

## Research Article



## Phytochemical and Biochemical Activity of Two Host Plants of Muga Silk Worm *Antheraea assamensis* Helfer. from Dibrugarh, Assam

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### ABSTRACT

*Persea bombycina* and *Litsea polyantha* belongs to the family Lauraceae has been traditionally used as host plants of muga silkworm, *Antheraea assamensis*. The present research work has been designed to evaluate the antioxidant and biochemical activity of organic extracts of these two plants along with the determination of nutritive value. The study revealed that cold methanolic extract (MC) exhibited higher amount ( $8.03 \pm 0.60$ ) of phenolic contents and hot methanol extract (MH) showed higher antioxidant activities ( $92.97 \pm 0.01$  and  $91.27 \pm 1.05$  respectively) against DPPH. Similarly, the cold petroleum ether extract (PEC) contained higher amount ( $3.72 \pm 0.01$ ) of flavonoid contents and exhibited lower antioxidant activity in DPPH model. Biochemical activity reported a higher quantity of reducing sugar (RS) and free amino acid (FAA) in the leaves of *P. bombycina* than *L. polyantha* ( $0.66 \pm 0.00$  mg/ml and  $0.14 \pm 0.01$  mg/ml;  $0.212 \pm 0.010$  mg/ml and  $0.023 \pm 0.004$  mg/ml) respectively. Regarding nutritive content, *P. bombycina* recorded greater value ( $345.34$  Cal/ 100gm) than *L. polyantha* ( $345.34$  Cal/ 100gm).

**Keywords:** Phenolic content, flavonoid content, antioxidant activity, biochemical property, nutritive value, *Persea bombycina*, *Litsea polyantha*.

### INTRODUCTION

North-eastern region of India is a homeland of various of sericigenous insects.<sup>1,2</sup> The golden coloured 'muga' silk is obtained from a semi-domesticated multivoltine insect called *Antheraea assamensis* Helfer. (muga silkworm), which is unique as well as prerogative to the North east India.<sup>3,4</sup> Being a polyphagous insect, it fed on the leaves of primary host plants namely, Som (*Machilus bombycina*) and Soalu (*Litsea polyantha*), but it also feeds on a wide range of other economically important food plants such as Dighloti (*Litsea salicifolia*) and Mejankari (*Litsea citrata*).<sup>5,6,7</sup>

Out of its diverse host plants, *P. bombycina* (= *Machilus bombycina* Linn.) is most prevalent in Assam because of its importance as a principal host of muga silkworm<sup>8, 9, 10</sup> followed by *L. polyantha* (= *L. monopetala* (Roxb.) Pers.)<sup>10</sup>. It is reported that, commercial parameters of this silkworm like shell ratio and reelability of muga silk are better in som leaves fed muga worms than soalu ones<sup>1</sup>. According to Neog *et al.*,<sup>3</sup>; Fraenkel,<sup>11</sup> and Lin, *et al.*,<sup>12</sup> the feeding preferences of the silkworms are largely mediated by the presence and distribution of secondary metabolites existed in plants. The leaf is harvested for silkworm rearing and so the quantities as well as quality of the host plant leaf yield directly affect the cocoon production by the silkworms<sup>13</sup>. Shimizu<sup>14</sup>, Ito and Arai<sup>15</sup> also described that quality of leaves is the most important factor for obtaining a good cocoon crop since it largely depends on developmental vigour of the silkworm breeds which in turn, is further influenced by the nutrient availability of the leaves fed. Therefore in order to isolate better quality muga silk from the cocoons, its host plants

claim such analytical study. So in this present study, we have aimed to analyze the qualitative and quantitative phytochemicals, antioxidant potentials, biochemical constituents and nutritive value of these two primary host plants of muga silkworm.

### MATERIALS AND METHODS

#### Plant material collection

Fresh mature leaves of *P. Bombycina* and *L. Polyantha* were collected from household premises of Dibrugarh District, Assam, India. The materials were shade dried at room temperature and grounded to fine powder using electric grinder.

#### Preparation of extract

The dried samples were macerated separately with methanol and petroleum ether under shaking conditions for 48 h (at 25°C) and later filtered through Whatman No. 1 filter paper. The extract was then concentrated with the help of rotary evaporator under pressure/evaporated at a constant temperature (60°C) until a semi dried powder/sticky mass of crude extract was obtained. After getting the dry mass, these were considered as cold methanol (MC) and petroleum ether (PEC) extracts and finally, the concentrated extract was stored in refrigerator for further experiment. Similarly, soxhlet extraction using these two solvents was considered as hot methanol (MH) and petroleum ether (PEH) extracts. Then the crude extracts were dissolved in Dimethyl sulphoxide (DMSO) - a neutral solvent to make final concentration for further analysis.



## Experimental

### Phytochemical analysis, total phenol and flavonoid content, antioxidant activity

Plant extracts were subjected towards the qualitative phytochemical analysis (Edeoga, *et al.*,<sup>16</sup>; Aja, *et al.*,<sup>17</sup>; Khandelwal,<sup>18</sup>; Majaw and Moirangthem,<sup>19</sup>; De, *et al.*,<sup>20</sup> and Ajayi, *et al.*,<sup>21</sup>) and quantification of total phenolic content (TPC) after the standard protocol of Malik and Singh,<sup>22</sup> and total flavonoid content (TFC) by Mervat and Hanan,<sup>23</sup> method.

Antioxidant activity was evaluated by using DPPH radical scavenging assay model as described by Anti-Stanojevic, *et al.*,<sup>24</sup>

### Biochemical estimation

The biochemical activity was examined by estimating the biochemical parameters like reducing sugar (RS) and free amino acid (FAA) in the leaves of both plants.

### Sample preparation

The powdered samples were homogenized to 10% (w/v) by using mortar- pestle in 80% ethanol for reducing sugar and chilled distilled water for free amino acid. The homogenate was then centrifuged at 10,000 rpm for 15 min at 4°C and supernatant was taken and finally, stored at -20°C until further utilization. The biochemical estimation of FAA was done following the methods of Moore and Stein,<sup>25</sup> and Miller,<sup>26</sup> for reducing sugar.

### Determination of nutrient content

Nutrient content of the leaves of both host plants was determined by using following formula (Indrayan, *et al.*,<sup>27</sup>):

Nutritive value = 4 x percentage of protein + 9 x percentage of fat + 4 x percentage of Carbohydrate.

### Statistical analysis

The results were generated from the record of three replicates for each observation and were expressed as their mean ± SD.

## RESULTS AND DISCUSSION

Table 1 presents the qualitative phytochemical investigation of both the plants. The plants recorded the presence of a variety of phytochemicals such as tannin, flavonoid, glycosides, phenol, reducing sugar, crude carbohydrate and crude protein content. Terpenoids and steroids are present in *L. polyantha* and absent in *P. bombycina*. The genus *Litsea* was reported to contain several secondary metabolites (Agrawal *et al.*,<sup>28</sup>; Choudhury *et al.*,<sup>29</sup>) and our study also proves their statements. According to Choudhury *et al.*,<sup>29</sup> methanol extract of *L. polyantha* recorded alkaloids, steroids, anthraquinone and glycosides with the absence of cardiac glycosides.

**Table 1:** Qualitative analysis of phytochemicals in leaves of *P. bombycina* and *L. polyantha*

Tests for phytochemicals	Host plants	
	<i>P. bombycina</i>	<i>L. polyantha</i>
Tests for Tannins		
1) Ferric chloride test	+	+
2) Lead Acetate test	+	+
Test for Phlobatannin	-	-
Tests for Flavonoid		
1) Ammonia solution - Conc. H <sub>2</sub> SO <sub>4</sub> test	+	+
2) NaOH test	+	+
Tests for Terpenoid		
Salwoski's test	-	+
Test for Steroid		
Salwoski's test	-	+
Test for Glycoside	+	+
Test for Cardiac glycoside		
Keller Killani test	-	-
Tests for Alkaloid		
1) Dragendroff reagent	-	-
2) Mayer's reagent	-	-
3) Picric acid test	-	-
Test for Saponin		
Frothing test	-	-
Test for Reducing sugar		
Fehling's test	+	+
Test for Carbohydrate		
Molisch's test	+	+
Test for Protein		
Picric acid solution	+	+
Test for phenol		
Ferric chloride test	+	+
Test for carotenoids		
1) Anthraquinone	-	-
2) Free anthraquinone	-	-
3) Carotenoid	-	-

(+) indicates presence of phytochemical constituents; (-) indicates absence of constituents

### Total Phenolic and flavonoid contents

The quantification data of total phenolic contents (TPC) and total flavonoid contents (TFC) of different solvent extracts of these two plants is given in table 2 and fig. 1. The results were expressed in catechol equivalent (mg/g of extract) and quercetin equivalent (mg/g extract) respectively. Methanolic extracts of *P. bombycina* and *L. polyantha* shows greater amount TPC and TFC than petroleum ether extracts. MC and MH of *L. polyantha* recorded more flavonoid content (3.65±0.35 and 2.80±0.00 respectively) than *P. bombycina* (1.18 ±0.01

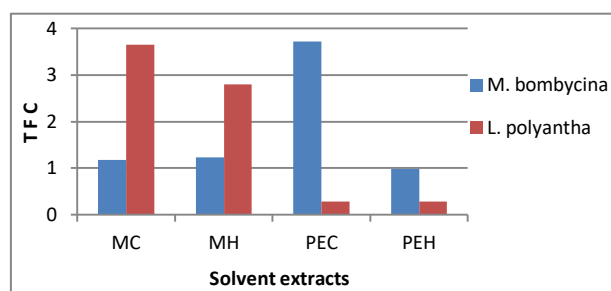
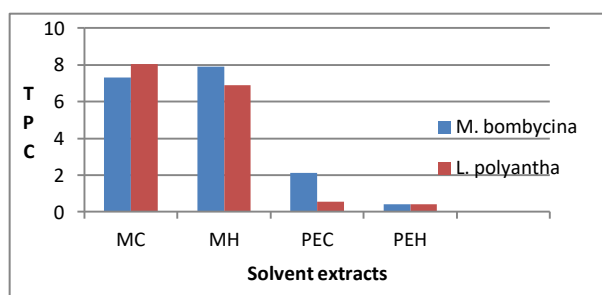


and 1.24±0.15 respectively). Choudhury *et al.*,<sup>29</sup> also recorded good quantity of phenol and flavonoid content in *L. polyantha*. Neog *et al.*,<sup>3</sup> reported more phenol in tender leaves of *P. bombycina* (1.946%) as compared to medium (1.182%) or mature leaves (0.712%). The phenol

groups are usually found in the form of polyphenols which serve as antioxidant by scavenging free radicals to stable form revealing a positive correlation between them.<sup>30-34</sup>

**Table 2:** Value of TPC and TFC of cold and hot extracts of *P. bombycina* and *L. polyantha*

Plant extract	TPC				TFC			
	MC	MH	PEC	PEH	MC	MH	PEC	PEH
<i>P. bombycina</i>	7.32 ±0.03	7.91 ±0.13	2.12 ±0.12	0.43 ±0.10	1.18 ±0.01	1.24 ±0.15	3.72 ±0.01	0.98 ±0.00
<i>L. polyantha</i>	8.03 ±0.60	6.88 ±0.22	0.57 ±0.02	0.42 ±0.08	3.65 ±0.35	2.80 ±0.00	0.29 ±0.34	0.28 ±0.00



**Figure 1:** Graphical presentation of TPC and TFC of the solvent extracts of *P. bombycina* and *L. polyantha*.

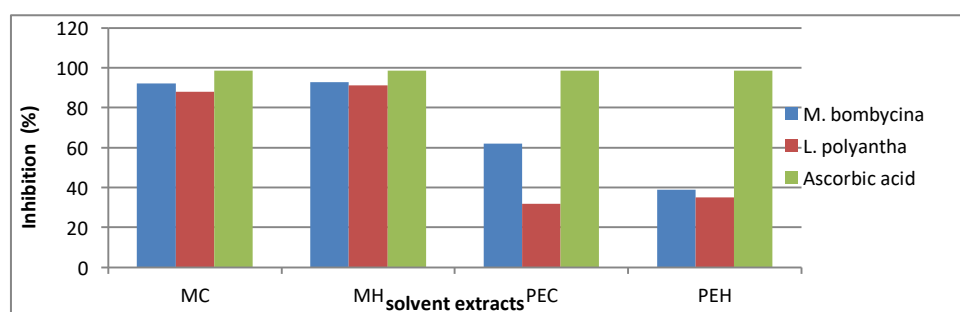
**Antioxidant activity**

The percentage of inhibition in DPPH model of different solvent extracts of both plants is given in table 3 and fig. 2. The scavenging activity of the extracts was studied by taking 0.5 ml of the plant extracts at 1 mg/ml concentration with reference to the standard ascorbic acid and the highest inhibition was exhibited by methanolic extract in both cold (92.24 ±0.22) and hot (92.97 ±0.01) extract. *P. bombycina* have more antioxidant activity than *L. polyantha* as compared to ascorbic acid (98.59±0.07). Plants having medicinal properties are considered as enriched with antioxidants due to the presence of bioactive polyphenols such as phenols and flavonoids and due to their antioxidant

effect, these phytochemicals are efficient in combating or inhibiting diseases caused by free radicals<sup>35,36,37,38,39</sup>. DPPH model is a widely accepted tool to determine the free radical scavenging activities of the antioxidants by producing a deep violet colour in solvent. The antioxidant potentiality can be measured by deduction of absorbance at 517nm with loss of violet colour<sup>40</sup>. The present study reveals that the cold and hot extracts of *P. bombycina* and *L. polyantha* have the potential to scavenge the DPPH free radical. Choudhury *et al.*,<sup>29</sup> reported that methanol extract of *L. Polyantha* have significant antioxidant activity. Agarwal *et al.*,<sup>28</sup> also suggested that the genus *Litsea* is the rich source of natural antioxidants.

**Table 3:** Antioxidant activity of cold and hot extracts of the plants

Samples	DPPH radical scavenging activity (% inhibition in mg/ml)			
	MC	MH	PEC	PEH
<i>P. bombycina</i>	92.24 ±0.22	92.97 ±0.01	62.16 ±0.09	38.90 ±0.09
<i>L. polyantha</i>	88.08 ±0.24	91.27 ±1.05	31.95 ±0.00	35.21 ±1.66
Ascorbic acid	98.59±0.07			



**Figure 2:** Antioxidant activity of cold and hot extracts of *P. bombycina*, *L. polyantha* and Ascorbic acid.

### Biochemical estimation

Reducing sugar and free amino acids were higher in *P. bombycina* (0.66±0.25 and 0.212±0.010 mg/ml respectively) whereas lower in *L. polyantha* (0.14±0.01 and 0.023±0.001 mg/ml respectively). The results are presented in table 4 and fig. 3. Biochemical constituents of leaves of silkworm host plants play a major role in producing healthier cocoons (Agarwal *et al.*,<sup>41</sup>). Present study recorded the higher level of FAA in *P. Bombycina* as compared to that of *L. polyantha* which follows the findings of Chaudhuri *et al.*,<sup>42</sup> described as when silkworm

larvae fed on leaves of *L. polyantha*, the concentration of protein, carbohydrate and lipid were higher in oocytes and FAA content was found to be higher when fed on *P. bombycina*. The concentration of protein, FAA and lipid were maximum in the eggs when the food plant was som (*P. bombycina*). Kakati and Kakati,<sup>43</sup> recorded higher (11.78±0.84%) carbohydrate in *P. bombycina* than *L. polyantha* (9.01±0.67%) and higher reducing sugar in *P. bombycina* (2.35±0.77%) than *L. polyantha* (2.12±0.31%) like our findings. Chaudhuri *et al.*,<sup>42</sup> recorded more carbohydrate when fed on soalu leaves.

**Table 4:** Reducing sugar (RS) and free amino acid (FAA) in the extracts of *P. bombycina* and *L. polyantha*.

Plant extract	RS (mg/ml)	FAA (mg/ml)
<i>P. bombycina</i>	0.66 ± 0.25	0.212 ± 0.010
<i>L. polyantha</i>	0.14 ± 0.01	0.023± 0.004

### Nutritive Value

Table 5 presents the nutrient content of the plants. *P. bombycina* showed more ash, fat, moisture and protein content along with nutritive value than *L. polyantha*. Alterations of nutritional values of host plants has been considered as one of the factor associated with physiological growth and development of their consumer silkworms and this alteration in nutritional values of foods is dependent on the amino acid availability and protein digestibility. Leaf moisture plays a vital role in the improving the nutrient level of leaves<sup>44</sup> which in turn, enhances the feeding efficiency of the larvae and thereby increases the growth rate as well.<sup>45</sup> It was reported by

Chaluvachari and Bongale<sup>46</sup> that higher larval weight and moulting ratio were associated with higher values of leaf moisture content. Dutta *et al.*,<sup>47</sup> recorded higher moisture content in *L. polyantha* (71.84%) than *P. bombycina* (67.02%). In contrast to our study, Kakati and Kakati,<sup>43</sup> from Lakhimpur reported that the moisture content was higher in *P. bombycina* (68.89±0.74%) than *L. polyantha* (64.84±1.29%). Sharma *et al.*,<sup>48</sup> showed that the leaves of *P. bombycina* have 2.708% of crude protein, 31.452% of carbohydrate content. Kakati and Kakati,<sup>43</sup> also recorded that the total ash content was found to be higher in *P. bombycina* (4.33±0.54%) than *L. polyantha* (4.13±0.65%).

**Table 5:** Nutritive value of *P. bombycina* and *L. polyantha* extract.

Plant extract	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Nutritive value (Cal/100gm)
<i>P. bombycina</i>	6.96±0.03	4.55±0.00	5.70±1.00	6.70±1.00	76.09±1.00	382.46
<i>L. polyantha</i>	10.45±1.00	4.34±1.09	0.90±0.01	1.65±0.04	82.66±1.01	345.34

### CONCLUSION

Secondary metabolites, antioxidants and biochemicals present in these two plant proves their use as host plant by silkworm because these phytochemicals play a key role in accepting their food or diet by the silkworms. *M. bombycina* is more efficient than *L. polyantha*.

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