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Note

Thin-layer chromatography–densitometry and high-performance liquid chromatography of catechin in cube gambir

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Cube gambir (*Pale catechu*) is obtained by drying the aqueous extract of twigs and leaves of *Uncaria gambir* (Hunter) Roxburgh (Rubiaceae); it is used in dyeing and tanning and as an astringent in pharmacy. Increasing interest in this drug arises from its high content in (2*R*,3*S*) (+)-catechin or (+)-cyanidanol which shows hepatoprotective and antiulcer activities, and fortifies connective tissues.

As cube gambir is used for industrial extraction of catechin and as it must be distinguished from black catechu or cutch (obtained from *Acacia catechu*, Fabaceae), it seems useful to compare thin-layer chromatography (TLC)–densitometry with high-performance liquid chromatography (HPLC) methods: (1) for separation of this major constituent from other components of cube gambir and from (2*R*,3*R*) (−)-epicatechin which occurs in black catechu, and (2) for catechin determination.

Gas chromatography requires a derivatization step and cannot be applied to thermolabile catechin polymers which are present in cube gambir.

EXPERIMENTAL

HPTLC pre-coated plates of silica gel 60 F_{254} and pre-coated plates of cellulose F were obtained from Merck (Darmstadt, F.R.G.). Catechin, epicatechin, 4-hydroxybenzoic acid and crude extracts were dissolved in methanol–water (1:1) containing 1% acetic acid; after filtration (for crude extracts), the solutions were applied at 15 mm from the lower edge of the chromatoplate with 1-μl capillaries, and immediately developed with chloroform–ethyl formate–formic acid (50:40:10); this mobile phase was allowed to travel, in unsaturated tanks, a distance of 100 mm. After evaporation of solvents, UV detection was performed at 277 nm with a Shimadzu high-speed TLC scanner CS-920 with linearizer at position 1.

The HPLC chromatograph (Waters Assoc., Milford, MA, U.S.A.) was equipped with one pump (Model 6000 A), a sample loop (Model U6K), an UV detector (Model 440) operating at 254 nm and a Hibar® column (250 × 4 mm I.D.) prepacked with LiChrosorb® RP-18 (mean particle size 5 μm) from Merck.

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RESULTS AND DISCUSSION

In regard to the low stability of catechin in solution (oxidation, oxidative polymerization), solvents suitable for catechin solubilization were first investigated. From this study it appeared that addition of 1% acetic acid to the solvent, water or methanol–water (1:1), prevented catechin degradation; these solutions were stable for several hours at ambient temperature.

Numerous studies on TLC, HPLC and metabolism of naturally occurring phenolic compounds, especially of catechin, have been reported[1–9]. Described TLC conditions allowed the separation on silica gel of catechin (R_F 0.34) from epicatechin (R_F 0.40). Alternatively, the use of cellulose thin layers and 1-butanol–acetic acid–water (40:10:50; upper phase) as mobile phase afforded a better separation (catechin, R_F 0.64; epicatechin, R_F 0.78)[10]; moreover, experiments performed with ^{3}H-labelled catechin and detection by a Geiger-Müller counting tube showed that catechin undergoes degradation during chromatography on silica gel, but not on cellulose. However, this degradation did not interfere with the determination when development was performed just after spot application.

Concentrations of catechin between 2.5 and 10.0 mg/ml afforded a linear calibration graph with a correlation coefficient (r) greater than 0.999. The presence of other constituents of cube gambir which could interfere in the catechin determination was excluded by densitometric absorption measurements at 260, 277, 290 and 300 nm in comparison with the standard. The use of spraying reagents for phenolic func-

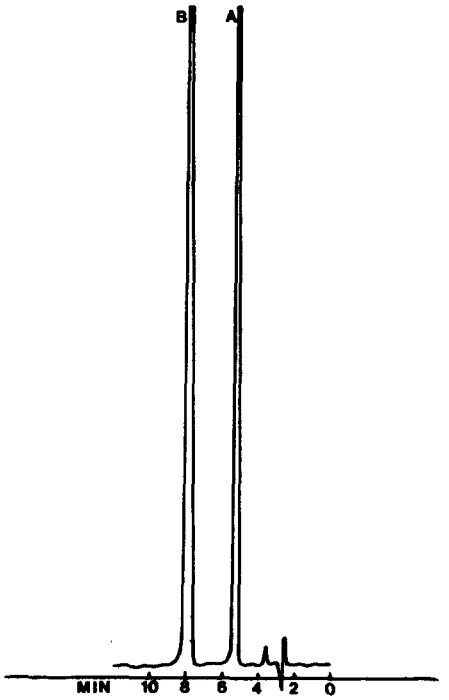

Fig. 1. Separation of catechin in cube gambir. Mobile phase: II, water–methanol–acetic acid (72.5:27.5:1). Peaks: A = catechin; B = 4-hydroxybenzoic acid.

TABLE I

COMPARISON OF RETENTION TIMES (t_R), CAPACITY FACTORS (k') AND RELATIVE RETENTIONS (α) OF CATECHIN, 4-HYDROXYBENZOIC ACID (INTERNAL STANDARD) AND EPICATECHIN IN THREE DIFFERENT MOBILE PHASES

I, water–acetonitrile–acetic acid (85:15:1); II, water–methanol–acetic acid (72.5:27.5:1); III, water–ethanol–acetic acid (80:20:1). Flow-rate: 1 ml/min. Injection volume and concentrations: 25 μl of a solution containing 5 mg of catechin and epicatechin and 4 mg of 4-hydroxybenzoic acid dissolved in 100 ml of mobile phase.

Mobile phase	*t_R (min sec), k'*			*α (epicatechin/catechin)*
	Catechin	*4-Hydroxybenzoic acid*	*Epicatechin*	
I	5 48, 1.32	7 09, 1.86	8 00, 2.20	1.67
II	5 12, 1.08	7 54, 2.16	9 06, 2.64	2.44
II	3 57, 0.58	7 06, 1.84	6 21, 1.54	2.65

tions, such as 2,6-dichloroquinone chlorimide, improved the sensitivity but dramatically increased the variation coefficient.

Typical TLC determination of catechin in cube gambir gave the following results: 48.21 ± 1.6% (C.V.) (six determinations, each yielding a mean value calculated from the integration results of eighteen spots corresponding to two spots of the unknown solution and four different standard concentrations repeated three times on the plate).

HPLC conditions were selected following two criteria:

(1) Separation of catechin from epicatechin and 4-hydroxybenzoic acid, chosen as internal standard because of its HPLC behaviour and possible extraction from crude material under the conditions used for catechin isolation.

(2) Analysis time less than 10 min.

Addition of acetic acid to the mobile phase decreased peak tailing by suppression of ionization of the acidic functions and prevented catechin degradation during chromatography. From a comparison of capacity factors (k') and relative retention values (α), it appeared that mobile phases II (Fig. 1) and III were quite similar with regard to selectivity and resolution but could be complementary because the reversal of the elution order of epicatechin and 4-hydroxybenzoic acid (Table I). This reversal had been previously observed when the methanol concentration in water was modified[1], and also with ethanol and acetonitrile; the influence of catechin,hydroxybenzoic acid and epicatechin in the organic part of the mobile phase on their relative retention times was thus confirmed.

The catechin content of the same sample determined by HPLC was 50.59 ± 0.58% (coefficient of variation) (mean value of ten determinations performed with mobile phase II). The results obtained from both HPLC and TLC determinations were in good agreement, the difference (*ca.* 2.4%) observed most probably indicating that a cube gambir constituent was not separated from catechin by HPLC. In summary, TLC–densitometry was found useful for rapid catechin determination in crude extracts and during the industrial processing of catechin from cube gambir.

REFERENCES

1 L. W. Wulf and C. W. Nagel, *J. Chromatogr.*, 116 (1976) 271.
2 I. C. Shaw and L. A. Griffiths, *Xenobiotica*, 10 (1980) 905.
3 T. S. Banerjee and K. C. Guha, *J. Food Sci. Technol.*, 18 (1981) 10.
4 E. L. Wilson, *J. Sci. Food Agr.*, 32 (1981) 257.
5 M. Samejima and T. Yoshimoto, *Mokuzai Gakkaishi*, 27 (1981) 658.
6 D. J. Daigle and E. J. Conkerton, *J. Chromatogr.*, 240 (1982) 202.
7 D. J. Daigle and E. J. Conkerton, *J. Liquid Chromatogr.*, 6 (1983) 105.
8 M. Wermeille, E. Turin and L. A. Griffiths, *Eur. J. Drug Metab. Pharmacokinet.*, 8 (1983) 77.
9 K. vande Casteele, H. Geiger, R. De Loose and C. F. van Sumere, *J. Chromatogr.*, 259 (1983) 291.
10 A. E. Bradfield and E. C. Bate-Smith, *Biochim. Biophys. Acta*, 4 (1950) 441.