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Bioefficacy of marine seaweed *Sargassum wightii* Greville ex J.Agardh extracts on the growth of *Bombyx mori* (L.) larvae

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Marine algae are renewable living resources that are a rich source of structurally important novel and biologically active secondary metabolites. These seaweeds are economically valuable resources, used as food, fodder, fertilizer, and medicine and thus useful to mankind in many ways. In the present study, the brown alga, *Sargassum wightii* Greville ex J. Agardh (SW) has been analyzed for secondary metabolites using three solvent extracts (acetone, ethyl acetate and water), and were tested for growth efficacy at five different concentrations (100, 200, 300, 400 and 500 ppm) on the mulberry silkworm *Bombyx mori* (L.) third instar larvae by leaf dip method. Extraction rate of *S. wightii* water extract was 1.3 g (F= 1399.29; df=3, 36; p=0.005); ethyl acetate extract was 0.7 g (F= 30.76; df=3, 36; p=0.005); and acetone extract was 0.4 g (F= 1399.29; df=3, 36; p=0.005). Results have shown the presence of steroids, such as tannins, phlobatannins and saponins; flavonoids, phenolic compounds and xanthoproteins in these extracts obtained by cold percolation method. The seaweed extract enhanced the growth of silkworm larvae *B. mori* with increased concentrations. The highest concentration (500 ppm) of water extract showed more growth rate than the control. The 100, 200, 300 and 400 ppm of water extract showed maximum growth comparable to the control at 96 h of our study period. This finding envisages that secondary metabolites could be extracted with the cold percolation method and can be used for biological and agricultural lead.

Keywords: Brown algae, Feed supplement, Mulberry silk moth, Silkworm

Sericulture is an agro-based industry in India with high employment potential and economic benefits to agrarian families[1]. Silk is a fibre produced mainly by the domestic silkworm or mulberry silk moth, *Bombyx mori* (L.), belonging to the Lepidopteran order, Bombycidae family, providing more than 99% of the world's silk[2]. India is the second largest producer of mulberry silk and is cultivated on around 282 thousand hectares of land[1]. The mulberry leaves are the sole source of food for the larval instars of the silkworm, *B. mori*. This insect is a typical monophagous insect[2]. Man has immensely benefited from the silk product by silkworms and subsequently, researchers have been trying to unveil the factors that can be manipulated to the benefit of the silkworm rears[3]. Silkworm pupae are used as food and as a source of oil and it reported to contain vitamin B1, B2 and vitamin E[4].

Macroscopic marine algae, popularly known as 'seaweeds', are one of the most important living resources of the ocean. They are found attached to the bottom in relatively shallow coastal waters. They grow in intertidal, shallow, and deep sea areas up to 180 meters depth or on a solid substrate such as rocks, dead corals, pebbles, shells, and other plant materials[5]. India, a tropical South Asian country, has a stretch of about 7,500 km of coastline, excluding its island territories by 2 million km[6]. Marine organisms produce different primary and secondary metabolites for their survival, defence and signal as well as to protect themselves from environmental pressures and attacks[7]. In this context, seaweeds are a promising source of natural products.

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Marine macroalgae are nature's most biologically active resources, as they possess a wealth of bioactive compounds like fatty acids[5], polysaccharides[8], alkaloids and phenolic compounds7. Furthermore, seaweed extracts offer a novel approach to growth management[9]. Though seaweeds have been used for many purposes, their secondary metabolites utilization for pest management is still at the infant stage. Seaweed dietary fibres perform a varied range of functions such as bactericidal[5], antioxidant, antimutagenic, anticoagulant, antitumor, fungicidal, medicine, antimicrobial[10] as well as source of food and feed[11]. Primary metabolites comprise common sugars, amino acids, proteins and chlorophyll while secondary metabolites consist of alkaloids, flavonoids, tannins, saponins, terpenoids, phenolic compounds, etc.[5].

As the rich source of various metabolites marine algae have been reported to influence the life and performance of different insects[12]. In the present study, we explored the impact of the brown alga seaweed *Sargassum wightii* extracts on the growth of the multivoltine, cross-breed silkworm, *Bombyx mori* (L.) (Race: PMX CSR2).

Materials and Methods

Collection and preparation of algal seaweed

The selected seaweed was collected by hand picking method from the submerged marine rocks at Idinthakarai (N 08°10'32.3" E 077°44'31.3") in Tirunelveli district, Tamil Nadu, India. The selected seaweed was collected during low tide in the intertidal and subtidal regions where the vegetation was discontinuous and occurring in patches. After collection, the collected seaweed was washed thoroughly thrice with tap water and once with sterile distilled water to remove salt, sand and epiphytes. A fresh sample was preserved in 4% formalin. The latitude and longitudes of the study area were recorded using GPSmap 76 (GARMAN). Cleaned seaweeds were shade dried for two weeks, partially powdered using a domestic blender (Preethi XL-7, Maya Appliances (P) Ltd, Madras), and used for the experiments.

Identification of algal seaweed

The selected alga (both preserved and herbarium) was identified by Dr. K Eswaran, Scientist-In-Charge at CSIR-Central Salt & Marine Chemicals Research Institute (CSMCRI), Mandapam, Ramanathapuram, Tamil Nadu.

Extraction of algal seaweed

For extraction of secondary metabolites by cold percolation methods was followed. For the cold percolation method, 250 g of the powdered algal material was soaked with 750 mL (1:3 w/v) of selected polar and nonpolar solvents (Acetone, Ethylacetate and Water) in an aspirator bottle for 48 h at room temperature of 27±2°C. The extract was filtered through a funnel with Whatman no. 1 filter paper. After filtration, the filtered extract was poured into the distillation unit at 20°C for separation of the solvent and the secondary metabolites residue (10 mL) was evaporated and dried over sodium sulphate in a desiccator under vacuum. The crude extracts were stored in the refrigerator (LG, India) for further use. The extraction rate was calculated by weighing the crude extracts obtained from 100 g of dry plant material after extraction with respective solvents.

Phytochemical screening

The qualitative phytochemical analyses of selected algal seaweed extracts were performed using the standard procedures of Mohanty & Adhikary[13].

Silkworm rearing and growth bioassay

For the present study, 4th instar larvae of multivoltine silkworm, *Bombyx mori* (L.) (Race: PMX CSR2) was obtained from the Department of Sericulture, Demonstration cum training center, Govt. of Tamil Nadu, V. M. Chatram, Tirunelveli, Tamil Nadu, India. The collected larvae were reared in the Pilot Silkworm Rearing Centre,Department of Zoology, Sadakathullah Appa College (Autonomous), Rahmath Nagar, Tirunelveli, Tamil Nadu, India. Fresh and healthy leaves of mulberry (*Morus alba*; MR2 variety) were used in the present study. An optimum temperature of 25±1°C and 70±5% relative humidity were maintained throughout the experimental period. The bottoms of the rearing trays were lined with paraffin paper and the edges were with wet foam rubber strips. Bed cleaning, spacing, and feeding tie were adopted carefully following the methods of Kumari *et al*.[14].

Fresh mulberry leaves were collected daily from the mulberry farm during the early hours of the day and stored in cool conditions to maintain their freshness using a wet gunny cloth. The mulberry leaves were separately soaked in the different concentrations (100, 200, 300, 400 and 500 ppm) of the selected seaweed extract solution for 15 min and then dried in air for 10 min following the method of He *et al*.[15] with slight modifications. The larvae were taken in equal numbers of 30 each in 5 trays and fed with soaked mulberry leaves. It was observed for 24, 48, 72 and 96 h. Control group larvae with the same number were maintained and fed with water-soaked mulberry leaves (without seaweed extract) and the larval weight was recorded in the control and experimental groups.

Statistical analysis

The extraction rate and growth rate results were expressed as Mean ± Standard error (SE). The data were analyzed by comparing the means of One-Way Analysis of Variance (ANOVA) and the p-value arrived at 5% to assess the statistical significance using the statistical package SPSS (20.0 version).

Results and Discussion

Seaweeds are a rich source of many nutrients such as phosphorus, potassium, zinc, calcium, iron, copper, magnesium, manganese, sodium, nickel, etc.[16] and the nutrient-rich seaweed extracts are found to be effective in increasing growth of plants and other organisms[17]. Enrichment of the mulberry leaves and seaweed extract by nutrient supplementation is one of the strategies by which the larvae's weight can be enhanced and maintained. The crude extract obtained from the brown alga, *S. wightii* water extract yielded 1.3 g (F= 1399.29; df=3, 36; p=0.005), ethyl acetate extract yielded 0.7 g (F= 30.76; df=3, 36; p=0.005), and acetone extract yielded 0.4 g (F= 1399.29; df=3, 36; p=0.005), respectively (Fig. 1). The results of the phytochemical analysis of *S. wightii* extracts revealed that steroids, tannins, and flavonoids were present in the acetone extract. Tannins, steroids, flavonoids, xanthoproteins, and phenolic compounds were present in the ethyl acetate extract. Tannins, saponins, phlobatannins, flavonoids, and phenolic compounds were present in the water extract of *S. wightii* (Table 1).

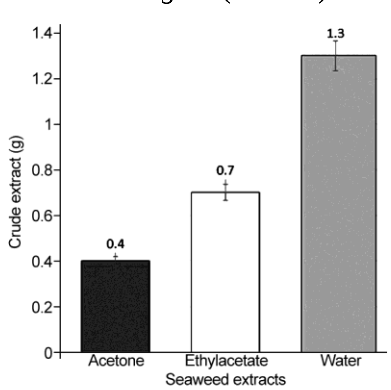

Fig. 1 — Weight of crude extracts (in gram) obtained from the brown alga, *Sargassum wightii*

In the present study, the *S. wightii* extracts significantly increased the growth of the silkworm larvae *B. mori* and the water extract of *S. wightii* showed the highest growth rate of 0.67 mg at 500ppm after 96 h of exposure time (Fig. 2). In the control group, the growth of the larvae was observed to be normal on mulberry leaves without seaweed extracts (Fig. 2). Similar to our study, He *et al.*[15] reported that 0.01, 0.1 and 1% lactic acid supplementation positively increased *B. mori* larval weight and female cocoon shell weight compared to the control group. Further, Bhatti *et al.*[18] reported that supplementation of 2% aqueous honey significantly increases larval weight and silk cocoon yield.

Seaweed also contains several compounds which are biologically active like antiviral, antibacterial, antifungal, antioxidant, hypertensive, and pesticidal properties[8]. Compounds of phenols, oxygen hetero-cyclic, terphenyls, sterols, polysaccharides, dibutenolides peptides, saponins, flavonoids, alkaloids, terpenoids, phlobatannins, tannins, alkaloids, anthraquinones,

Table 1 — Phytochemical analysis of different extracts of the brown alga, *Sargassum wightii*

Phytochemicals	*Sargassum wightii* extracts		
	Acetone	Ethyl acetate	Water
Alkaloids	-	-	-
Steroids	++	+	-
Reducing sugar	-	-	–
Tannins	++	++	+++
Phlobatannins	-	-	+
Saponins	-	-	++
Flavonoids	+	+	+
Terpenoids	-	-	-
Cardiac glycosides	-	-	-
Phenolic compounds	-	+	+
Amino acid	-	-	-
Essential oils	-	-	-
Aromatic acids	-	-	-
Xanthoprotein	-	+	-
Carbohydrate	-	-	-

+ indicates present; - indicates absent; + is intense; ++ is moderately intense; +++ is highly intense

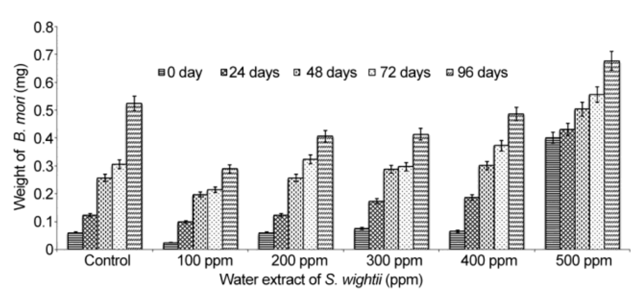

Fig. 2 — Effect of water extract of *Sargassum wightii* on the growth of the silkworm *Bombyx mori*

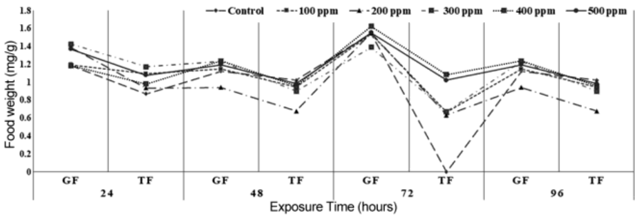

Fig. 3 — Food intake capability of *Bombyx mori* in *Sargassum wightii* water extract treatment

reducing sugars, cardioglycosides, amino acids, and proteins have been reported earlier from different seaweed extracts globally[8]. In the present study, screening of secondary metabolites from different extracts (acetone, ethyl acetate, and water) of *S. wightii* revealed the presence of steroids, tannins, and flavonoids were present in the acetone extract. In ethyl acetate extract tannins, steroids, flavonoids, phenolic compounds, and xanthoproteins were present. In the water extract, tannins, saponins, phlobatannins, flavonoids, and phenolic compounds were present in the water extract of *S. wightii*. Similarly, our result also revealed the presence of steroids, tannins, flavonoids, xantho-proteins, saponins, phlobatannins, and phenolic compounds in the extracts of seaweed *S. wightii*.

In the present study, we observed concentration-dependent growth. Water extract of *S. wightii* showed the highest growth rate in the mulberry silkworm *B. mori* in all the tested concentrations comparing other extracts (Fig. 2). At 500 ppm concentration, the water extract of *S. wightii* showed 0.67 mg growth rate in *B. mori* after 96 h of exposure. Similarly, the water extract of *S. wightii* showed 0.55, 0.50, 0.43 and 0.40 mg growth rates in *B. mori* at 400, 300, 200 and 100 ppm after 72, 48 and 24 h of exposure, respectively. The food intake capability of *B. mori* differed between the concentrations at 24, 48, 72 and 96 h of exposure. Initially, the silkworm larvae *B. mori* showed sluggish nature in the uptake of feed up to 24 h. However, the larvae started taking feed after 24 h and consumed maximum food at 96 h in all concentrations (Fig. 3).

The quantity and quality of dietary protein have long been considered to be important in the silkworm's growth. Higher growth rate, as well as weight gain, can be observed in the higher protein utilized group, and the relative growth rate varied among the different breeds of the silkworm[19]. In the present study, the difference in the growth rate of seaweed extract supplemented larvae from the control larvae indicates that the *S. wightii* water extract supplementation results in a higher growth rate compared to the control group at 500 ppm concentration after 96 h of exposure.

Conclusion

Results of this study have demonstrated a concentration-dependent growth in the mulberry silkworm *Bombyx mori* in the extracts of seaweed, the brown alga, *Sargassum wightii*. This seaweed supplementation will add to quality feed for larval culture in sericulture industry.

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