Research Article

Phytochemical and Biochemical Analysis of Two Host Plants of Eri Silkworm, *Samia ricini* (D.)

M. Changmai[1], J. Chetia[1]*, S. Upadhyaya[1], R. N. S. Yadav[1], M. Bhuyan[2]
[1]Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India.
[2]CSIR- NEIST, Jorhat, Assam, India.
***Corresponding author's E-mail:** junali.chetia@yahoo.com

Accepted on: 24-04-2015; **Finalized on:** 31-05-2015.

ABSTRACT

Ricinus communis Linn. and *Heteropanax fragrans (*Roxb.) Seem.- the primary and secondary host plants of Eri silkworm (*Samia ricini)* were selected to evaluate the total phenolic content, antioxidant as well as antimicrobial activity, biochemical analysis along with nutritive value. The study confirms the presence of various phytochemicals like-tannin, flavonoid, terpenoid, steroids, glycosides, saponin, phenol, reducing sugar, carbohydrate, protein and absence of phlobatanin, cardiac glycosides, alkaloids, anthraquinone, free anthraquinone and carotenoids. DPPH radical scavenging activity shows that both of these plants have good antioxidant activity in reference to the Ascorbic acid. It also shows that *R. communis* have more antioxidant power than *H. fragrans*. Methanol extracts showed higher phenol and flavonoid content than the petroleum ether extracts. Similarly biochemical constituent's like- reducing sugar (TRS) and free amino acid (FAA) are also higher in *R. communis* than *H. fragrans*. A little anti-bacterial activity is shown by the plants. Nutritive value of the plants is also significant.

Keywords: Phytochemicals, phenol, flavonoid, antioxidant, biochemical, antimicrobial activity.

INTRODUCTION

The medicinal plants are the major source of natural drug. The medicinal value of these plants typically results from the combined effect of some chemical substances which are known as phytoconstituents or secondary metabolites- such as alkaloids, steroids, tannins, phenolic compounds, flavonoid. These are capable of producing definite physiological action on body[1,2,3]. A large number of phytochemicals have inhibitory effects on all types of micro-organisms, *in-vitro*[4]. These are synthesized by primary or rather secondary metabolism of plants.

North-eastern region of India is a homeland of about a dozen of sericigenous insects[5]. The eri silkworm, *Samia ricini* D. is a domesticated multivoltine lepidopteran insect and is cultured for a period of over 5000 years[6]. The primary food plant of this economically important polyphagus insect is castor (*Ricinus communis* Linn.) but it also feeds on a wide range of food plants such as *Heteropanax fragrans* (Roxb.) Seem, *Manihot utilissima* Phol, *Evodia flaxinifolia* Hook, *Ailenthus gradulosa* Roxb. etc.[7] which are considered as secondary food plants.

R. communis L. (Euphorbiaceae), commonly known as 'Era goch' is an evergreen soft wooded small shrub often cultivated in Assam to rear Eri silkworm[8]. It is the best alternative food plant for eri silkworm[9]. It is reported to contain antioxidant, anti inflammatory, antidiabetic, antitumour, antiasthmatic and antibacterial activity and used for treatment of jaundice and hepatitis, skin and breast cancer in initial phase[10]. Ricin contained in *R. communis* is a well known poisonous compound that elicits violent purgative action in man[11]. *H. fragrans* Seem. (Araliaceae), commonly known as 'Kesseru' is a small soft wooded evergreen tree used to rear eri silkworm[8]. Udddin[12] investigated that the plant is used to treat cancer by the Chakma community from Bangladesh. Its roots and bark are used for detoxification, blood activation and detumescence, and pain easing.

Out of these two host plants; *R. communis* is mainly annual in nature and has to be grown fresh in every six months, so it is not available throughout the year. In contrast to that, *H. fragrans* is perennial in nature and is available throughout the year. Again host plants have significant effect on colour and compactness of silk[13].

With reference to the above, in the present study, we selected the leaves of these two plants to determine their qualitative and quantitative phytochemical analysis, antioxidant and antimicrobial activity, biochemical constituents and nutrient content.

MATERIALS AND METHODS

Sample Collection

Fresh leaves of *R. communis* and *H. fragrans* were collected from household premises of Dibrugarh, Assam, India. The materials were shade dried and grounded to fine powder using electric grinder.

Sample Extraction

Samples were macerated separately with methanol and petroleum ether for 48 hours and filtered through Whatman No 1 filter paper. The filtrate was then evaporated at a constant temperature (60°C) until a semi dried powder/sticky mass of crude extract was obtained. These were considered as cold methanol (MC) and petroleum ether (PEC) extracts. Soxhlet extraction using

methanol and petroleum ether were considered as hot methanol (MH) and petroleum ether (PEH) extracts.

The crude extracts were dissolved in Dimethyl sulphoxide (DMSO) as neutral solvent to make final concentration for further analysis.

Experimental

Phytochemical Analysis, Total Phenol and Flavonoid Content, Antioxidant and Antimicrobial Activity

The qualitative phytochemical analysis was performed following the standard laboratory methods described by Edeoga[14]; Aja[15] and Ajayi[10].

Quantitative estimation of total phenolic content (TPC) was done by the method described by Malik and Singh[16] and total flavonoid content (TFC) by the method described by Mervat and Hanan[17].

Antioxidant activity was studied by using DPPH radical scavenging method as described by Anti-Stanojevic[18].

Antimicrobial Activity Study

The antimicrobial test was carried out by agar well diffusion method described by Nair[19] using 6mm borer. The activity was determined by measuring the diameter of zone of inhibition (ZOI) exhibited by the extract.

Selected Strains for Antimicrobial Study

Two Gram Positive bacterial strains viz, *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 3160) and one Gram Negative strains viz, *Escherichia coli* (MTCC 443) were used for the study.

The strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference of bacterial strains were maintained on nutrient agar slants and stored in freeze. Strains were regularly sub cultured using nutrient broth.

Standard Antibiotics

Standard antibiotics- Ampicillin (AP) 10mcg and Streptomycitin (ST) 10mcg were taken for the selected bacterial strains and were employed for comparision of ZOI with the sample.

Biochemical Analysis

Plant Sample Preparation

The powdered samples were homogenized in hot 80% ethanol for reducing sugar and ice cold distilled water for free amino acid by using mortar- pestle to 10% (w/v) in cold condition.

The homogenate was centrifuged at 10,000 rpm for 15 min at 4^{o}C and supernatant was taken for biochemical estimation/ stored at -20^{o}C till utilize.

Free amino acids and reducing sugar were estimated following the methods of Moore and Stein[20] and Miller[21] respectively.

Determination of Nutrient Content

Moisture, ash and fat content were determined by the method described by Indrayan[22].

The nutritive value was determined by the following formula:

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

Statistical Analysis

All the experiments were performed in triplicate and the results were expressed in Mean ± SD.

RESULTS AND DISCUSSION

Table 1 presents the phytochemical analysis of the plants. Both of these plants shows the presence tannin, flavonoid, terpenoid, steroids, glycosides, saponin, phenol, reducing sugar, carbohydrate, protein and absence of phlobatanin, cardiac glycosides, alkaloids, anthraquinone, free anthraquinone and carotenoids. Presence of saponin and absence of alkaloids is identical to the results of Rao[23] from Jaipur but absence of reducing sugar, tannin, terpenoids and flavonoids is not similar to our result. Kensa and Yasmin[24] from Tamil Nadu also showed the presence of phytochemicals like- saponin, tannin, flavonoid, steroid, carbohydrate, alkaloids and phenol, which is also similar our results. Similarly Vandita[25] from Gujarat; Jean and Gupta[26] from Azamgarh; Naqvi[27] also tested the phytochemicals of *R. Communis* and showed similar kind of results. The results of TPC and TFC are presented in Table 2. In case of TPC, all the extracts of *R. communis* show higher value than *H. fragrans*. Among them, MH extract of *R. communis* shows the highest (6.94 ± 0.33) and PEC extract of *H. fragrans* shows the lowest (0.45 ± 0.34) phenolic content. But in case of TFC, MC and MH extracts of *H. fragrans* shows more flavonoid content then *R. communis* but this is opposite to PEC and PEH extracts. MC extract shows the highest (1.80 ± 0.10) and PEH extract shows the lowest (0.34 ± 0.01). The presence of good quantity of flavonoid results a good antioxidant activity[26]. Both of these plants showed a good antioxidant activity against DPPH and the results are presented in Table 3. From the results it is shown that *R. Communis* shows more antioxidant activity than *H. fragrans* in all cases. Highest antioxidant activity is shown by MC extract (91.49 ± 0.22) of *R. Communis* and lowest activity is shown by PEH extract (31.28 ± 0.66) of *H. fragrans*. All these results are lower than the standard ascorbic acid (98.59 ± 0.07). Several studies has described that higher the phenolic content results the higher antioxidant activity[28,29] and this is also shown by our results. Reducing sugar and free amino acid are presented in Table 4. For both of the cases *R. communis* showed more amount than *H. fragrans*. Reducing sugar and free amino acid of *R. Communis* (1.26mg/ml and 0.067 mg/ml) is higher than *H. fragrans* (0.31mg/ml and 0.016mg/ml) respectively. Naqvi[27] from Pakistan evaluated biochemical analysis from *R. communis* and significant quantity of

total sugar and reducing sugar were determined from the plant. Table 5 presents the nutritive value of the plants. It shows that the nutritive value of *H. fragrans* (341.80 cal/100gm) is slightly higher than *R. communis* (340.62 cal/100gm). Many researchers also reported favourable effect of total carbohydrate content in eri silkworm host plants[30-32]. Table 6 presents the antibacterial activity of the plant extracts and they showed good inhibition against *S. aureus* than the other strains. There are several reports in the literature regarding the antibacterial activity of crude extracts prepared from the both of these plants[33,2,14,34]. In this case petroleum ether extract do not show any activity against the selected strains while methanol extract shows a little activity. MH extract of *R. Communis* shows highest ZOI (12mm) against *E. coli*. Methanol extract of *H. fragrans* shows ZOI (10mm and 10mm) respectively against *S. aureus*. Rao[23] showed antibacterial activity against different bacteria using methanol extract of *R. Communis*. Naqvi[27] evaluated the anti-microbial efficacy from *R. Communis* and showed good inhibition against different gram positive and gram negative bacteria. Sharma[35] from Bhopal investigated the antimicrobial potential of different extracts of castor against several bacteria and revealed that the methanol and ethyl acetate extracts of *R. communis* leaves possess good inhibition than petroleum ether extract. Similarly Kota and Manthri[36] from Andhra Pradesh, evaluated antibacterial activity of ethanolic leaf extract of *R. communis* against different bacteria and found significant activity against the selected bacteria.

Table 1: Qualitative Phytochemical Analysis of the Plants.

Parameters Tested		Results	
		R. communis	*H. fragrans*
Tannins	1) Ferric chloride test	+	+
	2) Lead Acetate test	+	+
Phlobatannin		-	-
Flavonoid	1) Ammonia solution -Conc. H_2SO_4 test	+	+
	2) NaOH test		
Terpenoid	Salwoski's test	+	+
Steroid	Salwoski's test	+	+
Glycoside		+	+
Cardiac glycoside	Keller Killani test	-	-
Alkaloid	1) Dragendroff reagent	-	-
	2) Mayer's reagent		
	3) Picric acid test		
Saponin Frothing test		+	+
Reducing sugar	Fehling's test	+	+
Carbohydrate	Molisch's test	+	+
Protein	Picric acid solution	+	+
Phenol	Ferric chloride test	+	+
Carotenoids	1) Anthraquinone	-	-
	2) Free Anthraquinone	-	-
	3) Carotenoid	-	-

(+) indicates presence of constituents; (–) indicates absence of constituents.

Table 2: TPC and TFC of the Plants.

Samples	TPC (mg catechol equivalent/gm dry material)				TFC (mg quercetin equivalent/gm dry material)			
	MC	MH	PEC	PEH	MC	MH	PEC	PEH
R. communis	6.06 ± 0.03	6.94 ± 0.33	0.99 ± 0.10	1.81 ± 0.44	1.80 ± 0.10	0.95 ± 0.01	0.72 ± 0.01	1.08 ± 0.00
H. fragrans	4.50 ± 1.09	1.34 ± 0.10	0.45 ± 0.34	0.51 ± 0.30	1.97 ± 0.35	1.35 ± 0.01	0.35 ± 0.00	0.34 ± 0.01

Table 3: Antioxidant Activity of the Plants.

Samples	DPPH radical scavenging activity(% inhibition in mg/ml)			
	MC	MH	PEC	PEH
R. communis	91.49 ± 0.22	86.09 ± 0.01	40.67 ± 0.09	42.89 ± 0.09
H. fragrans	90.52 ± 0.44	32.02 ± 1.05	34.09 ± 0.08	31.28 ± 0.66
Ascorbic acid	98.59 ± 0.07			

Table 4: Reducing sugar (RS) and free amino acid (FAA) of the plants.

Sample	RS(mg/ml)	FAA(mg/ml)
R. communis	1.26 ±2.86	0.067 ±0.013
H. fragrans	0.31 ±0.54	0.016 ±0.010

Table 5: Nutritive value of the plants.

Sample	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Nutritive value (Cal/100gm)
R. communis	10.95	4.87	0.70	20.75	62.83	340.62
H. fragrans	11.30	4.50	1.00	4.65	78.55	341.80

Table 6: Anti-bacterial activity of the plants.

Samples		Diameter of inhibition of zone (mm)		
		B. subtilis	***S. aureus***	***E. coli***
R. communis	MC	-	10	-
	MH	10	-	12
	PEC	-	12	-
	PEH	-	-	-
H. fragrans	MC	-	10	-
	MH	-	10	-
	PEC	-	-	-
	PEH	-	-	-
Ampicillin (AP) 10mcg		-	-	10
Streptomycin (ST)10mcg		18	10	12

*- No activity; Zone of inhibition includes the diameter of well (6mm).

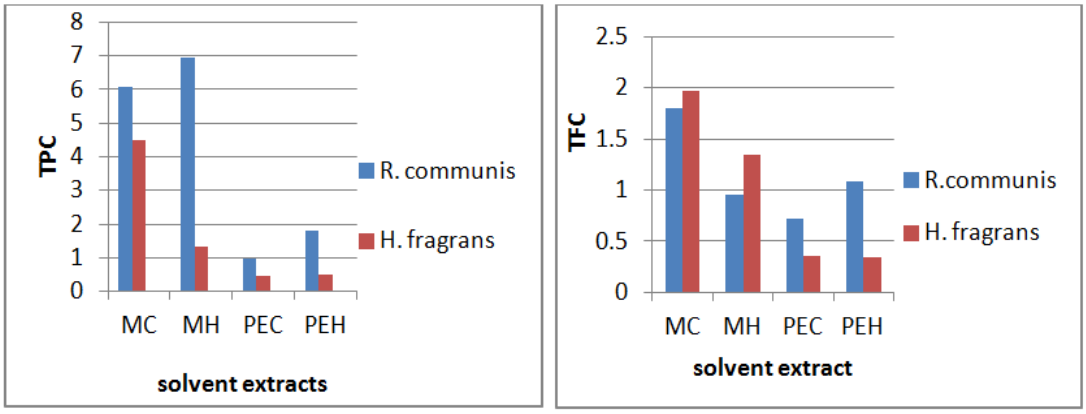

Figure 1: TPC and TFC of the plants.

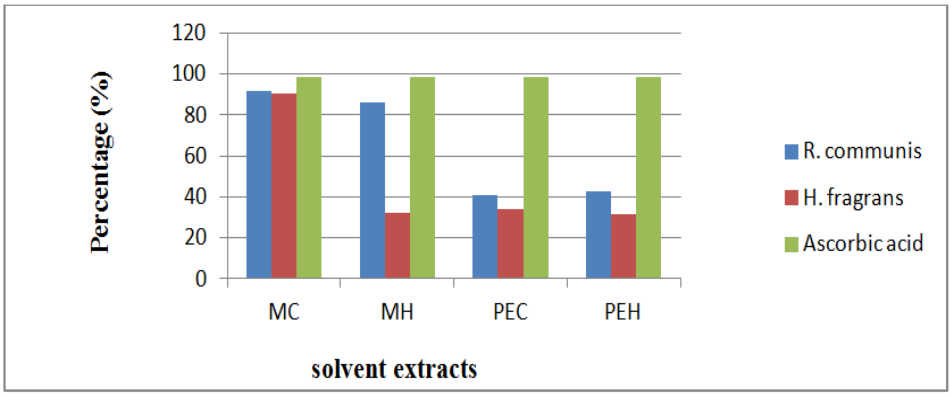

Figure 2: Percentage of antioxidant activity of 0.500ml of the plant extracts at 1mg/ml concentration with reference to the ascorbic acid.

CONCLUSION

The presence of various secondary metabolites and their antioxidant and antibacterial activity proves the medicinal uses of these plants. Further analysis for accurate quantification of such metabolites may help in developing artificial diet for them.

Acknowledgement: Authors like to acknowledge Department of Life Sciences, and Centre for Studies in Biotechnology, Dibrugarh University, Dibrugarh for their laboratory facilities. M. Changmai, J. Chetia and S. Upadhyaya also thankful to the DST for financial support.

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Source of Support: Nil, **Conflict of Interest:** None.