RESEARCH PAPER

Extraction and Functional Group Analysis of Gossypol from Cottonseed Meal

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Abstract

Gossypol is a polyphenolic aldehydic compound which has been studied for its versatile biological activities. The extraction of gossypol from cottonseed meal was attempted using acidified solvent system. Mixture of acetone: ethanol and water (70:20:10) at 4.5 pH with solid-to-liquid ratio of 1:20 can be considered as best conditions for the extraction of gossypol. The purity and presence of specific functional groups was confirmed using Fourier Transform Infrared (FTIR) spectroscopy. Study will give a basis to further optimize the protocol for efficient extraction of gossypol form cottonseed meal for possible applications in agriculture, medicine and pharmaceuticals.

Keywords : Gossypol, Extraction, FTIR, Cottonseed meal

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Introduction

Gossypol is a polyphenolic, yellow coloured toxic pigment present in cotton plant which helps it to survive against herbivores (Coutinho *et al*., 2002). Gossypol and gossypol analogs are reported to have various bioactivities viz. antioxidant, antiviral, anti-fertility, anti-oxidant, anti-cancer, anti-microbial and anti-parasitic (Keshmiri-Neghab and Goliaei, 2013). Further, it is reported to have various agricultural implications namely insecticidal and anti-feeding activity (Keshmiri-Neghab and Goliaei, 2013). The spectrum of various bioactivities and agricultural implications of gossypol has been displayed in the **Fig. 1.**

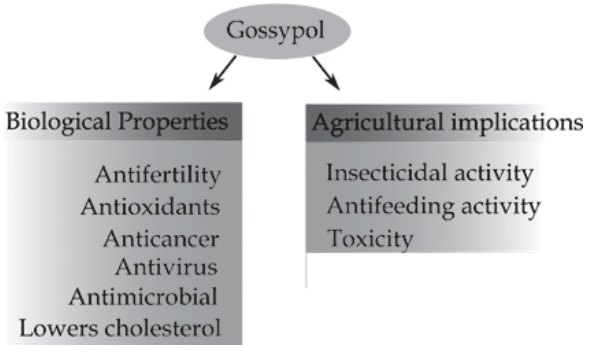

Fig. 1: Bioactivities and agricultural implications of gossypol

Cottonseed contains 0.002-6.64% of gossypol depending upon the variety and the climatic factors in various regions where the cotton crop is cultivated (Kumar *et al.,* 2018; Gadelha *et al.,* 2014). During the extraction of cottonseed oil from cottonseed, most of the gossypol is retained in cottonseed meal which makes it unsuitable for consumption by monogastric animals and humans. As the gossypol is soluble in various organic solvents, many researchers have attempted to remove gossypol from cottonseed and making it fit for consumption by non-ruminants. Besides, the extracted gossypol can be used for various biological and agricultural applications which remains non-utilized during the current processing of cottonseeds. Solvent-based extraction (Dechary *et al*., 1952; Kuk *et al*., 2005; Pelitire *et al*., 2014; Singh *et al*., 2015; Li *et al*., 2016) is the most frequently applied commercial technique for extraction and removal of gossypol from cottonseed or cottonseed meal. Hence, preliminary experiments have been conducted to extract and isolate gossypol from cottonseed meal by solvent extraction. Recovery of gossypol was evaluated and its identity was confirmed by functional group analysis. This study will provide information about utilization of gossypol in medical, health and agriculture fields.

Material and Methods

Extraction of gossypol

Cottonseed meal (CSM) was supplied by M/S Clean Cotton Impex (CCI), Tirupur. It was evaluated for free and total gossypol levels. CSM was first defatted using hexane to remove any residual oil and later on subjected to extraction of gossypol using solvent extraction. An acidified mixture (pH adjusted to 4.5 by using H_2SO_4) of (i) acetone: ethanol and water (40:40:20) and (ii) acetone: ethanol and water (70:20:10) was used for extraction of gossypol. Gossypol

is considered to be stable at a pH of 4.5. The mixture of solvent and cottonseed meal (CSM) in ratio of 20:1 was kept in a rotary shaker for 2 h at 150 rpm for complete extraction of gossypol into the solvent system. The solventized samples were collected and filtered through Whatman filter paper no. 1 and then the solvent was evaporated by using rotary evaporator to get crude gossypol. The gossypol thus obtained was then collected in a Petri dish and dried thoroughly by keeping in an incubator at 45 ºC for 48 h. It was preserved in dark at 4 °C for further evaluation.

Analytical methods

Free gossypol content

CSM was analyzed for free gossypol by slightly modified AOCS method used by Kumar *et al*. (2019).

Total gossypol content

CSM was analyzed for total gossypol by AOCS method with slight modification as followed by Kumar *et al*. (2019)

Functional group analysis by Fourier Transforms Infrared (FTIR) spectroscopy

FTIR spectroscopy of the extracted crude gossypol as well as standard gossypol was carried out by using IR Prestige 21, Shimadzu, Japan. A small amount of sample was ground together with dried potassium bromide (KBr) powder to form a pellet. After making the pellet by pressing, the infrared absorption of the sample was recorded at 400-4000 cm^{-1}. Pure dry potassium bromide was used as a control.

Statistical analysis

All the experiments were performed in triplicate. All values were reported as the mean (n = 3) ± standard deviation.

Results and Discussion

Extraction of gossypol

Cottonseed meal was evaluated for its free and total gossypol content and later on used for the extraction of gossypol. The meal was found to have free gossypol content of 0.07 ± 0.007% and total gossypol content of 2.09 ± 0.19% on dry weight basis. The extraction of gossypol was carried out using two solvent systems at acidic pH of 4.5, as gossypol is considered to be stable at this pH. The recovery achieved using acetone: ethanol and water (40:40:20) was 6.28 mg/g of cottonseed meal (on dry weight basis). Whereas, yield of crude gossypol was 8.76 mg/g for acetone: ethanol and water (70:20:10). Hence, acetone: ethanol and water (70:20:10) at 4.5 pH with solid-to-liquid ratio of 1:20 was considered better for the gossypol extraction. A flow diagram of gossypol extraction from cottonseed meal is presented in **Fig. 2.**

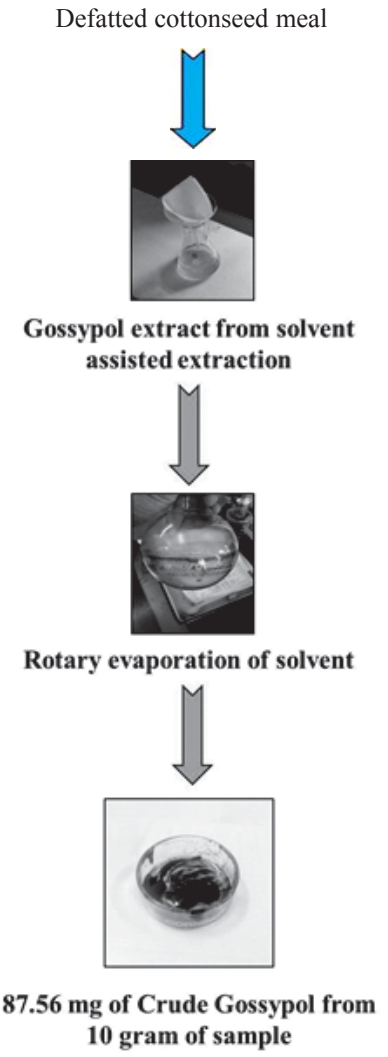

Fig. 2: Extraction of gossypol from cottonseed meal

FTIR spectroscopy analysis

FTIR spectrum of extracted and standard gossypol samples are presented in Fig. 3. It is observed that both spectrum in general show absorption bands at similar wavelengths. The bands at 1620 and 1551 cm^{-1}correspond to aromatic C=C, 1443, 1383 cm^{-1} for methyl bending vibrations, 1338 and 1303 cm^{-1} for the in-plane -CH bending of phenyl, 1124 and 1055 cm^{-1} for the in-plane -CH bending of 1, 2-substituted phenyl, 967 and 915 cm^{-1} for the two adjacent phenyls, 876 and 844 cm^{-1} for the -CH of p-disubstituted phenyl, and 772 and 700 cm^{-1} for out-of-plane -CH and ring bending. Similar results have been reported by earlier researchers (Nakanishi and Solomon, 1977; Bellamy, 1975; Stuart, 1996; Mirghani and Che Man, 2003). The results of FTIR analysis of extracted gossypol from cottonseed meal showed a very similar spectrum when compared with standard gossypol **(Fig. 3.).**

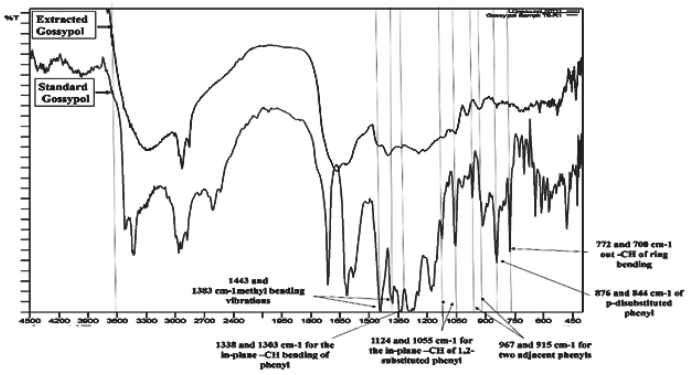

Fig. 3: FTIR spectra of standard (red) and extracted gossypol (blue) Conclusion

Conclusion

Gossypol is a polyphenolic aldehydic compound which has been reported to have versatile biological activities. Hence it is important to establish a suitable protocol for the extraction of gossypol. We found acetone: ethanol and water (70:20:10) at 4.5 pH with solid-to-liquid ratio of 1:20 to be the best conditions for the extraction of gossypol. The functional group analysis of extracted and standard gossypol using FTIR spectroscopy showed similar pattern which establish the correctness and efficacy of the extraction protocol.

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Cotton Facts: Pricing and Price Risk Management (ICAC, 2003)

In addition to consolidating price information, the cotton futures market provides a means for all sectors of the cotton trade to manage or hedge their exposure to the risk of unexpected price fluctuations. Dramatic fluctuations of cotton prices may be attributable to a number of factors, ranging from weather changes in cotton producing regions to government policies. Cotton producers, ginners, merchants, and textile mills employ the cotton futures market to achieve price protec- tion, reduce their effective purchasing costs or increase their ultimate selling price. By hedging the price of cotton they must buy and sell, they can avoid the potentially devastating effects of unexpected price fluctuations. This is possible because other market participants, professional traders and speculative investors are willing to assume the risk in return for the opportunity to profit should the market move in their favor. The following terms and definitions are used in the cotton price risk management.