Physicochemical and Phytochemical evaluation of Cocoon of *Bombyx mori* Linn. (Abresham)

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Abstract

De-wormed cocoon of *Bombyx mori* Linn. (Silkworm) of Bombycidae family, plays a foremost role in Unani system of medicine. Its decoction is used in the preparation of Unani compound formulations for the treatment of different diseases like cardiovascular and cerebro-vascular disorders. Because of these prominent medicinal properties, the drug was standardized according to WHO guidelines. The main aspects included in the study were organoleptic characters, morphological features, physico-chemical & phytochemical parameters, fluorescence analysis of decoction and infusion of the drug extracts and HPTLC profile. The study also included safety evaluation measures, such as heavy metal analysis, microbial load, aflatoxins which provide scientific means regarding the qualitative and quantitative aspects. They are widely accepted in the quality assessment of herbal drugs and also to lay down the standard for the genuine drug. Phytochemical screening was also carried out in the drug extracts. The present study is aimed to standardize and provide the scientific evidences for the decoction and infusion of the drug for its safe and effective therapeutic potential.

Keywords: *Bombyx mori,* Physicochemical, Phytochemical screening, HPTLC fingerprint, Standardization.

Introduction

Bombyx mori Linn. Syn. *Phalaena mori* Linn. is an economic insect whose silk is emerging as a source for solving a broad range of biological problems (Mondal, 2007). Its dietary flavonoids are metabolized and accumulate in cocoons, thereby causing green coloration. Flavonoids increase the UV-shielding activity of cocoons and thus could confer an increased survival advantage to insects contained in these cocoons (Daimona *et al*., 2010). Cocoon-making behavior is the most highly developed in Lepidoptera (moths and butterflies). The silkworm, *Bombyx mori*, is a monophagous insect whose only food is mulberry leaves. The cocoon shell of the silkworm consists mainly of proteins such as fibroin and sericin (Tamura *et al*., 2002). Silkworm cocoon colors are determined by two main pigments, carotenoids (Harizuka, 1953) and flavonoids (Tazima, 1978), which are derived from mulberry leaves (Fujimoto, 1959).

Flavonoids modified by *B. mori* may be useful as medicinal or cosmetic materials. The ethanolic extracts of yellow-green colored cocoon shells of a range of strains of *B. mori* have potent antibacterial activity (Kurioka *et al*.,

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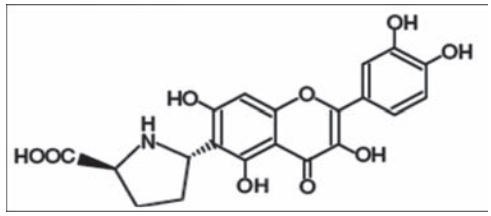
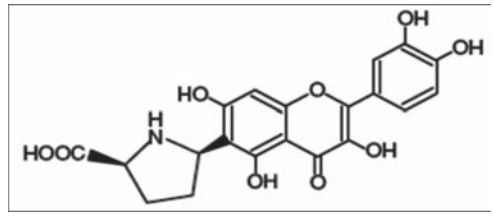
1999) and strong antioxidant activity (Yamazaki *et al*., 1999). Two flavonoids containing the L-proline moiety, 6-*C*-[(2*S*,5*S*)-prolin-5-yl] quercetin (prolinalin A) and 6-*C*-[(2*S*,5*R*)-prolin-5-yl] quercetin (prolinalin B), were isolated from the cocoon shell of the silkworm of *Bombyx mori* by Hirayama *et al*., 2006. Further research among silkworm strains for novel flavonoids towards possible biological activity reveal that the aqueous MeOH extract of the yellow cocoon shell of a Chinese race of Daizo contain novel flavonoids with an amino acid moiety (Hirayama *et al*., 2006).



Prolinalin A



Prolinalin B

Bombyx mori in Unani context

In Unani Medicine *B. mori* cocoon is known as 'Abresham' and popularly known as Abresham Muqriz; Muqriz means cut (Kabeeruddin, undated). It is used in various formulations as one of the ingredients like 'Khamira-e-Abresham Sada', 'Khamira-e-Abresham Hakeem Arshad Wala' etc. for many cardiac and neurological disorders (Khan et al., 2006). Therapeutic actions attributed to the drug are Muffareh (Exhillerent), Munaffis-e-Balgham (Expectorant), Jali (Detergent) and Muqawwi-e-Qalb (Cardiac tonic). Crude extract of *Bombyx mori* cocoons along with two other drugs also acts as protective remedy in hyperlipidemia. National Formulary of Unani Medicine (Anonymous, 2007) includes several formulations with large or small number of ingredients for the treatment of cardiovascular and cerebrovascular disorders. Some of the formulations are being used in Unani medicine with good results and efficacy (Goswami, 1977). But there is no definite data regarding dose and effect relationship for the extract of cocoons of *Bombyx mori*. This led to the logical study for evaluating the role of aqueous extract i.e., infusion and decoction of silk cocoons of *Bombyx mori* as a single drug for its safe therapeutic potential.

Properties of Bombyx mori silk (Abresham Muqriz)

Bombyx mori silk is attributed the qualities viz., 'Hot and dry' (Garm-o-Khushk) in its temperament. It is a cardiac tonic and a nervous stimulant. It is an

expectorant and removes excess 'Kapha' (Phlegm) from the blood (Hamdani, 1980). Recent advancement have shown that it is being used to treat palpitation, hypertension and heart diseases, which occur due to hardening of arteries.

Materials and methods

Collection of material

Dewarmed cocoons were procured from the pharmacy of Central Research Institute of Unani Medicine (CRIUM), Hyderabad. The present investigation on the drug include parameters such as morphological studies, physico-chemical parameters, Phytochemical screening and HPTLC fingerprint of infusion and decoction (Aqueous extracts), fluorescence studies and safety evaluation.

Chemical analysis

Physico-Chemical parameters of the cocoons were studied as shown in table 1, such as total ash, water and alcohol soluble matter, PH value and loss on drying at 1050C. Physico-chemical parameters were determined according to the methods described in 'The Unani Pharmacopoeia of India' (Anonymous, 2009). Fluorescence analysis was carried out as per the method described by Trease and Evans (1972) and GBC-908 AA model Atomic Absorption Spectrophotometer (AAS) was used to determine the concentration of heavy metals. Microbial load and aflatoxins contamination were analyzed as per the methods described in WHO guidelines (Anonymous, 1998).

Phytochemical screening was carried out in the aqueous extracts i.e., infusion and decoction as per the methods described by Trease and Evans (1972) to observe the nature of phyto-constituents present in the drug. Phyto-constituents such as flavanoids and proteins were detected as the major constituents in the cocoons.

HPTLC fingerprint profile

Preparation of extract of the sample drug

Cocoons in a quantity of 10 g were macerated in 100 ml of methanol and water separately in stoppered conical flasks and kept for 2 hours while shaking at regular intervals. Later the contents were filtered through whatman No. 41 filter paper and evaporated the solution to 10 ml. The solutions thus obtained, were used as samples for separation of components.

Infusion preparation

Infusion was prepared by macerating 10 g of Cocoons for a short period of time with either cold or boiling water.

Decoction Preparation

To obtain the decoction, 10 g of Cocoons were boiled in specified volume of water for defined time, cooled and strained. This procedure was carried out for extracting water soluble or heat stable constituents.

Development and determination of the solvent system

The samples were spotted as 6mm band on Pre-coated Aluminum sheets of Silica Gel 60 F_{254} (Merck). After trying with various solvent systems with variable volume ratios, the suitable solvent system as stated in table 2 was selected in its proportional ratio, and developed in the Twin through TLC chamber to the maximum height of the plate.

Detection system

After developing, the TLC plate was dried completely and detected by spraying Ninhydrin reagent on the plate heated at 110^0C for 5 minutes and then observed in visible region and photographed as shown in figure 5.

Results and Discussion

Organoleptic Characters

The crude drug consists of de-wormed cocoons of *Bombyx mori* (Silkworm) were yellow in colour.

Identification

Macroscopy: Cocoons yellowish, oblong-ovate, 3-4.5 cm long and 2-3 cm in diameter; with very fine silk threads. Outer surface rough, fibrous, shining; inner surface smooth, shining; taste and odour indistinct. Macroscopical features of Cocoon of *Bombyx mori* as observed under visible light and UV at 254nm & 366nm are shown in figure 1.

Microscopy: Microscopical study reveals that Cocoon wall is made up of silk fibres of 35-50µ thickness. The fibres are translucent, silk threads solid, cylindrical or slightly flattened; highly refractive threads from 10-25µ (Fig. 2, 3 & 4).

Powder characters: The Powder is golden yellowish and contains pieces of silk fibres.

Physico-Chemical Standards

The Physico-chemical parameters data in the study shows the mean values of three readings and depicted in table 1.

Chemical Analysis: (TLC analysis, Heavy metals, microbial load, Aflatoxins, fluorescence behavior).

Infusion and decoction of Cocoons of aqueous extract of the drug whose chromatogram was developed using the solvent n-butanol: Glacial Acetic Acid: Water (4:1:1) and detected by spraying with ninhydrin reagent and heating the plate at 110^0C was observed as given in table 2. Under visible region it showed various spots with Rf values as given in table 3-5. Densitogram obtained from the HPTLC system for infusion, decoction and methanolic extract of cocoon at 580 nm were observed as shown in figures 6-8. Fluorescence study of infusion and decoction pertaining to their colour in daylight i.e, visible region and under ultra-violet light were noticed as presented in table-6. The preliminary phytochemical screening for nature of compounds present were carried out in infusion and decoction of cocoon of *Bombyx mori,* revealed the presence of alkaloids, carbohydrates, flavonoids, proteins. In aqueous and alcoholic extracts it revealed the presence of Glycosides, flavanoids and proteins as shown in the table 7 and 8. For safety evaluation studies of the drug, estimation of heavy metals such as cadmium, lead, mercury and arsenic were carried out and found to be absent except the presence of mercury which was within the permissible limits as given in table 9. Similarly, Aflatoxins were analyzed and found to be absent as given in the table 10. Microbial load were analyzed and found to be within the permissible limits as given in the table 11, inferring the drug to be safe and non toxic.

Table 1: Physico-chemical parameters of the de-wormed Cocoon

Parameters	Results in average (n=3)	Limits
Total ash (% w/w)	1.20%	(Not more than 1.5%)
Acid insoluble ash (%w/w)	0.25%	(Not more than 1.0%)
Alcohol sol. Matter (%w/w)	0.85%	(Not less than 0.5%)
Water sol. matter (% w/w)	5.23%	(Not less than 5%)
Loss of weight on drying at 1050C	6.38%	(Not more than 7.0%)
P^H of Infusion	6.7	
P^H of Decoction	6.3	

Table 2: TLC profile of infusion and decoction of cocoon of Bombyx mori along with Rf values and detection system

S. No.	Name of the extract	Solvent system	Detection	No. of spots	Rf values
1.	Infusion	n-butanol: Glacial acetic acid: water =4:1:1	Ninhydrin reagent	5	0.01, 0.31, 0.36, 0.56, 0.67
2.	Decoction	n-butanol: Glacial acetic acid: water =4:1:1	Ninhydrin reagent	3	0.01, 0.30, 0.37

Table 3: Peak list of Infusion of cocoon of Abresham at 580 nm

Peak no.	Y-Pos	Area	Area (%)	Height	Rf value
1	10.8	20.90	3.0	11.95	0.01
2	26.8	142.31	20.6	33.60	0.31
3	29.5	148.99	21.6	34.34	0.36
4	40.0	77.57	11.2	19.57	0.56
5	46.1	299.84	43.5	70.05	0.67

Table 4: Peak list of Decoction of cocoon of Abresham at 580 nm.

Peak no.	Y-Pos	Area	Area (%)	Height	Rf value
1	10.8	40.85	28.2	20.12	0.01
2	26.2	42.12	29.1	14.38	0.30
3	30.2	61.83	42.7	15.64	0.37

Table 5: Peak list of methanolic extract of cocoon of Abresham at 580 nm

Peak No.	Y-Pos	Area	Area (%)	Height	Rf value
1	26.2	78.15	33.0	24.55	0.30
2	29.8	54.45	23.0	17.19	0.37
3	44.3	104.11	44.0	27.58	0.64

Table 6: Fluorescence analysis of powdered drug

S. No	UV 254 nm	UV 366nm	Visible region
Infusion	Black	Blue	Pale yellow
Decoction	Blue	Blue	Pale yellow

Table 7: Phytochemical screening of the nature of compounds present in the infusion and decoction of cocoon of *Bombyx mori.*

S. No.	Phyto constituents	Infusion	Decoction
1.	Alkaloids	-	+
2.	Carbohydrates	+	+
3.	Flavonoids	+	+
4.	Glycosides	-	-
5.	Phenols	-	-
6.	Proteins	+	++
7.	Saponins	-	-
8.	Steroids	-	-
9.	Tannins	-	-

Table 8: Phytochemical screening of the nature of compounds present in the aqueous and alcohol of cocoon of *Bombyx mori.*

S. No.	Phyto constituents	Aqueous	Alc
1.	Glycosides	-	+
2.	Flavonoids	+	+
3.	Proteins	+	+
4.	Alkaloids	-	-
5.	Tannins	-	-

Table 9: Heavy Metal Analysis

S. No.	Parameter analyzed	Results	Permissible limits as per WHO
1	Arsenic	Nil	Not more than 3.0 ppm
2	Cadmium	Nil	Not more than 0.3 ppm
3	Lead	Nil	Not more than 10.0 ppm
4	Mercury	0.206 ppm	Not more than 1.0 ppm

Table 10: Aflatoxin Contamination

S. No.	Parameter analyzed	Results	Permissible limits as per WHO
1	B1	Nil	Not more than 0.50 ppm
2	B2	Nil	Not more than 0.10 ppm
3	G1	Nil	Not more than 0.50 ppm
4	G2	Nil	Not more than 0.10 ppm

Table 11: Safety evaluation

S. No.	Parameter analyzed	Results	Permissible limits as per WHO
1	Total Bacterial Load	8 x 102	Not more than 105/g
2	Total Fungal count	Nil	Not more than 103/g
3	E.Coli	Nil	Nil
4	Salmonella Spp	Nil	Nil

Conclusion

The drug under study was subjected to Physico - chemical analysis, which is supportive in establishing the standards along with other parameters such as macroscopic, microscopic and fluorescence behavior as reported in the present investigation. The study on heavy metals, microbial and aflatoxins found within the permissible limits, indicating the drug is safe. Phytochemical screening also reveals the presence of nature of compounds which play the prominent role in the therapeutic efficacy of the drug as reported in the literature. Consequently the drug was brought up in determining and ascertaining its quality standard and also developed HPTLC fingerprint profile which helps in identification and assessment of the quality of drug. Therefore, it may be concluded that the study is an attempt to lay down quality parameters of the drug used in Unani System of Medicine.

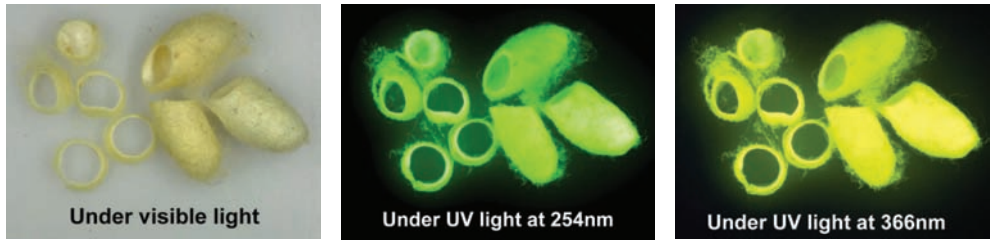


Fig. 1. Macroscopical feature of Cocoon of Bombyx mori under visible light and UV at 254nm & 366nm

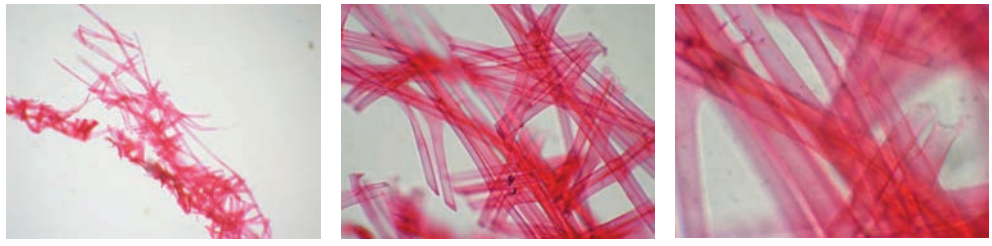


Fig. 2. Microscopical feature of shell of Bombyx mor showing the fibres of silk

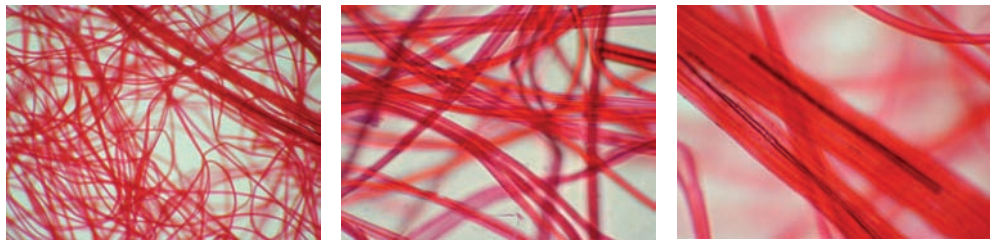


Fig. 3. Microscopical feature of silk fibres of cocoon of Bombyx mori with staining of fibres

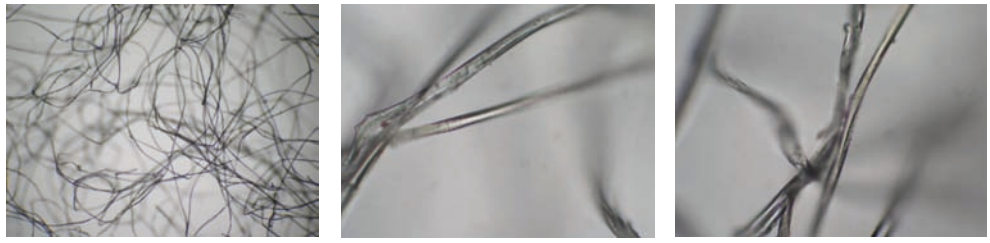
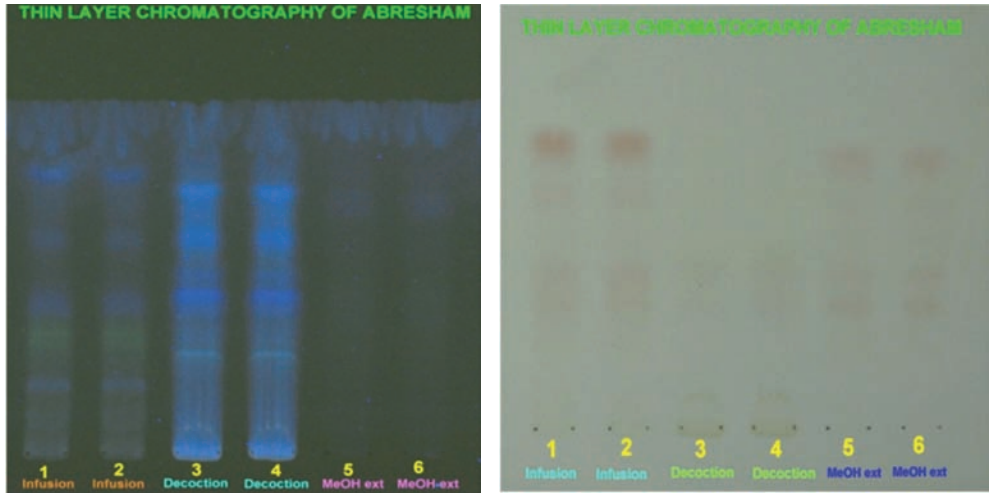


Fig. 4. Microscopical feature of silk fibres of cocoon of Bombyx mori with out staining of fibres



Before spraying at UV 366nm

After spraying under visible region

Fig. 5. TLC plates shown for the Infusion, decoction and methanolic extract of Cocoon of Bombyx mori (Abresham) before observed at UV 366nm and after spraying with ninhdyrin reagent and observed under visible

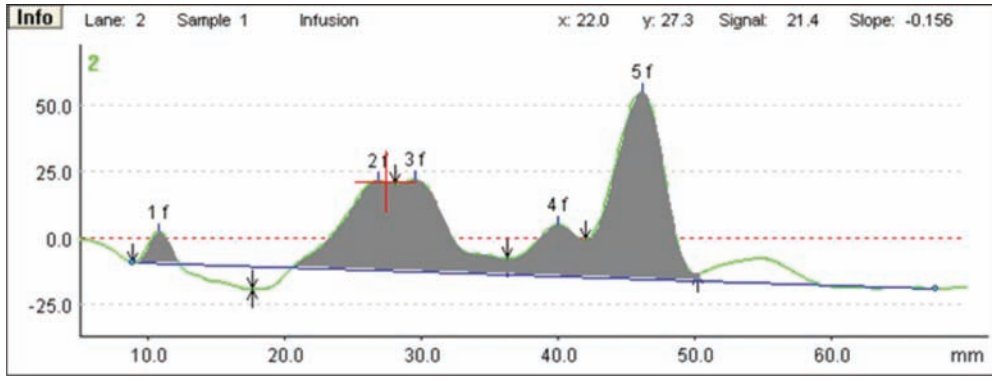


Fig. 6. Densitogram of Infusion of cocoon at 580 nm

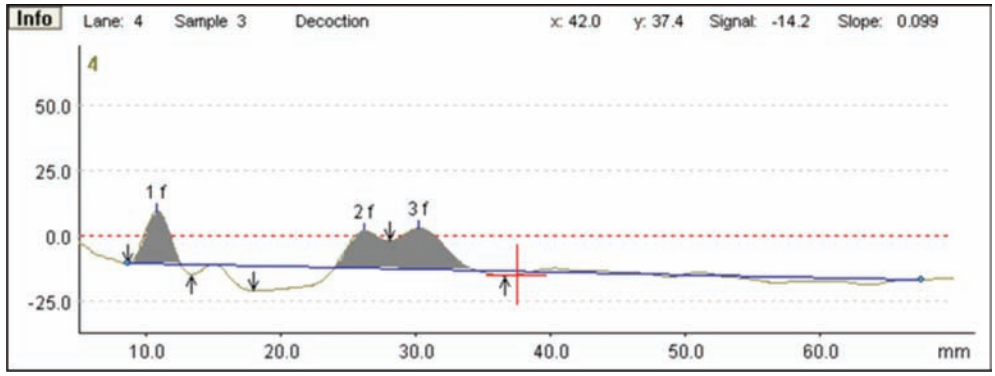


Fig. 7. Densitogram of Decoction of cocoon at 580 nm

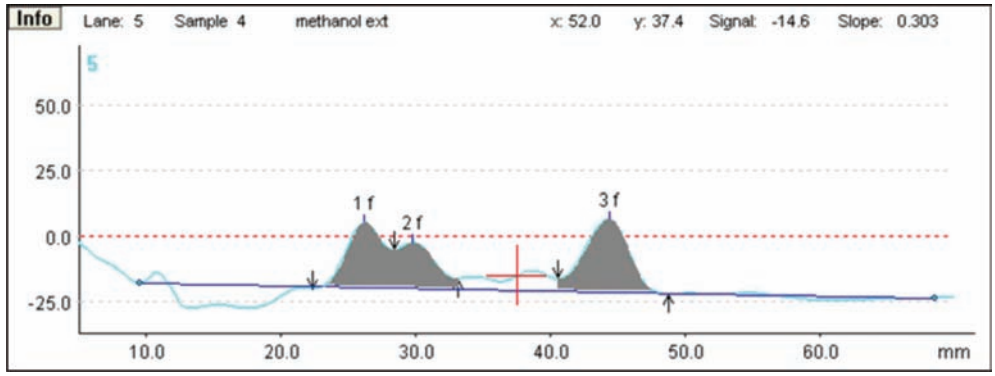


Fig. 8. Densitogram of methanolic extract of cocoon at 580 nm

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