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Short Communication

Application of two rapid techniques of column chromatography to separate the pungent principles of ginger, *Zingiber officinale* Roscoe

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ABSTRACT

Two rapid techniques of column chromatography, flash chromatography and vacuum chromatography, together with solvent systems differing in polarity, have been used to separate the pungent principles (gingerols and shogaols) of an extract of ginger powder. Gingerols consist of a homologous series of aldols each containing a phenolic group which together with shogaols, showing similar structure, give the pungency of ginger. Thin-layer chromatographic analysis of the fractions collected from both chromatographic techniques showed that the main gingerol homologues are separated satisfactorily from the other compounds only using vacuum chromatography. These compounds were eluted with hexane–diethyl ether (30:70) and (20:80) respectively. The results obtained with flash chromatography, on the other hand, were not satisfactory.

INTRODUCTION

The rapid and successful separation of selected compounds from a plant extract containing a myriad of substances is a problem facing the plant biochemist. It is therefore important to effect a crude separation of the plant components before more sophisticated procedures are employed. Such separation may include extraction using selected solvents, partition into two immiscible solvents (liquid–liquid extraction) and vacuum or flash chromatography [1–4]. Apart from the speed of the chromatographic process, vacuum and flash chromatography also offer a high loading capacity. Their use is of course not limited to the crude separation of the components of plant extracts, as they have been used successfully for the purification and separation of compounds in mixtures containing few components, such as products of chemical synthesis. A wide range of packing materials have been employed with both techniques, with silica gel used most frequently, but others, such as cellulose, polyamide, and bonded silica (reversed-phase particles) have also been used [5].

Attempts have been made to isolate gingerols (Fig. 1) from a crude extract of ginger by Shoji *et al.* [6] and Farthing and O'Neill [7]. Shoji *et al.* [6] partitioned the methanol extract between ethyl acetate–water, and *n*-hexane–methanol, followed by repetitive column chromatography (six times) on

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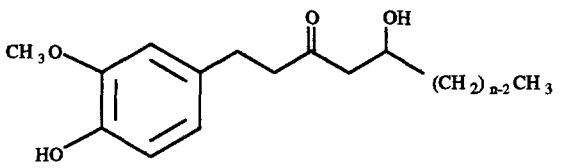
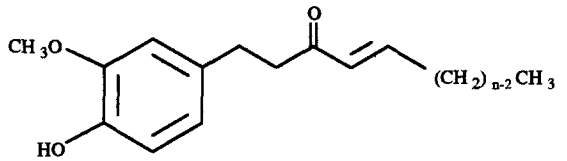


[n]-Gingerol



[n]-Shogaol

Fig. 1. Molecular structure of gingerol and shogaol.

silica gel eluted with benzene–ethyl acetate, while Farthin and O'Neill [7] used the same liquid–liquid extraction [6], followed by counter current chromatography, which gave good separation of the gingerol homologues.

In this paper a comparison is made between flash and vacuum column chromatography and their effectiveness assessed in separating the gingerols the main components of the pungent principle of *Zingiber officinale* Roscoe.

EXPERIMENTAL

Preparation of extract (stock solution)

Freeze-dried ginger rhizome powder (20 g) was extracted with acetone (analytical-reagent grade; BDH, Poole, U.K.) according to the method described by Spiro and Kandiah [8]. The acetone extract was evaporated to dryness on a water bath at 35°C using a rotary evaporator and the residue dissolved in 50 ml of 80% methanol. The resultant solution was subjected to liquid–liquid extraction 3 times with 50 ml hexane. The hexane fraction which contains mainly waxy materials, volatile oils and higher amount of shogaols than in the ether phase, was analysed on thin-layer chromatography (TLC) (see Fig. 3) and then discarded. To the 80% methanol fraction, a mixture of diethyl ether and water (2:1, v/v) was added to produce two layers, the organic and aqueous phases. The pungent principles were mainly present in the ether fraction which was then dried with anhydrous sodium sulphate, evaporated to dryness as before, dissolved in 10 ml metanol and stored as the stock solution.

Thin-layer chromatography

TLC was used to evaluate the products of fractionation and eluates of flash and vacuum column chromatography. TLC was carried out on Silica gel 60, 20 × 20 cm plates, layer thickness 0.2 mm (Merck No. 5553, Darmstadt, Germany), under saturated conditions in a glass tank (Panglas, Shandon, TLC chromatank, Runcorn, UK). The solvent system was toluene–methanol (80:5), a variation of that of Bhagya [9]. Visualization of the compounds on the plates was achieved after spraying with Folin–Ciocalteu reagent (BDH). Using this method gingerols, which constitute the main part of the pungent principle in ginger, displayed R_F values of 0.24–0.29 and shogaols, the minor component, showed R_F values of 0.45–0.50 as shown in Fig. 3.

Flash chromatography

The equipment used for flash chromatography was a glass chromatography column 135 cm in length with an inside diameter of 2.0 cm, supplied by Aldrich (Gillingham, UK), as decribed by Still *et al.* [3]. To prepare the column 9.5 g of silica gel 60 (40–60 μm, Merck 9385) was added slowly while tapping continuously to produce even packing, to give and effective column length of 15 cm, then an 0.8-cm layer of 40–100 mesh sand (BDH) was carefully placed on the flat top of the dry gel bed. Initially, 40 ml of the solvent system, as used for TLC, was added to the column and pressure applied from a cylinder containing nitrogen to remove the solvent from the silica, remove any trapped air and produce a compact column. Finally, an extra 200 ml of solvent was run through, to achieve an even compact column. To the column a 2-ml sample, obtained by evaporating 2 ml of stock solution in methanol in a stream of nitrogen and redissolving the residue in 2 ml of eluent, was applied. The sample was drawn into the silica by applying pressure from a nitrogen

cylinder. After the whole sample had been adsorbed by the column, 200 ml of eluent was added, pressure applied to produce a flow-rate of 50 mm per min (read from the decrease in the level of the solvent above the column bed) and 20 fractions of a volume of 10 ml collected. The compounds in each fraction were analysed by TLC following the method described above.

Vacuum chromatography

A Buchner flask (100 ml) fitted with a sintered filter funnel with an inside diameter of 3.5 cm was connected to a water pump. To the funnel, a similar amount (9.5 g) of silica gel 60, as used in flash chromatography, was added slowly to obtain an evenly packed silica column, 3 cm in length.

A 3-ml volume of the stock solution of ginger extract was added to 2 g of silica gel 60 and dried by means of a rotary evaporator over a warm water bath at 35°C. The dried powder containing the sample was then spread evenly over the top of the silica column. A piece of filter paper (Whatman No. 1, Maidstone, UK) with the same diameter as the inside diameter of the funnel was placed on top of the sample to prevent damage to the column during addition of the solvent. Hexane (25 ml) was added slowly, and the solvent allowed to penetrate the whole column, then the atmosphere in the flask was sucked out to facilitate rapid elution and the eluent collected as the first fraction. Further elution was carried out using 20 aliquots of 25 ml of solvent mixtures with increasing polarity, composed of hexane–diethyl ether and diethyl ether–methanol, as shown in Fig. 2. The individual eluates of 25 ml were transferred to a test tube, evaporated to a volume of 10 ml in a stream of nitrogen and then analysed by TLC.

RESULTS AND DISCUSSION

Preliminary separation

The TLC chromatograms of the fractionation

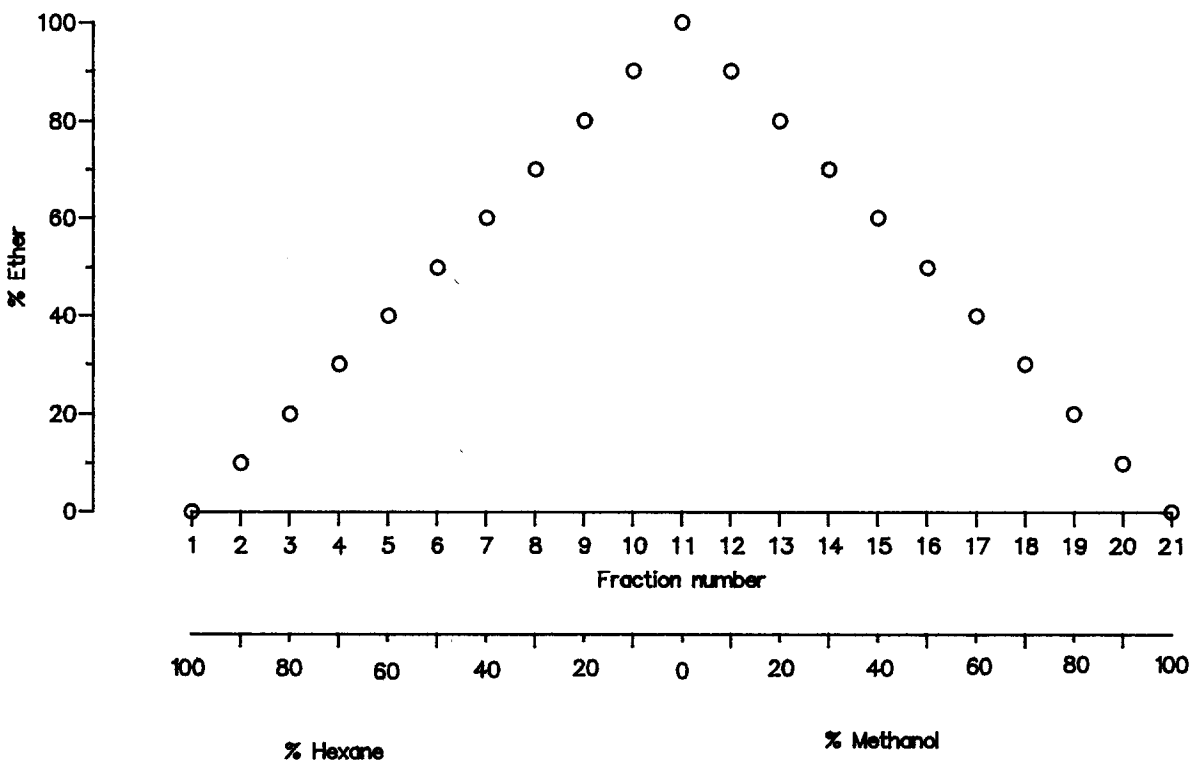


Fig. 2. Composition of the solvent system used in vacuum chromatography (21 different aliquots).

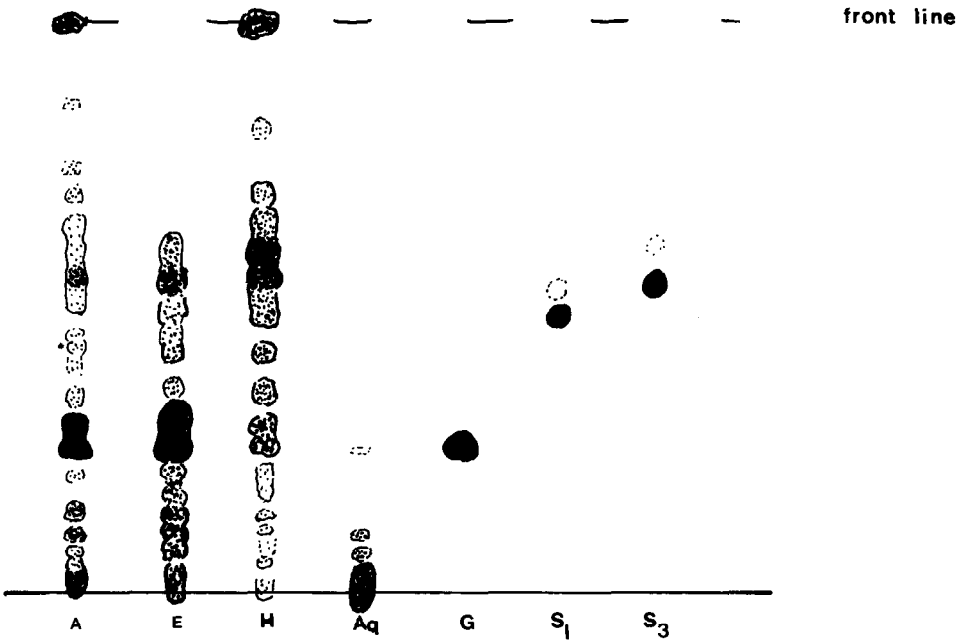


Fig. 3. TLC of the fractions obtained from the liquid–liquid extraction. A = total acetone extract (5 μl), B = ether phase (10 μl), H = hexane phase (10 μl), Aq = aqueous phase (10 μl), G = [6]-gingerol (2 μl = 70 μg), S_1,S_3 = shogaol homologues (2 μl = 25 μg). The amount of sample loaded is indicated in brackets, as for Figs. 4–6.

products are presented in Fig. 3. As predicted, the pungent principles are present mainly in the ether fraction, with a higher amount of shogaol in the hexane phase. In the aqueous fraction, compounds which react with Folin–Ciocalteu reagent were also present. These compounds, however, are unlikely to be the pungent components of ginger, since they do not run far from the origin in the TLC system used.

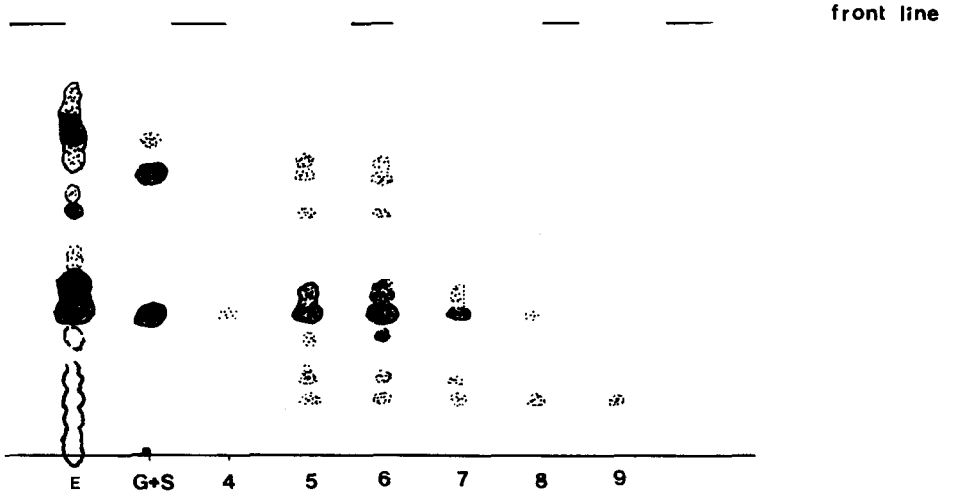


Fig. 4. TLC of the 10-ml fractions obtained using flash chromatography with toluene–methanol (80:5) as solvent. Numbers indicate the different fractions collected (10 μl), E = ether phase (10 μl), G + S = [6]-gingerol (2 μl = 70 μg), shogaol (2 μl = 25 μg). Only fractions which were known to contain the compounds under investigation were loaded onto the TLC plates.

Flash chromatography

This is a moderate-resolution, preparative technique usually carried out in a column-overload condition, using the same solvent system as with TLC. It has been reported that compounds wich differ in R_F value by only 0.15 can be separated satisfactorily using flash chromatography [3,5]. Therefore, it was expected that, using this technique, gingerols the major components of the pungent principle in ginger and shogaols, would be clearly separated because the difference between their R_F values is greater than 0.15 (*ca.* 0.20–0.25).

The results, however, presented in Fig. 4 show that most of the components of the pungent principle (gingerols, shogaols) were present in the same fractions (5 to 7). Overloading does not seem to be the cause, because the loading capacity of the column used [3] is approximately 400 mg, while the amount of sample loaded was less than 200 mg. Different lengths of the bed column (10–15 cm) and different flow-rates (50–100 mm per min) were tested, as well as the solvent system hexane–diethyl ether (30:70) employed in vacuum chromatography which gave clear separation of the gingerols. However, less satisfactory results were obtained as all the components of the pungent principle appear in fractions H_2–H_4 (see Fig. 6). The silica gel employed for the development of the TLC was 15 μm (Merck No. 5553), while for flash chromatography it was 40–60 μm. (Merck No. 9385). If the silica gel used for both forms of chromatography had been the same, as suggested by Majors and Enzweiler [5], it would have simplified comparison of the results. It is also possible that the number of compounds present in the plant extract may also affect the efficiency of the column.

Vacuum chromatography

It was surprising that although the technique of vacuum chromatography is much simpler than flash chromatography, the main pungent components of ginger, the gingerols, are almost completely resolved from the shogaols, present in fractions 8 and 9 (see Fig. 5). Using this technique the length of the column was only 3 cm, much shorter than the column used for flash chromatography (15 cm), with a wider inside diameter of the column which provides a high loading capacity. It appears that the solvent

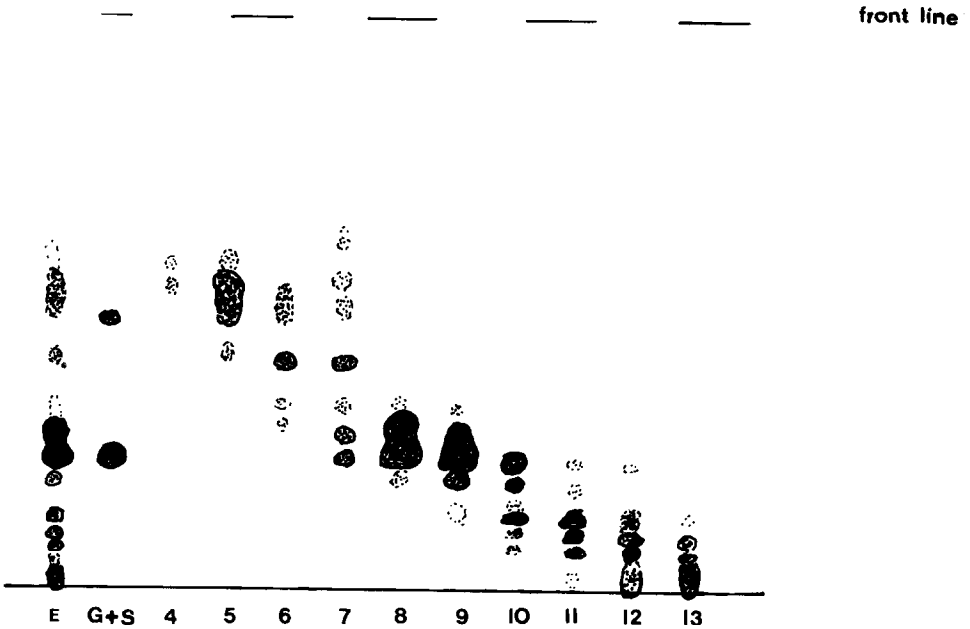


Fig. 5. TLC of the 10-ml fractions obtained using vacuum chromatography, when a solvent gradient was employed. Numbers indicate the different fractions collected, 4–13 (10 μl); E = ether phase (10 μl); G + S = [6]-gingerol (2 μl = 70 μg); shogaol (2 μl = 25 μg). Only fractions which were known to contain the compounds under investigation were loaded onto the TLC-plates.

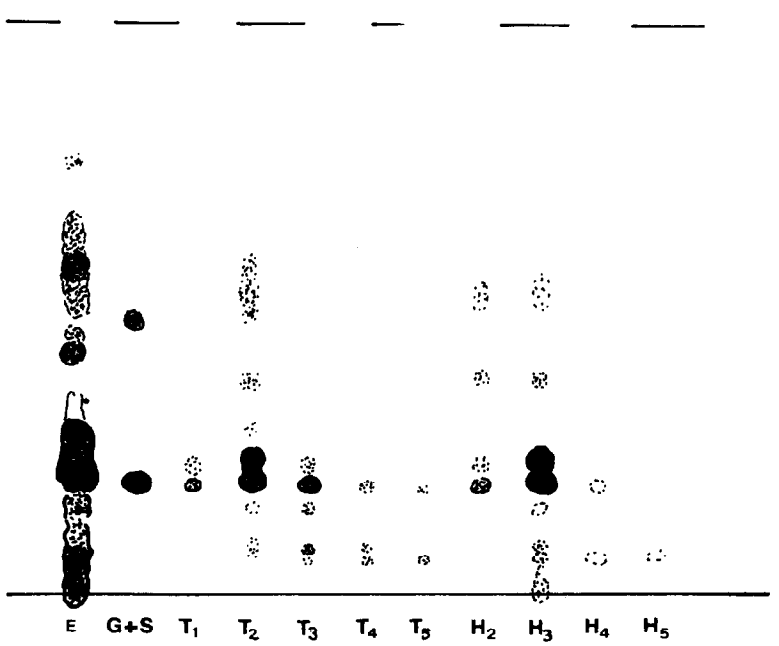


Fig. 6. TLC of the 10 ml fractions obtained using flash chromatography with hexane–diethyl ether (30:70) as solvent system, fractions H_2–H_4 (10 μl) and the 10-ml fractions collected in vacuum chromatography when an isocratic solvent system toluene–methanol (80:5) was used, fractions T_1–T_5 (10 μl); E = ether phase (10 μ); G + S = [6]-gingerol (2 μl = 70 μg); shogaol (2 μl = 25 μg). Only fractions which were known to contain the compounds under investigation were loaded onto the TLC plates.

gradient plays a major part in this chromatographic technique. Indeed, isocratic elution using the solvent system developed for TLC has also been tried. However, a satisfactory separation was not achieved (see Fig. 6). Similar results were obtained in an attempt to isolate the steroid (23,24-dimethylcholesta-5,22-dien-3β-ol) from a petroleum ether extract of *Cresentia cujete* bark (unpublished observation). It is unlikely that the method of application of the sample to the column has any significant effect on the efficiency of separation because the results presented in Fig. 6 (fractions T_1–T_5) show that the separation achieved using an isocratic system was inferior to that with a gradient (see Fig. 5) with the same loading arrangement used for both solvent systems.

Of course, solvent gradients can also be employed in flash chromatography, but then the technique becomes long and tedious, with the simple equipment employed for the above experiments. These results suggest that a number of advantages are offered by vacuum column chromatography. 1) The equipment is of low cost and easily assembled using components available in most laboratories. 2) A solvent system can easily be developed by mixing two solvents with different polarities in different proportions. 3) This method may also be used for purification by increasing the polarity of the gradient more slowly and eluting smaller volumes for each fraction.

A much more complicated system of vacuum liquid chromatography has been developed by Targett *et al.* [4] using a longer column, maintained under vacuum, with isocratic elution as for flash chromatography. Therefore, results similar to those of flash chromatography might be expected since isocratic elution is used, but would appear to be less favourable for a preliminary separation of the component of plant extracts.

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