Commercially available withania somnifera and spirulina fusiformis biology essay

Science, Biology

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Abstract

Herbal medicine and their preparations have been widely used for thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. Herbal drugs are one of the most widely used in ayurvedic medicine. Active constituents of herbal drugs can be alkaloids, steroids, lactones etc. which play major role in modulating the pharmacological activities of the drug. So, they are being isolated and being studied extensively. In the present study various active components of the herbal preparations Withania somnifera and spirulina fusiformis will be identified and analysed using HPLC techniques. Keywords: Herbal medicine, synthetic drug, Alkaloid, Steroid, Lactones, HPLC, Withania somnifera, spirulina fusiformis

Introduction

Withania somnifera, most commonly called as Ashwaghanda, has been reported to be an important herb in indigenous medical systems and in ayurvedic systems. It is a shrub , with ovate leaves and greenish-yellow flowers which is found in western India. It is also commonly referred as Indian gensing or winder cherry. . Historically , the plant has been used as an aphrodisiac, liver tonic, anti-inflammatory bronchitis, asthma, ulcer, senile dementia. Patients who undergoing radiation and chemotherapy, they are also Ashwaghanda as a medicine. Ashwaghanda products are available

throughout US as dietary supplements. The activities are mainly attributed towards the presents of different withenoides mainly withaferine A.. Human health disorders such as Central nervous system disorder , particularly in epilepsy , stress and new generative stress, Withania somnifera is used for treatment. It has been found to have important healing effects on solid tissues, skin support , connective tissues , lymph tissues , blood vessels and mucous membrane. Steroidal alkaloids and steroidal lactones in a class of constituents called withanolide. It is the major biochemical constituents of Ashwaghanda root. The withanolides serve as important hormone precursors that can convert into human physiologic hormones as needed. Ashwaghanda is thought to be amphoteric; i. e., it can help regulate important physiologic processes. The theory is that when there is an excess of a certain hormone, the plant-based hormone precursor occupies cell membrane receptor sites so the actual hormone cannot attach and exert its effect. Spirulina fusiformis is blue-green algae , which contain rich amount of raw proteins and major vitamins such as A1, B1, B2, B6, B12. Nowerdays the important of Spirulina fusiformis is brightened due to its pharmaceutical properties. Its tremendous nutritional potential and therapeutic impacts have led to several clinical studies on its different chemopreventive effects The effect of Spirulina fusiformis such as hepatoprotective and antioxidant are react against acetaminophen-induced hepatotoxicity in mice. C: UserskuttuDesktopdownload. jpgFig 1: Structure of Withaferin A

Materials and methods

Sample: Commercially available Withania somnifera obtained from IMCOPS and Spirulina fusiformis obtained from Acuman Pharmaceutical Pvt. Ltd, Pondichery was used for the analysis.

Preparation of standard and sample for HPLC analysis of Withania somnifera

Standard : 10mg of Withaferin A working standard was dissolved in 50ml of Methanol which was further diluted by dissolving 1ml of this solution to 5ml Methanol. Sample preparation : 1mg of root sample were accurately weighted and dissolved in 50ml of Methanol. Further diluted 1ml of this solution to 50ml using methanol HPLC grade. Mobile phase : The choice of mobile phases is based on the desired retention behaviour and the physicochemical properties of the analyte. Acetonitrile and water at the ratio of 80: 40 at 35°C , with a flow rate of 1. 0Ml/min is used as mobile phase .

Preparation of standard and sample for HPLC analysis of Spirulina fusiformis

Standard : 10mg of Spirulina fusiformis working standard was dissolved in 50ml of Methanol which was further diluted by dissolving 1ml of this solution to 5ml Methanol. Sample preparation : 1mg of root sample were accurately weighted and dissolved in 50ml of Methanol. Further diluted 1ml of this solution to 50ml using methanol HPLC grade. Mobile phase: The mobile phase components were (A) Acetone (B) acetonitrile, and (C) orthophosphoric acid , in the ratio of 70: 20: 10 . The flow rate was 1, ml /min at 27°C.

Result and discussion

Under optimized condition, the separation of these metabolic Withania somnifera extract shown in figure 2. Withaferin-A biologically active and is a major component of sample Withania somnifera . Withaferin-A which are also commercially available, where chosen as marker compounds of similar polarity in less than 20 minute after all separation parameters were carefully. C: UserskuttuDesktopCamerasample 2. pngFig 2: Auto-scaled chromatogram- Withaferin AC: UserskuttuDesktopsample 1. pngFig 3: Auto-scaled chromatogram- Withania somniferaFig 4 : HPLC analysis of Spirulina (200nm. 254nm, 330nm)Fig 5: HPLC profile of the standards (Thiamine , Ascorbic acid and Betacarotene at 200 nm)SL NO. NAMERTAREAHEIGHT1Withaferin A1. 795206088208255757 92Withania somnifera1. 796248765991254963 5Table 1: Peak value of HPLC chromatogram-Withania somnifera and Withaferin AThe right mobile phase was crucial for a satisfactory result as well. Only by using methanol, compound 2 could be well resolved from the signal of the unidentified substance. In combination with reagent alcohol the separation was further improved, whereas the addition of acid, advantageous. Performing the separation time and the column backpressure, without any decrease in peak resolution. The HPLC chromatogram of root of standard Withaferin A showed at a retention time of 1. 75 minute with an area of 206088208 and absorbance unit 2. 59 at wavelength 225nm (fig 2) and Withania somnifera was showed at a retention time of 1. 75 minute with an area of 248765991 and absorbance unit 2. 58 at wavelength 225nm (fig 3). The HPLC conditions were slightly changed as the separation had to be performed at room

temperature and flow rate of 1. 0 ml compounds with modifications in the solvent composition and flow rate, the MS signals were readily assignable. Prior the analysis of Ashwaghanda sample and products, the efficiency of the extraction procedure was verified. Phytochemical screening of spirulina fusiformis has done by HPLC analysis and it revealed the presence of beta carotene, Ascorbic Acid, Thiamine and Vitamin B 12 . The HPLC profile of spirulina fusiformis was acquired at three wavelengths, 200, 254, and 330 nm (Fig. 3). Comparisons with standard betacarotene, Ascorbic Acid, and Thiamine, were performed at 200 nm, and 254 nm and Vitamin B 12 at 330 nm because those standards were best detected at this wavelength (Fig. 2, 3 and 4). Based on wavelength comparison between the major peaks in spirulina fusiformis and the standards, the spirulina fusiformis was found to contain, beta carotene at (peak with retention time (t R) of 4. 645 min), Ascorbic acid at (peak with retention time (t R) of 2. 634 min)and Thiamine at(peak with retention time (t R) of 1. 95 min) . Interestingly, the spirulina fusiformis bioactive compounds were best separated at 200 nm and 254 nm for beatcarotene , Asorbic acid and thiamine . For Vitamin B12 at (peak with retention time (t R) of 2. 608 min)as indicated by an increase in the number of peaks detected.