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In vitro study on the transfer of volatile oil components

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Abstract

The following volatile oils were tested in vitro: chamomile (*Matricaria recutica* L.), peppermint (*Mentha piperita* L.) and sage (*Salvia officinalis* L.) to obtain information on which components of volatile oils or minerals are able to pass through the membranes under different conditions. The transfer of chamomile and peppermint oil from aqueous volatile oil to the stomach (pH = 1.1) and then to the plasma (pH = 7.5) was studied, and the transfer of sage oil through the skin (from pH = 5.5 to pH = 7.5) was examined. The transfer of some components was more favorable than that of others. The transfer of chamomile oil was faster to buffer pH = 1.1 than from buffer pH = 1.1 to buffer pH = 7.5 and most of the components, except for chamazulene, passed through the membranes. In the case of peppermint the components went through the membranes in the first 15 min although the main components mostly remained in the initial solution. The sage oil transferred showed the same characteristics as the starting oil. A small amount of metal present in the volatile oils also passed through the membranes. The transfer of metals varied, depending on the time, type of the oil, metal quality and the conditions applied.

Keywords: Volatile oil; *Matricaria recutica* L.; *Mentha piperita* L.; *Salvia officinalis* L.; In vitro transfer

1. Introduction

Volatile oil containing drugs and volatile oils have been used for a long time both in folk medicine and in therapeutics. Recently, the use of volatile oils has increased, since some part of folk medicine, for instance therapy with aromatic materials, depend mainly on the type of oil applied. The therapeutic effect of many volatile oils has already been proved [1,2].

The application of volatile oils can be internal or external. In gastroenterology volatile oils are often used for their appetizing, choleric, anti-spasmodic, anti-inflammatory, etc., effect [3–6]. The components of oils, due to their lipid solubility, can easily pass through the membranes of the mouth, the nose, the respiratory system and through the skin. After percutaneous administration of volatile oil, the components of oils appear in the air exhaled [2].

In spite of the great significance of volatile oil in phytotherapy and in medical science, it has not been examined so far whether the different com-

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ponents are able to pass through the different membranes. It has not been clarified either what components of the volatile oil are able to penetrate and show biological activity.

Most aromatic components of the volatile oils examined were terpene derivatives: chamazulene, (−)-α-bisabolol, α-farnesene, β-farnesene, matricin in chamomile [7–9], (−)-menthol, (+)- and (−)-menthone, (+)-isomenthone, (+)-neomenthone, (+)-menthofuran, eucalyptol and other monoterpenes in peppermint [10], borneol, bornyl acetate, α-pinene, β-pinene, α-thujone, β-thujone, myrcene, eucalyptol, cineol as well as other mono- and sesquiterpenes in sage [11,12].

The therapeutic effect of oil components differs and the effect varies as a function of time [13]. There is hardly any data on absorption of oil components in the human body and on the effect of the different solutions (acidic moiety, plasma) on the terpene derivatives. Since the components of volatile oils differ in their number of double bonds and functional groups, a certain order of transfer may evolve during the absorption.

In our experiments the absorption of the following volatile oils was tested in vitro: chamomile (*Matricaria recutica* L.), peppermint (*Mentha piperita* L.) and sage (*Salvia officinalis* L.) in order to determine the components of volatile oils or minerals capable of passing through the membranes under different conditions.

2. Experimental

2.1. Materials

The volatile oils of chamomile (*M. recutica* L.): Aetherolum chamomillae, peppermint (*M. piperita* L.): Aetherolum menthae piperitae and sage (*S. officinalis* L.): Aetherolum salviae were purchased from the commercial network.

2.1.1. Buffer solutions

Stomach (pH = 1.1): 1 N HCl (94 g), NaCl (0.35 g) and glycocoll (0.5 g) in water (1000 ml). Plasma (pH = 7.5): Na_2HPO_3 (20.5 g) and KH_2PO_4 (2.8 g) in water (1000 ml). Skin (pH = 5.5): Na_2HPO_3 (1.5 g) and KH_2PO_4 (8 g) in water (1000 ml). ICP standards were purchased from Merck.

2.2. Methods

2.2.1. Stability measurements

The oil sample (1 g) was allowed to remain for 2 h in a buffer solution in an ultrasound water bath.

2.2.2. Transfer of volatile oils in vitro

The transfer of chamomile and peppermint oil from aqueous volatile oil to the stomach (pH = 1.1) and from the stomach to plasma (pH = 7.5) was studied on a membrane diffusion model (Fig. 1) at 37°C. Volatile oil (0.5 g) was dissolved in water or in acidic solution (10 ml) and the solution was passed into an inside container. The outside container was filled with 100 ml of buffer solution (pH = 1.1 or 7.5). Fractions (10 ml) were taken from the outside container at the following times: 15, 30, 60 and 120 min. The aliquot taken was supplied with buffer solution (10 ml, pH = 1.1 or pH = 7.5 depending on the experiment).

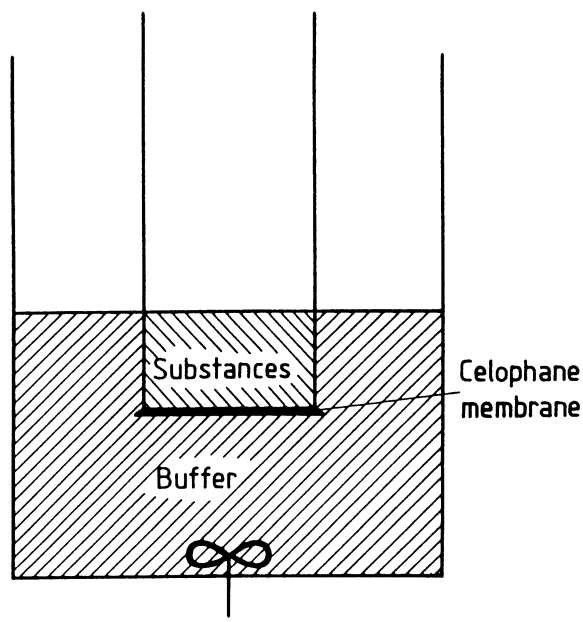

Fig. 1. Diffusion model

The transfer of sage oil through the skin from pH = 5.5 to pH = 7.5 was examined (Sartorius SM 16750) as follows: The volatile oil (1 g) was dissolved in a buffer solution (pH = 5.5, 10 ml) and the solution was placed in the equipment. Fractions (10 ml) were taken at the following time periods: 15, 30 and 60 min.

2.2.3. Measurements of organic components:

Quantitative analysis of the volatile components was carried out by gas chromatography and gas chromatography-mass spectrometry (GC-MS). Sample preparation: the fraction (10 ml) was extracted with petroleum ether (boiling range 30–40°C) and the organic solution was concentrated to 1 ml.

Gas chromatograhic measurements were carried out with a Bush 610 instrument equipped with a capillary silicon column (Quadrex GC, column number 51130D, 25 m, 0.32 mm ID). Temperature program: 2 min. at 60°C, then heated at 8°C/min, up to 230°C.

GC-MS parameters were as follows: Finnigan GCQ instrument, column: 30 m, ID: 0.22 mm, film thickness: 0.25 mm, stationary phase: BPX5 (non polar), column temperature: 60°C, heated at 8°C/min., 230°C isotherm for 3 min, carrier gas: helium at a linear velocity of 40 cm/s, injector temperature: 200°C, injected solution: 0.4 μl, Finnigan MS detector, MS parameters: start: 3 min after injection, electron-impact-ionisation (E^+) mode, mass range 40–650, scanning rate 1 analysis/s.

Evaluation of the results was made according to Finningan GCQ 2.2 software.

2.2.4. Measurements of inorganic components

Concentrations of the elements of samples were determined by on a inductively coupled plasma optical emission spectrometer (ICP-OES). Type of instrument: Atom Scan 25 (Thermo Jarrell Ash), a sequential plasma emission spectrometer. Sample preparation for element measurement: the samples (0.5 g oil and 10 ml of evaporated solution) were digested with HNO_3 (5 ml) in a microwave digestion machine (MarsX, CEM). After digestion, the samples (three parallel) were diluted to 10 ml, from which the following elements were determined in three parallel measurements: Al, Ca, Cr, Cu, Fe, K, Mg, Mn, P, S and Zn.

Mean values and S.D. were calculated from parallel measurements.

3. Results and discussion

3.1. Stability measurements

In order to examine the behaviour of oils under different conditions, the oils were allowed to stand in different buffer solutions. After a lapse of 2 h chamomile in the acidic buffer solution showed no change, while in plasma pH the ratio of the oil components changed. The amount of α-bisabolol, the main component of the original chamomile oil, decreased and the amount of chamazulene increased as was verified by gas chromatographic measurements (Fig. 2).

In the case of peppermint oil similar results were found as above. After 2 h in acidic buffer solution neither the components nor the ratio of components changed. Upon the effect of basic buffer solution, the menthol component totally disappeared and a new component, isomenthone, appeared which was only a trace component in the original oil (Fig. 3). The components of oils were also identified by GC/MS.

Sage oil kept in buffer solution (pH = 5.5 and pH = 7.5) showed no change. The chromatogram of sage oil is shown in Fig. 4.

3.2. Transfer of total amount of volatile oil

In vitro transfer of volatile oil was examined first by gravimetry. The transfer of chamomile and peppermint oil from aqueous volatile oil to buffer pH = 1.1 and then from buffer pH = 1.1 to buffer pH = 7.5 was studied. The transfer of sage oil from buffer pH = 5.5 to buffer pH = 7.5 was examined. The results show that the transfer of chamomile oil to the acidic moiety is faster than its transfer from buffer pH = 1.1 to buffer pH = 7.5. The transfer was steady in both experiments (Fig. 5). In the case of transfer from aqueous solution to buffer pH = 1.1, 36.4% of the initial amount of volatile oil passed through the mem-

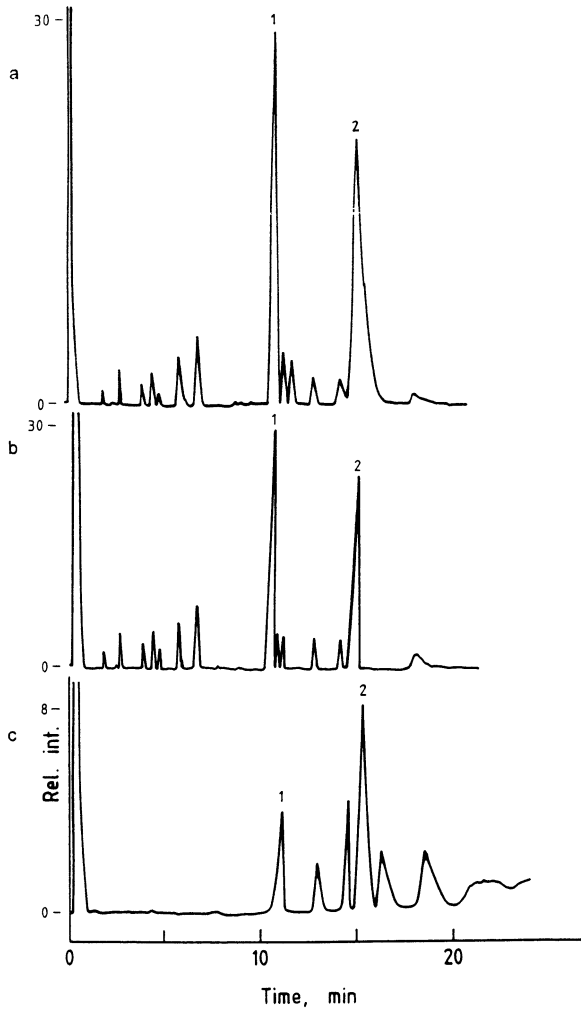

Fig. 2. Chromatogram of chamomile oil (a) and after being kept at different conditions for 2 h: pH = 1.1 (b) and pH = 7.5 (c). 1, α-Bisabolol; 2, chamazulene.

brane, while this value was found to be 13.7% in the case of transfer from buffer pH = 1.1 to buffer pH = 7.5.

First the transfer of peppermint oil from the aqueous solution to buffer pH = 1.1 is relatively fast. After 60 min a higher amount of oil is transferred from buffer pH = 1.1 to buffer pH = 7.5 (Fig. 6). Only about 10% of the initial amount of oil could pass through the membranes: 11.8% from the aqueous solution to buffer pH = 1.1 and 12.9% from buffer pH = 1.1 to buffer pH = 7.5.

The transfer of sage oil from pH = 5.5 to pH = 7.5 was examined in a Sartorius instrument. Only a small portion of the initial oil (6.1%) passed through the membrane (Fig. 7) in steady transfer.

3.3. Transfer of volatile oil components

The transfer of some components was found to be more favorable than that of other components. The effect of volatile oils also depends on both the

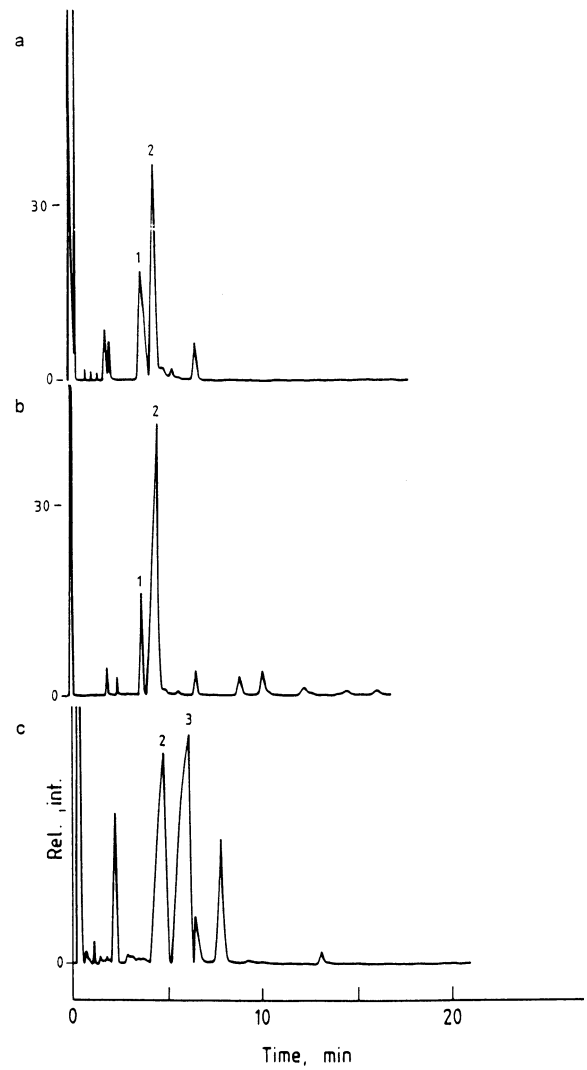

Fig. 3. Chromatogram of peppermint oil (a) and after being kept at different conditions for 2 h: pH = 1.1 (b) and pH = 7.5 (c). 1, Menthol; 2, menthone; 3, isomenthone.

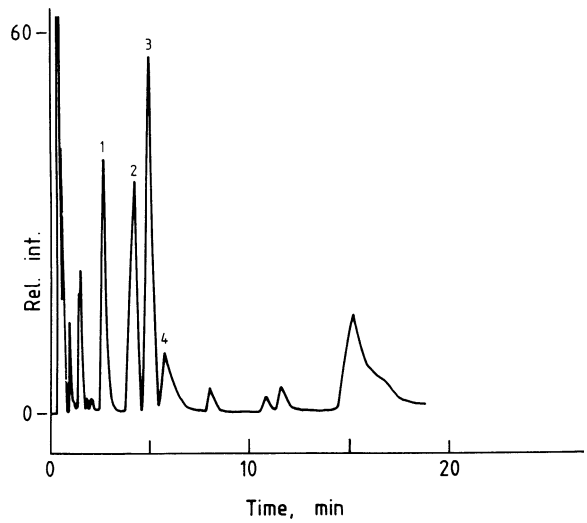

Fig. 4. Chromatogram of sage oil. 1, Eucalyptol; 2, α-thujone; 3, β-thujone; 4, champora.

amount and ratio of components. A change in the components, naturally, involves a change in the biological effect.

The transfer of some chamomile oil components is more favorable to buffer pH = 1.1 than from pH = 1.1 to pH = 7.5 and most of the components, except for chamazulene, passed through the membranes. The pattern of the chromatogram of chamomile volatile oil transferred from aqueous solution to pH = 1.1 was equal to that of the initial oil, although the relative intensity of the components decreased against time. The chromatogram of the oil transferred from a buffer solution of pH = 1.1 to pH = 7.5 in the first 15 min was similar to that of the original oil. Later, the amount of α-bisabolol decreased and other minor components appeared in the oil transferred. The residual oil contained almost only chamazulene. According to the above, chamomile oil exerts its effect at two different sites. α-Bisabolol transferred to the plasma may have an antispasmodic effect on muscle cells [14] and the residual chamazulene may exert an antiflogistic effect in the stomach [5].

In the transfer of peppermint oil from aqueous solution to pH = 1.1 steady penetration of the characteristic components could be observed (in a similar ratio to that in the original oil), while the residue contained mostly main components. The chromatogram of the oil transferred from the

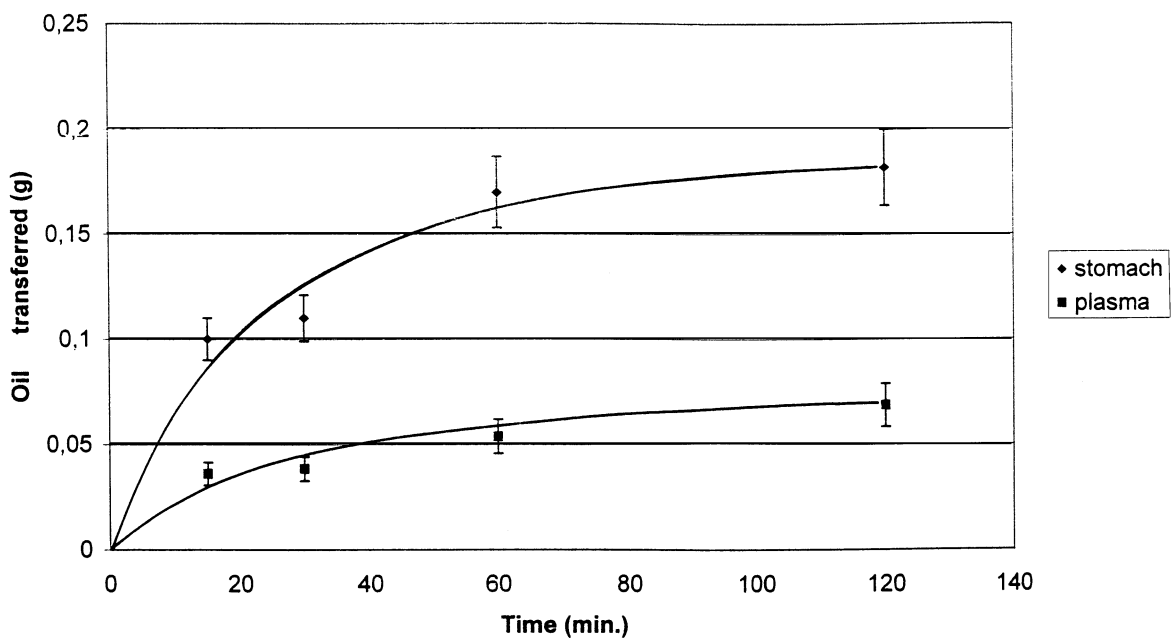

Fig. 5. Transfer of chamomile oil to the stomach and from the stomach to plasma.

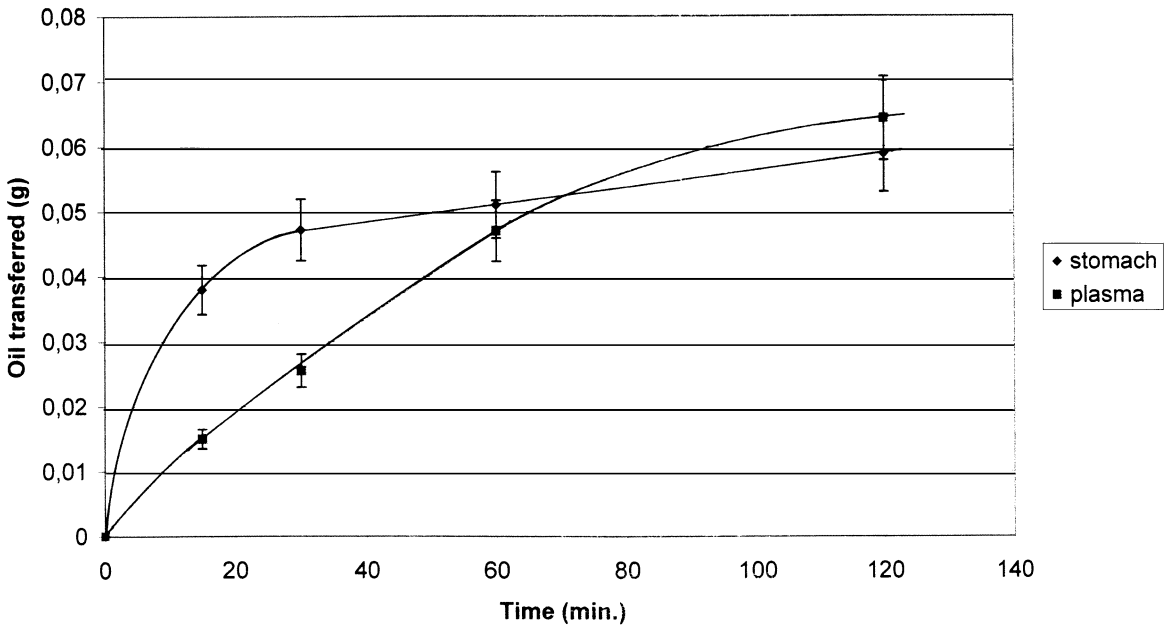

Fig. 6. Transfer of peppermint oil to the stomach and from the stomach to plasma.

stomach to the plasma in the first 15 min was nearly identical than that of oil kept in a buffer solution of pH = 7.5. The menthol component disappeared and isomenthone appeared. It may be concluded, therefore, that the effective components are menthone and isomenthone.

The transfer of sage oil was examined by resorption from pH = 5.5 to pH = 7.5. The chromatogram of the sage oil transferred showed similar spectra to that observed for the initial oil. The only difference was a slight change in the ratio of the components and that no eucalyptol could be detected.

The metal ion content of oils and the fractions were measured by ICP-AES. Element concentration in the oils was found to be relatively low (Table 1). In most cases element concentration of the fractions was below the detection limit and evaluable results were obtained only for calcium and magnesium. It has also been determined that a small amount of metals present in the volatile oils passed through the membranes. The transfer of metals changed as a function of time depending also on the type of oil, the quality of the metal and the conditions applied (Table 2). As can be seen in Table 2, volatile oils affect the penetration of ions through the membranes [15]. Contraction of the smooth muscle cells requires increased calcium concentration

4. Conclusion

Although the experiment was carried out with the use of a simple diffusion model, the results do not show only distribution based on diffusion.

Our membrane diffusion experiment and study with the Sartorius instrument are good models for the examination of volatile oil transfer. Although these in vitro investigations do not always cover processes in the human body, this method may offer some valuable information on the behaviour of oils and oil components under different conditions.

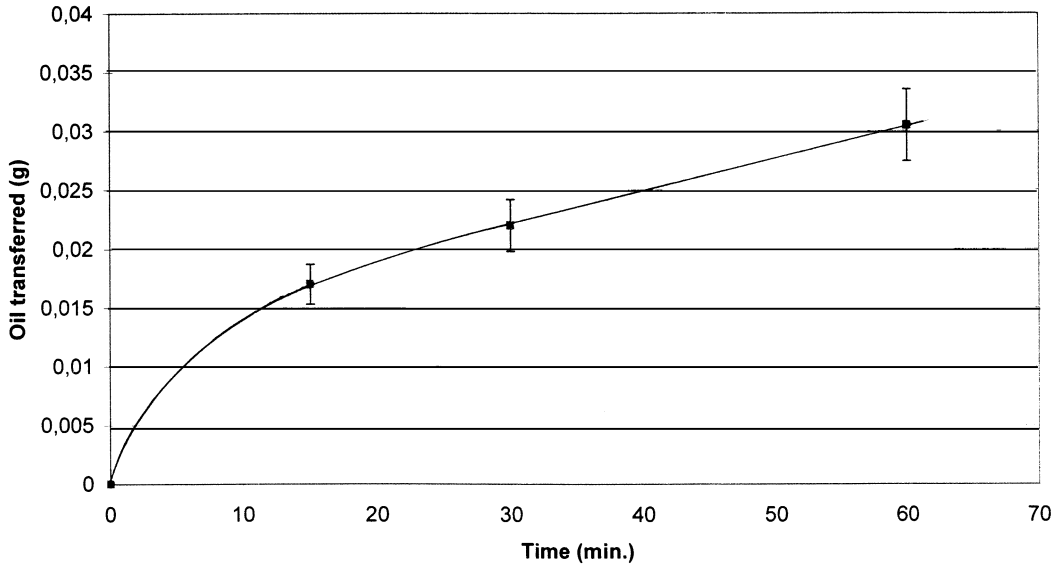

Fig. 7. Transfer of sage oil through the skin (from pH = 5.5 to pH = 7.5).

Table 1
Element content of oils in μg/g and ± S.D. ($n = 3$)

Elements	Chamomile	Peppermint	Sage
Al	<0.02	4.56 ± 0.37	<0.02
Ca	2.28 ± 0.005	6.86 ± 0.19	2.05 ± 0.56
Cr	<0.004	0.320 ± 0.058	0.047 ± 0.005
Cu	<0.002	<0.002	<0.002
Fe	0.182 ± 0.063	1.194 ± 0.007	0.401 ± 0.001
K	4.73 ± 0.77	2.745 ± 1.116	7.01 ± 0.69
Mg	1.37 ± 0.04	2.37 ± 0.02	0.868 ± 0.015
Mn	0.0087 ± 0.0005	0.0295 ± 0.0006	<0.001
Na	2.76 ± 0.16	37.15 ± 0.32	1.11 ± 0.08
P	2.50 ± 0.013	<0.05	1.26 ± 0.51
S	7.69 ± 0.61	10.9 ± 0.2	18.61 ± 1.5
Zn	0.248 ± 0.029	0.325 ± 0.005	0.204 ± 0.014

Table 2
Transfer of elements under different conditions in percentage of the initial oil

Element	15 Min	30 Min	60 Min	120 Min
Chamomile oil, from aqueous solution to pH = 1.1				
Ca	3.3	3.6	6.7	7.3
Mg	2.6	3.8	4.3	3.7
Chamomile oil, from pH = 1.1 to pH = 7.5				
Ca	4.7	5.1	6.6	7.4
Mg	<2.5	<2.5	<2.5	3.4
Mentha oil, from aqueous solution to pH = 1.1				
Ca	2.2	3.5	4.4	6.2
Mg	1.7	2.0	2.6	3.2
Mentha oil, from pH = 1.1 to pH = 7.5				
Ca	1.9	2.7	3.3	5.9
Mg	1.5	2.0	2.4	4.5
Sage oil, from pH = 5.5 to pH = 7.5				
Ca	<1.0	1.7	3.3	–
Mg	<2.0	<2.0	2.0	–

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