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Short Communication

Determination of the anticancer drug bruceoside-A in the Chinese drug Yadanzi (*Brucea Javanica* Merr.)

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ABSTRACT

A high-performance liquid chromatographic method was developed for the determination of bruceoside-A in the Chinese drug Yadanzi (*Brucea javanica* Merr.). After being boiled in ethanol for the lysis of enzyme, nuts of the fruits of Yadanzi were ground and refluxed with light petroleum (b.p. 60–90°C) to remove the oil, then sonicated in methanol for 1 h and the methanolic solution was left overnight. The resulting solution was analysed using a high-performance liquid chromatograph equipped with a reversed-phase ODS-bonded column and eluted with methanol–water (41:59, v/v) at a flow-rate of 1 ml/min with UV detection at 254 nm.

INTRODUCTION

The fruits of Yadanzi (*Brucea javanica* Merr.) are a common drug in Chinese traditional medicine. Bruceoside-A (Fig. 1) extracted from the fruits has been reported to have anticancer activity [1–3]. Several studies have been reported on the identifi-

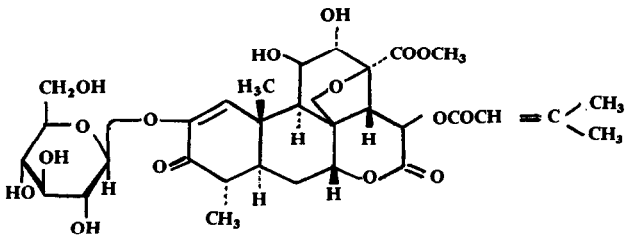

Fig. 1. Structure of bruceoside-A.

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cation of chemical components in the fruits [1–7]. However, to our knowledge, no method has been reported for the determination of bruceoside-A in the fruits. The quality of the fruits of Yadanzi is dependent on the place of production and storage conditions. Information about the bruceoside A content in the fruits is needed for the correct administration of the drug. This paper describes a high-performance liquid chromatographic (HPLC) method for the determination of bruceoside-A in the fruits of Yadanzi.

EXPERIMENTAL

Materials

Bruceoside-A standard was a kind gift from Professor Xian Li (Shenyang Pharmaceutical Institute, Shenyang, China) and Professor Jing-Xi Xie, (Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China). Samples of fruits of Yadanzi were purchased from Beijing, Tianjin, Yunnan, Haikang and Hainandao (China). Chemicals were of analytical-reagent grade.

High-performance liquid chromatography

A Waters Model 510 pump was used together with a 25 cm × 5 mm I.D. reversed-phase bonded column packed with 10-μm ODS material (C_{18}-YWG, Tianjin, China). The sample loop had a volume of 10 μl and the Shimadzu SPD-2A ultraviolet detector was set at 254 nm (0.02 a.u.f.s.). The mobile phase was methanol–water (41:59, v/v) at a flow-rate of 1 ml/min.

Analytical procedure

The shells of Yadanzi fruits were removed and 1.3 g of the nuts was boiled in 10 ml of ethanol for the lysis of enzyme on the surface of the fruits. After grinding, 0.25 g of the nuts was wrapped in a piece of filter-paper and refluxed with 30 ml of light petroleum (b.p. 60–90°C) for 3 h to remove the oil. The nuts were then sonicated in 15 ml of methanol for 1 h and the solution was left overnight. A 5-μl volume of the methanolic solution was injected into the HPLC system and the amount of bruceoside-A in the nuts was calculated from a calibration graph. The ethanolic solution used for the lysis of enzyme was also injected into the HPLC system and the amount of bruceoside-A obtained from this solution was added to that obtained from the methanolic solution to give the total amount of bruceoside-A in the nuts.

RESULTS AND DISCUSSION

Optimization of HPLC conditions

A column packed with reversed-phase ODS C_{18} was tried with eluents consisting of different combinations of methanol and water for the separation of bruceoside-A from the fruits of Yadanzi. Bruceoside-A was well separated with methanol–water (41:59, v/v), as shown in Fig. 2. When the proportion of methanol was decreased, the separation could be further improved. In such a case, more sample could be injected for the collection of bruceoside-A from the outlet of the column.

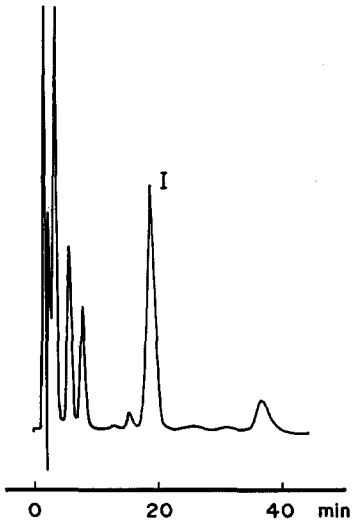

Fig. 2. HPLC of the extracts from the nuts of Yadanzi fruits obtained from Tianjin. For chromatographic conditions, see Experimental. Peak I: bruceoside-A.

Identification of chromatographic peak corresponding to bruceoside-A

The eluate corresponding to peak I in Fig. 2 was collected and evaporated to dryness under vacuum with gentle heating, and further dried in a bottle over phosphorus pentoxide for 3 days. The IR spectrum of the dried powder shown in Fig. 3 was identical with that obtained by Li and Xian, who reported the spectrum for the structural elucidation of bruceoside-A [4], and the NMR spectrum in Fig. 4 coincided

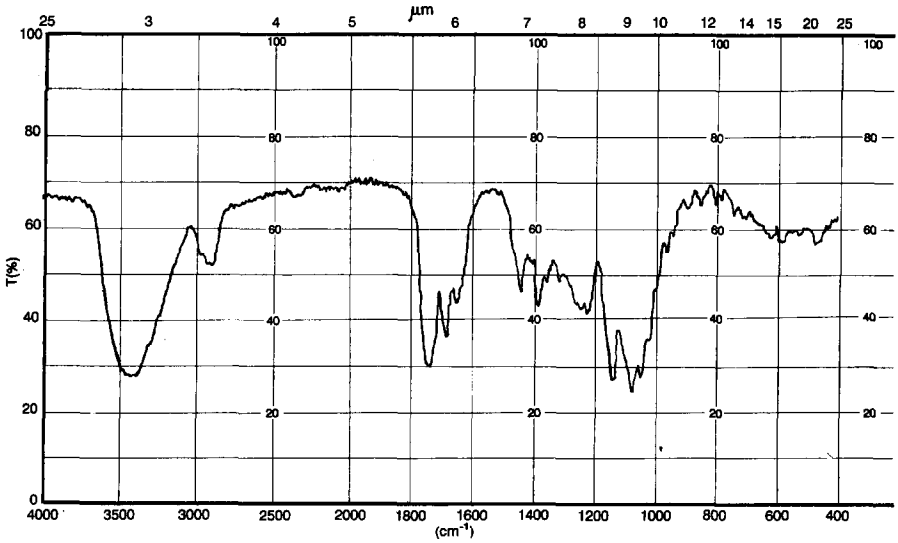

Fig. 3. IR spectrum of bruceoside-A (KBr pellet).

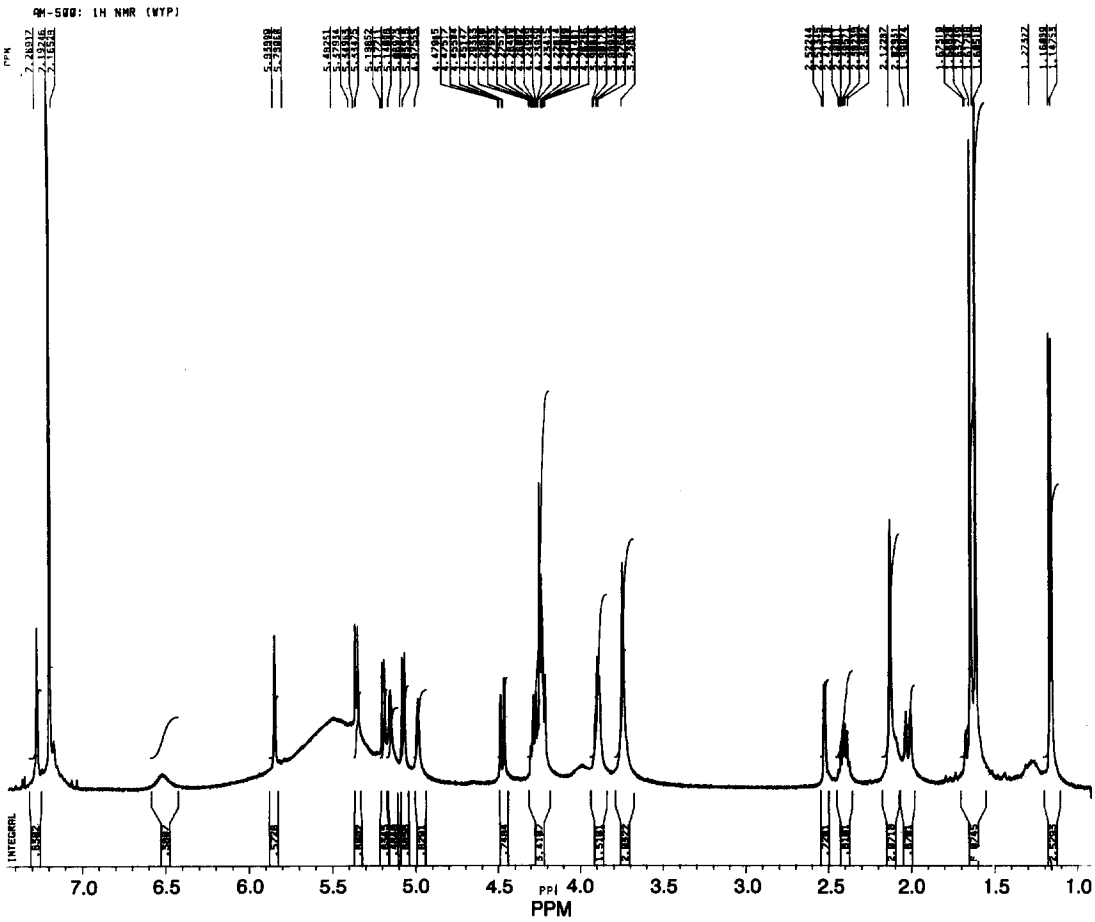

Fig. 4. NMR spectrum of bruceoside-A. Solvent: deuterated pyridine. Reference: tetramethylsilane.

with that obtained by Lee *et al.* [1] for the identification of bruceoside-A. The mass spectrum obtained with fast atom bombardment yielded an ion of *m/z* 705, which might be from $M^+ + 23$ (23 is the atomic weight of sodium), and addition of potassium to the sample yielded an ion of *m/z* 721, which might be from 682 + 39 (39 is the atomic weight of potassium). The molecular mass of bruceoside-A is 682. The retention time of the component collected corresponding to peak I in Fig. 2 was identical with that of the bruceoside-A standard obtained from Professor Jing-Xi Xie and Professor Xian Li. The compound collected from the eluate corresponding to peak I in Fig. 2 was thus identified as bruceoside-A.

Calibration

The calibration graph for bruceoside-A was prepared by injection of 0.22–1.10 μg of bruceoside-A. The resulting graph was linear within this range, with a correla-

tion coefficient of 0.998 ($n = 5$). The regression equation is $y = 0.2 + 65.1x$, where y is the amount of bruceoside-A, x is the peak height.

Optimization of experimental conditions for the removal of oil in the fruits of Yadanzi

A 700-mg amount of ground nuts of Yadanzi fruits was placed in 30 ml of light petroleum (b.p. 60–90°C), followed by refluxing, sonication or being left at room temperature. The weights of the nuts were checked to calculate the amount of oil removed. It was found that refluxing for 3–4 h was the most efficient method for the removal of the oil, and a period of 3 h was adopted.

Comparison of different methods of extraction

Methanol and ethanol were tried for the extraction fo bruceoside-A from the nuts of Yadanzi fruits. After refluxing for 30 min, the amount of bruceoside-A extracted by ethanol was 89% of that extracted by methanol. Extraction with methanol was further studied with different treatments. As shown in Table I, bruceoside-A was stable during reflux and after being refluxed for 1 h the peak height of bruceoside-A ceased to increase. Extraction by sonication followed by leaving the solution overnight yielded the same results as those obtained by refluxing for 1 h. The latter was simpler and therefore used in later experiments.

Recovery

The recovery of bruceoside-A in the extraction procedure was studied by the addition of bruceoside-A to Yadanzi nuts from which the oil had been removed, then the extraction was carried out by adding 15 ml of methanol to the samples and sonication for 1 h and the solutions were left overnight. The resulting solutions were examined by HPLC to check the content of bruceoside-A. As shown in Table II, five runs yielded an average recovery of 98.2% with a relative standard deviation of 4.0%.

Determination of bruceoside-A in samples obtained from different districts in China

Yadanzi fruit samples obtained from Beijing, Tianjin, Yunnan, Haikang and Hainandao were analysed according to the analytical procedure. The results are shown in Table III. The chromatograms were very similar to each other, as shown in Fig. 2.

TABLE I

COMPARISON OF DIFFERENT METHODS FOR THE EXTRACTION OF BRUCEOSIDE-A FROM THE NUTS OF YADANZI FRUITS

Method	Peak height of bruceoside-A (mm) ($n = 2$)
Sonicated with methanol for 1 h	40.0, 41.0
Left in methanol overnight	40.0, 40.0
Sonicated in methanol for 1 h then left overnight	48.0, 49.0
Refluxed in methanol for 30 min	45.0, 50.0
Refluxed in methanol for 1 h	46.0, 51.0
Refluxed in methanol for 2 h	46.0, 51.5
Refluxed in methanol for 3 h	45.0, 51.0

TABLE II

RECOVERY OF BRUCEOSIDE-A IN THE EXTRACTION PROCEDURE

No.	Content of bruceoside-A (mg)	Bruceoside-A added (mg)	Bruceoside-A found (mg)	Recovery (%)
1	4.042	2.754	6.918	104.43
2	4.042	2.550	6.444	94.20
3	4.042	2.562	6.538	97.42
4	4.042	2.225	6.257	99.55
5	4.042	2.290	6.226	95.37

Five analyses of bruceoside-A in the sample obtained from Tianjin yielded an average value of 1.43% with a standard deviation of 0.06%. As shown in Table III, the amounts of bruceoside-A in samples from different sources varied widely.

TABLE III

DETERMINATION OF BRUCEOSIDE-A IN THE FRUITS OF YADANZI OBTAINED FROM DIFFERENT DISTRICTS IN CHINA

Source	Bruceoside-A found (%)	*n*
Beijing	0.50, 0.47	2
Hainandao	1.04 ± 0.05	3
Haikang	1.11 ± 0.04	3
Yunnan	0.85 ± 0.04	4
Tianjin	1.43 ± 0.06	5

CONCLUSION

A proposed HPLC method for the determination of bruceoside-A in the fruits of Yadanzi provides a means of elucidating the quality of the Chinese drug Yadanzi and would be useful for guidance regarding administration of the drug in the correct dosage.

ACKNOWLEDGEMENTS

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