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# LARVAL GROWTH AND COCOON TRAITS IN BOMBYX MORI L (PM×CSR$_2$) (LEPIDOPTERA: BOMBYCIDAE) RAISED ON MULBERRY LEAVES FORTIFIED WITH ALPINEA GALANGA (L) RHIZOME EXTRACT

**J. P. JESPA [a#] AND F. BRISCA RENUGA [a*†]**

[a] Department of Zoology, Holy Cross College, Nagercoil, Affiliated to Manonmaniam Sundaranar University Abishekapatti, Tirunelveli, Tamil Nadu, India.

**AUTHORS' CONTRIBUTIONS**

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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## ABSTRACT

The mulberry silkworm *B. mori* feeds exclusively on mulberry leaves and the leaf quality is as a sole factor governing the production of good cocoon crop. Improvement in the nutritional quality of mulberry leaves can enhance the efficiency of cocoon and seed production in silkworm. They act as growth promoting factors, indirectly helps in reducing further spread of diseases. Therefore the present work is selected to analyse the efficacy of aqueous extract of medicinally important plant *Alpinia galanga* on larval growth and cocoon traits. Larval duration, Larval weight (g), Cocoon, pupal and shell weight (g), Cocoon Shell Ratio (CSR) (%) were measured. From the present investigation it was understood that its application is an inexpensive source of fortificant for silkworm rearing. In this study plasmatocytes was increased during the concentration increased. The increased number of granulocytes and plasmatocytes can be related with the defense mechanism in *B. mori*, as both the haemocytes functions as phagocytes.

**Keywords:** *Alpineagalanga*; cocoon shell ratio; growth efficiency; haemocyte count.

## 1. INTRODUCTION

The culture of monophagous *Bombyxmori* L. larvaehas becomeone of the most important cottage industries in all over the world. China and India are the two main producers of silk, manufacturing more than 60 percent of total world turnover. Sericulture an agro based plays a vital role in the improvement of

*[#]Research Scholar;*
*[†]Associate Professor;*
**Corresponding author: Email: brisren@gmail.com;*

rural economy of India. Various measures like supplementation of *B. mori* larvafed with micro and macro nutrients, plant bioactive compounds have been successfully carried out [1]. Recently many attempts have been made to fortify mulberry leaves with botanical extracts so as to improve the quality of feed efficiency of the silkworm which inturn helps to increase cocoon production and silk quality (Prabhu et al. 2012). The quality of mulberry leaves the sole natural food of the silkworm *B.mori* L. has a direct bearing on the quality of cocoon produced [2]. Thus nutritionis the singlemost important factor that influences the growth and development of *B. mori* [3].

Botanicals have immense ability to influence the metabolic activities of insects. Hence, mulberry leaves fortified with botanical extracts enhance thenutritional quality of mulberry leaf and improve the feeding efficiency of silkworm and subsequently increase silk production. Fortification of mulberry leaves with plant products including medicinal plant extracts and essentialoils alter the physiology of the silkworm. Various botanicals increase the food consumption of silkworms and biomass, thereby enhancing cocoon yield [4]. Products from the plants are easily available, less expensive, safe and efficient and rarely have side effects [5]. Khanikor and Dipsikha [6] observed the silkworms treated with essential oil of *O. sanctum*, *O. gratissimum* and *A. conyzoides* increased THC and improves the immune response at the lowest dose.

The rhizomes and flowersof *Alpineagalanga*belonging to the familyZingiberaceae iscommonly known as "Greater galangal" were used as edible items by the traditional people . They are mainly cultivated in South East Asia, distributed in Himalayas and Southern region of Western Ghats in India. The most important bioactive constituents of *A.galanga* are alkaloids, tannins, flavonoids, and phenolic components phytosterols, essential oil, α-glucosidase and α amylase [7,8]. These bioactive constituents showed good developmental, antioxidant, anti-inflammatory and antimicrobial activities. There has been no attempt so far to study the *A. galanga* plant extract on the silkworm *B.mori*. The aim of this study is to evaluate the influence of various concentrations of the aqueous plant extract of *A.galanga* on the growth and economic performance of silkworm, *Bombyxmori* L.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Extraction of *A. galanga*

The Rhizomes ofA.galanga were collected from certain areas of Kanyakumari district, Tamil Nadu, India. Collected plant part were washed in tap water, shade dried and powdered in an electrical blender [9]. Fifty grams of plant powder were mixed with 350 ml distilled water and vortexed in an orbital shaking incubator (REMI ) for about 11 hrs at 30˚C. The extract was filtered and is reduced to a paste using rotary evaporator. The reduced extract was stored at 4℃ and was used for further analyses.

### 2.2 Phytochemical Screening of the Plant Extract

Phytochemical tests were performed to analyse the presence ofalkaloids, glycosides, flavonoids,phenols, amino acid, protein,tannins**,** saponins, phlobatannins and terpenoids. The presence of a variety of phytochemical compounds in the extract was determined by using the methods of Brindha et al. [10] and Harborne [11].

### 2.3 Test for Alkaloids (Wagner's Reagent)

A small amount of extract was treated with 2mL of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100ml of water) and observed the formation of reddish brown precipitate (or colouration).

### 2.4 Test for Carbohydrates (Benedict's Test)

A few drops of Benedict's reagent were added to 2 mL of extract, boiled in water bath for 5 min, cooled and observed for a reddish brown precipitate.

### 2.5 Test for Cardiac Glycosides (Keller Kelliani's Test)

5ml of extract was treated with 2ml of glacial acetic acid in a test tube and 1 mL ferric chloride solution was added to it. This was carefully heated and then cooled. This was transferred to a test tube containing 2 mL concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

### 2.6 Test for Flavonoids (Alkaline Reagent Test)

Few drops of 20% sodium hydroxide solution was added to 2mL of extracts. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, revealed the presence of flavonoids.

### 2.7 Test for Phenols (Ferric Chloride Test)

A fraction of the extracts was treated with aqueous 5% ferric chloride and noticed for formation of deep blue or black colour.

### 2.8 Test for Amino Acids and Proteins (1% Ninhydrin Solution)

2ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

### 2.9 Test for Saponins (Foam Test)

To 2mls of extract, was added 12 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam.

### 2.10 Test for Tannins (Braymer's Test)

2mls of extract was treated with 10% ferric chloride solution and observed for formation of blue or greenish colorsolution.

### 2.11 Test for Phlobatannins

Deposition of a red precipitate when 2 ml of extract of plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

### 2.12 Test for Terpenoids (Salkowki's Test)

Two mL extracts was treated with Chloroform (1 mL) followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoid.

### 2.13 Fourier Transform Infrared Spectrophotometer (FTIR)

10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan) with a Scan range from 400 to 4000 cm $^{-1}$with a resolution of 4 cm $^{-1}$ (Joshi 2012).

### 2.14 Treatment of *B. mori* with *A. galanga* Rhizome Solvent Extract

The disease free layings of L x CSR2 hybrid of *B. mori*was procured from the Government SericultureCenter, Konam, Nagercoil. Twenty third instar larvae were fed with mulberry leaves treated with different concentrations (0.01%, 0.1%, 1.0%, 1.5% and 2.0%) of aqueous solvent extracts of *A. Galangal* rhizome.Thus treated mulberry leaves were shade dried and fed to the larvae. The control group was fed with fresh mulberry leaves treated with distilled water. Three replicates were maintained for the control and experimental groups. The cocoons were harvested on the fourth day after spinning to find the economical and biological parameters such as larval weight, cocoon weight, shell weight and shell ratio for each experimental and control categories.Ten cocoons were cut open after removing the floss without causing any damage to the pupae. The weight of pupae and the shellsweremeasured using electronic balance [12]. Theeconomical and biological parameters calculated using the following formula,

**TG =** Final body weight – Initial body weight

**% increase/ decrease in TG =** $\frac{TGE - TGC}{TGC}\ x\ 100$

**Shell ratio(SR) =** SW/CW ×100

**% increase/ decrease in SR =** $\frac{SRE - SRC}{SRC}\ x\ 100$

TG- Tissue growth;
TGE- Tissue growth in experiment;
TGC - Tissue growth in control;
SR- Shell ratio;
SW- Shell weight;
CW – Cocoon weight.

### 2.15 Haemocyte Count

The haemolymph was collected in pre chilled tubes containing a few crystals of phenylthioureato arrest the activity of prophenol oxidase followed by melanization of the haemolymph samples [13]. The samples were stored at -20°C for further use. For total haemocyte count (THC) and differential haemocyte count (DHC), fresh haemolymph was diluted with Ringer's solution. The haemolymph was placed on the Neubauers double lined haemocytometer and allowed to settle for a minute and the haemocytes were counted under the light microscope. While making the observations of Total Haemocytes count,four big corner squares of 1mm size were counted. For calculation of the total haemocyte (THC) method of Jones [14] was used. Differential haemocyte count (DHC) was estimated by counting different haemocytes from the population of 150 cells, based on the morphological features as described by Nittono [15]. THC and DHC was determined in at least 10 larvae both in control and experimental animal separately.

**Total haemocyte count** = (No of cells counted / Volume of small square) X 1

## 3. RESULTS AND DISCUSSION

### 3.1 Preliminary Analysis of Phytochemical

The results of phytochemical analysis ofaqueous extract of*A. galangal* showedpositiveresults forconstituents such as glycosides, flavanoids, phenol, amino acids and tannins. The secondary metabolites which are produced through metabolic pathways of plant cells exhibited many biological activities. The flavonoids are a common group of phytochemicals in all plants and they play an important role in the metabolism. Phenolsare very wide spread in nature consist of a single phenolic ring and often possess alcoholic, aldehydic and carboxylic acid groups [16].

### 3.2 Growth Efficiency

The dietary supplementation with various concentrations of *A. galanga* aqueous extract to the silkworm larvae resulted in an increase in larval weight, cocoon weight and shell ratio. Maximum increase in tissue growth (62.27%) was observed in 1.5 % of.*A.galanga* extract treated categories. Maximum cocoon and shell weight (28.57%) wereobserved in 2.0% treated categories of *B. mori*.The increase of larvae, pupae and shell weight was concentration dependent ie increased with the increasingconcentration. In the present study, the treatment of larvae at different concentration of extract have beneficial effects on the growth of the silkworm *Bombyxmori*larval weight, cocoon weight, pupal weight, shell ratio, by enhancing feed efficacy when compared to control (Table 1).

A strong correlation was observed between growth of silkworm and silk production after leaf treatment with extracts of plants like *Tribulusterrestris, Boerhaviadiffusa* and *Phyllanthusniruri* which showed growth promoting effects due to presence of active compounds, which may enhance the bioavailability of nutrients for better digestibility [17]. Nutrition plays an important role in improving the growth and development of *B. mori* [18]. The growth promoting effect and economic performance of *Bombyxmori* after the treatment of plant extract was also observed by different authors [19].

### 3.3 Total and Differential Haemocyte Count

The change in the THC and DHC indicates the effects of plant extracts on cellular immunity. There are plenty of reports by various scientists, both in reducing or increasing cell numbers [20] were available. The increased number of THC in all treated categories than the control indicates the defence response of silkworm by boosting immunity level. The data of the present study agreed with that of the earlier investigator.

*Alpenia galanga* fed silkworm larvae showed increased number of total hemocytes count and is concentration dependent (Table 2). The haemocytes increasing trend is responsible to the immune resistance to various physiological changes due to infection. In the present study five different types of hemocytes were reported in the Silkworm, *B. mori*based on their morphology viz., granulocytes, plasmatocytes, oenocytoids, prohaemocytes and spherulocytes (Fig. 1). Present result corroborates the earlier reports of Balavenkatasubbaiah et al. [21]; Ling et al. [22]; Kerenhap et al., [23] and Nakahara et al., [24]. Decreased per cent Plasmatocytes (PL) (18.95), Prohemocytes (PR) (21.05) and Spherulocytes (SP) (22.07) were observed in all treated categories against PL- 20, PR & SP- 30 in control group. These three types of hemocytes decreases with the increasing concentrationof *A.galanga* extract where as maximum of 20.93% Granulocytes and 22.98% Oenocytes were observed at 2% plant extract treated categories. The reduction in plasmatocytes and prohemocytes may be due to the haemolytic activity of the plant extracts. Further the reduction in their number may be attributed to the inhibition of their mitotic division, their conversion to other types of cellsor the inhibitory activity of hematopoietic organs by plant extracts. Plasmatocytes were responsible for cellular immune responses in many insect [22].

Being phagocytic in nature the plasmatocytes and sperulocytes may not be available freely as reported by Sharma et al. (2003). Increased number of oenocytoids represents its involvement in the complex phenol metabolism as reported by Ling et al. [25]. The number of haemocytes in circulation can change rapidly in response to environmental stress, wounding or infection. It is possible that the number of haemocytes was directly altered by the change in food. Present result showed that significant change in the number of treated haemocytes than the control, because of presence of secondary metabolites in plant extracts [26]. The increased number of granulocytes and plasmatocytes were reported by Al-Attar [27] and Anandkumar and Micchael [28].

**Table 1. Growth efficiency of V$^{th}$ instar *B. mori* treated with different concentration aqueous extract of *A. galangal***

| Treatment (%) | Cocoon weight (CW) (g) | %increase/ Decrease (CW) | Pupal weight(PW) (g) | %increase/ Decrease (PW) | Shell weight (SW) (g) | %increase/ decrease (SW) | Tissue growth (TG) (g) | % increase/ decrease (TG) | Shell ratio(SR) | % increase / decrease (SR) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 1.17±0.03 | - | 0.96±0.05 | - | 0.21±0.02 | - | 0.91 ± 0.53 | - | 17.9 | - |
| 0.01 | 1.21 ±0.08 | 3.42 | 1±0.045 | 4.17 | 0.21±0.04 | 1.59 | 1.28 ± 0.08 | 41.03 | 17.36 | -3.02 |
| 0.1 | 1.20 ±0.01 | 2.57 | 0.98±0.09 | 2.08 | 0.22±0.001 | 4.76 | 1.43 ± 0.13 | 57.51 | 18.33 | 2.40 |
| 1 | 1.23±0.01 | 5.13 | 0.99±0.001 | 3.13 | 0.24±0.008 | 14.29 | 1.47 ± 0.06 | 61.9 | 19.51 | 8.99 |
| 1.5 | 1.25±0.01 | 8.84 | 1.0±0.85 | 4.17 | 0.25±0.02 | 19.05 | 1.48 ± 0.03 | 62.27 | 20 | 11.73 |
| 2 | 1.24±0.004 | 5.98 | 0.98±0.03 | 2.08 | 0.26±0.03 | 23.81 | 1.46 ± 0.08 | 60.44 | 20.97 | 17.15 |

*'-'value indicates decrease and '+' value indicates increase*

**Table 2. Total and per cent increase and decrease of haemocyte of V$^{th}$ instar *B. mori*multivoltine breed after treated with aqueous extracts of *A. galanga*rhizome**

| Treatment | Total No.of cells $10^3$/mm$^3$ | % increase /decrease |
|---|---|---|
| Control | 12.80 ±0.41 | 6.25 |
| 0.01 | 13.6 ± 0.48 | 6.25 |
| 0.1 | 13.6 ± 0.29 | 25 |
| 1 | 16.0 ± 0.29 | 49.8 |
| 1.5 | 19.18 ± 0.29 | 50 |
| 2 | 19.20 ± 0.25 | 62.5 |

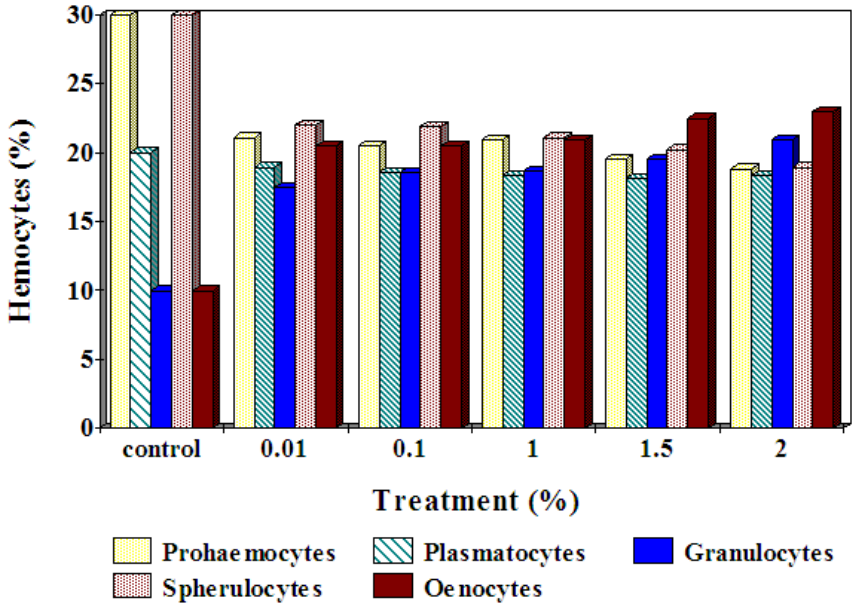


**Fig. 1. Differential hemocyte count of V$^{th}$ instar *B. mori*multivoltine breed after treated with aqueous extracts of *A. galangal* rhizome**

### 3.4 Fourier Transform Infrared Spectroscopic Analysis

Fourier Transform Infrared Spectroscopy (FTIR) is a high-resolution analytical technique to identify the chemical constituents or functional groups and elucidate the structural compounds [29]. The results of FTIR analysis confirmed the presence of Alcohol, Phenol, Alkanes, flavanoids, aldehydes, amines and Carboxylic acid. Das & Santhy [30] also reported alkanes, amides, carboxylic acids, epoxides, alcohols, aliphatic amines, aromatics and phenol compounds after FTIR analysis of *A. galanga*. In the present work the result of the FTIR also correlate with the preliminary phytochemicalsanalysis of *A. galanga*. A broad absorption band between 3650 and 3250 cm-1 ranges indicating the presence hydrogen bond. This band confirms the existence of hydroxyl (- OH) compound, by the presence of spectra at frequencies 1600–1300, 1200–1000 and 800–600 cm-1. This band is confirmed as alcohol due to the lack of aromatic rings as there is no narrow band above 3000 cm$^{-1}$. These results are in accordance with the reports on selected Indian medicinal plants, which showed similar characteristic vibration modes in the same transmittance range. Presence of phenol in agreement of earlier reports of Raina et al. [31], and Nampoothiri et al. [3].

Presence of absorption band below 3000 cm-1 showinga long chain linear aliphatic compounds at 2935 and 2860 cm$^{-1}$indicates presence of alkenes which is followed by peaks between 1470 and 720 cm-1. In the triple bond region i.e. between 2000-2500 cm-1**,** appearance ofa peak at 2200 cm-1 should be absorption of C≡C and this peak is usually followed by the presence of additional spectra at frequencies of 1600– 1300, 1200–1000 and 800–600 cm-1 .Absence of absorption band between 2220to 2260 indicates absence of cyanide groups which means that the extract not contains any toxic substances (Fig. 2) [33].

**Table 3. Functional group of compounds present in aqueous extract of A. *galangal* rhizome**

| Frequency ranges (cm-1) | Frequency peak value (cm-1) | Stretching vibration and specific functional groups. | Intensity | Relevant chemical compounds | |
|---|---|---|---|---|---|
| 3500 to 4000 | 3894.28<br>3849.92<br>3745.76 | Medium sharp, O-H stretching vibration indicating presence of phenol | 28.24<br>28<br>29.8 | Di-(p-hydroxy-cis-styryl)]methane Di hydroxyl 1'-acetoxyeugenol acetate.<br>trans-p-hydroxycinnamaldehyde and trans-p-hydroxycinnamyl acetate | Exhibited potent inhibitory activities against the autoxidation [34] |
| 3550 to 3200 | 3446.79<br>3427.51 | Strong and broad wave;<br>O- H stretching vibration. Intermolecular H bonding indicating presence offlavanoids | 10.07<br>10.21 | Galangin (3,5,7-trihydroxyflavone)<br>Non methylated flavanoids- Kaempferol , Quercetin, Myricetin.<br>Methylated flavanoids- Galangin 3- methyl ether, Kaempferide, isokaempferide, Kumatakenin, isohamnetin , Quercetin 3- methyl ether , methoxylatedflavonols.) | Suppress the proliferation of cells [35] (Luo et al. 2013)<br>[36] |
| 3000 to 2840 | 2956.87 | Medium, C-H stretching asymmetric vibrations indicating presence of Alkane (terpenoids) | 37.7 | α-humulene and caryophyllene oxide | Antimicrobial properties [37,38] |
| | 2926.01<br>2856.56 | Strong to Medium, C- H stretching symmetric vibrations indicating presence of indicating presence of Alkane (terpenoids) | 35.42<br>42 | 1,8-cineole; β-caryophyllene; β-bisabolene; β-selinene; β– farnescene; α- terpineol; α- pinene; chavicol; eugenol; chavicol acetate; β- sesquiphellandrene; methyl eugenol and farnesol. | [29] |
| 2400 to 2350 | 2364.73 | Medium; O═C═O asymmetric stretching indicating presence of Carbon di oxide. | 41.11 | | Anirban Chouni and Santanu Paul [39] |
| 2349 to 2322 | 2333.87 | Weak, C═N symmetric stretching vibrations indicating presence of Amines. | 45.86 | Difluoromethanimine, Proteins and amino acids | Highly responsive to developmental and environmental signals [40] |
| | 2270.22 | Weak, C═N symmetric | 62.49 | | |

| **Frequency ranges (cm-1)** | **Frequency peak value (cm-1)** | **Stretching vibration and specific functional groups.** | **Intensity** | **Relevant chemical compounds** | |
|---|---|---|---|---|---|
| | | stretching vibrations indicating presence of Amines. | | | |
| 1440 to 1395 | 1398.39 | Strong O-Hasymmetrical bending vibration indicating presence of Carboxylic acid. | 64.23 | Carboxylic acids are used as precursors to form other compounds such as esters, aldehydes, and ketones. | -- |
| 1120 to 11120 | 1118.71 | Strong C=O stretching vibration indicating presence of Secondary alcohol. | 64.29 | p-acetoxycinnamyl alcohol, and p-coumaryl alcohol ethyl ether | Acting against detoxifying enzymes (Sukhirun et al . 2011) |

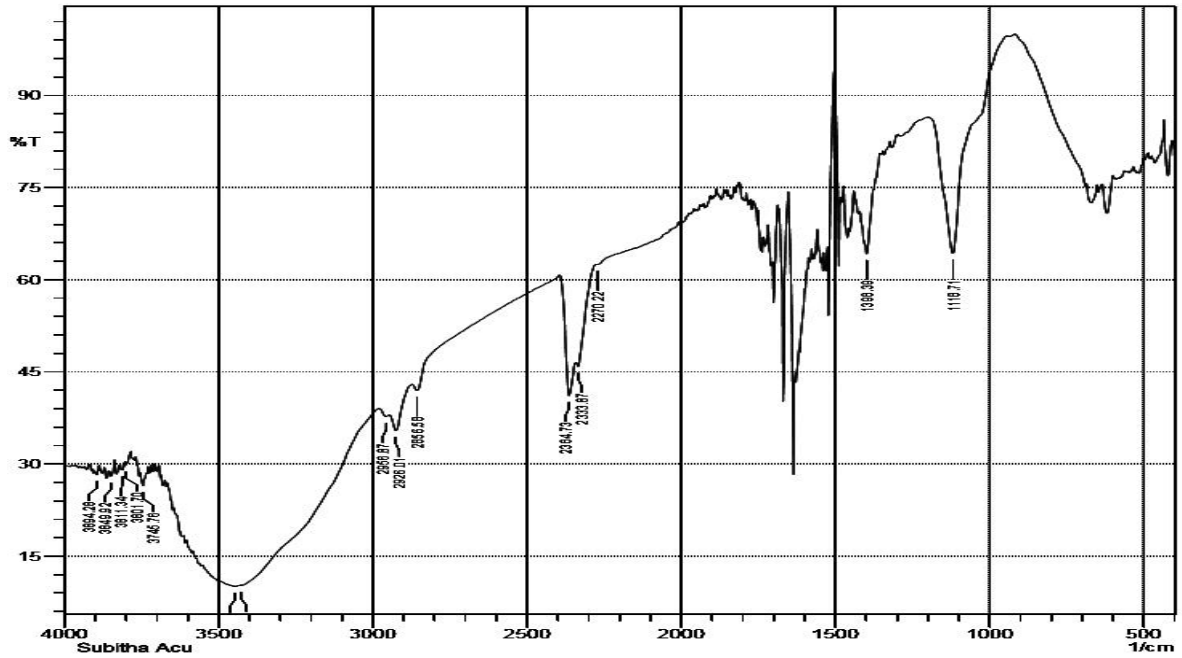


**Fig. 2. Fourier transform infrared (FTIR) spectroscopic analysis of aqueous extract of *A. galangal* rhizome**

In the present study presence of asymmetric and symmetric strong and medium bonds at C= C double bond region (1500-2000 cm-1) represent C = O bonds of weak stretching non conjugated compounds between 1700 to 1900 and C= C carbonyl compound 1850 - 1650 cm-1 ranges describing simple carbonyl compounds such as ketones, aldehydes, esters, or carboxyl compounds. The peaks lower than 1700 cm-1 replying amides or carboxylate functional group which was confirmed by the reduced intensities of the peak due to the conjugated functional groups such as aldehydes, ketones, esters, and carboxylic acids which reduces the absorption frequency of carbonyl compounds (Fig. 2).

Strong intensity peak between 1650 and 1600 cm-1, weak bonds between 1615 and 1495 cm-1 and multiple bands with a weak intensity between 2000 and 1700 cm-1 informing the presence of aromatic rings. Presence of aromatic rings also supported by the presence of absorption band between 1600/1500 cm-1 namely C-H bending vibration with the intensity of medium absorption to strong and single or multiple absorption bands found in the area between 850 and 670 cm-1 and single and strong absorption band around 750 cm-1(Fig. 2). Presence of many major bands in the spectral range of 1600–600 cm–1 reflects that some bioactive molecules of significance have been detected with nutraceutical effect.

The functional group of the compounds were given in Table 3.

## 4. CONCLUSION

From the present work it is concluded that *Alpiniagalanga* possesses rich phytochemical and pharmacological potentials. Compiling all the current knowledge so far we have regarding rhizome of *Alpiniagalanga* it is evident that the plant is a potential powerhouse of several lead molecules which are responsible for numerous bioactivities beside their medicinal importance. More research and evaluation needs to be done to isolate and identify different chemicals present in the plant which will be used to enhance the commercial characters of silkworm which can improve silk yield.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Pardeshi AB, Barge SB. Impact of oral supplementation of *Alternantherasessilis* plant extract on the economic parameters of silkworm, *Bombyxmori* L, Bioscience Discovery Journal. 2017;8(3):596-601.
2. Horie Y, Inokuchi T, Watanabe K. Quantitative studies of food utilization by the silkworm Bombyxmori L. through the life cycle II. Economy of nitrogen and amino acids. Bulletin Sericultural Experimental Station, Japan. 1978;27(2),3(2):531-578.
3. Chakrabarty S, Kaliwal BB. Supplementation with Potassium Carbonate, Magnesium carbonate and their synergetic effects on the economic traits of the silkworm, Bombyxmori L. World J. Science and Technology. 2011;1:10-23.
4. Maribashetty VG, Gayathri MV, Chandrakala MV, Gururaj CS, Shivakumar C. 'Utilization of botanicals for improvement in economic traits and control of pests and diseases in silkworm, *Bombyxmori*, Indian Journal of serIculture 2010;49(1):1-11.
5. Merlin Rose C, Catherine L. Preliminary phytochemical screening and Antibacterial Activity on *Vitexnegundo.* International Journal of Current Pharmaceutical Research; 2011.
6. Bulbuli Khanikor, Dipsikha Bora. Effect of Plant Based Essential Oil on Immune Response of Silkworm, Antheraeaassama Westwood (Lepidoptera: Saturniidae) Int. J. Indust. Entomol. 2012;25(2):139-146.
7. Rini CS, Rohmah J, Widyaningrum. Te antibacterial activity test Galanga on the growth of bacteria *Bacillus subtilis* and *E. coli* Materials science and Engineering. 2018;420:012142.
8. Srividya AR, Dhanabal SP, Misra VK, Suja G. Antioxidant and Antimicrobial Activity of Alpiniaofficinarum Indian J Pharm Sci. 2010;72(1):145–148.
9. Beula Rani KR, Sundar SK, Murugan M. Antimicrobial activity and phytochemical study of medicinal plant *Alpinea galanga* Asian J. of Pharma. Clinical Res. 2016;9:3.
10. Brindha P, Sasikala Purushoth. Preliminary Phytochemical studies of higher plants. Ethnobot. 1977;3: 84-96.
11. Harbone JB. Phytochemical Methods, London Chapman and Hall Ltd. 1973;49-188.
12. Sonwalker TN. Hand book of Silk Technology, Wiley Eastern Limited, New Delhi; 1993.
13. Takeda H, Kawakuchi Y, Ohsika T, Maekawa H, Tsuchida K. Impaired yolk protein uptake by oocytas of a Bombyxmori mutant, Insect Biochemistry and Molecular Biology. 1996;26:607-616.
14. Jones JC. Current concepts concerning insect haemocytes American Zoology. 1962;2:209-246.
15. Nittono Y. Studies on the blood cells in the silkworm, *Bombyxmori* L. Bull. Seric. Expt. Stn. 1960;16:261-266.
16. Sonnenbichler J, Sonnnenbichler I, Sealkera F. ACS SympSer (Phytomedicines of Europe). 1998;691:263. K. Tawaha FQ. Alali M. Gharaibeh, M. Mohammad El-Elimat,.T. Food Chem. 2007;104:1372.
17. Murugan K, Jeyabalan D, Senthikumar N, Senthilnathan S, Sivaprakasam N. Growth Promoting effect of plant products on silkworm: A Biotechnological Approach. Jour. of Sci. and Ind. Res. 1998;57:740-745.
18. Kanafi RR, Ebadi R, Mirhosseini SZ, Seidavi AR, Zolfaghari M, Eteban K. A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm, *Bombyxmori*L. Indian Sericulture Journal. 2007;4:86-91.
19. Pardeshi AB, Bajad PN. Effect of *Xanthium indicum*Linn.plant extract on the economic parameters of silkworm, *Bombyxmori*L. Int. Jour. of Rec. Sci. Res. 2014b;5(3): 683-686.
20. Hassan HA, Bakr RFA, Abd El-Bar MM, Nawar GA, Elbanna HM. Changes of cotton leaf worm haemocytes and esterases after exposure to compounds derived from urea and rice straw. Egypt. Acad. J. Biolog. 2013;5:35–48.
21. Balavenkatasubbaiah M, Nataraju B, Thiagrajan V, Datta RK. Haemocytes counts in different breeds of silkworm *Bombyxmori* L. and their changes during progressive infection of Bm NPV. Indian J. Seric. 2001;40:158-162.
22. Ling E, Yu XQ. Hemocytes from the tobacco hornworm Manducasexta have distinct functions in phagocytosis of foreign particles and self dead cells, Develop. Comp. Immunol. 2006;30:301- 309.
23. Kerenhap W, Thaigarajan V, Kumar V. Biochemical and bioassay studies on the influence of different organic manures on the growth of mulberry variety V, and silkworm, *Bombyxmori* Linn. Caspian J. Environ. Sci. 2007;5(1):51-56.
24. Nakahara Y, Shimura S, Ueno C, Kanamari V, Mita K, Kiuchi M, Kamimura M. Purification and characterization of silkworm haemocytes by flow cytometry. Dev. Comp. Immunol. 2009;33:439-448.
25. Ling Erjun, Koji Shirai, Rensuke Kanekatsu, Kenji Kiguchi. Hemocyte differentiation in the

hematopoietic organs of the silkworm, Bombyxmori: Prohemocytes have the function of phagocytosis Cell Tissue Res. 2005;320(3):535-43.

26. Chavan JA, Bhawane GP. Study of Haemocyte Count In Silkworm Bombyx mori on 5th Day Of 5th Instar larvae inoculated with of bmnpnv infection and subsequent oral treatment of ehtanolic plant extracts in pm and CSR2 Races, International Journal Of Researches In Biosciences, Agriculture And Technology. 2017;V(3),
27. Al-Attar AM. Haematological, biochemical and histipathological studies on marsh frog, *Ranaridibunda*, naturally infected with *Waltonelladuboisi*. Int. J. Zool. Res. 2010;6:199-213.
28. Anand Kumar MD, Michael AS. Haematology and haemochemistry of silkworm, *Bombyxmori* L. infected with *Bacillus thuriengensis*. Int. J. of Env. Sec. 2011;2(2):451-457.
29. Archana P Raina, Abraham Z. Essential oil profiling of Alpinia species from southern India, Indian Journal of Experimental Biology. 2017 ;V55:776- 781.
30. Archana Das, Kumaran Sivanandan Santhy. Chemical caharacterisationn of *Alpineagalanga* (L) Willd by GC-MS, XRD, FTIR and UV-VISN spectroscopic methods International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7(9).
31. Raina PA, Verma SK. Abraham, Z volatile constituents of essential oil isolated from Alpinea galangal wild(*L) and A. officinarum rhizomes from North East India. J. Essential Oil Res. 2014;26:24.
32. Nampoothiri SV, Menon AN, Esakkidurai T, Pitchumani K. Essential oil composition of Alpineacalcarata and Alpinea galangal rhizomes A comparative study. J. Essential Oil Bearing Plants. 2016;19:82.
33. Samir Felhi, Amal Daoud, Hafedh Hajlaoui, Kais Mnafgui, Néji Gharsallah, Adel Kadri. Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of Ecballium elaterium seeds and peels fruits Food Science and Technology, Campinas. 2017;37(3):483-492.
34. Yu CL, Hsu GC, Yen J. Agric. Food Chem. CrossRef CAS PubMed Dorman HJD, Deans SG 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol. 2009;88:308-316.
35. Luo X, Lv X, Wang, Kong L. Phytochem. Lett. 2012;5:134.
36. Duangjai Tungmunnithum, Nobuyuki Tanaka, Ayumi Uehara, Tsukasa Iwashina. 0 Flavonoids Profile, Taxonomic Data, History of Cosmetic Uses, Anti-Oxidant and Anti-Aging Potential of *Alpiniagalanga* (L.) Willd Cosmetics. 2020;7:89.
37. Dorman, HJD & Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils Journal of Applied Microbiology. 2000;88(2):308-16
38. Pichette A, Larouche PL, Lebrun M, Legault J. Composition and antibacterial activity of *Abiesbalsamea* essential oil. Phytother Res. 2006;20:371-373.
39. Anirban Chouni, Santanu Paul. A Review on Phytochemical and Pharmacological Potential of Alpinia galangal, Pharmacogn J. 2018;10(1):9-15.
40. Bouchereau A, Guénot P, Larher FJ. Analysis of amines in plant materials. Chromatogr B Biomed Sci Appl. 2000;747(1-2):49-67.
41. Sukhirun N, Pluempanupat W, Bullangpoti V, Koul O. Bioefficacy of *Alpiniagalanga* (Zingiberaceae) Rhizome Extracts, (*E*)-*p*-Acetoxycinnamyl Alcohol, and (*E*)-*p*-Coumaryl Alcohol Ethyl Ether Against *Bactroceradorsalis* (Diptera: Tephritidae) and the Impact on Detoxification Enzyme Activities, Journal of Economic Entomology. 2011;104 (5):1534–1540.