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Effect of Herbal Plant Extracts on the Economic Parameters of Silkworm *Bombyx mori* L.

Sithi Jameela Muthu Mohamed[1*], Ponananthi Karnan[2], Chitra Devi Murugesan[3], Ramesh Kumar Senthur Pandi[2]

[1]Department of Zoology and Research Centre, Sadakathullah Appa College (Autonomous), Rahmath Nagar, Tirunelveli 627 011. Affiliated by Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli-627012, TamilNadu, India.
[2] Department of Zoology and Research Centre, Sadakathullah Appa College (Autonomous), Rahmath Nagar, Tirunelveli 627011, India

*Email: sithijameela57@gmail.com

Abstract

Background: In the unorganized sector, the silk industry has grown to become a well-liked cottage business that employs more than 10 million rural residents. The development of new technologies that are efficient, labor-saving, and environmentally benign is being pursued.
Objectives: To evaluate the secondary metabolites present in the selected *Cucurbita moschata* and *Ipomoea batatas* herbal plant (Leaf) extracts with five solvents namely ethanol, chloroform, acetone, petroleum ether, and water. To determine the antimicrobial activity of plant extracts against selected bacterial strains and assess the effect of fortified mulberry leaves with plants on the growth of silkworms and their weight of cocoon, pupa, shell, and shell ratio of silkworm *Bombyx mori*.
Method: In this study, the selected herbal plants (*Cucurbita moschata* and *Ipomoea batatas*) were collected by hand-picking method from Singaneri village, Nanguneri Taluk, Tirunelveli districts, Tamil Nadu, India. The qualitative phytochemical analyses of selected herbal plant extracts were performed using standard procedures.
Results: Phytochemicals results revealed that the different extracts (petroleum ether, chloroform, acetone, ethanol, and water extracts) revealed that, phenolic compounds, steroids, saponins, and xanthoproteins were present in the acetone extract. In petroleum ether tannins, saponins, pholobatanins, cardiac glycosides, phenolic compounds, flavanoids, carbohydrates, and xanthoproteins were present. In the antibacterial activity, ethanol and acetone extracts showed significant inhibitory effects against the pathogens viz., *Enterococcus*, *E.coli*, *Aceto bactor* and *Bacillus cereus* extracts did not show any activity. Moderate activity was expressed by ethanol extracts of *I. batatasa gainst E.coli* and *Acetobactor* (8.1±0.3mm diameter) concerning petroleum ether extracts. *C. moschata* revealed high activity against *E.coli* and Acetobactor with diameter of inhibition greater than 13.0±0.2 mm while *C. moschata* exhibited only moderate activity against *E.coli* and *Acetobactor*.
Conclusion: The selected herbal plant extracts could increase some biological characteristics of silkworm *B. mori*, but this enhancement could economically improve the sericulture goals. The growers might be offered this substance to help them produce more silk.

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Keywords

Phytochemical study, Anti bacterial activity, Supplementary feed, Herbal plant, Economic parameters

Introduction

A typical monophagous insect, the silkworm *Bombyx mori* only eats mulberry leaves. The nutritional quality of the leaves supplied to silkworms has a significant impact on their healthy growth and, eventually, their economic features (Krishna Swamie et al., 1971; Ravikumar, 1988). According to Rajashekaragouda et al. (1997), plants are the world's richest source of organic compounds, and phytochemicals from plants can affect the lives and behavior of certain insects. Mulberry leaf quality and silkworm feed efficiency have recently been improved by numerous initiatives to strengthen mulberry leaves with botanical extracts, which in turn aids in increasing cocoon production and silk quality. Previous research (Shivakumar et al., 1995; Murugan et al., 1998) supported the effect of numerous extracts on a variety of metabolic processes that increased the development and spinning of silk cocoons. As a beneficial modern practice, feeding silkworms, and fortifying mulberry leaves with additional nutrients has boosted the cocoon's economic value. The primary determinant of successful cocoon and crop production is thought to be the caliber of leaves supplied to the silkworms. Higher leaf quality would increase the likelihood of a successful cocoon harvest.

The enrichment of mulberry leaf with nutrient supplementation such as probiotics, antibiotics, vitamins, and amino acids is one of the strategies by which cocoon, and silk productivity can be increased and the quality can be hanced. India is the largest producer of medicinal plants, and it is rightly called the “Botanical Garden of the World”. Medicinal plants have natural therapeutic values against different diseases. The medicinal plants may promote host resistance against the infection by rest stabilizing body equilibrium and conditioning our body tissues. Medicinal plants are also easily available, and cheaper and they possess no toxicity as compared to that drug (Uma devi et al., 2013). This finding helps in further research in the investigation of other herbal plant extracts for their biological activities and also for the possibility of their being used as a source of feed for silkworms.

Methodology

Collection and preparation of herbal plants

The selected herbal plants (*Cucurbita moschata* and *Ipomoea batatas*) were collected by handpicking method from Singaneri village, Nanguneri Taluk, Tirunelveli districts, Tamil Nadu, India from March 2022. After collection, the collected herbal plants were washed thoroughly thrice with tap water and once with sterile distilled water to remove dust and sand particles. Cleaned herbal plants 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. The powdered herbal plant material was extracted by the soxhlation method using polar and non-polar solvents.

Extraction of herbal plants

For the extraction of secondary metabolites,250gm of powdered herbal plant power was extracted by soxhlation method using Petroleum Ether (PE), Chloroform (CH), Acetone (AC), Ethanol (EL), and Water (WA) separately and subsequently by hot continuous extraction (24hrs) (40-50º C) at room temperature in 750 ml capacity Soxhlet apparatus. After the solvent removal, the residue (10 ml) was evaporated and dried over sodium sulfate in a desiccator under a vacuum. The extracts were concentrated under reduced pressure by a desiccator, collected in air-tight glass vials (9.4 cm), and stored in our fridge at or (LG, India) for further use.

Phyto chemical screening

The qualitative phytochemical analyses of selected herbal plant extracts were performed using the standard procedures of Brindha et al. (1981) and Harbone (1984).

Antibacterial activity of the herbal plant extracts

The petroleum ether, chloroform, acetone, ethanol, and water extracts of different herbal plant extracts were used throughout the study. The condensed extracts were dissolved in 4% DMSO4 (dimethyl Sulfoxide). The petroleum ether, chloroform, acetone, ethanol, and water extracts of *Cucurbita moschata* and *Ipomoea batatas* were tested against different bacterial test pathogens for their antibacterial activity. The antibacterial activity of the herbal plant extracts was tested using the good diffusion method. The prepared culture plates were inoculated with different selected bacterial strains using the streak plate method. Wells were made on the agar surface with a 6mm cork borer. The extracts were poured into the well using a sterile syringe. The plates were incubated at 37±2ºC for 24 hours for bacterial activity. The plates were observed for the zone formation around the wells.

Silkworm rearing

For the present study, the third instar larvae of multivoltine silkworm, *Bombyx mori* (L.) Race (PMX csR2) was obtained from the Department of Sericulture, Demonstration cum training center, Government of Tamil Nadu, V. M. Chatram, Tirunelveli- 627 011, 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 (MR2 variety) of mulberry were used in the present study. An optimum temperature of 25±10 C and 70±5% relative humidity was maintained throughout the experimental period. The bottoms of the rearing trays were lined with paraffin paper and the edges with wet foam rubber strips (Jolly,1986). Bed cleaning, spacing, and feeding time were adopted carefully following the methods of Krishnaswami (1978).

Fresh MR2 mulberry leaves (*Morus alba*) 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 (2.5, 5.0, 7.5, and 10%) of the selected herbal plant extract solution for 15 minutes and then dried in air for 10 minutes. The larvae were taken in equal numbers of 30 each in three trays and fed with soaked mulberry leaves. It was observed for 24, 48, 72, and 96hrs. Control group larvae with the same number are maintained and fed with water-soaked mulberry leaves and the larval, pupal, cocoon, and shell weights were recorded in control and experimental groups.

Results and Discussion

Phytochemicals results revealed that the different extracts (petroleum ether, chloroform, acetone, ethanol, and water extracts) of *Ipomoea batatas* and *Cucurbita moschata* revealed that, phenolic compounds, steroids, saponins, and xanthoproteins were present in the acetone extract. In petroleum ether tannins, saponins, pholobatanins, cardiac glycosides, phenolic compounds, flavonoids, carbohydrates, and xanthoproteins were present. In chloroform extract steroids, tannins, saponins, pholobatanins, cardiac glycosides, flavonoids, phenolic compounds, carbohydrates, aromatic acids, and xanthoproteins were present. In ethanol extract steroids, tannins, terpenoids, phenolic compounds, aromatic acids, flavonoids, reducing sugars and xanthoproteins were present. In water extract, alkaloids, tannins, saponins, flavonoids phenolic compounds, aromatic acids, carbohydrates, pholobatanins, reducing sugar, terpenoids, cardiac glycosides, amino acids, essential oil, and xanthoproteins were present in the water extract of *Ipomoea batatas* (Table 1and 2). These sterols are widely distributed in the plant but in trace quantity in algae (Saeidnia et al., 2012).
However, terpenoids (cardiac glycoside, steroids, saponins), phenolics (flavonoids, tannins), xantho protein were recorded in the qualitative analyses indicating that all kinds of secondary metabolites can be extracted using the universal solvent water. However, if we keep the water extracts for four to five days, an inevitable problem arises due to bacterial and fungal growth, which often degrades the active constituents or gives false results in bioassays due to endotoxins produced by microorganisms (Tiwari et al., 2011). To avoid this problem, we analyzed the water extracts immediately after the extraction.

The antibacterial activity exhibited against selected Gram-positive and Gram-negative bacteria of *C. moschata* and *I. batatas* was summarized in Table 3. Ethanol and acetone extracts showed significant inhibitory effects against the pathogens viz., *Enterococcus, E.coli, Acetobactor*, and *Bacillus cereus*. extracts did not show any activity. Moderate activity was expressed by ethanol extracts of *I. batatas* against *E.coli* and *Acetobactor* (8.1±0.3mm diameter) concerning petroleum ether extracts. *C. moschata revealed high activity against E.coli and Acetobactor with a diameter of inhibition greater than 13.0*±0.2 mm while *C. moschata* exhibited only moderate activity against *E.coli* and *Acetobactor* and the other two Gram-positive bacteria. The antibacterial activity of water extracts extracted from *C. moschata* and *I. batatas* was studied and the result showed that only a slight inhibition zone was noticed with (4mg/ml) extracted from *C. moschata* and *I .batatas* respectively (Table 3).

In sericulture, nutritional requirements and conversion efficiency contribute directly or indirectly to the cost-benefit ratio of silkworm rearing. It was regarded as a crucial physiological factor for determining which silkworm breeds were superior. In silkworms, 97% of the total food intake during the last two instars and the feed utilization study confined to V^{th} instar larvae as 80-85% of the total leaves consumed in this instar as silkworms very active metabolically at this stage.

The silkworm's growth and development are continuously influenced by both internal and external influences (Murugan *et al*., 1998). The growth of silkworms was impacted by ascorbic acid (Javed and Gondal, 2002). Mulberry leaves with a combination of Nitrogen (0.2%) which enhances the growth and silk production (Javed and Gondal, 2002). Amway protein supplemented

(10%) mulberry leaf significantly improved larval growth and economic characteristics of silkworms (Amala Rani et al.,2011).

In this study, *C. moschata* and *I. batatas* water extract enhanced the growth of silkworm larvae *B. mori* followed by 2.5%, 5% and 7.5% when compared to control at different exposure times moderately increased the growth rate of the *B. mori*. The weight of the mulberry silkworm gradually increased from lower concentrations to higher concentrations (2.5%, 5%, 7.5%, and 10%). The results of *C. moschata* water extract enhanced the growth of silkworm larvae *B. mori* followed by 2.5%, 5%, and 7.5% when compared to control at different exposure times moderately increased the growth rate of the *B. mori* (Figure 1.). The weight of the mulberry silkworm gradually increased from lower concentrations to higher concentrations (2.5%, 5%, 7.5% and 10%). When compared to the control the higher concentration (10%) showed more growth rate compared to other concentrations. The herbal plant extracts enhanced the growth of silkworm larvae *B. mori* with the increase of concentrations. The 5%, 7.5%, and 10% showed maximum growth and also increased the cocoon weight, shell weight, and shell ratio when compared to the control of our study period (Figure 1). The herbal plant extracts enhanced the growth of silkworm larvae *B.mori* with the increase of concentrations. The 5%, 7.5% and 10% showed maximum growth and increased the cocoon weight, shell weight, and shell ratio when compared to the control of our study period (Figure 2). The nutritive value of mulberry leaves depends on various agro-climatic factors and any deficiency of nutrients in leaves affects silk synthesis by the silkworm.

Table 1. Qualitative phytochemical analysis of *Ipomoea batatas*

Phytochemicals	***Ipomoea batatas***				
	Acetone	**Chloroform**	**Ethanol**	**Petroleum ether**	**Water**
Alkaloids	–	–	–	–	+
Steroids	+	+	–	–	–
Reducing sugar	+	–	+	–	–
Tannins	+	++	+	+	++
Pholobatanins	–	–	–	+	+
Saponins	–	+	–	+	++
Flavonoids	+	+	+	+	+
Terpenoids	–	–	–	–	–
Cardiac glycosides	–	+	–	+	–
Phenolic compounds	+	+	–	+	+
Amino acid	–	–	–	–	–
Essential oils	–	–	–	–	–

Aromatic acids	_	_	_	_	+
Xanthoprotein	+	+	_	+	+
Carbohydrate	_	+	_	+	+

(+indicates present and–indicates absent; +-intense,++-highly intense)

Table 2. Qualitative phytochemical analysis of *Cucurbita moschata*

Phytochemicals	***Cucurbita moschata***				
	Acetone	**Chloroform**	**Ethanol**	**Petroleum ether**	**Water**
Alkaloids	_	_	_	_	+
Steroids	+	+	_	_	_
Reducing sugar	_	_	+	_	+
Tannins	_	++	+	_	++
Pholobatanins	_	+	_	_	+
Saponins	+	+	_	_	++
Flavanoids	_	_	+	_	_
Terpenoids	_	_	_	_	+
Cardiac glycosides	_	+	_	_	+
Phenolic compounds	_	_	_	_	_
Amino acid	_	_	_	_	+
Essential oils	_	+	_	+	+
Aromaticacids	_	+	_	_	_
Xanthoprotein	_	+	_	+	+
Carbohydrate	_	_	_	_	_

(+indicates present and–indicates absent; +-intense,++-highly intense)

Table 3. Antibacterial activity of different solvents extracts of *Cucurbita moschata* and *Ipomoea batatas* against Gram positive and Gram negative bacteria

Bacterial Pathogens	Control (mm)					Test (CM)(mm)					Test (IB) (mm)				
	WA	CH	AC	ET	PE	WA	CH	AC	ET	PE	WA	CH	AC	ET	PE

Enterococcus	-	-	-	-	10	-	11	12	12	-	-	13	12	14	18
E. coli	-	-	9	-	11	-	8	11	16	-	-	9	10	10	20
Acetobacter	-	-	-	11	8	-	12	13	15	-	-	11	10	15	20
B. cereus	-	-	-	-	-	-	11	9.5	11	11	-	11	9	9	10

WA-Water, CH-Chloroform, AC-Acetone, ET-Ethanol, PE-Petroleum Ether

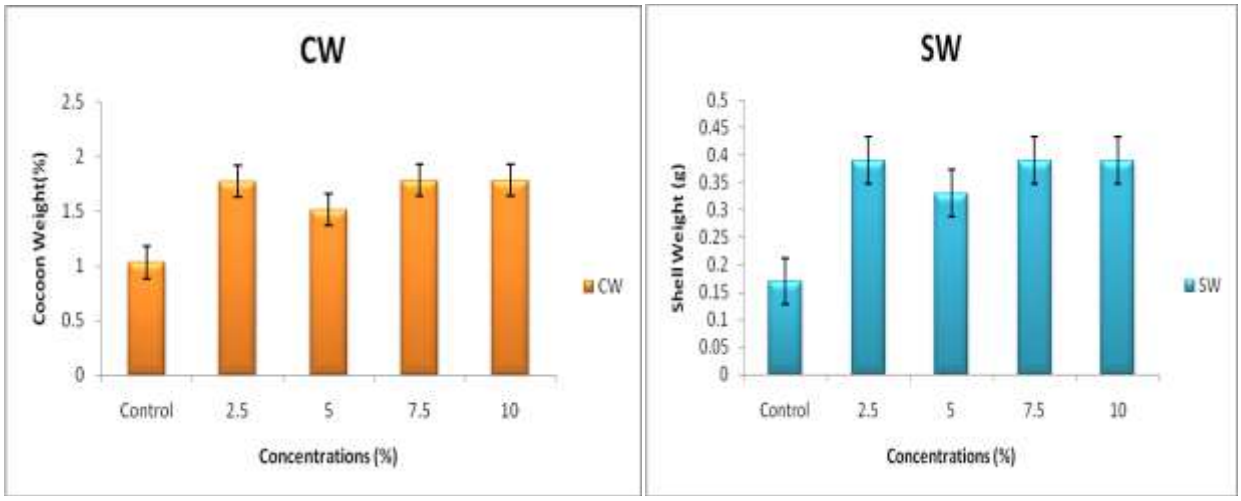


Figure 1. Different concentrations(2.5%,5%,7.5%and10%)of selected *Cucurbita moschata* water extract treated against mulberry silkworm *Bombyx mori* (Cocoon weight (A), Pupal weight(B),Shell weight(C))and Shell ratio (D).

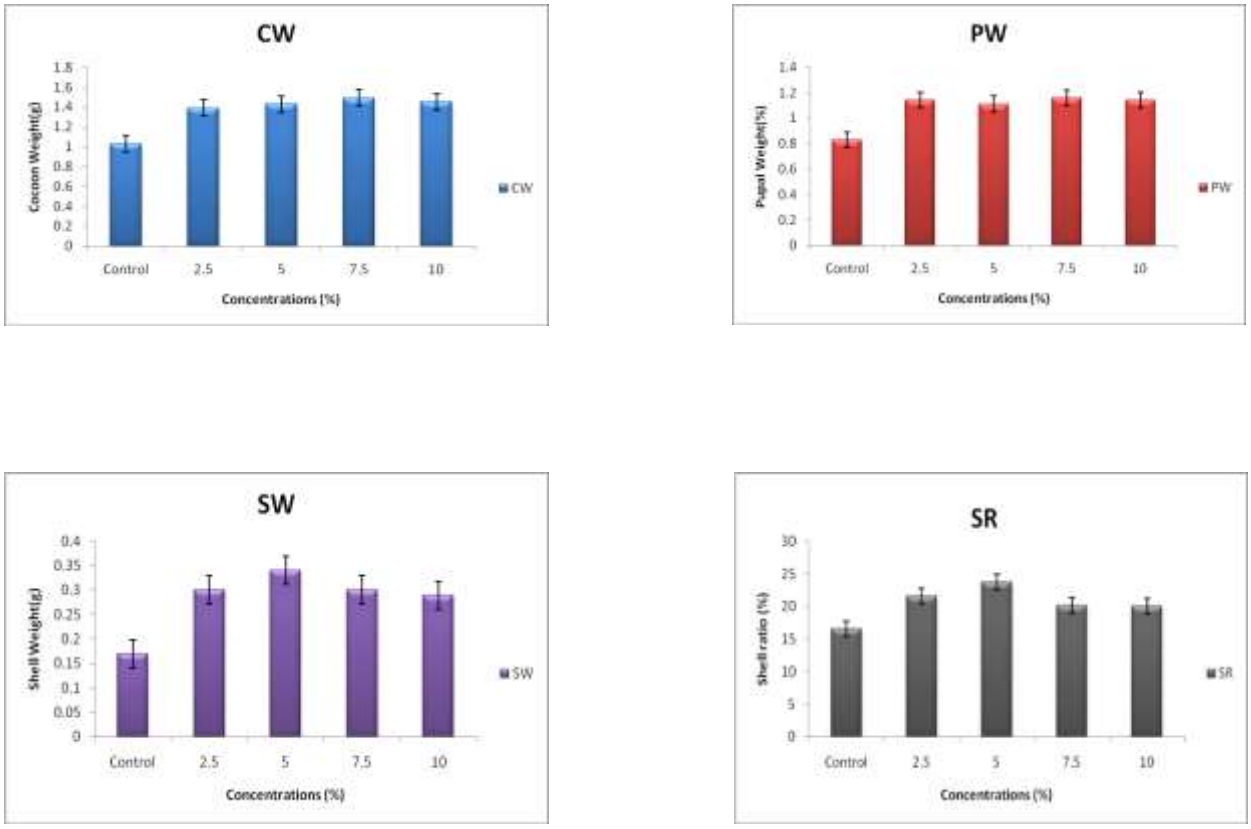


Figure 2. Different concentrations (2.5%, 5%, 7.5% and 10%) of selected *Ipomoea batatas* water extract treated against mulberry silkworm *Bombyx mori* (Cocoon weight (A), Pupal weight(B),Shell weight(C))and Shell ratio(D).

Conclusion

In the present study, the treatment of selected herbal plant extracts (*Cucurbita moschata* and *Ipomoea batatas*) at the concentration of 7.5% and 10% respectively had significant beneficial effects on the growth of the silkworm, cocoon weight, shell weight, pupal weight and shell ratio parameters and also increase the quantity of silk by enhancing the feed efficacy. This supplementation could be prescribed to the farmers to get a larger quantity of silk.

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