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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2017, Vol. 33, No. (3):
Pg. 1550-1554

Extraction and Isolation of Withaferin A (Steroidal Lactone) from Withania somnifera Leafs and It's TLC and HPLC Analysis

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http://dx.doi.org/10.13005/ojc/330360

(Received: April 06, 2017; Accepted: May 02, 2017)

ABSTRACT

Withania Somnifera (*Ashwagandha*) is one of the most important ancient medicinal plant of Unani and Ayurvedic system of traditional medicine which is extensively used by tribal people world wide for many ailments. The leaves of the plant are bitter in taste and are applied for carbuncles, inflammation, swellings and conjunctivitis. In the present study phytochemical constituents present in withania somnifera leaf is studied by applying process of extraction and isolation and is analyzed by using TLC and HPLC method. The results show the presence of phytochemical ingredients like withanoside IV, withanoside V + VI, withaferin A, with astramonolide, withanolide A, withanone, withanolide B.

Keywords: Withanolides, Withania somnifera, HPLC, Withaferin A.

INTRODUCTION

Withania Somnifera , commonly known as *Ashwagandha* belong to family Solanaceae and is one of the most important ancient medicinal plant of Unani and Ayurvedic system of traditional medicine.[1] The plant is mainly grows in drier parts of India and also cultivated for medicinal purpose. Withania Somnifera has wide range of bioactive compounds and extensively used by tribal people worldwide to cure many ailments. Traditionally , the plant is used to enhance energy, vigour, endurance, strength, health, vital fluids, muscle fat, blood, lymph, semen and cell production[2]. The plant is also useful in the treatment of burns, wounds, dermatological and gastrointestinal disorders, asthma, bronchitis, cancer and geriatric problems. It is called "Indian Ginseng" for its great rejuvenating properties[3]. The leaves of the plants are applied for carbuncles, inflammation and swelling and its juice is useful in conjunctivitis.[4,5]

Withanolids are steroidal compounds and mainly found in the leaves and roots of withania

somnifera[6,7]. The leaves of Withania Somnifera are reported to contain a number of withanolides and alkaloids[8]. Withaferin A is the most important withanolide which is found in the leaves and roots of the plant.

EXPERIMENTAL

Extraction and Isolation

200 gram leaf powder were extracted in 2 Liter round bottom flask with reflux condenser at 70^{0}C by (80:20) methanol : water three times, first extraction is 800ml for 3hrs second is 600 ml for 3 hrs and third extraction is 600 ml 3hrs collected all extracted material in solvent in 5 litre round bottom flask with distillation setting, distilled out solvent at 60^0 C under reduced pressure to a volume of 40 ml and put it in a beaker kept overnight material were separated from liquid portion decant the liquid we get the material about 8 gram dry it under vacuum at 60^{0}C get 6 gram[9].

6 gram material from above refluxes in 50 ml pet ether 60-80 two times and decant the pet ether material is dry on silica for column chromatography 20 gram in air load on column with 25 gram silica gel for column chromatography eluted with Chloroform 150 ml first fraction $CHCl_3$: CH_3OH (99:1) 300 ml second fraction , $CHCl_3$:CH_3OH (98.5: 1.5) third fraction and check on TLC show withaferin A in the second and third fraction, Concentrated the second and third fraction under vacuum crystallized in methanol get 150 mg of 85 % purity.

Analytical: TLC System

Sample preparation

i) 150 mg standardized sample dissolve in 25 ml methanol and apply about 10 micro litre on TLC plate
ii) 100 mg sample before eluted column
iii) 2 to 3mg sample dissolve in 10 ml methanol and apply about 10 micro litre on TLC plate

Solvent system - Chloroform : Methanol (9.5:0.5) Detection – Spray Ethanol sulphuric acid reagent[10] black spot of withaferin A appeared

HPLC analysis

Solution A: Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL of water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

Solution B: Filtered and degassed acetonitrile

Standard solution 1

Dissolve, using gentle heat, a quantity of standardized RS in methanol to obtain a solution having a known concentration of about 1.5 mg/mL.

Sample solution 2

Dissolve, using gentle heat, a quantity of in methanol to obtain a solution having a known concentration of about 6 mg/mL. (before eluted on chromatographic column).

Sample solution 3

Transfer about 2 mg of isolated crystallized Powdered of withaferin weighed, to a 25-mL volumetric flask and make up with methanol to volume, and mix.

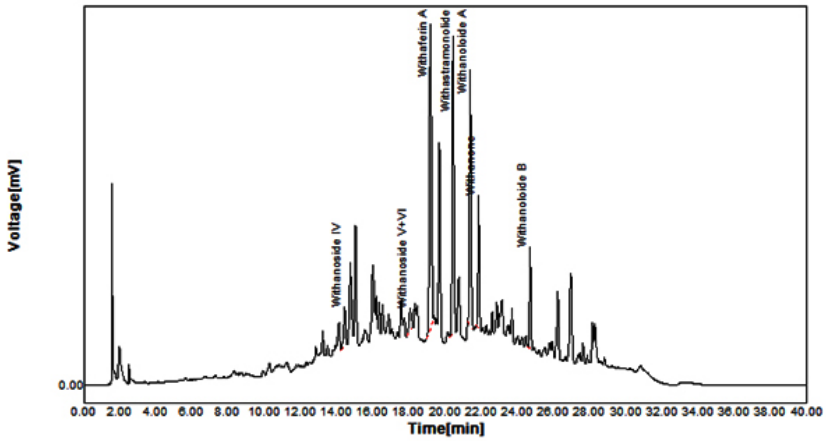
Before injection, all solution passes through a membrane filter having a 0.45- µm or finer porosity, discarding the first few mL of the filtrate[11].

Mobile phase: See the gradient table below.

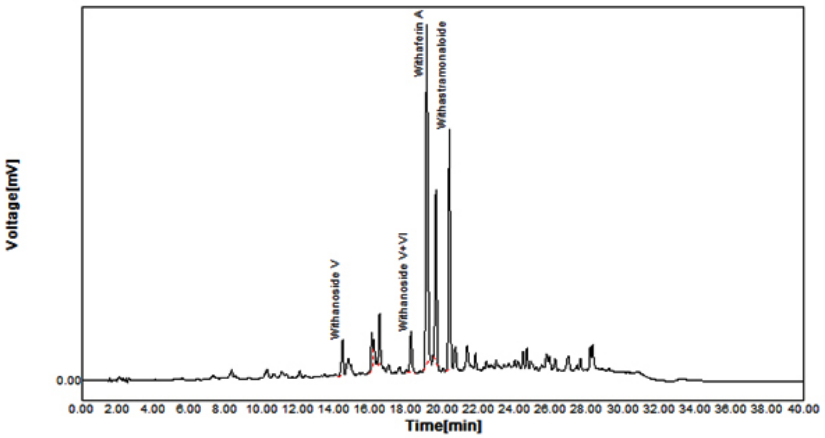
Time(min)	Solution A(%)	Solution B (%)
0	95	5
18	55	45
25	20	80
28	20	80
30	95	5
40	95	5

Chromatographic system

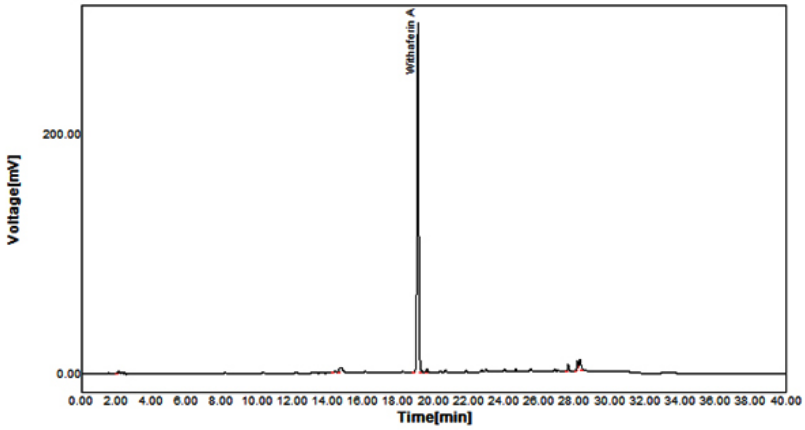
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 25-cm, end-capped; packing



No.	Name	RT[min]	Area[mV*s]	Height[mV]
1	Withanoside IV	14.4167	77.8135	10.8373
2	Withanoside V+VI	18.0500	56.4334	6.2853
3	Withaferin A	19.1667	619.9073	81.2658
4	Withastramonolide	20.4167	551.8586	79.9317
5	Withanoloide A	21.3667	440.8266	68.3315
6	Withanone	21.8333	184.5255	35.2502
7	Withanoloide B	24.6833	148.1876	26.9866



No.	Name	RT[min]	Area[mV*s]	Height[mV]
1	Withanoside V	14.4500	114.7165	16.0431
2	Withanoside V+VI	18.2333	140.4946	18.8618
3	Withaferin A	19.1000	1188.5211	158.2922
4	Withastramonaloide	20.3500	773.3888	112.1440
Sum			2217.1211	305.3411



No.	Name	RT[min]	Area [mV*s]	Height [mV]
1	Withaferin A	19.0833	1913.4049	292.0541

L1
Flow rate: 1.5 mL/min
Injection size: 20 µL

Sample Name:1- *ASHWAGANDHA*
Sample ID: NRPL STANDARDIZED
Date: 2016-10-17 PM 12:00:59
Channel:1. YL9120 UVD A

RESULT

Sample 2: Before eluted
Date: 2016-10-17 PM 12:41:59
Channel:1. YL9120 UVD A

CHROMATOGRAM

RESULT

Sample- 3
Isolated Withaferin A

RESULT

DISCUSSION

Sample 1,2,3 were analyzed as per standard conditions mentioned. Different components in sample of Withania somnifera had been estimated using HPLC, the active ingredients withanoside IV, withanoside V+VI, withaferin A, withastramonolide, withanolide A, withanone and withanolide B had been marked in the chromatogram obtained, sample 2 as given above is also run before eluted, their active ingredients and it's sum is mentioned in chromatogram, by analyzing sample-3 withaferin A is isolated.

CONCLUSION

Plant material obtained is authenticated first for its identification by taxonomist. It's extract is prepared , as mentioned earlier and phytochemical ingredients like withanoside IV, withanoside V + VI, withaferin A, withastramonolide, withanolide A, withanone, withanolide B are studied using HPLC analysis, different peaks obtained confirm the presence of these active ingredients in sample, further it is attempted to isolate withaferin A, in it's pure form.

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