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Report on Occurrence of Rhein in the Leaves of *Senna obtusifolia* (L.) Irwin and Barneby

Nandi, Asis Kumar and Maiti, Aparna*

Department of Botany and Forestry, Vidyasagar University, Midnapore, WB 721102, India

*Corresponding Author E-mail: to.aparna.maiti@gmail.com

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ABSTRACT

Senna obtusifolia (L.) Irwin and Barneby of the tribe Cassinae under family Leguminosae is growing in wild in different parts of India and abroad. Rhein, an anthraquinone, is a biochemical of multifarious medicinal properties like, correction of maladies of liver, kidney, anti-oxidant, anti-obesity and even anti-cancer. The presence of this anthraquinone from the leaves of the species has been reported in the present paper. The extraction process through hydrolysis of sennosides yielded a mixture of anthraquinones from which the rhein was isolated. With the preliminary assurance of the chemical through Thin-layer chromatography quantification of the chemical was done by high-performance-liquid chromatography.

Key words: *Senna obtusifolia, Rhein, Sennosides, Thin-layer chromatography, High performance liquid chromatography.*

INTRODUCTION

Anthraquinone is a kind of phenolic compound having rather limited distribution in the plant kingdom and responsible for imparting colours of different shades. Rhein is a lipophilic anthraquinone available from various plant species[3]. It occurs in a free state as well as a glucoside. The chemical structure of rhein is 1, 8-dihydroxyanthraquinone-3-carboxylic acid. Rhein is claimed to have hepatoprotective, chondroprotective, neuroprotective, antidiabetic, antioxidant, anticancer and antimicrobial activities[5]. It is the precursor compound of commercial diacerein, a medicine used for the treatment of osteoarthritis, and inflammation. The structure of rhein and its variant are shown in Figures 1 and 2, respectively.

Earlier literature reports on the isolation of rhein from the species of *Aloe*, *Cassia*, *Rheum*, *Rhubarb*, and *Senna*[1,3,4]. Rhein has been reported from *Senna tora*[2,6]. *S. obtusifolia* is a very close congeneric species of *S. tora* with much morphological consanguinity. However, no presence of rhein has been so far reported from *S. obtusifolia*. *S. obtusifolia* is an annual foetid herb with a wide galore of medicinal and economic importance. Growing as an ignored weed in wild in a varied range of habitat across India and other tropical and subtropical countries.

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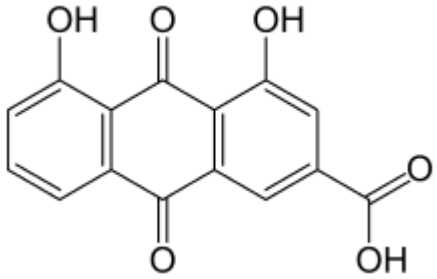

Fig. 1: Structure of Rhein

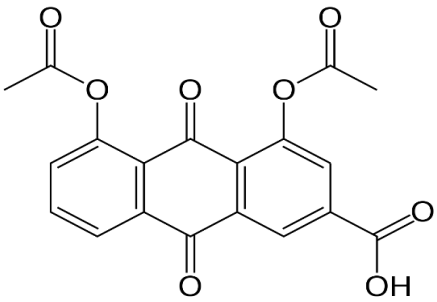

Fig. 2: Structure of 1,8-diacyl derivative

MATERIAL AND METHODS

The healthy matured leaves of *S. obtusifolia* were procured from wild. The collected specimen of *S. obtusifolia* was identified at the CNH of Botanical Survey of India, Shibpur, Howrah. The leaves were dried in an oven at 50^{o}C, for 5 hours. The dried leaves were powdered and used for the extraction of rhein.

The procedure used for extraction of rhein is as follows. Fifteen (15) grams of powdered leaves were added to a 100 ml mixture of 25% methanol in water. The mixture was warmed at 45°C and thereafter 5 ml of concentrated hydrochloric acid was administered. Then, 100 ml of toluene was added to that mixture and refluxed for 8 hours at 100°C. For removing crude drug, if any, the mixture was cooled and filtered. The organic and aqueous layers were then separated by applying gravity separation process and the filtrate containing the anthraquinones was taken. Extra anthraquinones, if any, were washed by filtering the aqueous layers and the crude drug with toluene twice. After combining the filtrate with additional toluene, the organic layer was taken. This organic layer was partitioned with 75 ml (taking 25 ml for 3 times) of aqueous sodium hydrogen carbonate solution (10%) and continued till the aqueous layer stopped showing the characteristic pink color. 10% aqueous ice-cold hydrochloric acid (approximately 95 ml) was added to the aqueous layer until the solution turned acidic and was taken up in 75 ml of ethyl acetate. The ethyl acetate layer was washed until it was free from acid and then dried over anhydrous sodium sulphate. Later, ethyl acetate was concentrated at reduced pressure and a dark yellow mass was obtained. Thin-Layer Chromatography (TLC) and High-Performance-Liquid Chromatographic (HPLC) analysis were performed for the identification and quantification of rhein.

RESULTS AND DISCUSSION

TLC was done on pre-coated silica gel G60 F254 plate (E. Merck) with n-hexane: ethyl acetate, used as the mobile phase. The compound appeared as a single band corroborating with the band obtained in respect of running standard chemical Rhein (Technical Grade) purchased from Sigma-Aldrich. The band also showed pink colour with Borntrager's reagent.

High performance liquid chromatographic analysis was carried out with HPLC system of Agilent Technologies using C 18 column. The extracted sample was carried out with n-hexane and ethyl acetate in a ratio of 4:1, as the mobile phase. A sharp peak was obtained at 307 nm with retention time of 1.62 min in both the standard as well as in the isolated sample and the isolated compound was detected as rhein corroborating with the peak of the standard chemical. The close peak at 1.62 min purports a very high confidence level of the presence of rhein in the isolated and extracted sample from *S. obtusifolia* leaves. The calibration of the standard sample was done by the regression analysis from where the quantity of rhein was determined. In the sample of *S. obtusifolia* the quantity of rhein was found to be 1.26 mg per gram of dried leaves, which showed a significant amount of rhein in the leaves of the plant.

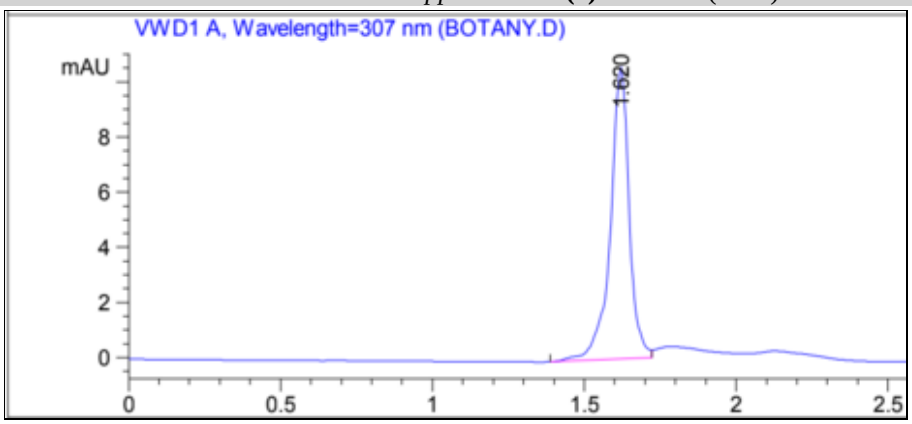

Fig. 3: HPLC chromatogram of standard Rhein

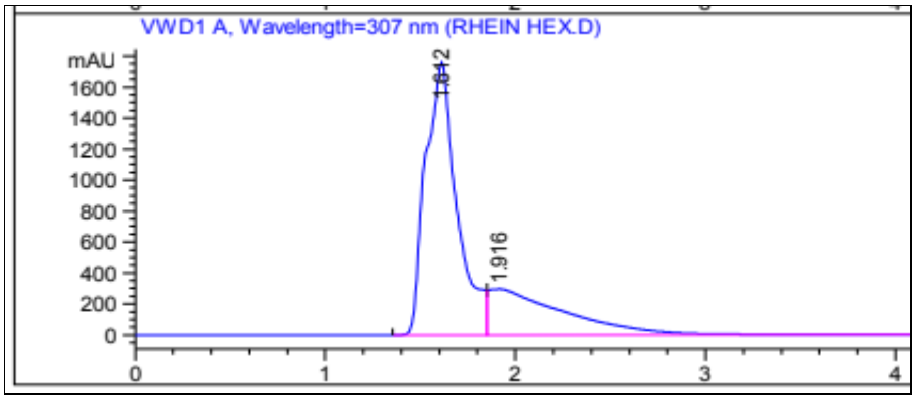

Fig. 4: HPLC chromatogram of rhein extracted from *Senna obtusifolia*

CONCLUSION

The present work expresses the potential of *S. obtusifolia* as a prospective source of the medicinally important compound rhein. So, the species available in abundance in the wild can be paid much care by growing in mass on adopting suitable cultural practices. Proper optimization and scaling-up methods may be utilized for large scale extraction from the leaves of the plant. Rhein thus obtained can be used largely in pharmaceutical industries and as a precursor of diacerein.

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