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## A MODIFIED EFFICIENT METHOD FOR ISOLATION OF RHEIN FROM CASSIA ANGUSTIFOLIA (SENNA)

Subodh A. Gangurde <sup>\*1</sup>, Kirti S. Laddha <sup>1</sup> and Shreerang V. Joshi <sup>2</sup>

Medicinal Natural Product Laboratory <sup>1</sup>, Department of Pharmaceutical Chemistry <sup>2</sup>, Department of Pharmaceutical Science and Technology, Institute of Chemical Technology, Matunga, Mumbai - 400019, Maharashtra, India.

### Keywords:

*Cassia angustifolia*, Rhein, Sennoside, Oxidative hydrolysis

### Correspondence to Author:

**Subodh Anil Gangurde**

Research Scholar,  
A-286, Medicinal Natural Product  
Laboratory, Department of  
Pharmaceutical Science and  
Technology, Institute of Chemical  
Technology, Nathal Parekh Marg,  
Matunga (E), Mumbai - 400019,  
Maharashtra, India.

**E-mail:** Subodh.gangurde007@gmail.com

**ABSTRACT:** Chemically, rhein is 9, 10-Dihydro-4, 5-dihydroxy-9, 10-dioxo-2-anthracenecarboxylic acid, and use as an intermediate to synthesize diacerein which widely used in the treatment of degenerative diseases like, osteoarthritis, osteoporosis, and rheumatoid arthritis and hence has commercial importance. Rhein occurs in free state in nature in different plant families like Cassia, Rhamnus, and Rheum but in very less concentration and as it is currently subject of interest because of its anti-tumor, anti-viral and anti-oxidant properties, therefore, there is need for simple and efficient isolation and extraction method to obtain rhein from available indigenous plant sources. A modified method developed for the isolation of rhein from *Cassia angustifolia* leaves is described, the method involves oxidative hydrolysis of sennoside with help of an oxidizing agent followed by the isolation of the rhein from reaction mixture, and with substantial yield improvement is achieved. The objective of this study to optimize yield of the rhein from senna semisynthetically. The isolated rhein was analyzed by spectral analysis for its confirmation.

**INTRODUCTION:** Senna (*Cassia angustifolia*) consists of the dried leaflets of cassia senna, which are also known in commerce as Alexandrian or *Khartoum senna*, also known as *Tinnevelly senna*. The senna plants are small shrubs of the family Leguminosae, about 1 m high, with peripinnate leaves. *Cassia senna* is indigenous to tropical Africa and Sudan also Somali land, Arabia, Sind, and Punjab. Senna has been used since ninth century and also mentioned in Ayurveda.

Senna contains aloe-emodin and rhein in free state and other two active constituent's sennoside A and sennoside B. They both hydrolyze to give two molecules of glucose and an aglycones sennidin A and B <sup>1</sup>. The leaves and pods of senna plant contain not less than 2.5% of anthraquinone glycoside mainly sennoside A and sennoside B, which are dianthrone glucosides derived from rhein and aloe-emodin.

This makes senna plant an important source of aloe-emodin and rhein which also serve as intermediate for the diacerein synthesis through acetylation of rhein <sup>2</sup> viz. acetyl derivative of rhein i.e. diacetyl rhein for the treatment of rheumatoid and osteoarthritis. Diacerein is used to treat degenerative diseases of joints and connective tissue matrix substances disease, rheumatoid

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arthritis. Diacerein also has antipyretic and analgesic effects results in the attention of the medical and academic medicine market <sup>3</sup>.

Rhein (9, 10-Dihydro-4, 5-dihydroxy-9, 10-dioxo-2-anthracenecarboxylic acid) also known as cassic acid, which is the substance of anthraquinone group and is found in the free state and as a glucoside in Rheum species and senna leaves; also in several species of cassia <sup>4</sup>. It is an anthraquinone compound currently have great interest by the researchers and numerous areas of ongoing research notably include antiviral, antitumor and antioxidant activities of rhein as well as its derivatives. The rhein is used as intermediate for synthesis of Diacerein, which is used to treat many degenerative diseases such as osteoporosis, rheumatoid arthritis, multiple myeloma <sup>5, 6</sup>. Due to great pharmacological value, efforts have been expanded over the years improving the techniques of extracting and purifying rhein <sup>7</sup>.

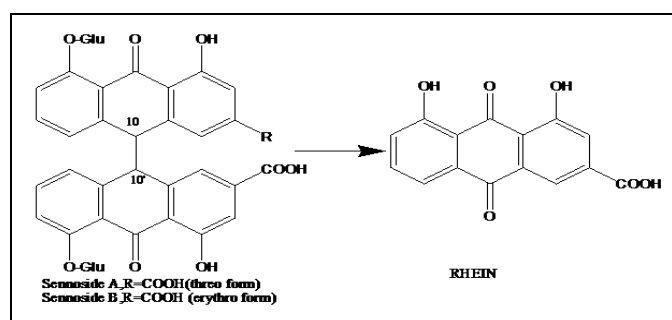


FIG. 1: CONVERSION OF SENNOSIDE TO RHEIN

## MATERIALS AND METHODS:

**Materials:** The crude senna powder was procured from yucca enterprises, Mumbai. All the chemicals and solvents were GR/LR grades and purchased from SD Fine Chemicals, and were used without purification. Thin-Layer chromatography was performed on pre-coated silica gel G60 F254 (E. Merck) by using ethyl acetate: methanol: water (100:13.5:10) as a mobile phase. Melting points of the compound were recorded by Analab Thermo Cal melting point apparatus in a capillary tube and uncorrected. IR spectra (KBr) were recorded on Perkin Elmer Fourier Transform Infrared spectroscopy. <sup>1</sup>H NMR spectra were recorded on MR400 Agilent Technology NMR spectrometer using Tetramethylsilane (TMS) as an internal standard and DMSO-d<sub>6</sub> as a solvent. Mass spectra were recorded on a direct insertion probe on Agilent Technologies 5975 series.

**Experimental Procedure:** 50 g senna powder was weighed and extracted with acetone to remove color pigment after extraction the marc was air-dried and filled in one liter round bottom flask containing 200 ml of 70% methanol. The flask was shaken properly to moisten all powder. Then 1 g of potassium permanganate (KMnO<sub>4</sub>) solution prepared in 20 ml of water is then added to a reaction mixture, followed by 1 g of ferric chloride solution in 10 ml water was added. In order to prepare acidic reaction medium 50 ml of 15 % HCl (v/v) was then added. The reaction mixture was stirred vigorously and allow to cool for some time as the temperature may get rise instantly. The resulting reaction mixture then refluxed for 1.5 h not more than 100 °C.

At the end of the reaction, the mixture was cooled slightly and filtered to remove senna crude powder. The water layer was discarded and crude powder was washed again with water and dried in vacuo. The dried crude powder weighed about 35 g which was then extracted with 300 ml toluene in a Soxhlet apparatus in order to recover anthraquinone. The toluene layer was then concentrated up to 100 ml and extracted with 10% sodium bicarbonate solution until the aqueous layer ceased to show the characteristic pink color. Combined sodium bicarbonate aqueous layer and was acidified with dilute hydrochloric acid with stirring, the precipitate of crude rhein was formed, and upon standing, the precipitate got settled down, filtered and dried in vacuo and product recrystallized with glacial acetic acid.

**RESULTS AND DISCUSSION:** The literature survey revealed that the efforts have been made previously to isolate rhein from senna, which involves column chromatography and the concentration of the free rhein is very low so as per the structure of the sennoside A & B it is possible to break adianthrone ring at 10, 10 position to some extent which results in the formation of rhein. And the effort has been made for the same to improved yield of the rhein. The present study were contemplated efficient method for the extraction of the rhein from senna leaves which contains sennoside A & B by the following methods *viz.* a) removal of color pigment with acetone treatment b) Oxidising the sennoside containing plant material by using oxidizing agents c) extraction of formed

rhein by using toluene as solvent and d) separation of the rhein from toluene by using sodium bicarbonate followed by acidifying alkali layer to precipitate out rhein. It has been seen that 50 gm of senna powder contains approximately 1.5 gm sennoside anthraquinone from which about 0.6 gm of rhein obtained, which is improved yield of rhein produced in comparison with some previous methods.

Thus, rhein obtained was preliminarily identified with the Borntrager's reagent which gave pink color and also by thin-layer chromatography on precoated silica gel G60 F254 plate using ethyl acetate: methanol: water (100:13.5:10). The isolated rhein showed melting point of 321-323 °C (Reported 320-322 °C)<sup>8</sup>. The confirmation of the isolated rhein were done by Infra-red (IR) spectra which showed broad peak at 3405 cm<sup>-1</sup> (O-H stretch aromatic) indicate hydroxyl group, 1692 cm<sup>-1</sup> (C=O stretch) indicate carboxyl group, 1571 cm<sup>-1</sup> (C=C stretch), 1452 cm<sup>-1</sup> (C-O Stretch, COOH), 1268 (C-O stretch, ester) and 1632 cm<sup>-1</sup>

peak showed chelated carbonyl and hence, IR spectra clearly indicate presence of hydroxyl and carboxyl group of rhein and other functional groups **Fig. 2**. The mass spectrum showed a molecular ion peak at 284.3 (M<sup>+</sup>) which exactly matches with molecular weight of rhein **Fig. 3**. <sup>1</sup>H NMR spectrum was in conformity with assigned spectra *via* Rhein **Fig. 4**<sup>9</sup>. Full characterization is as follows.

M.P: 321-323 °C (Reported M.P: 320-322 °C)<sup>6</sup> IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3405, 1692, 1632, 1571, 1452, 1268, 1189 <sup>1</sup>H NMR (400 MHz, DMSOd6)  $\delta$  11.85 (s, 2H), 8.07 (d, J = 1.5 Hz, 1H), 7.79 (t, J = 7.9 Hz, 1H), 7.74 - 7.64 (m, 2H), 7.36 (d, J = 8.1 Hz, 1H). MASS: ESI-MS m/z: 284.3 (M<sup>+</sup>).

Thus, Rhein obtained was purified by crystallization and was fully characterized by recording its Melting point, FTIR, proton NMR, and MASS Spectrum. On the basis of spectral analysis the isolated compound was identified as Rhein.

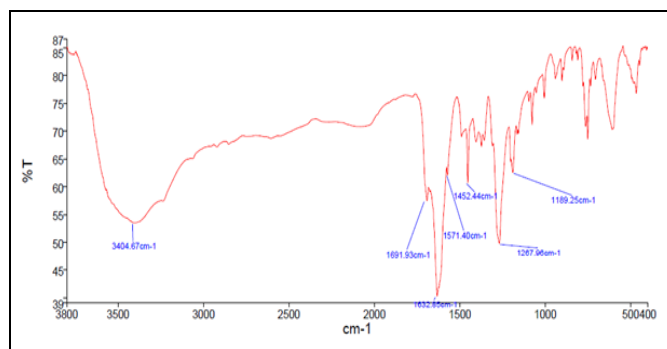


FIG. 2: IR SPECTRA OF RHEIN

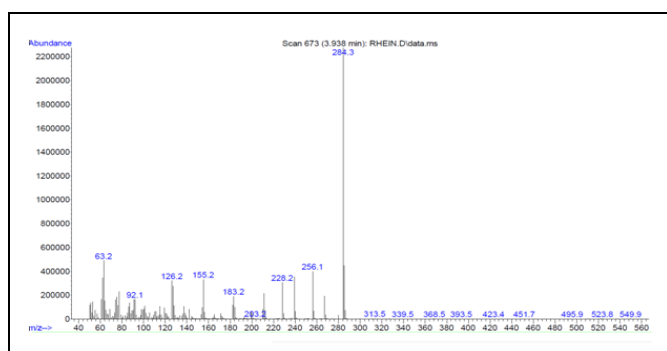


FIG. 3: MASS SPECTRA OF RHEIN

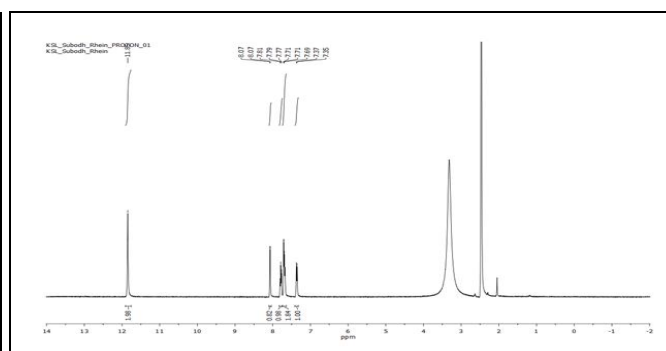


FIG. 4: NMR SPECTRA OF RHEIN

**CONCLUSION:** The semisynthetic isolation method developed involved two-step extraction method which gave an improved yield than previous extraction methods, and *Cassia angustifolia* (senna) should be a further good

source for the rhein production. This method was found efficient on the laboratory scale, and further optimization on kilo scale can be done to make it suitable for the large scale production of rhein.

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**CONFLICTS OF INTEREST:** We declare that we have no conflict of interest.

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