvent extract of *E. crassipes*, and *S. cristaefolium*, was independently incorporated with the basal diet at 0.5, 1.0, and 1.5%. Thus, 12 such experimental diets were formulated.

Experimental animal

The post larvae (PL-12) of the freshwater prawn, *Macrobrachium rosenbergii* were procured from prawn culture nursery pond, Singanallur (Lat.10.99^{o}N; Lon. 77.02^{o}E), Coimbatore, Tamil Nadu, and India. They were transported to the laboratory in polythene bags half filled with oxygenated water. They were acclimated to ambient laboratory conditions for 2 weeks in cement tank (6 × 3 × 3 feet) with ground water (temperature, 27±1.0; pH, 7.0±0.15; total dissolved solids, 950±16.0 mg L^{-1}; dissolved oxygen, 7.10±0.30 mg L^{-1}; BOD, 32.0±3.0 mg L^{-1}; COD, 135.0±10.00 mg L^{-1}; ammonia, 0.030±0.005 mg L^{-1}). During acclimation the prawns were fed with boiled egg albumin and artificially formulate feed of our own. The half of the tank water was routinely changed every day to maintain a healthy environment devoid of accumulated metabolic wastes and aerated to ensure sufficient oxygen availability, respectively. The unfed feeds, faeces, moult and dead prawns were removed by siphon method without disturbing the prawns.

Feeding trial

Thirteen groups of *M. rosenbergii* PL (initial length and weight of 2.55±0.11cm and 0.15±0.02g, respectively) each with 30 prawns were maintained in 30 L plastic tanks in a triplicate experimental set-up. They were starved for 24 h and then, the feeding trials were begins. One group served as control and fed with feed formulated using without incorporation of *E. crassipes* and *S. cristaefolium* in any form, and the remaining twelve groups were fed with experimental diets prepared by incorporation of each weed powder (at 5, 10, and 15%), and each solvent extract (at 0.5, 1.0, and 1.5%) for 60 days. Again, on the final day of experiment, the length and weight of PLs were measured to calculate the nutritional indices, and the PLs were sacrificed for estimations of basic biochemical constituents.

Evaluations of nutritional indices

The growth parameters, such as survival rate (SR), length gain (LG), weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER) were calculated by adopting the equations of Tekinay and Davies [45]. 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 w2 – log w1 / t × 100 (where, w1 & w2 represents initial and final weight (g) respectively, and, 't' is the total number of experimental days). Food conversion ratio, FCR (g) = Total quantity of feed intake (g) / Weight gain of the prawn (g). Protein efficiency ratio, PER (g) = Weight gain (g)/ Protein intake.

Estimations of biochemical constituents

The contents of basic biochemical constituents, such as total protein [46], total carbohydrate [47], total lipid [extracted by using chloroform–methanol mixture method of Folch *et al.* [48] and estimated by following the method of Barnes and Blackstock [49]**,** ash and moisture [41] of individual diet fed PLs were estimated.

Statistical analysis

Data between control versus experiments and between experiments were subjected to statistical analysis through one-way ANOVA and subsequent *post hoc* multiple comparison with DMRT by adopting SPSS (v20). All the details of statistical analyses were given in respective tables. The *P* values less than 0.05 ($P<0.05$) were considered as statistically (95%) significant.

RESULTS AND DISCUSSION

The proximate composition of *E. crassipes* was found as follows, crude protein (11.81%), crude fat (1.55%), crude fibre (14.20%), total ash (23.72%) and total nitrogen free extract (11.38%). It has 3276 k.cal of gross energy. Similarly, *S. cristaefolium* contains 12.80% of crude protein, 1.05% of crude fat, 15.03% of crude fibre and 11.28% of total nitrogen free extract. It has 3281 k.cal of gross energy. In addition these weeds contain sand-and-silica, calcium, phosphorus and salt. The formulated basal diet contains 34.0% of crude protein, 5.51% of crude fibre, 3.53% of total lipid, 9.40% of total ash, 8.96% of moisture, 31.10% of carbohydrate and 4324 kcal/kg of gross energy (Table 1).

Table-1: Proximate composition of *E. crassipes* and *S. cristaefolium*, and the basal diet

Proximate Composition (%)	*E. crassipes*	*S. cristaefolium*	Basal Diet
Crude protein	11.81±1.30	12.80±1.56	34.0±3.35
Crude fibre	14.20±1.41	15.03±1.72	5.15±0.52
Etheric extract	1.55±.0.19	1.05±0.10	3.53±0.50
Total ash	23.72±2.76	28.19±2.30	9.40±1.50
Moisture	--	--	8.96±1.00
Total nitrogen free extract	11.38±0.87	11.28±1.02	31.10±3.52
Sand and silica	1.08±0.09	0.97±0.08	--
Calcium	0.24±0.06	0.19±0.04	--
Phosphorus	1.38±0.13	1.68±0.17	--
Salt	1.06±0.09	1.20±0.18	--
Gross energy (kcal/kg)	3276±10.50	3281±11.90	4324±10.65

Each value is mean ± standard deviation of three individual observations

Primary phytochemicals of *E. crassipes* and *S. cristaefolium*

The petroleum etheric extracts of *E. crassipes* and *S. cristaefolium* showed the presence of 5 different primary compounds, such as alkaloids, terpenoids, flavonoids, tannins and polyphenols (Table 2). The ethanolic extracts of *E. crassipes* and *S. cristaefolium* showed the presence of 5 different compounds, such as Tannins, polyphenols, saponins, cardiac glycosides and quinones (Table 2). Similarly, the presence of alkaloids, flavonids, terpenoids, phenols, saponins, quinines, glycoside and anthocyanins has been reported in ethanolic extract of *E. crassipes* [50-52]**.** The presence of alkaloids, terpenoids, flavonoids, tannins, phenols, steroids, sterols, saponins and glycosides have been reported in different solvents extract of *Sargassum ilicifolium*, *Sargassum wightii*, *T. ornate*, *G. corticata*, *Sargassum polycystem* [37, 42, 53-55].

Table-2: The primary phytochemicals present in *E. crassipes*and *S. cristaefolium* extracts

Phytochemicals	*E. crassipes*		*S. cristaefolium*	
	Petroleum etheric	Ethanolic	Petroleum etheric	Ethanolic
Alkaloids	+++	--	++	--
Terpenoids	++	--	+++	--
Flavonoids	++	--	+	--
Tannins	+	++	++	++
Polyphenols	+	+++	++	+++
Saponins	--	+++	--	+++
Cardiac gylcosides	--	++	--	++
Quinones	--	++	--	+

+, poorly present; ++, moderately present; +++, luxuriantly present; --, absent

Secondary phytoconstituents of *E. crassipes* and *S. cristaefolium*

GC-MS analysis of the petroleum etheric extract of *E. crassipes* revealed the presence of 5 different secondary metabolic compounds {2-Methyl-2-[2-dimethyl (phenyl) silylprop-2-en-1-yl] tetrahydrofuran; Z-Phenyl (4-pyrimidinyl) methanoneoxime; Dodecanoic acid; 6,7-Dimethoxy-2-tetralone; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol}, of which 3 compounds {Dodecanoic acid; 6,7-Dimethoxy-2-tetralone; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol} are possessed biological properties (Table 3; Fig. 1).

The ethanolic extract of *E. crassipes* showed presence of 9 different secondary metabolic compounds {Phosphonic acid, phenyl-,methyl phenyl ester; 5-hydroxy-1-deutero-1,2-pentadiene; Phenol, 2-methoxy-5-(1-propenyl)-,(E); 2-Bromolauric acid; Cycloheptane, 1,3,5-tris(methylene); 4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol; 5,5,5-Trifluoro-2-methyl-4-trifluoromethyl-1,3-pentadiene; Tetradecanoic acid; Loliolide}, of which 5 compounds {Phenol, 2-methoxy-5-(1-propenyl)-, (E); Cycloheptane, 1,3,5-tris(methylene); 4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol;

Tetradecanoic acid; Loliolide} are possessed biological properties (Table 4; Fig. 2).

The petroleum etheric extract of *S. cristaefolium* revealed the presence of 6 different compounds {5,5-Dideuteriomethoxycyclohexane; Piperidine, 1,4-dimethyl; Docosane; (S)-(4S,5S)-4-Methoxymethyl-5-phenyl-2-{(Z)-[2-(N-1-phenylethylamino)-2-phenyl] ethenyl}-2-oxazoline; 5-Iodo-5-(1'-naphthyl)-1-phenoxypent-4-en-2-ol; 8,9:14,15-dibenzo-2,4,6,16,18,20-docosahexaene-10,12-diynedial}, of which one compound {Docosane} possesses biological property (Table 5; Fig. 3).

In the ethanolic extract of *S. cristaefolium*, the presence of 7 different compounds {1,2-Dihydro-1,4-diphenylphthalazine; Methyl hydrogen 2,2'-dimethoxy-1,1'-binaphthalene-3,3'-dicarboxylate; 4-Benzyl-2,4,6-triphenyl-4H-thiopyran; 2',5'-Bis(bromomethyl)-1,1':4',1''-terphenyl; 1,3,4-Thiadiazol-2-amine, 5-(pentylthio); cis-2-[2-(hydroxymethyl)cyclopentyl] ethanol; Dethiobiotin} have been detected, of which one compound {cis-2-[2-(hydroxymethyl)cyclopentyl] ethanol} possesses biological property (Table 6; Fig. 4).

Table -3: GC-MS profiles of secondary phytochemical compounds detected from petroleum etheric extract of *E. crassipes*

RT	Name of the compound	MF	MW	SI	RSI	Biological properties by literature only
10.62	2-Methyl-2-[2-dimethyl (phenyl)silylprop-2-en-1-yl] tetrahydrofuran	$C_{16}H_{24}OSi$	260	999	990	--
15.02	Z-Phenyl(4-pyrimidinyl) methanoneoxime	$C_{11}H_9N_3O$	199	442	695	--
17.85	Dodecanoic acid	$C_{12}H_{24}O_2$	200	743	791	Anti-microbial,nematicide and pesticide (Prabhadevi*et al.*, 2012; Markkas and Govindharajalu, 2015)
21.72	6,7-Dimethoxy-2-tetralone	$C_{12}H_{14}O_3$	206	549	600	Antiseptic and anesthetic (Sulochana*et al.*, 2016)
28.80	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	773	884	Antimicrobial, anti-inflammatory, anti-cancer, diuretic, anti-tuberculosis, insecticidal, anti-oxidant (Raman *et al.*, 2012; Das and Himaja, 2014; Parthipan*et al.*, 2015)

RT, Retention time; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar index

Table-4: GC-MS profiles of secondary phytochemical compounds detected from ethanolic extract of *E. crassipes*

RT	Name of the compounds	MF	MW	SI	RSI	Biological properties by literature only
3.91	Phosphonic acid, phenyl-, methyl phenyl ester	$C_{13}H_{13}O_3P$	248	687	735	--
7.14	5-hydroxy-1-deutero-1,2-pentadiene	C_5H_7DO	84	636	858	--
10.02	Phenol, 2-methoxy-5-(1-propenyl)-, (E)	$C_{10}H_{12}O_2$	164	756	789	Anesthetic, allergenic, antibacterial, anti-inflammatory, antioxidant,anti-pyretic, anti-bacterial, anti-septic and anti-cancer activity (Sathish*et al.*, 2012; Hadi*et al.*, 2016)
15.00	2-Bromolauric acid	$C_{12}H_{23}BrO_2$	278	383	431	--
18.13	Cycloheptane, 1,3,5-tris(methylene)	$C_{10}H_{14}$	134	796	829	Aroma chemical and aroma precursor (deJong and Heijmen, 1980)
22.24	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol	$C_{14}H_{22}O$	206	626	652	Melamine, dyes (Kumar *et al.*, 2014)
26.91	5,5,5-Trifluoro-2-methyl-4-trifluoromethyl-1,3-pentadiene	$C_7H_6F_6$	204	588	722	--
30.05	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	778	882	Antioxidant, anticancer, hypercholestrolemic, larvicidal repellent activity, nematicide, (Sivakumar*et al.*, 2011; Diana and Parthipan, 2015; Priya and Subhashini, 2016)
32.90	Loliolide	$C_{11}H_{16}O_3$	196	632	720	Antioxidant and cell protective(Yang *et al.*, 2011)

RT, Retention time; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar

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Table-5: GC-MS profiles of secondary phytochemical compounds detected from petroleum etheric extract of *S. cristaefolium*

RT	Name of the compound	MF	MW	SI	RSI	Biological properties by literature only
13.02	5,5-Dideuteriomethoxycyclohexane	$C_7H_{12}D_2O$	114	876	956	--
17.58	Piperidine, 1,4-dimethyl	$C_7H_{15}N$	113	337	530	--
22.15	Docosane	$C_{22}H_{46}$	310	475	547	Antibacterial activity (Waage and Hedin 1985)
31.09	(S)-(4S,5S)-4-Methoxymethyl-5-phenyl-2-{(Z)-[2-(N-1-phenylethylamino)-2-phenyl] ethenyl}-2-oxazoline	$C_{27}H_{28}N_2O_2$	412	574	935	--
33.78	5-Iodo-5-(1'-naphthyl)-1-phenoxypent-4-en-2-ol	$C_{21}H_{19}IO_2$	430	575	775	--
36.12	8,9:14,15-dibenzo-2,4,6,16,18,20-docosahexaene-10,12-diynedial	$C_{30}H_{22}O_2$	414	791	945	--

RT, Retention time; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar index

Table-6: GC-MS profiles of secondary phytochemical compounds detected from ethanolic extract of *S. cristaefolium*

RT	Name of the compounds	MF	MW	SI	RSI	Biological properties by literature only
13.00	1,2-Dihydro-1,4-diphenylphthalazine	$C_{20}H_{16}N_2$	284	521	768	--
18.52	Methyl hydrogen 2,2'-dimethoxy-1,1'-binaphthalene-3,3'-dicarboxylate	$C_{25}H_{20}O_6$	416	741	834	--
29.08	4-Benzyl-2,4,6-triphenyl-4H-thiopyran	$C_{30}H_{24}S$	416	561	777	--
32.18	2',5'-Bis(bromomethyl)-1,1':4',1"-terphenyl	$C_{20}H_{16}Br_2$	414	414	628	--
35.04	1,3,4-Thiadiazol-2-amine, 5-(pentylthio)	$C_7H_{13}N_3S_2$	203	263	673	--
36.10	cis-2-[2-(hydroxymethyl)cyclopentyl] ethanol	$C_8H_{16}O_2$	144	423	811	Antimicrobial activity (Ramya*et al.*, 2015)
42.43	Dethiobiotin	$C_{10}H_{18}N_2O_3$	214	359	781	--

RT, Retention time; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar index

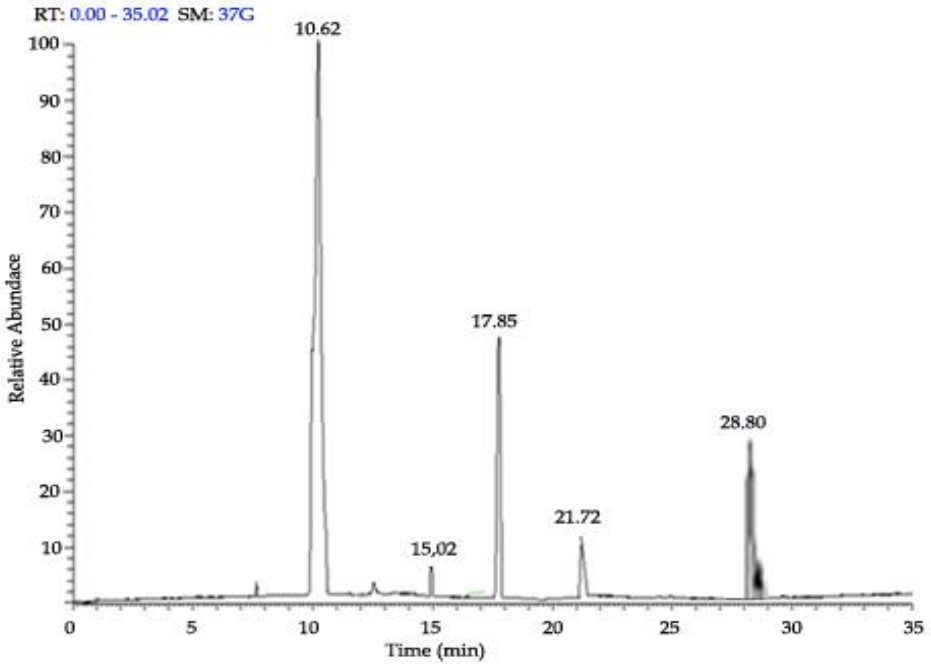

Fig-1: GC-MS chromatogram of petroleum etheric extract of *E. crassipes*

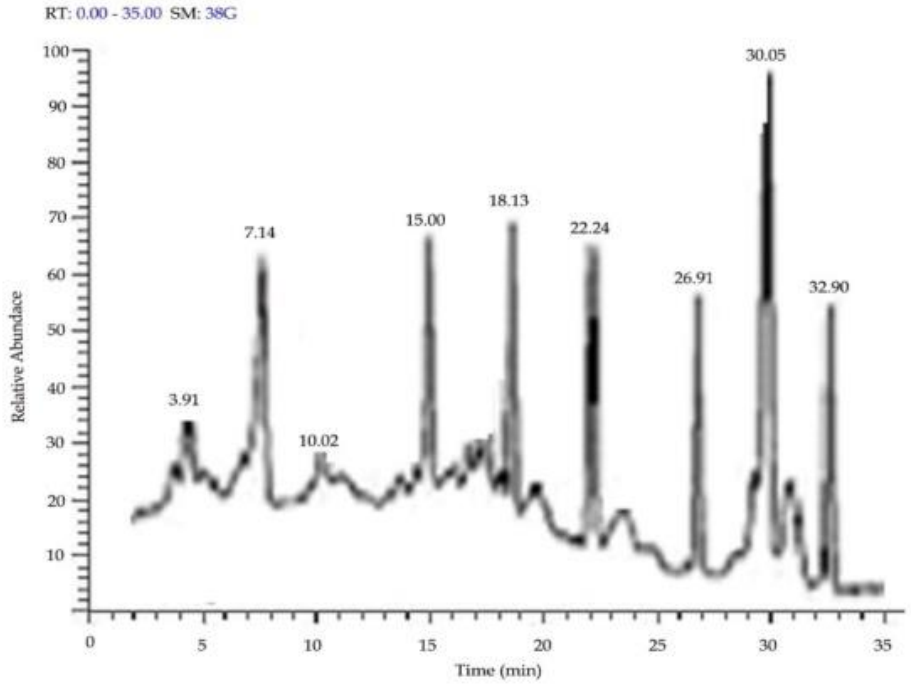

Fig-2: GC-MS chromatogram of ethanolic extract of *E. crassipes*

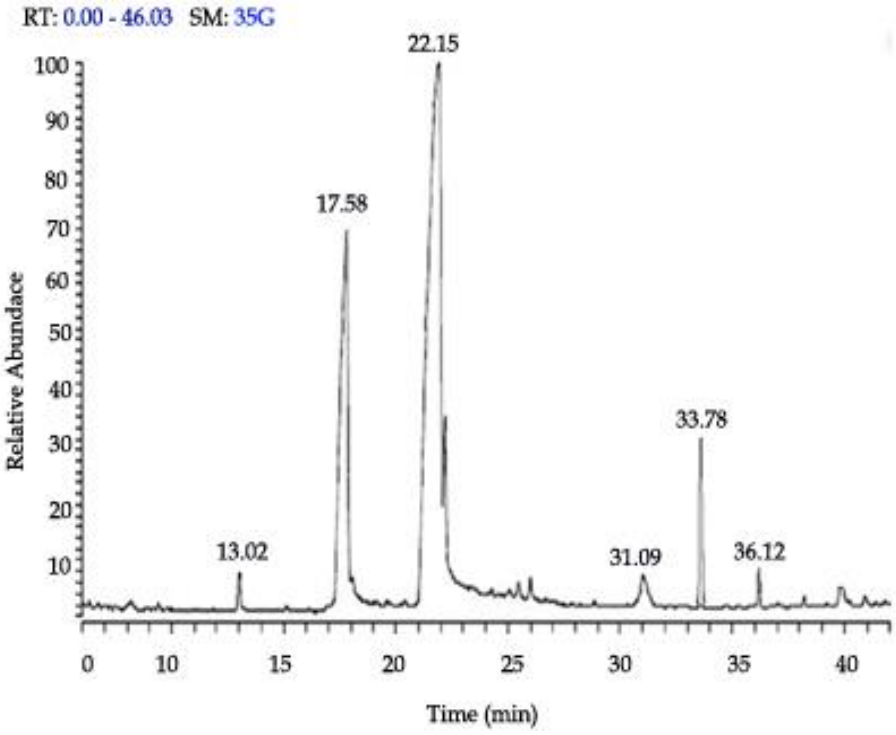

Fig-3: GC-MS chromatogram of petroleum etheric extract of *S. cristaefolium*

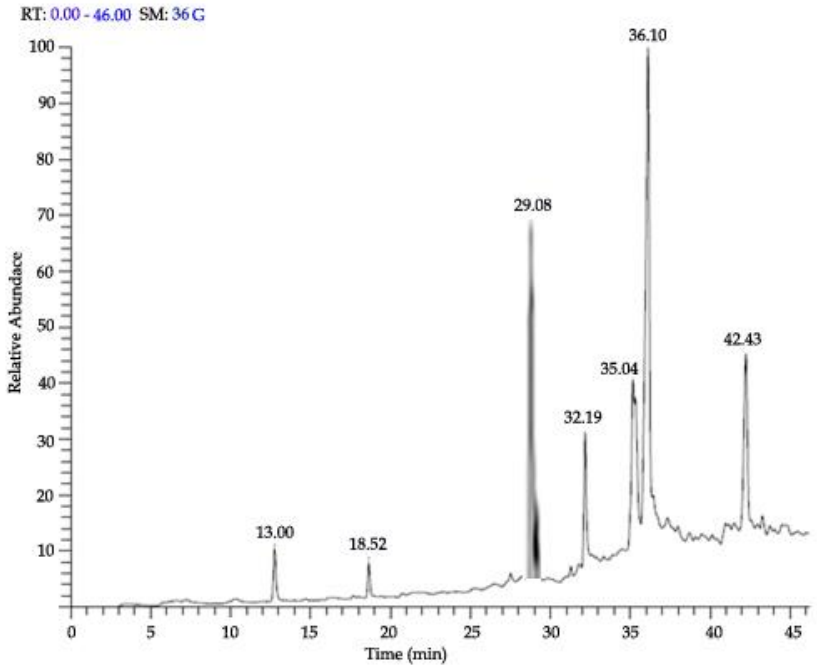

Fig-4: GC-MS chromatogram of ethanolic extract of *S. cristaefolium*

Nutritional indices and basic biochemical constituents

Significant improvements in survival rate, weight gain, food conversion ratio, protein efficiency ratio, concentration of total protein and ash content were recorded (P<0.05), particularly in PL fed with 10% raw powder and 1.5% of petroleum etheric, and ethanolic extracts of *E. crassipes* and *S. cristaefolium* (Tables 7-10). Among the two weeds, *E. crassipes* was produced little out-performence than that of *S. cristaefolium*. Among the two solvents, ethanolic extract performed better than that of petroleum etheric extract. This may be due to presence of more number of bioactive components in ethanolic extract of *E. crassipes*, or due to the presence of a particular bioactive component. In the category of *E. crassipes*, the concentration of carbohydrate showed significant increase when compared with control, whereas, the contents of lipid and moisture did not showed significant difference (Table 9). In the category of *S. cristaefolium*, the concentrations of carbohydrate and lipid showed significant increases when compared with control, whereas, the content of moisture showed significant decrease (Table 10).

Table-7: Nutritional indices of *M. rosenbergii* PL fed with fishmeal replaced with *E. crassipes* powder and its extracts incorporated diets (Initial morphometric measurement: length, 2.55±0.11cm, and weight, 0.15±0.02g)

Parameter	Control	Fishmeal replaced with *E. crassipes*			Petroleum etheric extract of *E. crassipes*			Ethanolic extract of *E. crassipes*			F-value
		5%	10%	15%	0.5%	1.0%	1.5%	0.5%	1.0%	1.5%	
SR (%)	73.00± 3.00^{b}	73.00± 4.00^{b}	80.00± 4.00^{b}	75.00± 4.00^{b}	74.00± 3.00^{b}	77.00±4 .00^{ab}	80.00±4 .00^{ab}	74.00± 3.00^{b}	77.00±4 .00^{ab}	83.00± 5.00^{a}	2.13
LG (cm)	0.54± 0.03^{b}	0.56± 0.04^{b}	0.64± 0.04^{a}	0.56± 0.02^{b}	0.54± 0.03^{b}	0.57± 0.02^{b}	0.57± 0.03^{b}	0.53± 0.02^{b}	0.57± 0.02^{b}	0.65± 0.04^{a}	6.24
WG (g)	0.40± 0.03^{f}	0.42± 0.02^{ef}	0.56± 0.03^{bc}	0.44± 0.04^{de}	0.42± 0.04^{ef}	0.46± 0.02^{de}	0.53± 0.03^{ab}	0.48± 0.03^{cd}	0.55± 0.03^{ab}	0.59± 0.04^{a}	15.44
SGR (%)	0.48± 0.18^{a}	0.54± 0.20^{a}	0.68± 0.23^{a}	0.61± 0.22^{a}	0.50± 0.21^{a}	0.57± 0.15^{a}	0.62± 0.21^{a}	0.56± 0.15^{a}	0.65± 0.22^{a}	0.71± 0.23^{a}	0.41
FCR (g)	5.60± 0.42^{a}	5.50± 0.43^{ab}	4.80± 0.44^{ab}	5.30± 0.44^{ab}	5.42± 0.33^{ab}	5.30± 0.43^{ab}	5.12± 0.41^{ab}	5.30± 0.35^{ab}	5.08± 0.44^{ab}	4.78± 0.41^{ab}	1.34
PER (g)	0.35± 0.02^{g}	0.45± 0.03^{de}	0.60± 0.05^{ab}	0.50± 0.04^{cd}	0.41± 0.04^{ef}	0.42± 0.06^{ef}	0.55± 0.04^{bc}	0.50± 0.05^{cd}	0.55± 0.04^{bc}	0.63± 0.05^{a}	12.63

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).

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

Table -8: Nutritional indices of *M. rosenbergii* PL fed with fishmeal replaced with *S. cristaefolium* powder and its extracts incorporated diets (Initial morphometric measurement: length, 2.55±0.11cm, and weight, 0.15±0.02g)

Parameter	Control	Fishmeal replaced with *S. cristaefolium*			Petroleum etheric extract of *S. cristaefolium*			Ethanolic extract of *S. cristaefolium*			F-value
		5%	10%	15%	0.5%	1.0%	1.5%	0.5%	1.0%	1.5%	
SR (%)	73.00± 3.00^{bc}	77.00±4 .00^{abc}	80.00± 3.00^{a}	78.00± 3.00^{ab}	74.00±5 .00^{abc}	75.00±2 .00^{abc}	77.00±5 .00^{abc}	73.00± 2.00^{bc}	75.00± 3.00^{c}	77.00±3 .00^{abc}	2.20
LG (cm)	0.54± 0.03^{bc}	0.45± 0.03^{e}	0.52± 0.04^{bcd}	0.46± 0.02^{e}	0.47± 0.03^{de}	0.55± 0.03^{bc}	0.57± 0.03^{b}	0.50± 0.02^{cde}	0.56± 0.03^{b}	0.66± 0.03^{a}	13.70
WG (g)	0.40± 0.03^{d}	0.41± 0.02^{d}	0.51± 0.03^{ab}	0.46± 0.03^{bcd}	0.43± 0.02^{cd}	0.46± 0.04^{bcd}	0.54± 0.04^{a}	0.45± 0.04^{bcd}	0.48± 0.03^{bc}	0.56± 0.04^{a}	7.84
SGR (%)	0.50± 0.18^{a}	0.52± 0.19^{a}	0.67± 0.24^{a}	0.56± 0.22^{a}	0.54± 0.20^{a}	0.60± 0.23^{a}	0.68± 0.20^{a}	0.56± 0.23^{a}	0.62± 0.22^{a}	0.70± 0.24^{a}	0.31
FCR (g)	5.60± 0.42^{a}	5.50± 0.46^{a}	5.06± 0.43^{a}	5.10± 0.45^{a}	5.60± 0.40^{a}	5.40± 0.43^{a}	4.90± 0.30^{a}	5.59± 0.43^{a}	5.00± 0.55^{a}	4.83± 0.53^{a}	1.43
PER (g)	0.38± 0.02^{a}	0.40± 0.04^{a}	0.50± 0.05^{a}	0.48± 0.03^{a}	0.44± 0.03^{a}	0.50± 0.05^{a}	0.51± 0.03^{a}	0.46± 0.06^{a}	0.55± 0.30^{a}	0.57± 0.05^{a}	1.04

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).

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

Table-9: Basic biochemical constituents of *M. rosenbergii* PL fed with fishmeal replaced with *E. crassipes* powder and its extracts incorporated diets

Parameters (mg/g wet wt.)	Control	Fishmeal replaced with *E. crassipes*			Petroleum etheric extract of *E. crassipes*			Ethanolic extract of *E. crassipes*			F-value
		5%	10%	15%	0.5%	1.0%	1.5%	0.5%	1.0%	1.5%	
Protein	78.12± 3.09^{ef}	80.20± 2.89^{def}	92.21± 3.93^{b}	84.16± 3.67^{cde}	76.84± 4.12^{f}	85.25± 3.87^{cd}	92.15± 4.56^{b}	79.75± 3.12^{def}	90.50± 3.75^{bc}	102.56± 3.29^{a}	14.73
Carbohydrate	25.12± 2.25^{b}	27.04± 2.54^{ab}	28.42± 2.95^{ab}	28.21± 2.69^{ab}	28.54± 2.43^{ab}	29.16± 2.56^{ab}	7.04± 3.02^{ab}	28.76± 2.76^{ab}	29.28± 2.65^{ab}	30.86± 3.01^{a}	0.999
Lipid	16.29± 1.05^{a}	16.10± 1.14^{a}	16.70± 1.66^{a}	16.25± 1.17^{a}	16.17± 1.12^{a}	16.46± 1.45^{a}	17.16± 1.61^{a}	16.76± 1.43^{a}	17.28± 1.55^{a}	17.95 ± 1.78^{a}	0.762
Moisture (%)	67.23± 2.10^{a}	68.50± 2.45^{a}	67.60± 2.26^{a}	68.00± 2.31^{a}	68.10± 2.23^{a}	67.5± 2.34^{a}	66.30± 2.14^{a}	67.60± 2.43^{a}	67.30± 2.18^{a}	65.10± 2.56^{a}	0.574
Ash (%)	11.27± 1.16^{b}	12.87± 1.50^{ab}	14.98± 1.39^{a}	13.92± 1.36^{ab}	13.65± 1.23^{ab}	13.72± 1.32^{ab}	13.90± 1.41^{ab}	13.10± 1.57^{ab}	13.42± 1.56^{ab}	13.98± 1.64^{ab}	1.379

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).

Table-10: Biochemical constituents of *M. rosenbergii* PL fed with fishmeal replaced with *S. cristaefolium* powder and its extracts incorporated diets

Parameters (mg/g wet wt.)	Control	Fishmeal replaced with *S. cristaefolium*			Petroleum etheric extract of *S. cristaefolium*			Ethanolic extract of *S. cristaefolium*			F-value
		5%	10%	15%	0.5%	1.0%	1.5%	0.5%	1.0%	1.5%	
Protein	68.12± 3.09^{de}	74.12± 2.58^{de}	90.24± 4.75^{bc}	84.10± 4.50^{cd}	76.75± 4.20^{e}	85.20± 3.80^{bcd}	92.10± 4.34^{b}	78.50± 3.34^{de}	89.00± 3.75^{bc}	100.20± 3.50^{a}	11.42
Carbohydrate	24.12± 2.25^{b}	26.20± 1.94^{ab}	28.20± 1.85^{ab}	27.20± 2.75^{a}	28.40± 2.58^{ab}	28.90± 2.10^{ab}	29.25± 2.20^{a}	29.12± 2.52^{a}	29.29± 2.40^{a}	30.15± 2.95^{a}	1.70
Lipid	14.29± 1.05^{b}	15.75± 1.10^{ab}	16.55± 1.20^{ab}	16.10± 1.10^{ab}	16.02± 1.25^{ab}	16.15± 1.55^{ab}	17.16± 2.10^{ab}	16.10± 2.05^{ab}	16.60± 1.71^{ab}	17.51 ± 1.70^{a}	0.97
Moisture (%)	70.52± 2.10^{a}	68.10± 2.00^{ab}	67.00± 2.10^{ab}	67.50± 2.43^{ab}	68.50± 2.30^{ab}	67.80± 2.40^{ab}	66.00± 2.45^{b}	69.10± 2.41^{ab}	68.20± 2.11^{ab}	65.00± 2.51^{b}	1.37
Ash (%)	11.27± 1.16^{b}	12.52± 1.25^{ab}	15.00± 1.46^{a}	13.00± 1.29^{ab}	13.59± 1.18^{ab}	13.75± 1.15^{ab}	13.90± 1.48^{ab}	13.00± 1.75^{ab}	13.50± 1.50^{ab}	13.75± 2.00^{ab}	1.35

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).

It has been reported that the marine macro algae, *T. ornata*, *G. corticata* and *S. polycystem* incorporated feed produced significant improvement in survival rate, weight gain, food conversion ratio, protein efficiency ratio and basic biochemical constituents, such as total protein and ash contents in *M. rosenbergii* [36, 37, 39, 56]**.** The enhanced growth performance have also been reported in *Penaeus monodon, Penaeus indicus, Papeneopsis stylirostris, Litopenaeus vannamei* and *M. rosenbergii* due to the green seaweed, *Enteromorpha* sp [57], in *Penaeus indicus* juveniles fed with seaweeds, *Ulva lactuca* and *S. wightii* enriched *Artemia* nauplii [58]**,** in *M. rosenbergii* PL fed with the freshwater microalgae, *Spirulina platensis*, *C. vulgaris* and *Azolla pinnata* incorporated feeds [33, 34], in the red sea bream, *Pagrus major* due to the brown seaweeds, *Undaria pinnatifida* and *Ascophyllum nodosum* [59], and in the fish Gibel carps, *Carassius auratus gibelio* due to *Chlorella* [60].

The algae incorporations have served as appetizer due to their active principles, which stimulate secretions of gut, resulted in improved digestibility, absorption and accumulation of nutrients, which in turn induce transcription and ultimately protein synthesis in prawns [24, 25, 33, 34, 36-39, 56]. Therefore, the results in the present study indicate that the available protein in the diets was efficiently utilized for biomass production. Thus, the raw powders of *E. crassipes* and *S. cristaefolium* are recommended as ingredients, and their ethanolic extracts are recommended as feed additives in aqua feed formulations for sustainable culture of *M. rosenbergii*.

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