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Fast Chromatographic Study of Propolis Crudes

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ABSTRACT

A rapid analytical chromatographic method for screening the components of a propolis extract has become necessary as increasing use of this substance demands a greater understanding.

As the method only requires thin layer chromatography (TLC) equipment, it could be useful for testing crude preparations and their popular ethanolic extracts. Using this method, significant differences in the general TLC pattern have been found between propolis crudes from different geographical origins.

INTRODUCTION

Propolis is a resinous material produced by bees, containing resins, waxes, flavonoids and some 'impurities' (e.g. pollen, wood, fragments of bees), and used by the bees to seal the hives.

Propolis was found to show antibacterial activities (Grange & Davey, 1990) directly or synergistically (Madarova, 1980), to promote the regeneration of bone (Stojko *et al.*, 1979), cartilage (Scheller *et al.*, 1977) and dental pulp (Scheller *et al.*, 1978), to stimulate the formation of collagen (Havsteen, 1983); its extract was found to be non-toxic in experimental animals (Kleinrok, 1978); a dermatological allergy was, however, reported when used in local application (Marchenay, 1977).

Propolis is now found in tooth-pastes, chocolates, shampoos, creams, tablets, etc. and world production has increased to several thousands of kilograms per year; as the composition of the crude material may vary

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depending on its geographical origin, fast thin layer chromatography (TLC) systems seem to be useful for rapid quality control.

MATERIALS AND METHODS

Propolis from Pennsylvania (USA) was purchased from the Sigma Chemical company (St Louis, Missouri, USA) in two forms: the crude preparation (P-8904) and its purified extract (P-1010). Crude propolis from Alsace (France) was also obtained, diluted in cold methanol and extracted by reflux, filtered and concentrated with a Buchi apparatus and dried. Silica TLC plates containing a fluorescent indicator were purchased from E. Merck (Darmstadt, Germany). All the solvents for TLC tanks were purchased from Fluka (Buch, Switzerland). Photographs were obtained using a Polaroid DS34, a Polaroid T-667 film, using an exposure time of $\frac{1}{30}$ s and an aperture of 32.

Crude materials and purified extracts were all dissolved in 10 mg/ml ethanol, and these solutions were used for the further TLC study.

The solutions of the purified alcoholic extracts from both sources were clear dark yellow, when the crude materials gave turbid tan suspensions with precipitating insolubles. These suspensions were allowed to stand, in order to permit the decantation of the insoluble material, and filtered for TLC spotting.

A 10–20 μl sample of each of the four solutions was spotted on the silica plates, and two developing systems were used.

(1) Toluene/chloroform/acetone (40/25/35),
(2) Hexane/ethyl acetate/acetic acid (60/40/3).

The plates were developed, sprayed with sulphuric acid in methanol (v/v), and heated at 100°C. Observation was done under a UV lamp at 254 nm, and photography was done as described above.

RESULTS

Photographs of separations are presented in Figs 1 and 2.

CONCLUSIONS

(1) Although these systems only mobilize some of the components, they suffice as a 'fingerprint' analysis for the purpose of comparing crudes or extracts.

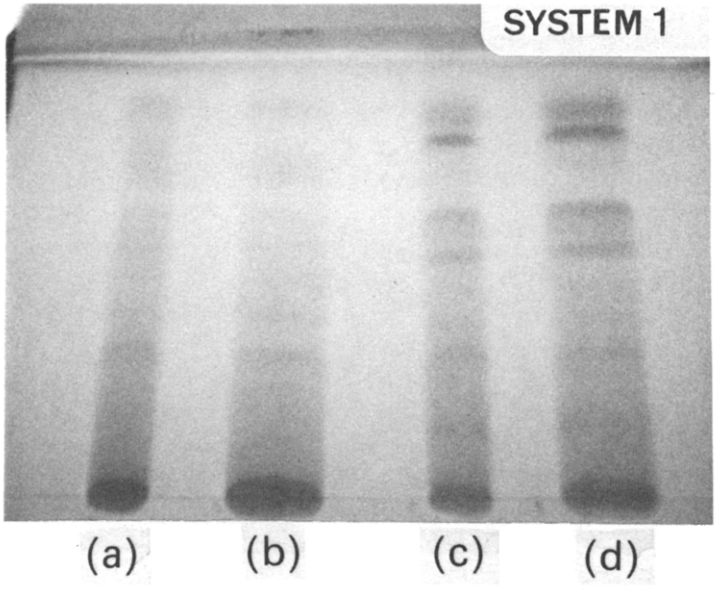

Fig. 1. Photograph for the TLC plate for system 1. (a) Purified extract from B (Sigma), dissolved in 10 mg/ml EtOH, sample spot—10 μl. (b) Crude propolis (Sigma), dissolved in 10 mg/ml EtOH, sample spot—20 μl. (c) Purified extract from D, dissolved in 10 mg/ml EtOH, sample spot—10 μl. (d) Crude propolis from Alsace, dissolved in 10 mg/ml EtOH, sample spot—20 μl.

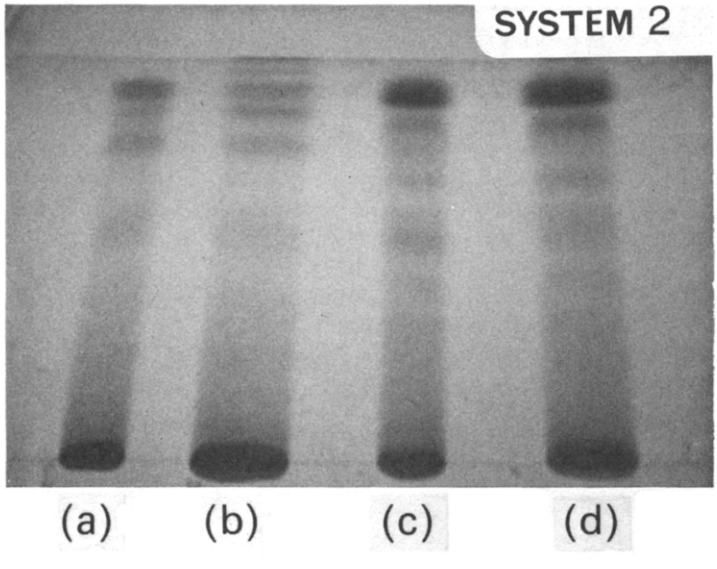

Fig. 2. Photograph for the TLC plate for system 2. (a) Purified extract from B (Sigma), dissolved in 10 mg/ml EtOH, sample spot—10 μl. (b) Crude propolis (Sigma), dissolved in 10 mg/ml EtOH, sample spot—20 μl. (c) Purified extract from D, dissolved in 10 mg/ml EtOH, sample spot—10 μl. (d) Crude propolis from Alsace, dissolved in 10 mg/ml EtOH, sample spot—20 μl.

(2) Significant differences in TLC pattern are observed between propolis from the two origins.
(3) Under these screening systems, extracts show the same patterns as their crude starting material. Only a minor band running with the solvent front can be observed in both crudes lanes but not in extract lanes.
(4) For further identification of propolis components, these systems might be useful for their isolation.

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