



Antibacterial activity and efficient use of acetone leaf extract of *A. Muricata* for improvement of commercial traits in silkworm, *B. mori*, L

Janga Sathis¹, Kuntamalla Sujatha²

^{1,2} Sericulture Unit, Department of Zoology, Kakatiya University, Warangal, Telangana, India

Abstract

In this experiment utilization efficiency of mulberry silkworm was studied. Acetone extract of *Annona muricata* was evaluated on rearing performance, nutritional efficiency, commercial characters of silkworm in vitro (24±1⁰, 75±5% RH and 16L:8D). In this study, the antibacterial activity of sour sap of five different solvents were evaluated using disk diffusion method against two colonies of bacteria (colony-1 and colony-2). The highest zone of inhibition (19.33mm) was observed with acetone leaf extract against both colonies. The efficiency of conversion of ingested food (ECI), conversion of digested food (ECD), digesta, approximate digestibility (AD), reference ratio (RR) after treatment with *A. muricata* at T3 treatment was high when compared to other treatments and control which may be due to growth stimulation factors and also the presence of secondary metabolites.

It can be concluded from above mentioned studies the silkworm feed on mulberry leaves fortified with *A. muricata* at different concentrations influenced the larval growth, nutritional efficiency and cocoon characters which are beneficial to the farmers.

Keywords: *Annona muricata*, nutritional efficiency, commercial characters, antibacterial activity

Introduction

Sericulture is a cottage industry occupying a unique position in agriculture for hundreds of years being practiced in China, India and Japan for the upliftment of down trodden people. From last few decades, silk production had increased drastically, due to adaptation of new technologies in both mulberry, non-mulberry and effective control of pests and diseases. It is reported that flacherie among the major diseases of silkworm is causing crop loss to a tune of 30-40% in silk industry. Many bactericidal chemicals have been used for the control of flacherie disease which are not only expensive but also cause environmental pollution hence as an alternative medicine, plant-based medicines are in usage which are not only cheap but eco-friendly. One such plant-based medicine is *Annona muricata*.

Annona muricata L belongs to unique family of Annonaceae, which is having 2300-2500 species and more than 130 genera in Annonaceae.

Annona muricata is also called as sour sap or guanabana, found to growing throughout the World. In India, it is distributed in Andaman & Nicobar Island, Telangana, Andhra Pradesh, Assam, Karnataka, Kerala, Maharashtra, Tamil Nadu and Uttar Pradesh.

Vernacular names

Telugu-Laxmanaphal
English-Prickly custard apple
Kannada-Mulluvaramaphala
Tamil-Mullu-sita-pazham

Plant description

Sour sap trees are bushy, low reaches a height of 8.5-10 m, bark is dark brown in colour and young branches are rusty-hair, smooth and glossy. Dark green leaves are oblong to elliptical and pointed at both ends, 6.25-20cm X 2.5-6.25cm wide. The leaves of *Annona muricata* contain more than 50

mono-THF acetogenins.

Material and Methods

The fresh leaves of *Annona muricata*, L weighing 10 kgs were collected from Achyuthapuram forest region, Badradri Kothagudem district, Telangana, India in the month of September. The leaves were washed and shade dried for 30 days, finely powdered and kept in air tight bottles for further experimental use.

The finely powdered leaf material was initially extracted with five different solvents such as acetone, chloroforms, ethanol, methanol, and water, crude extracts were collected, were used for further experiments.

Antibacterial activity

Multivoltine Pure race, 4th instar mulberry silkworm cadavers were collected from rearings that were conducted in the lab (Kakatiya University, Warangal and Kothakonda farms of Karimnagar, Telangana, India). The silkworms were surface sterilized with 0.1% mercuric chloride solution, further washed with distilled water. After sterilization, the silkworms were crushed in mortar and pestle and then with the help of innoculum needles, a loopful of the innoculum was taken and streaked on the nutrient agar media, incubation at room temperature for 24 hours. The next day, different colonies were identified and from these colonies, the bacteria was picked and streaked on the nutrient agar slants.

Screening of *Annona muricata* leaf extracts by paper disc diffusion method

Antibacterial activity of five different solvents of *Annona muricata* leaf extracts were identified by paper disc diffusion method at 5% concentration (5gm leaf extract dissolved in 100 ml of solvent). Whatman No1 filter paper was cut into circular discs of 5mm diameter. The small discs

were sterilized in autoclave, then dipped in five different solvent leaf extracts of *A. muricata* at 5% concentration. After that these discs were placed on the agar media already sealed with two identified colonies collected from two different farm areas of Telanagana, isolated from silkworm cadavers and plates were incubated at room temperature. The next day, clear inhibition zone were observed around the discs which were measured by scale to nearest mm and compared with control (Streptomycin).

Table 1

Sl No	Different Solvents of Leaf Extracts	Inhibition Zone (In mm)	
		Colony-1	Colony-2
1	Ethanol	4.9	4.9
2.	Ethanol extract	9.7	9.6
3	Methanol	5.3	5.4
4	Methanol extract	12.4	12.4
5	Acetone	7.2	7.5
6	Acetone extract	19.2	19.3
7	Chloroform	6.1	6.1
8	Chloroform extract	13.6	13.5
9	Water extract	3.4	3.4
10	Streptomycin (control)	17.8	17.9

Data presented as mean of 3 readings
Descriptive statistics (Min, Max, Mean, CV%)

Result

The results of the activity of different solvent extracts of *Annona muricata* leaf are shown in Table.1
The highest antibacterial activity of 19.3mm and 19.2mm inhibition zones were observed in colony-2 and colony- 1 respectively (The colonies were sent for identification at NCBI, Pune) and lowest activity of 3.4mm in colony-1 and colony-2 of water extract of *A. muricata* leaf was observed

Rearing of Silkworm

12 Diseased Free Layings (DFLs) of multivoltine type (Kolar gold) procured from Grainage centre, (NSSP Central Silk Board, Vijayawada-52008, Andhra Pradesh, India) were selected for the study and reared in rearing house as per the standard rearing method ^[1] (Dandin *et al.*, 2005). The 1st day larvae of 3rd instar were selected for the treatment and were divided into 7 groups, each group consisting of 3 replicates with 100 larvae in each. The test solution of 5% acetone extract was selected on basis of antibacterial activity conducted and diluted into five different concentrations (T₁-1:1, T₂-1:2, T₃-1:3, T₄-1:4 T₅-1:5) were prepared while control (acetone) and normal (water) were also maintained.

The solutions were sprayed on Victory 1 variety of mulberry plant, shade dried and fed to each of the 7 batches daily along with the feed. The observations on nutritional and economic parameters of mulberry silkworm studied.

Nutritional efficiency

The effect of acetone extract of *Annona muricata* leaf on mulberry silkworm *B.mori.L* on nutritional efficiency (Ingesta, Digesta, Aproximate Digesta %, Reference Ratio % and Efficiency Conversion of Ingesta %) is given in Table-2.

The amount of Ingesta (2051 gm) and Digesta (1560) recorded were maximum at T₃ concentration when compared to other six batches and followed by T₄

concentrations.

Table-2 indicates the Ingesta, Digesta, Approximate Digesta%, Reference Ratio % and Efficiency Conversion of Ingesta % values which increased from T₁-T₃ thereafter gradually decreased where as a hyper bola curve was identified in the values. Whereas lowest Ingesta (1974), Digesta (1471), AD% (74%), RR (3.29%) and ECI (12.99%) values were recorded in control batch.

Commercial Characters of Silkworm

The result of the commercial parameters of silkworm fed with different concentrations of acetone extract of *Annona muricata* leaf are presented in Table 3. A clear difference was observed in seven batches.

The cocoon and shell weights increased in treated batches when compared to control and normal batches. Among the treatments, T₃ batch showed significantly high values in cocoon weight, shell weight and shell ratio percentage

Table 2

Treatments	Ingesta	Digesta	AD%	RR%	ECI	ECD	RCR	RGR
T1	1974	1501	74.02	3.87	16.71	21.44	0.96	0.075
T2	1987	1515	74.8	3.96	16.89	21.98	0.97	0.076
T3	2051	1560	76.06	4.19	17.46	22.85	1.85	0.084
T4	2004	1509	75.6	4.08	16.77	21.98	1.22	0.079
T5	1990	1501	75.23	3.87	15.74	20.7	0.9	0.075
Normal	2008	1504	74.88	3.85	14.65	19.49	0.82	0.073
Control	1974	1471	74.51	3.29	12.99	17.44	0.77	0.072
Minimum	1949	1460	73.81	3.18	12.58	17.05	0.74	0.071
Maximum	1989	1571	76.63	4.26	18.24	23.96	1.91	0.76
Std Deviation	29.06	29.53	0.8	0.3	1.54	1.89	0.37	0.149
Std Error	6.34	6.44	0.17	0.06	0.33	0.41	0.08	0.032
Significance	0.001	0	0.074	0	0	0	0	0.466

Table 3

Treatments	ECI to Cocoon	ECD to Cocoon	ECI to Shell	ECD to Shell
T1	6.6	8.71	1.05	1.07
T2	8.27	10.73	1.07	1.2
T3	8.83	12.07	1.14	2.01
T4	7.37	10.19	1.09	1.52
T5	7.08	9.83	1.08	1.35
Normal	6.32	8.55	1.04	1.13
Control	6.15	8.1	1.02	0.98
Minimum	6.15	8.1	1.02	0.98
Maximum	8.83	12.07	1.14	2.01
Std Deviation	0.95	1.34	0.03	0.33
Std Error	0.2	0.29	0.0084	0.07
Significance	0	0	0	0

Discussion

Through the present work it is clear that acetone leaf extract of *Annona muricata* posses promising antibacterial properties against colony-1 and colony-2 when compared to

the control (Streptomycin)

The antibacterial activity of the crude solvent extracts of *Annona muricata* showed inhibitory effect in range of 3.4mm to 19.7mm against both colony-1(19.2mm) and colony-2(19.3mm). The highest zone of inhibition (19.3mm) was observed with acetone leaf extract against colony-1 and colony-2 (19.7 mm) respectively.

The phytochemicals derived from natural products in nature have antibacterial activity, one among them is *Annona muricata*, which displayed pharmacological and biological activities such as antiviral, antibacterial and antiparasitic properties. Based on the research work undertaken it can be confined that acetone is a superior extractant to water

According to [2] Dzoyem *et al.*, 2016 acetone extract has been mentioned as best extractant for antimicrobial activity in many plants. It also reported that the methanolic and aqueous leaves extract of *A. muricata* have been used against bacterial strains such as *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus subtilis* [3] (Rajeshwari *et al.*, 2012) [4] Mathinpai *et al.*, 2016 and [5] Pathalo *et al.*, 2010 also reported that the leaf extract of *A. muricata* showed high effective on candida species at all concentrations

The data on nutritional parameters for the silk worm fed with mulberry leaves fortified with *A. muricata* at different ratios (1:1,1:2,1:3,1:4, and 1:5) and control has been presented in Table-2 and studied in terms of grams(green weight / larval weight)

A food consumption results showed that ingesta is high in all treatments when compared to control. The lowest ingesta was recorded with *A. muricata* acetone leaf extract at concentration 1:1 and in control. Food consumption (ingesta) value recorded at T₃ (1:3) is significantly high when compared to other treatments and control. Ingesta values were found in descending order at T₁, T₂, T₅, T₄ and T₃ concentrations of *A. muricata*

The high value of Approximate Digestability was observed at T₃ treatment. The high value indicates the suitability of this food for the silkworm. Significant difference in the efficiency of conversion of Ingesta food is measure which shows the ability of larval to utilize of the ingested food which is more at T₃ when compared to other treatments and control.

The high nutritional values are necessary for the larvae to maintain its metabolic demand during metamorphosis similar results were also reported [6] in eri silkworm. There is a narrow difference in AD % among the treatments.

In the present investigation, T₃ treatment showed increase in Ingesta, Digesta, and Approximate Digestability when compared to other treatments and control. This may be due to feeding of mulberry leaf fortified with *A. muricata* to silk worm, which made the larvae to feed more quantity of leaves, increased the passage of food through the gut, thereby facilitating increase in digestion and assimilation which are ultimately regulated in other corresponding efficiency parameters as stated by [7, 8]. It can be interpreted that from the above data that ingestion, digestion and growth in the larval stages are inter related and the role of digestion in silk worm increases with concentrations which was highest in T₃ treatment and also with advance in age. These are in close agreement with observations of [9, 10, 11].

Throughout the world *A. muricata* has been found as folk loric herbal medicine as it contain secondary metabolites like tannin, steroids, flavonoids, anthraquinone that had been reported by [12] the flavonoids are reported to have

antioxidative activity and immunity properties. The tannins, flavonoids and phenolic acids are known to effect protein digestability and absorption which were reported in development stages of silkworm derived from mulberry leaves that are consumed by the silkworms

The efficiency of conversion of ingesta to cocoon (ECI to cocoon) and efficiency of conversion of digesta to cocoon revealed significant difference, it was high in T₃ treated batches followed by T₂, T₄ and T₅. Among the treatments, Digesta, Approximate Digestability, Reference Ratio as well as Efficiency of Ingesta and Digesta to Cocoon and Shell when fed to mulberry silkworm showed highest feeding efficiency data at T₃ treatment when compared to other treatments and control. However further research is ongoing to determine which bioactive compound in *A. muricata* showed enhanced feeding efficiency and antibacterial activity.

Commercial parameters of silk worm

The highest antibacterial activities are believed to be due to presence of acetogenins in sour sap. Very few studies have been found on this extract against bacteria and fungi in other organisms

In the present study, *A. muricata* exhibited antibacterial activity against two identified colonies of bacteria. The antimicrobial activity may be due to the presence of secondary metabolites like tannins, steroids, saponins, flavonoids and anthraquinones as reported by [12, 13]

From the present study undertaken, it is observed that acetone crude of *A. muricata* showed highest antibacterial activity when compared to other solvent crude extracts of the plant hence it can be recommended as alternate therapeutic agent for control of silkworm pathogens. Further studies are being carried out to elucidate the structure and mechanism of the action of this plant. Furthermore, silkworm larvae fed with treated leaves also showed difference in nutritional efficiency

The present work is comparable with results of [13] the diet supplemented with plant extracts at different concentrations not only improved the larval growth but also the economic traits as it acted as feeding stimulant at low concentrations and thereby improved the nutritional intake and disease resistance, ultimately the commercial characters. These findings are in agreement with the findings of [14, 15] who observed increase in silk productivity when mulberry leaves were fortified with certain botanicals. The increase in silk productivity may be due to biochemical constituents such as alkaloids, tannins, steroids, saponins, flavonoids, anthraquinones and acetogenins present in the *A. muricata* which plays a vital role due to availability of the above-mentioned nutrients in the silkworm feed

Anti-oxidative activity of the medicinal plant is mainly due to the presence of secondary metabolites like tannins, steroids, alkaloids present in trace amount in leaves of *A. muricata*. Such activity is of great interest for application against human and other related useful insects also. Further research work is being carried out to elucidate the structure.

Conclusion

The silkworm feed on mulberry leaves fortified with *A. muricata* at different concentrations influenced the larval growth, nutritional efficiency and cocoon characters which are beneficial for sericulture rearers.

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