Vol. 1/1 (2001) 67 - 69

Short Communication

JOURNAL OF NATURAL REMEDIES

Screening of pyrrolizidine alkaloids in some herbal drugs

P.C. Arun, B. Murali, M.S. Anand, M. Deepak*

Research & Development Centre, Natural Remedies Pvt. Ltd., Plot No. 5B, Veerasandra Indl. Area, Bangalore, 561 229,

Received 15 July 2000; Revised and accepted 15 September 2000

Abstract

Objective: Detection of pyrrolizidine alkaloids (PA) in some polyherbal formulations. Materials and methods: Silica gel TLC with specific spray reagent for PA. Results: None of the herbal formulations tested were positive for PA. *Phyllanthus amarus* roots were found to contain PA. Conclusion: TLC method is recommended for routine detection of PA in plant drugs.

Key words: *Phyllanthus amarus.* Pyrrolizidine alkaloids, TLC, polyherbal formulations.

1.Introduction

Pyrrolizidine alkaloids [PA] constitute a large group of natural products with over 200 known examples [1]. These alkaloids, of late, have generated much interest due to their hepatotoxicity in humans and animals. Plants containing hepatotoxic PA are present in most parts of the world and often cause poisoning to grazing livestock. PA poisoning is a considerable economic problem with losses due to the death of livestock and to the general debilitating effects of chronic PA intoxication [2].

Keeping this in view, investigations were carried out on some polyherbal formulations *viz*., Zigbir, Zeetress, Zist and Involon (the marketed products of M/s Natural Remedies Pvt. Ltd., Bangalore) to detect the presence of PA. Major herbal ingredients of these formulations are given in Table 1. PA have been reported to be present in the roots of *Phyllanthus niruri.* There has been a controversy within the scientific community over the actual botanical identity of the popular Indian crude drug called Bhumyamalaki (Kevenelly) [3,4]. The sample of two different species of *Phyllanthus* sold as Kevenelly in the market were also included in the study.

2. Materials and Methods

All the plant raw materials used in the study were authenticated in the pharmacognosy section of Natural Remedies R&D Centre, Bangalore and specimen samples have been kept in the museum. The two different species of Kevenelly were identified as *P. amarus* and *P. maderaspatensis* which was confirmed by National Institute of Science Communication, New Delhi.

PA were extracted using standard alkaloid extraction procedure [5]. Each sample (50 g) was extracted

* Corresponding Author
E-mail: deepak.12345@usa.net

Table 1
Composition of tested herbal formulations and content of alkaloids

Formulation	Major ingredients	Part used	% alkaloids	PA
Zigbir	*Andrographis paniculata*	Leaves	0.28%	Not detected
	Phyllanthus maderaspatensis	Whole plant		
	Solanum nigrum	Whole plant		
Zeetress	*Withania somnifera*	Roots	0.24%	Not detected
	Ocimum sanctum	Leaves		
	Emblica officinalis	Fruits		
Involon	*Gloriosa superba*	Tubers	1.33%	Not detected
	Plumbago zeylanica	Roots		
	Gossypium herbaceum	Roots		
	Lepidium sativum	Seeds		
	Peganum harmala	Seeds		
	Adhatoda vasica	Leaves		
Zist	*Withania somnifera*	Roots	0.06%	Not detected
	Ocimum sanctum	Leaves		
Crude drugs-tested	*Phyllanthus amarus*	Roots	0.03%	Detected
	Phyllanthus maderaspatensis.	Roots	0.03%	Not detected

* Under patent process (details cannot be revealed).

with methanol (250ml x 3) and the combined methanolic extract was concentrated under reduced pressure and dried in vacuum. The dried extract was slurried in water (250ml), acidified with 0.1N HCl to a pH of 2.0 and extracted with chloroform (250 ml x 3). The water layer was basified (pH-9.5) with dilute ammonia solution and then again extracted with chloroform (250 ml x 3). The latter was concentrated to get the alkaloid fraction. Percentage of alkaloid fraction obtained from different formulations are also given in Table 1.

Estimation of PAs was done using a thin layer chromatographic method given in the literature [6,7]. The alkaloid extracts in each case were dissolved in chloroform and made upto known volumes (1ml for Zigbir, Zeetress and 5ml for *P. amarus* & *P. maderaspatensis*; 10ml for Involon; 20 ml for Zist). 10µl and 20 µl of each solution were spotted on a TLC silica gel 60 F254 pre-coated aluminium sheet.

A reference PA (monocrotaline, 2mg, Sigma, USA) was dissolved in 5 ml of chloroform and different volumes (5µl, 10µl and 20µl) were also spotted on the TLC plate along with samples. The plate was developed in the mobile phase comprising of chloroform: methanol: conc. ammonia (85:14:1). The TLC plate was sprayed successively with the following reagents, and the plates dried at 140^{0}C for 5 min after each spray.

1. Hydrogen peroxide (36% solution): diethylene glycol dimethyl ether: acetic anhydride (1:1:2)
2. Acetic anhydride: petroleum ether (1:4)
3. Dimethylamino benzaldehyde (0.5g) dissolved in ethanol (35ml) and diethylene glycol dimethylether (15ml). HCl (1ml) is to be added immediately before use.

The PAs gave magenta colour on TLC plates and were scanned using densitometer at 550nm.

3. Results and discussion

In view of the possible acute and chronic hepatotoxic effects of the medicinal plants possessing PA it becomes highly necessary for the herbal drug manufacturers to ensure the absence of PA in their products. Although other chromatographic methods based on PC, HPLC, and GC are available in the literature [3] we found the TLC procedure simple and suitable for routine detection of PAs in herbal products. All the herbal formulations tested were found to have alkaloids with Involon showing the maximum (1.33%). However, none of the

formulations gave positive test for PA (Table 1).

Amongst the two raw materials tested *viz.*, roots of *P. amarus* & *P. maderaspatensis*, only the former gave positive test for PA. Since extracts of aerial parts of *P. amarus* are being used extensively in India for the treatment of ailments related to liver, herbal drug manufacturers should ensure the absence of roots in the raw material of *P. amarus*.

It can be concluded that since plants containing PA are considered a health hazard for both humans and livestock [3] a test for the absence of PA would contribute to the safety data of herbal products generated by other direct means like toxicity studies. The TLC method for detection of PA being simple can therefore form a part of routine quality control protocols of every plant based drug.

References

1. Robins DJ. (1998) *Chem. Soc. Rev.* 18: 375-408.

2. Mattocks AB, Juker R. (1987) *J. Nat. Prod.* 50: 161-166.

3. Robins DJ. (1993) *Methods in Plant Biochemistry*, Academic Press Limited: London; 24-28,

4. Bagchi GD, Srivastava GN, Singh SC. (1992) *Int. J. Pharmacog*. 30 : 161-168.

5. Trease GE, Evans WG. (1997) *Pharmacognosy*, 14th edn., Harcourt Brace & Co.: India; 343-345.

6. Mierendorff HJ. (1995) *Food Sci. Technol.* 97: 33-37.

7. Sharma KK, Khajuria GS, Atal CK. (1965) *J. Chromatogr.* 19:433-434.