A STUDY OF THE PHOTOTOXICITY OF ORANGE FLOWER ABSOLUTE

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ABSTRACT

We have confirmed the phototoxicity of orange flower absolute, studied the phototoxic ingredient and developed a method to eliminate it. To confirm the phototoxicity, we tested French orange flower absolute, Moroccan absolute and Egyptian absolute with Hartley strain guinea pig under UV irradiation ranging from 320-400 nm supplied with fluorescent lamps.

Using a combination of isolation techniques including HPLC and IR, we were able to confirm that the phototoxic agent was bergapten. The development of a non-phototoxic orange flower absolute using ion exchange chromatography is described.

1. INTRODUCTION

It is well known that bergamot expressed oil, lime expressed oil, bitter orange oil and other cold pressed oils are phototoxic. However, there is no report that orange flower absolute is phototoxic. Orange flower absolute is an important natural oil which is used for the formulation of perfume and cosmetic products. As a result, it is important to know whether orange flower absolute is phototoxic or not.

In this study, we confirmed the phototoxicity of orange flower absolute and identified the derivatives bergapten and bergamottin epoxide, the former of which is considered to be responsible for its phototoxicity This consideration was made because bergapten is a well known phototoxic substance, so we assumed that it is the phototoxic substance in orange flower absolute.

To eliminate the phototoxic substances found in orange flower absolute, vacuum distillation, column chromatography and ion exchange chromatography were performed.

2. EXPERIMENTAL

2-1. Identification of Phototoxicity

a. Materials: Egyptian orange flower absolute, French orange flower absolute, Moroccan orange flower absolute, Italian bergamot oil, 8-methoxy psoralen.
b. Animal: Hartley strain guinea pig (400 g)
c. Light sources: Toshiba FL-40S BLB lamp (Toshiba Co.)
d. Light Measurement: Topcon (Model UVR-365)
e. Procedure: 0.02 ml test solution (acetone solution) was topically applied to two sites on the shaven and depilated dosal skin. The half row was covered with aluminum foil. The other was exposed to UV irradiation ranging from 320 to 400 nm supplied with a fluorescent lamp for 40 to 60 min (1.41×10^8 ergs/cm^2). Guinea pigs were exposed to UV Irradiation under the bank of six fluorescent lamps. The concentration of test materials were 10% in accetone.
f. Evaluation: The evaluation of skin reaction was done after 48 hours of irradiation by the following criteria.

Evaluation of Skin Reaction

Erythema		Edema	
No erythema	0	No edema	0
Very slight erythema	0	Slight edema	1
Well defined erythema	2	Moderate edema	2
Moderate erythema	3	Severe edema	3
Severe erythema	4		

$$\text{Fraction Number} = \frac{\text{Number of Positive reactions}}{\text{Number of Subjects}}$$

$$\text{Average Score} = \frac{\text{Total Score of Reaction Intensity}}{\text{Number of Subjects}}$$

Table 1. Phototoxicity of Orange Flower Absolute

Sample (10% in acetone)	Content in Oil Bergapten (ppm)	Phototoxicity UV (+)	UV (–)
Egyptian	2650	9/9 (3.7)*	0/9 (0.0)
French	545	10/10 (1.0)	0/10 (0.0)
Moroccan	680	10/10 (3.3)	0/10 (0.0)
Bergamot oil	2180	10/10 (3.5)	0/10 (0.0)
8-methoxy psoralen (200 ppm)		2/2 (5.5)	0/2 (0.0)

Legend: * = parentheses indicate the average score.

Above results show that orange flower absolute has phototoxicity.

2-2. Identification and Removal Procedure of Phototoxic Agent

A schematic diagram of the methods used to remove the phototoxic agent of orange flower absolute can be seen in Figure 1.

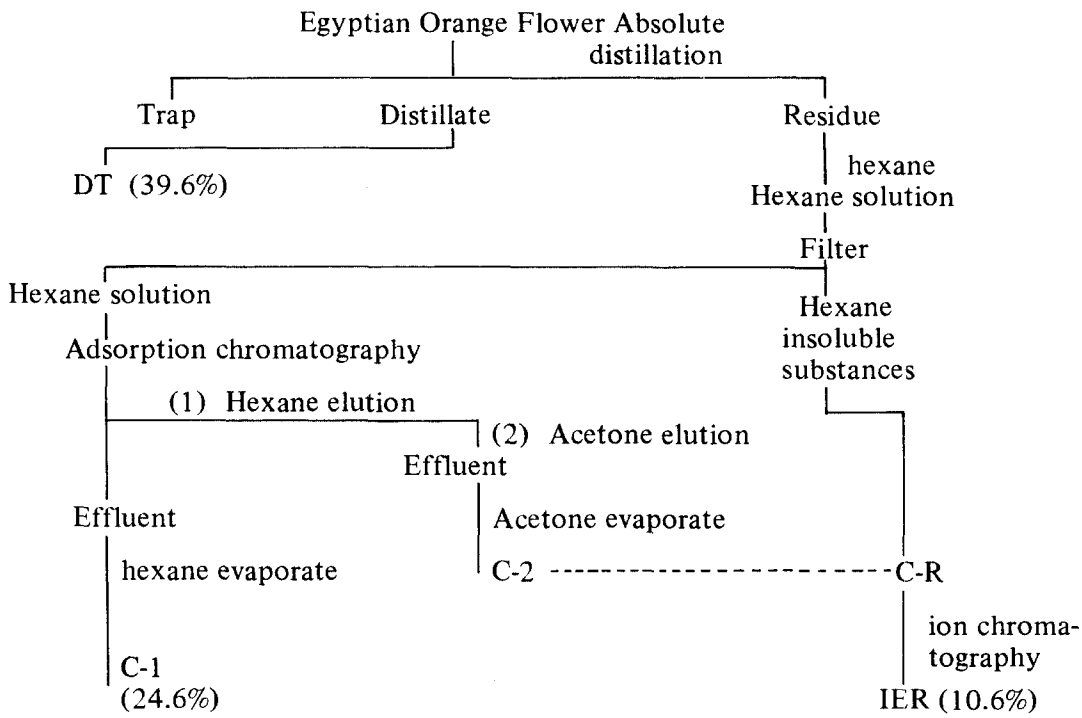

Figure 1. The fractionation of Egyptian orange flower absolute

As can be seen in Figure 1, the distillate (DT) and residue were obtained through distillation from 25°C to 150°C under the reduced pressure of 0.3 mm Hg. The residue was dissolved in hexane, to separate the hexane soluble substances from the hexane insoluble substances. Column chromatography (Amberlite XAD-7) was used to separate polar substances (C-2) and non-polar substances (C-1) from hexane soluble substances.

Polar substances obtained from hexane soluble substances were added to hexane insoluble substances obtained from distillation residue. We called this mixture C-R. We tested the phototoxicity of the mixture of C-1 and DT; the results are shown in Table 2.

Table 2. Phototoxicity of Egyptian Orange Flower Fractions

Sample (10% in acetone)	Phototoxicity UV (+)	UV (-)
DT + C-1	0/10 (0.0)*	0.10 (0.0)
DT + C-1 + IER	0/9 (0.0)	0.9 (0.0)
Bergamottin epoxide	0/9 (0.0)	0/9 (0.0)

Legend: * = parentheses indicate the average score.

As we can see from the data presented in Table 2, the mixture of C-1 and DT is not phototoxic. Consequently, we can conclude that the phototoxicity is concentrated in the C-R fraction.

By HPLC using a 8mm x 25cm Develosil column, a hexane-tetrahydrofuran (80:20) eluent with a flow rate of 2 ml/min and the UV detector setting on 310 nm on each fraction, bergapten and bergamottin epoxide were both detected in the complete Egyptian orange flower absolute, the C-R fraction but not in fractions DT and C-1. Bergapten and bergamottin epoxide were isolated by preparative HPLC from fraction C-R and identified by comparison of their spectra with the IR and NMR spectra of authentic samples. An authentic sample of bergapten was isolated from bergamot oil and that of bergamottin epoxide was obtained by oxidation of bergamottin which was also isolated from bergamot

oil. Bergamottin epoxide was found to be non-phototoxic as shown in Table 2.

To eliminate bergapten and bergamottin epoxide from C-R, ion exchange chromatography (Amberlite A-27) was used as shown schematically as follows:

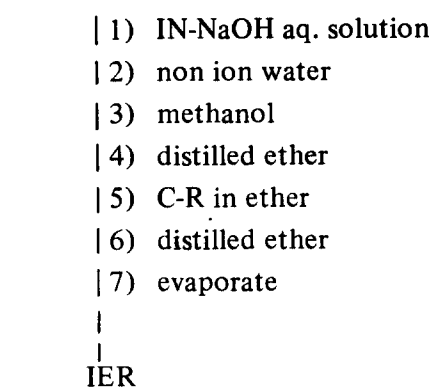

Fig. 2. Ion exchange chromatography

By using HPLC, we confirmed that there is no bergapten or bergamottin epoxide in IER fraction. We added IER fraction to the mixture of C-1 and DT and we tested the phototoxicity of the mixture of IER, D-1 and DT. As can be seen in Table 2, the phototoxic reaction was not found in the mixture of IER, C-1 and DT.

We show the gas chromatogram of the mixture of IER, C-1, and DT and Egyptian orange flower absolute in Figures 3 and 4 respectively. A comparison between the chromatograms presented in Figures 3 and 4 shows that the mixture is similar to the complete orange flower absolute.

3. CONCLUSION

We confirmed the phototoxicity of orange flower absolute and identified bergapten as the phototoxic agent of orange flower absolute. Bergamottin epoxide was also identified. To remove these furocoumarins and obtain non-

phototoxic orange flower absolute, we used vacuum distillation, column chromatography and ion exchange chromatography. The yield of non-phototoxic orange flower absolute was 74.8 percent, as can be seen in Figure 1. A product approaching orange flower absolute was obtained. If closer matching in aroma is desired, compounding techniques would have to be employed.

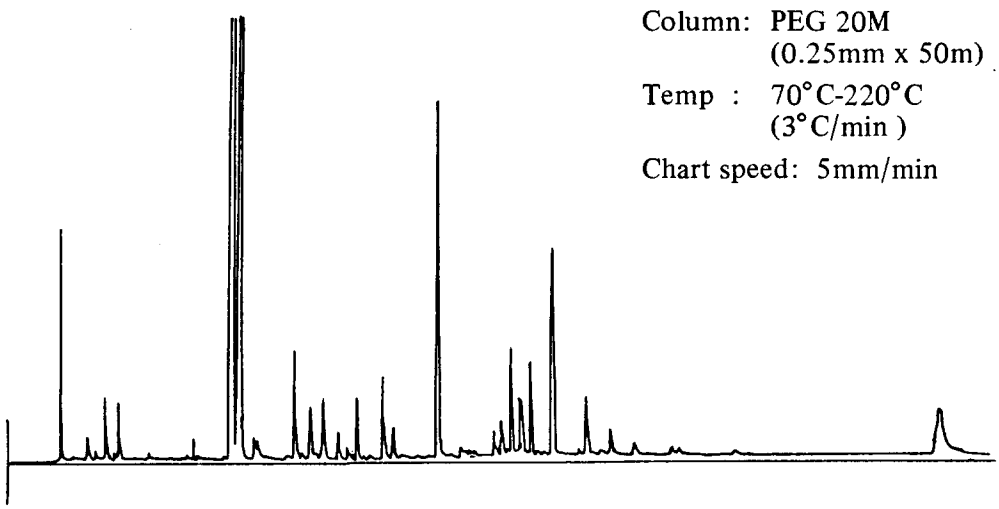

Figure 3. Gas chromatogram of orange flower absolute

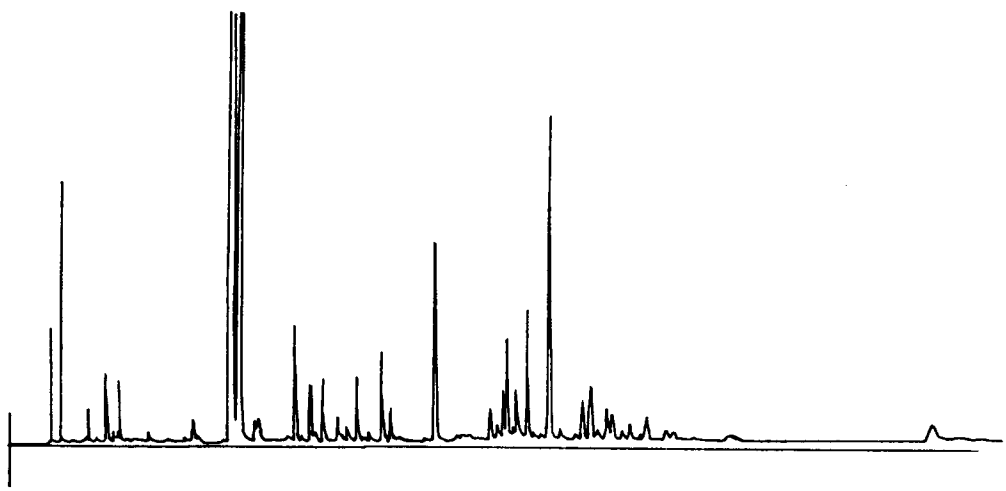

Figure 4. Gas chromatogram of IER, C-1 and DT