

Polysaccharides From *Hygrophila Auriculata* And Interaction With Bovine Serum Albumin

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Abstract

The plant materials of *Hygrophila auriculata* (K.Schum) Heine, (Acanthaceae), has been traditionally used for the treatment of inflammation, pain. However, no phytochemical study on its high molecular weight compound has so far been performed. To study the interaction with polysaccharides and bovine serum albumin present, I have isolated polysaccharides from depigmented plant materials of *H. auriculata* by extraction with cold water. The cold water extracted materials, which were isolated in 0.63 % yield, have been designated as WE. Ultraviolet spectrometric analyses showed that the studied water extracted carbohydrate polymer (WEp) interacts with bovine serum albumin.

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DOI: <https://doi.org/10.53555/AJBR.v27i4S.4276>

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1. Introduction

Hygrophila auriculata (K. Schum)Heine (synonym: *Asteracantha longifolia* Nees, *Barleria auriculata* schum, *Barleria longifolia* linn) Acanthaceae, is a wild herb commonly found in moist places on the banks of rivers, ditches and paddy fields throughout India, Sri lanka, Burma, Malaysia, and Nepal. The plants are described in the ayurvedic literature as Ikshura, Ikshagandha and Kokilasha having eyes like the kokila or the Indian cuckoo. It is classified in the ayurvedic system of medicine as Seethaveryam, mathuravipaka and is used for the treatment of a number of conditions including Premeham (diabetes) and athisaram (dysentery).The plant *H.auriculata* (K. Schum) Heine has been reported to contain flavonoids (apigenin 7-O- glucuronide, apigenin 7-Oglucoside),[Bairaj, Nagarajan,n 1982] alkaloids (asteracanthine and asteracanthicine),[Parashar , Harikishan 1965] aliphatic esters (25- oxo - hentricontyl acetate, methyl-8-hexyltetracosanoate),minerals(Fe,Cu, Co), sterols(Stimagsterol),[Quasim,Dutta 1967] triterpenes (lupeol, hentricotane, betulin, luteolin, luteolin -7-O- rutinoides) [Govindachari, Nagarajan ,Pai

1957;] and essential oils. [Prashar, Harikishan 1965]Earlier scientific investigation of *H. auriculata* (K. Schum) Heine showed that the crude extract has antinociceptive,[Sanmugapriya, Shanmugasundaram, Venkatraman 2003] antitumor, antibacterial, antioxidant, hepatoprotective, hypoglycemic, haematinic, diuretic,[Hussain , Nazeer Ahamed , Ansari 2009] anabolic and androgenic activities, anthelmintic activity,[Patra , Jha , Murthy , Vaibhav 2008] anti-inflammatory and antipyretic activity[Nadkarni, 1978; Chopra, Nayer, and Chopra, 1956]. Many polysaccharides stimulate a range of biological effects, [Chattopadhyay, Nosalova, Saha, Bandyopadhyay, Fleskova, Ray, 2011; Ghosh, Chattopadhyay, Marschall, Karmakar, Mandal, Ray, 2009; Mantovani, Bellini, Angeli, Oliveira, Silva, Ribeiro, 2008] but no report exists on the carbohydrate polymer from *H. auriculata*. Complex formation between macromolecules such as anionic polysaccharides and proteins finds their application in a diversity of fields including pharmacy. [Schmitt, Sanchez, Desobry-Banon, Hardy, 1998].

We report herein the extraction of carbohydrate polymer

from *H. auriculata* plant material. In addition, the interaction between bovine serum albumin (BSA) and carbohydrate polymer (CP) has been studied.

2. Experimental Section

Plant material.

Powdered plant materials of *H.auriculata* were collected from the ayurvedic medical store of Burdwan, West Bengal, India.

General procedures.

The chemicals used were of an analytical grade or the best available. All experiments were conducted at least in duplicate. Evaporations were carried out under reduced pressure at around 50°C (SB 1100 Rotary Evaporator; Eyela, Tokyo, Japan). Toluene was used to inhibit microbial growth. Small volumes of samples are freeze-dried (Cool Safe 55-F freeze drier; Scanvac, Lynge, Denmark). Total carbohydrate content was determined by the phenol-sulfuric acid using galactose as standard. UV-VIS spectra were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan). GC performed with a Shimadzu GC-17A chromatograph (Shimadzu, Kyoto, Japan), fitted with a flame ionization detector, and a DB-225 column (30 m x 0.53 mm i.d.), using a program that maintained an isocratic temperature of 210 °C for 18 min and helium as gas vector. GC-MS was performed with a Shimadzu QP 5050A GC-MS instrument (Shimadzu) at 70 eV. Conditions for GC-MS were as described previously.

Isolation carbohydrate polymer.

The powdered material of *H.auriculata* (10 g) was extracted with distilled water (3 × 100mL, pH 5) at 4-8 °C for 6 h. The insoluble residue was removed by centrifugation (10,000 g, 15 min) and filtration of the supernatant through a glass filter (G 2). The extract was then diluted with 4 volume cold acetone (× 4), evaporated to a small volume, and EtOH was added (x3 volumes). The resulting precipitates were dissolved in H₂O and lyophilized to yield the water extracted carbohydrate polymer (CP, ~63 mg).

FT-IR Analysis

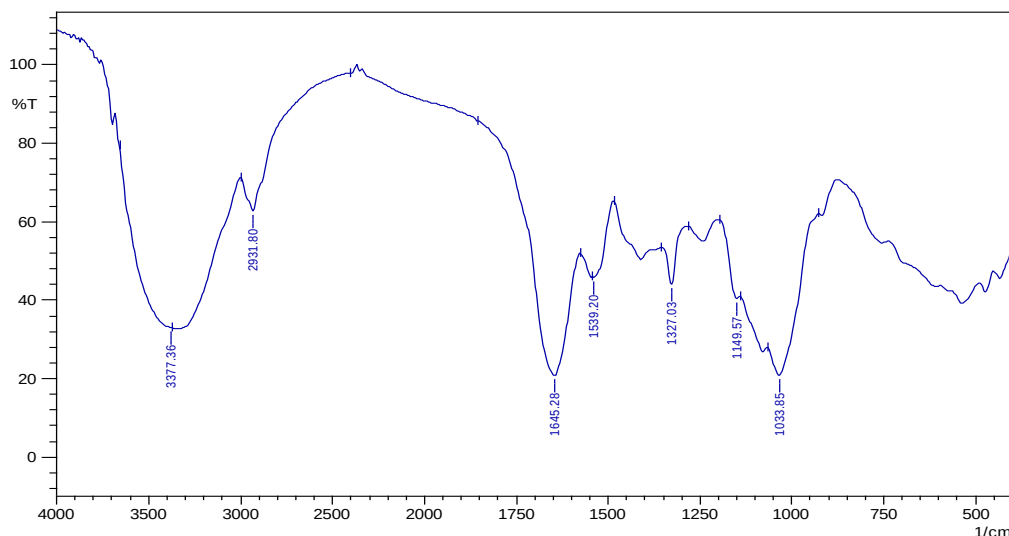


Fig. 1: FT-IR spectrum of WE_p fraction

Monosaccharide Analysis

Polysaccharide fractions were hydrolyzed either with 2M tri fluoro acetic acid for 6 hr at 100°C, centrifuged. Then TFA was removed on rotator evaporator. The presence of monosaccharide in hydrolysate in WE was also indicated by presence spots on TLC. Monosaccharides in the acid hydrolysate were also analyzed by thin layer chromatography on kieselgel 60F plate (Merck) using EtOH/PhOH/pyridine/0.1 M H₃PO₄(5/1/1/2 v/v) as eluent. Carbohydrates were then detected by heating at 100°C after treatment with a saturated solution of ethanol and H₂SO₄(8μL:2.5μL).

FT-IR Analysis

IR spectra were recorded on a Fourier transform IR spectrophotometer (FT-IR spectrometer, Spectrum RX 1, PerkinElmer, USA) using a KBr disc. Ultraviolet-visible (UV-vis) spectra were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan).

Bovine serum albumin (BSA)-Carbohydrate Polymer interaction.

UV-VIS Spectra. UV-VIS absorption spectra of 1mg/mL BSA alone and in presence of 0.05-1.0 mg/mL WE in a 10 mM phosphate buffer of pH 7.4 were recorded on a UV-VIS spectrophotometer at room temperature (298 K). The spectra of 0.05-1.0 mg/mL CP were also recorded under similar condition as blank in order to eliminate the spectral inferences on the BSA-CP system from CP.

3. Results and discussion

Chemical Characterization.

H.auriculata was extracted with H₂O, and the extract was subjected to fractional precipitation with acetone to obtain a precipitate of polysaccharide, precipitate was then lyophilized giving the water extracted carbohydrate polymer (WE_p).

Chromatographic analyses indicate the presence of a heteropolysaccharide made up of rhamnose, arabinose and galactose in the water extracted polymer.

FT-IR spectrum (Fig. 1) of WE_P fraction showed bands characteristic of polysaccharide. It consisted, inter alia, of (i) a broad band around 3377 cm⁻¹ originated from stretching vibrations of OH groups in sugar moieties, (ii) a band at 2931 cm⁻¹ derived from (C-H) stretching vibrations of methylene or methyl groups, and (iii) a band at 1533 cm⁻¹ related to the carbonyl stretching of the carboxylate anion (Fig. 1).

Moreover, the carbohydrate composition and the shape of the main bands appeared at 1033 indicated the presence of arabinogalactan type of polysaccharide.

Effect of carbohydrate polymer on BSA.

The UV-VIS spectra of BSA (1.0 mg/mL) alone and in the presence of CP (0.05-1.0 mg/mL) are indicated in Figure 4 at pH7.4. BSA has two absorption peaks. The peak at 228 nm and 277.5 nm is attributed to the π - π^*

transition of characteristic polypeptide backbone structure and n- π^* transition of aromatic amino acids.[Shu, Liu, Chen, Chen, Wang, 2011]At pH 7.4 with gradual addition of CP to BSA solution, the intensity of the peak at 228 nm decreases and the λ_{max} for the particles in the solution shifted towards longer wavelength feebly (7.5 nm). These spectral changes might arise from the disturbance of the microenvironment around the polypeptide caused by the binding of CP with BSA. Such complex formation occurs above the isoelectric point (Ip) of BSA (Ip=4.7) may be attributed to the electrostatic interaction between locally positively charged patches on the protein [Park, Muhoherac, Dubin, Xia, 1992] and negatively charged polysaccharide because of the presence of galacturonic acid.

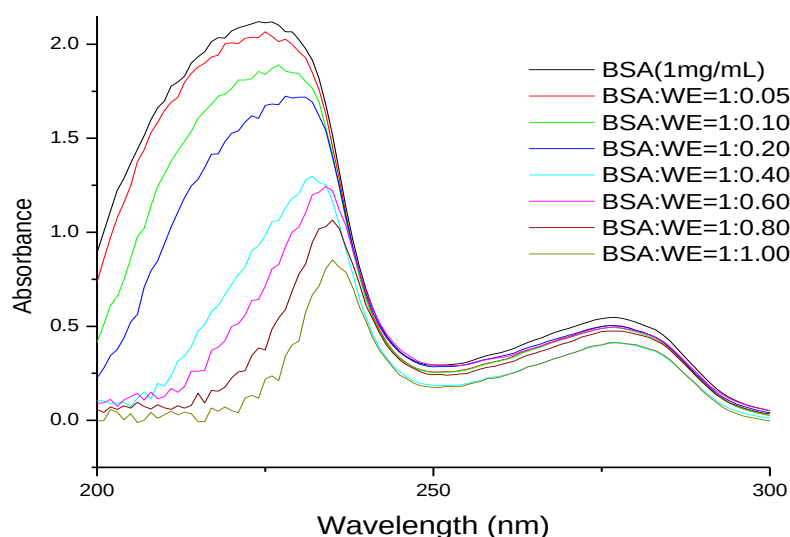


Fig. 2: Interaction of WE_P fraction with BSA

4. Conclusion

In conclusion, this study has shown that about 0.63 % of WE_P of *H.auriculata* could be extracted by water. Chromatographic analyses indicate the presence of a heteropolysaccharide made up of rhamnose, arabinose and galactose in the water extracted polymer. Further research will be directed towards a more detailed characterization of the purified polysaccharides and their biological activity study will be of interest. In addition, the interaction between bovine serum albumin (BSA) and carbohydrate polymer (CP) has been studied.

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