Asian Journal of Chemistry Vol. 18, No. 2 (2006), 1243–1247

Isolation of Withaferin-A from *Withania somnifera* Dun Leaves and its Antibacterial Activity

SHANAZBANU*, S. SHASHIDARA†, V.L. ASHOKA BABU and R. DHANAPAL**

Department of Pharmacognosy, Dayanand Sagar College of Pharmacy
Bangalore-560 078, India
E-mail: shanaz29@yahoo.com

Withaferin-A is a steroidal alkaloid with an unsaturated lactone obtained as an important constituent of *Withania somnifera* Dun. A new method, was developed for the isolation of withaferin-A from methanolic extract of *Withania somnifera* Dun leaves. The isolated compound was compared and confirmed with standard withaferin-A by using thin layer chromatography and various spectroscopic techniques (HPLC, UV-spectroscopy and IR). It was found that the method developed was simple, faster, easy to perform and reproducible. Further the isolated compound was screened for its antibacterial activity. The isolated compound showed significant activity when compared with standard streptomycin sulphate.

Key Words: Withaferin-A, *Withania somnifera*, Fractional crystallization, antibacterial activity.

INTRODUCTION

Withania somnifera Dun (Solanaceae) is a branched shrub, distributed throughout India. The leaves of the plant are in use in indigenous system of medicine for the treatment of ulcers, carbonals, septic wounds, helminthic, respiratory, nervous and gynecological disorders and tuberculosis etc.[1–3]. The leaves of the plant are reported to possess steroidal lactones and alkaloids[4–6]. Many workers have isolated Withaferin-A by different methods and reported it to have antibacterial activity[1, 7–9]. In the light of the above fact, in our investigation, we developed a simple method, fractional crystallization, for the isolation of Withaferin-A from the methanolic extract of leaves of *Withania somnifera* Dun. The isolated compound was screened for its antibacterial activity and is being reported here.

EXPERIMENTAL

About 1 kg of the leaves of *Withania somnifera* were procured from Anju Phytochemical, Singasandra, Hosur Road, Bangalore, India, dried and powdered.

About 1 kg of the powdered leaves was extracted with petroleum (60–80°C) in order to remove chlorophyll and fatty substances. The extract was refluxed with 5 L of methanol in a water bath for 7–8 h; methanol was separated and concentrated. The remaining concentrated mass was washed with petroleum ether again by decantation process. The defatted methanolic extract was dissolved in methanol : water (3 : 7). It was then washed with petroleum ether. The petroleum ether

†Department of Pharmacognosy, Government College of Pharmacy, Bangalore-560 027, India.
‡Department of Pharmacognosy, Rural College of Pharmacy, Devanahalli, Bangalore, India.
**Department of Pharmaceutics, Rural College of Pharmacy, Devanahalli, Bangalore, India.

layer was discarded and the remaining solution was extracted with diethyl ether. The ether layer was washed with water and dried over sodium sulphate to remove the remaining moisture. The solution was then kept overnight at room temperature. Precipitate of withaferin-A separated at the bottom of the beaker. The withaferin-A precipitate obtained was further purified to yield pure compound. The precipitate obtained was washed with ether about 4–5 times. It was then washed with acetone to yield pure crystals of withaferin-A. Yield about 2.2 g from 1 kg of the powder.

The isolated compound was characterized on TLC using silica gel as an adsorbent, chloroform : methanol (9 : 1) as solvent system and anisaldehyde sulphuric acid (violet colour spot) as the developing agent. A single component was observed to have has R_f value of 0.73 corresponding to the R_f value of standard Withaferin-A (procured from quality control department, Sami Labs Ltd., Peenya, Bangalore, India).

Melting points were determined in open capillaries and are uncorrected. UV-spectra were recorded on Schimadzu UV-160 spectrophotometer and it was scanned in the range between 200–400 nm. The IR spectra (KBr) were recorded on Perkin-Elmer-881 (Perkin-Elmer, USA). For HPLC analysis, Schimadzu model SPD-M10A with VP software was employed; chromatogram was performed on a C-18 ODSm, 2 × 0.4 cm-μ bonda pack (5 μ particle size) column. The mobile phase composed of acetonitrile and water was prepared and pumped at the rate of 1 mL/min and photo diode array at 254 nm as detector.

Melting point of the isolated withaferin-A was found to be 215°C. UV spectra of the isolated compound showed peak at 215 nm corresponding to standard withaferin-A at 214 nm (Fig. 1).

IR (KBr) showed IR absorption in the region of 2930 ν(C—H), 1620 ν(C═C), 1286 ν(C—O—C), 1678 ν(C═O) and 3415 ν(O—H) (Fig. 2).

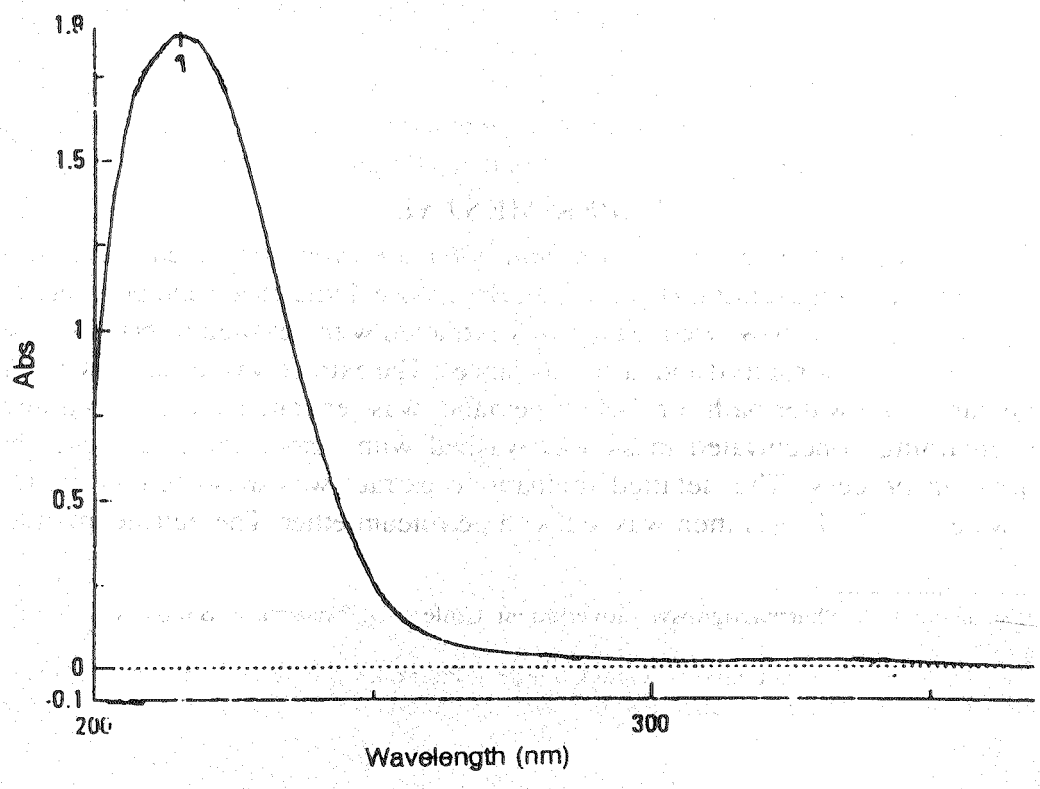

Fig. 1. UV-spectrum of isolated withaferin-A

HPLC and typical chromatogram are illustrated in Fig. 3. It shows that the retention times of isolated compound and the standard withaferin-A were about 20.215 s and 20.285 s, respectively.

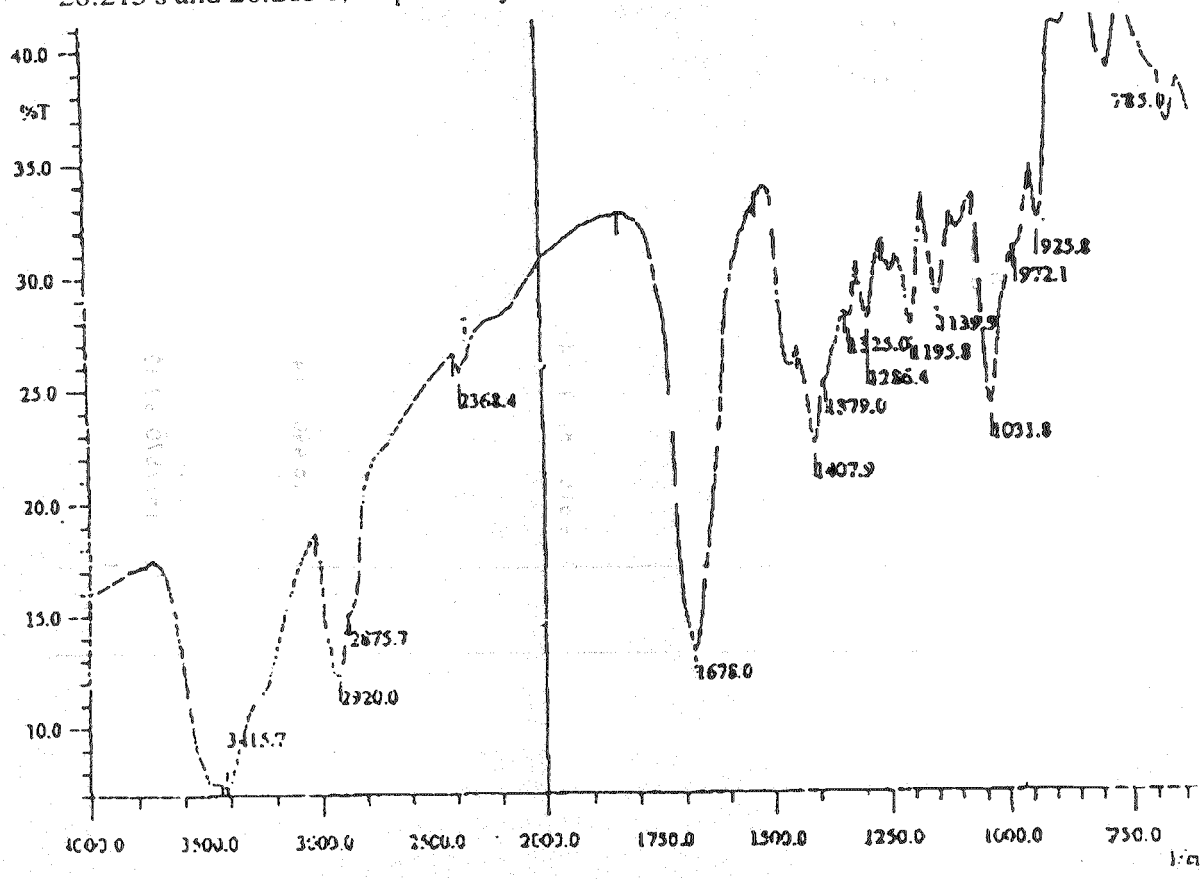

Fig. 2. IR spectrum of isolated Withaferin-A

Antibacterial activity

Test organism: Authentic cultures of *S. Aureus* (MTCC 96) and *B. subtilis* (MTCC 619) were used. All strains used were pure cultures preserved in slant agar culture at 4°C.

Determination of MIC by Broth dilution method[10]

5 mg/mL stock solution of withaferin-A was prepared in DMSO. It was then subsequently diluted to obtain a concentration of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 μg/mL. 350 mL of nutrient agar media was prepared in distilled water and was taken in a 500 mL conical flask. 10 mL of the media was taken in a 20 mL test tube and plugged with cotton. The media was sterilised by autoclaving at 15 lb/sq. in for 20 min at 121°C. The media was cooled at room temperature and inoculated with 0.1 mL of inoculum and then 0.2 mL of the test sample from individual concentration was added to the corresponding test tubes under sterile condition. The test tubes were incubated for 16 h and observed for the presence of turbidity. The concentration at which no turbidity was found was considered as MIC. Similar procedure was adopted for the extract of *Withania somnifera* leaves.

Determinaton of zone of inhibition by agar cup method[11]

Pure streptomycin sulphate was taken as standard antibiotic for comparison of the results. 30 mL of the sterilized nutrient agar media was inoculated with the test

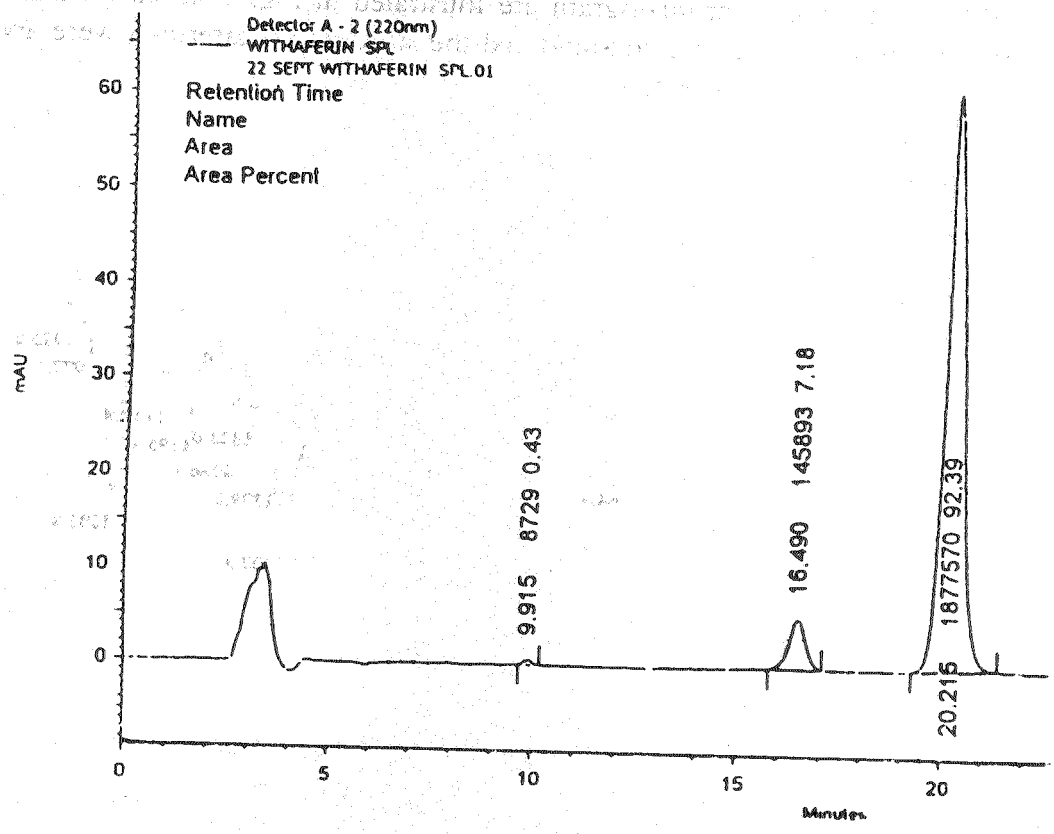

Fig. 3. HPLC Profile of isolated withaferin-A

Detector A-2 (220 nm)

Retention time	Area	Area (%)	Height
9.915	8729	0.43	584
16.490	145893	7.18	5364
20.215	1877570	92.39	60583
Total	2032192	100.00	66531

organism and poured aseptically into the petri dishes. Four holes were made with the help of sterile borer of 6 mm to accommodate 0.1 mL of the different samples of *Withania somnifera* leaves extract (20 mg/mL), isolated compound (100 µg/mL) and a blank (dimethyl sulfoxide). 2 mg/0.1 mL of *Withania somnifera* leaves extract, 10 µg/0.1 mL of isolated withaferin-A were used in the experiment. The plates were then incubated for 16–18 h and zone of inhibition was measured. Similar procedure was adopted for pure streptomycin sulphate (50 µg/mL) and the corresponding zone diameters were compared accordingly.

RESULTS AND DISCUSSION

TLC, HPLC and spectrophotometric techniques confirmed the isolated compound as withaferin-A. The spectra were found to be matching with that of the reference spectra of standard withaferin-A. This new method is found to be simple, economic, easy to perform and reproducible.

The results of comparative MIC values of both the isolated compound and the methanolic extract of *Withania somnifera* have been presented in Table-1. It clearly depicts that the isolated compound is comparatively more active against the tested bacteria as compared to the tested methanolic leaves extract of *Withania somnifera*.

TABLE-1
MINIMUM INHIBITORY CONCENTRATION OF ISOLATED WITHAFERIN-A

Microorganisms	MIC (μg/mL)
S. aureus (MTCC 96)	2.0
B. subtilis (MTCC 619)	2.5

The results of zone of inhibition of the isolated compound, methanolic leaves extract of *Withania somnifera* and their comparison with standard streptomycinsulphate is recorded in Table-2; the values indicate the significant activity of the isolated compound.

TABLE-2
ANTIBACTERIAL ACTIVITY OF ISOLATED WITHAFERIN-A AND *WITHANIA SOMNIFERA* EXTRACT

Microorganisms used	Zone of inhibition (mm)		
	Withania somnifera extract (20 mg/mL)	Withaferin-A (100 μg/mL)	Streptomycin sulphate (50 μg/mL)
S. aureus (MTCC 96)	22	12	24
B. subtilis (MTCC 619)	16	11	20

From the above observations, it was concluded that the isolated withaferin-A exhibited significant antibacterial activity against *S. aureus* (MTCC 96) and *B. subtilis* (MTCC 619).

ACKNOWLEDGEMENTS

Authors are sincerely thankful to Prof. S.S. Dhir, Principal, Dayanand Sagar College of Pharmacy and Sami Labs, Bangalore for providing instrumentation facilities.

REFERENCES

1. P.A. Kurup, *Antibiotics and Chemotherapy*, **8**, 511 (1958).
2. M.E. Williamson, *Withania somnifera*, in: Major Herbs of Ayurveda, Elsevier Science Ltd., pp. 321–325 (2002).
3. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, Vol. III, p. 1774 (1933).
4. B. Mukherji, *Indian J. Pharm.*, **22**, 94 (1960).
5. D.N. Majumdar, *Curr. Sci.*, **21**, 46 (1951).
6. D. Lavie, S. Greenfield and E. Glotter, *J. Chem. Soc. (C)*, 1753 (1966).
7. N.S. Dhalla, M.S. Sastry and C.L. Malhotra, *J. Indian Chem. Soc.*, **50**, 876 (1963).
8. D. Lavie, E. Glotter and Y. Shvo, *J. Chem. Soc. (C)*, 7517 (1965).
9. H.J. Jaffer, M. Jawad, H.S. Saber and A. Naib, *Fitoterapia*, **59**, 497 (1988).
10. F. Kavanagh, Dilution Methods in Antibiotic Assays, Academic Press, London, pp. 125–127 (1963).
11. H. Collins, Antibiotics and antibacterial substances, in: Microbiological Methods, 2nd Edn., Butterworth & Co., London, pp. 356–365 (1967).

(*Received*: 13 June 2005; *Accepted*: 22 December 2005) AJC-4559