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IMPROVED ISOLATION OF 4-HYDROXYISOLEUCINE FROM *Trigonella foenum graecum* SEEDS

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Fenugreek (*Trigonella foenum graecum* L.) has a long tradition of being used to treat diabetes in Iran, India, and other parts of the world [1]. Modern studies have demonstrated the antidiabetic effects of fenugreek in diabetic rats, including modulation of postprandial glucose levels, insulin secretion, and blood cholesterol [2–4]. The hypoglycemic effects of fenugreek seeds have been attributed to alkaloid components and also to the modified amino acid 4-hydroxyisoleucine (4-OH-Ile) [5, 6]. Currently 4-OH-Ile is prepared from dried fenugreek seeds [7–9] and out of eight possible stereoisomers only one form, 2*R*, 3*S*, 4*R*, predominates [8]. The complex stereochemistry of 4-OH-Ile precludes facile chemical synthesis. We describe significantly improved yields of 4-OH-Ile from fenugreek through extraction of germinated seedlings compared with dried seeds, which can be greatly enhanced by supplementing with isoleucine in the feed water.

Yields of 4-OH-Ile from dried seeds were about 280 mg per 100 g of seeds (Table 1) and were comparable to previous reports [7–9]. Yields from germinating seeds were markedly (30–90%) higher compared with dried seeds and could be increased several fold by inclusion of isoleucine supplement in the irrigation water. The marked improvement of yield of 4-OH-Ile following supplementation with isoleucine suggests that either isoleucine may induce the dioxygenase that catalyses the key step in the biosynthesis of 4-OH-Ile [10] or that endogenous levels of substrate are limiting. Seed variety from northeastern Iran show a moderately higher amount of 4-OH-Ile in comparison with that from Central Iran, which may be related to different irrigation methods and/or climate. Furthermore, it could be related to different amounts of dioxygenase in varieties, which results in differences in 4-OH-Ile content after germination (Table1).

Fenugreek seeds were obtained from northeastern or Central Iran and were characterized by the Pharmacognosy Department Herbarium, Tehran University of Medical Sciences, Iran. For germination, seeds were placed on a layer of sponge moistened with water in 50 × 50 cm boxes and incubated at 25°C for 6 days. Boxes were irrigated with 125 mL plain water or water supplemented with 5 mM isoleucine [10], which was changed everyday.

Extraction of 4-OH-Ile followed the method of Broca et al. [6]. Briefly, seeds or plantlets were crushed and defatted with petroleum ether and the powder extracted with 70% EtOH. After vacuum concentration, the extract was passed over Amberlite IR (H^+ form) and eluted with a 0.01 N to 1 N gradient of NH_3. Fractions containing 4-OH-Ile (determined by TLC using *n*-BuOH–AcOH–H_2O, 3:1:1, as solvent) were passed through a column of silica gel, eluted with 70% EtOH and recrystallized from MeOH. The structure was confirmed by IR spectroscopy, NMR at 500 MHz [8], and GC analysis, reported here for the first time, following derivatization with chloroformate [11] and using a purified sample of 4-OH-Ile obtained from Toronto Research Chemicals (Toronto, Canada) as standard. Analytical gas chromatography was carried out on a 6890 Series Gas Chromatograph (Agilent Technologies, Wilmington, DE, USA) using a Zebron ZB-PAAC column,10 m × 0.25 mm (Phenomenex, Torrance, CA, USA) equipped with a flame ionization detector (FID). The carrier gas was helium. The column temperature was programmed at 110°C, heating up to 320°C with a 35°C/min rate, and then keeping constant at 320°C for 1 min. Injector and detector temperatures were 250°C and 320°C. Copies of the original spectra and GC chromatograms can be obtained from the corresponding author.

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TABLE 1. Yields of 4-OH-Ile from Different Sources of Fenugreek Raw Material

Material	Yield of 4-OH-Ile (mg per 100 g material)	
	Northeastern Iran	Central Iran
Dried seeds	279	276
Plantlets	532	369
Plantlets + Ile	1779	

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REFERENCES

1. E. Basch, C. Ulbricht, G. Kuo, P. Szapary, and M. Smith, *Altern. Rev. Med.*, **7**, 20 (2003).
2. R. D. Sharma, T. C. Raghuram, and N. S. Rao, *Eur. J. Nutrn.*, **44**, 301 (1990).
3. M. Al-Habori and A. Raman, *Phytother. Res.*, **12**, 233 (1998).
4. Z. Madar, *Nutrn. Rep. Int.*, **29**, 1276 (1984).
5. J. Shani, A. Goldschmid, Z. Ahronson, and G. Sulman, *Arch. Int. Pharmacodyn. Ther.*, **210**, 27 (1974).
6. C. Broca, M. Manteghetti, R. Gross, Y. Baissac, M. Jacob, P. Petit, Y. Sauvaire, and G. Ribes, *Eur. J. Pharmacol.*, **390**, 339 (2000).
7. L. Fowden, H. M. Pratt, and A. Smith, *Phytochemistry*, **12**, 1707 (1973).
8. N. W. Alcock, D. H. G. Crout, M. V. M. Gregorio, E. Lee, G. Pike, and C. J. Samuel, *Phytochemistry*, **28**, 1835 (1989).
9. R. Hardman and I. M. Abu-Al-Futuh, *Planta Med.*, **36**, 79 (1979).
10. C. Haefele, C. Bonfils, and Y. Suavaire, *Phytochemistry*, **44**, 563 (1997).
11. P. Husek, *FEBS Lett.*, **280**, 354 (1991).