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# In vitro permeation through porcine buccal mucosa of *Salvia desoleana* Atzei & Picci essential oil from topical formulations

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**Abstract**

In the light of recent studies, which have shown that the essential oil derived from some Lamiaceae species has appreciable anti-inflammatory activity, moderate anti-microbial action and the ability to inhibit induced hyperalgesia, an assessment of the diffusion and permeation of *Salvia desoleana* Atzei & Picci (*S. desoleana)* essential oil through porcine buccal mucosa was considered useful for a possible application in the stomatological field. Topical formulations (microemulsions, hydrogels and microemulsion–hydrogels) were prepared for application to the buccal mucosa. The mucosa permeation of the oil from the formulations was evaluated using Franz cells, with porcine buccal mucosa as septum between the formulations (donor compartment) and the receptor phase chambers. The study also aimed at optimising the permeability of the *S. desoleana* essential oil by means of an enhancer, the diethylene glycol monoethyl ether Transcutol®. The diffusion of the oil through the membrane was determined by evaluating the amount of essential oil components present in the receiving solution, the flux and the permeation coefficient (at the steady state) in the different formulations at set intervals. Qualitative and quantitative determinations were done by gas chromatographic analysis. All the formulations allow a high permeability coefficient in comparison with the pure essential oil. In particular, the components with a terpenic structure (β-pinene, cineole, α-terpineol and linalool) have the highest capacity to pass through the porcine buccal mucosa when compared to the other components (linalyl acetate and α-terpinil acetate). Moreover, the enhancer, diethylene glycol monoethyl ether largely increases the permeation of the essential oil components in relation to the concentration. 

*Keywords:* *Salvia desoleana* Atzei & Picci essential oil; Buccal mucosa permeation; Enhancer effect; Diethylene glycol monoethyl ether; Transcutol®

## 1. Introduction

In previous studies (Peana and Satta, 1993; Peana et al., 1996, 1999; Moretti et al., 1997) we

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found that the essential oils of some Lamiaceae were able to produce marked inhibition of carrageenin- and histamine- induced oedemas in laboratory animals, to reduce hyperalgesia caused by the administration of formic acid and also to have moderate anti-microbial action. These activities suggested their possible use in the local treatment of inflammatory conditions, especially in the stomatological field.

The present work, as part of a wider research project shared between different groups, considers the capacity of the *S. desoleana* essential oil components to permeate a porcine buccal mucosa and the influence of the vehicle and of the enhancer diethylene glycol monoethyl ether Transcutol® on the permeation of the different components. The porcine buccal mucosa is largely used for in vitro experiments because the permeability of this membrane is very similar to the human buccal tissue (Squier and Rooney, 1976; Squier and Philip, 1996; De Vries et al., 1991).

Table 1
Concentration of essential oil permeant components in the *S. desoleana* essential oil[a]

| Components | % w/w |
|---|---|
| β-Pinene | 1.99 |
| Cineole | 10.20 |
| Linalool | 14.46 |
| α-Terpineol | 0.18 |
| Linalyl acetato | 26.76 |
| α-Terpinil acetate | 17.00 |

[a] The other essential oil components are a series of terpenes which are not able to permeate the porcine buccal mucosa.

Table 2
Compositions of the tested formulations

| Components | Formulations | | |
|---|---|---|---|
| | A (%) | B (%) | C (%) |
| *Microemulsions* | | | |
| *S. desoleana* | 5 | 10 | 15 |
| Isostearate isostearyle | 14.89 | 13.78 | 12.69 |
| PEG-8 caprylic/capric glicerydes | 33.52 | 31 | 28.55 |
| Polyglyceryl-6 isostearate | 19.08 | 17.67 | 16.26 |
| Water | 27.5 | 27.5 | 27.5 |
| *Gel* | | | |
| | D (%) | | |
| *S. desoleana* | 5 | | |
| Carbopol 974 P | 1 | | |
| Water | 94 | | |
| *Microemulsions–gel* | | | |
| | E (%) | F (%) | G (%) |
| Carbopol 974 P gel | 70 | 70 | 70 |
| Formulation A | 30 | – | – |
| Formulation B | – | 30 | – |
| Formulation C | – | – | 30 |
| *Microemulsions–gel and diethylene glycol monoethyl ether* | | | |
| | H (%) | I (%) | |
| Diethylene glycol monoethyl ether | 10 | 20 | |
| Carbopol 974 P gel | 60 | 50 | |
| Formulation B | 30 | 30 | |

## 2. Materials and methods

### 2.1. Materials

Isostearate isostearyle, PEG-8 caprylic/capric glicerydes (Labrasol®)), polyglyceryl-6 isostearate (plurol isostearique®) and diethylene glycol monoethyl ether Transcutol® were supplied by Gattefossé while Carbopol 934 P by BFGoodrich.

*S. desoleana* grows wild in Sardinia; fresh leaves collected during the fluxering season (late spring) were distilled in a Clevenger-type apparatus; the boiling range for distillation was 80–100°C at 1 atm.

The essential oil consists of a complex mixture of different characteristics (Peana and Satta, 1993). Table 1 shows the permeant essential oil components.

### 2.2. Developed formulations

In order to study the influence of formulations and of the enhancer diethylene glycol monoethyl ether on in vitro *S. desoleana* essential oil permeation, four different preparations were prepared at different concentrations in pure essential oil and tested against the pure oil.

The compositions of the various formulations are reported in Table 2. We tested the microemulsions at three different concentrations; the gel was tested only at a concentration of 5% because of

the low blending capacity of the oil in the hydrophilic phase; as a third formulation a pure Carbopol 934 P gel was added to the three microemulsions.

Moreover, we added the enhancer diethylene glycol monoethyl ether at 10 and 20% of concentration to the microemulsion at 10% of concentration with gel.

### 2.3. Tissue preparation

Porcine buccal mucosa with a fair amount of underlying connective tissue was surgically removed from the oral cavity of a freshly killed male pig (30–50 kg) obtained, on each study day, from a local slaughter house (CLAI Imola, Bologna). The buccal mucosa was placed in ice-cold phosphate buffer 0.15 M. The connective tissue of the mucosa was carefully removed using fine-point forceps and surgical scissors.

The cleaned buccal mucosa membrane was then placed in an ice-cold pH 7.4 phosphate buffer 1/15 M until it was mounted in the diffusion cells. The thickness of the porcine buccal mucosa was measured by means of an electronic callipers. The mucosa used in the experiments was $1.0 \pm 0.1$ mm thick.

### 2.4. In vitro diffusion study

The in vitro diffusion studies were carried out in standard Franz diffusion cells having 0.64 $cm^2$ diffusion area (Franz, 1975; Friend, 1992). The receptor compartment has a volume of 4.8 ml and was maintained at 37°C by means of a water bath, circulator, and a jacket surrounding the cells. The cells were filled with freshly prepared solution of water–ethanol 3:2 v/v. Water–ethanol was used in the receptor compartment in order to solubilize the essential oil components. The solution in the receptor compartments was continuously stirred at 600 rpm using a Teflon coated magnetic stirrer.

The porcine buccal mucosa was clamped between the donor and receiving compartments. A volume of 1 ml of the tested formulations and of the pure *S. desoleana* essential oil was placed in the donor compartment.

The amount of the essential oil components diffused through porcine buccal mucosa was determined by removing aliquots of 1 ml from the receptor compartments using a syringe and immediately replacing the same volume of solution (kept at 37°C).The samples were transferred to volumetric flasks, and stored in a refrigerator until they were analysed. Sampling schedule was 0.5, 1, 2, 4, 8, 12 and 24 h. All experiments were carried out in triplicate.

### 2.5. Quantitative analyses

The analyses of the samples was carried out by GC. GC analyses were performed using a Carlo Erba HRGC 5300 Mega series gas-chromatographer with FID detector.

Two columns with different polarity were employed. A Supelco Inc fused silica SPB % (biphenyl–dimethyl–vinylpolysiloxane (5:94:1) bonded phase) column (30 m $\times$ 0.32 mm, film thickness 0.25 μm) was used with a temperature program of 60–150°C at 5°C $min^{-1}$, with 20 min final hold; then 150–220°C at 5°C $min^{-1}$, holding the final temperature for 20 min. The carrier gas was helium (1 ml $min^{-1}$). The detector temperature was 250°C. The injection system was 'on column'.

A Supelco Inc fused silica Supelcowax 10 (Carbowax 20 M bonded phase) column (15m $\times$ 0.25 mm, film thickness 0.25 μm) was used with a temperature programme ranging from 50–180°C at 3°C $min^{-1}$, holding the initial temperature for 8 min and the final temperature for 20 min. The carrier gas was helium (0.6 ml $min^{-1}$). Injector and detector temperatures were 200 and 220°C, respectively. The injection was performed as 'split mode' (ratio: 1/40).

Identification of the single components of the essential oil was done by the method described in a previous work (Peana and Satta, 1993).

Quantitative determinations of the composition of the essential oil and the samples of receptor solution were performed by the internal standard method after adding known quantities of ethylene glycol monobutyl ethers.

### 2.6. Data analysis

For the essential oil components, absorption is a passive diffusion process and can be described by Fick's second law equation:

$$J_s = dQ_r/A\ dt \quad (1)$$

where $J_s$ is the steady-state buccal mucosa flux in μg $cm^{-1}$ $h^{-1}$, $dQ_r$ is the change in quantity of material passing through the membrane into the receptor compartment expressed in μg, $A$ is the active diffusion area in cm and $dt$ is the change in time. The steady-state flux of the *S. desoleana* essential oil components through the porcine buccal mucosa was calculated from the slope of the linear portion of the cumulative amount permeated through the membrane per unit area versus time plot.

To determine the permeability coefficient, we used the equation:

$$K_p = J_s/C_d, \quad (2)$$

where $K_p$ is the permeability coefficient, $J_s$ is the flux calculated at the steady-time and $C_d$ is the donor concentration (Zhang and Robinson, 1996).

The effectiveness of the enhancer diethylene glycol monoethyl ether was evaluated using enhancement ratio (Babar et al., 1990):

$$ER = K_{p_E}/K_{p_S}, \quad (3)$$

where $ER$ is the enhancer ratio, $K_{p_E}$ is the permeability coefficient of the formulation with the enhancer and $K_{p_S}$ is the permeability coefficient of the same formulation without the enhancer.

## 3. Results and discussion

Permeation profiles of the sum of the *S. desoleana* essential oil components trough porcine buccal mucosa from the pure oil and from different formulations are shown in Fig. 1. In Table 3, the flux $J_s$ and the permeability coefficient $K_p$ of the different essential oil components are represented.

The essential oil component $J_s$ from the pure essential oil is higher when compared to the formulations, while the $K_p$ is lower. The essential oil $J_s$ from the pure essential oil is high because of the concentration; the low $K_p$, instead, means that the formulations enhance the permeability with respect to the oil. The formulations which presented the best $K_p$ were the microemulsions–gel with diethylene glycol monoethyl ether, followed by microemulsions–gel without enhancer. This was then followed by the microemulsions and then by the gel.

The behaviour of the essential oil components $J_s$ and $K_p$ of the studied formulations compared to pure oil is however variable.

The β-pinene $K_p$ decreases with the increase of the essential oil concentration in the microemulsions and in the microemulsions–gel; this means that the microemulsion matrix plays a negative effect on the permeation of this essential oil component. There is no permeation of β-pinene from the gel because of a negative effect of the gel on the absorption through the skin.

The cineole $K_p$ is costant in all the studied formulations. This means that the formulations influence the permeation of this component in the same way.

The α-terpineol $K_p$ remains steady in the studied microemulsions; the $K_p$ in the microemulsions–gel is still steady but higher when compared to that of the microemulsions; this means that the microemulsions–gel enhance the permeation with respect to the simple microemulsions. This effect is independent of the essential oil concentration. The α-terpineol $K_p$ in the gel is higher when compared to the microemulsions but lower when compared to the microemulsions-gel.

The linalool does not cross the membrane from the microemulsions and from the gel but crosses the membrane from the microemulsions–gel. Moreover, in the microemulsions–gel, the $K_p$ decreases with the increase of the essential oil concentration.

The linalyl acetate crosses the membrane from the microemulsion at 15% of concentration and from the microemulsions–gel; in the microemulsions–gel the $K_p$ increase with the increase of the essential oil concentration.

The terpinil acetate doesn't cross the membrane from the gel or from the microemulsion at 5% of

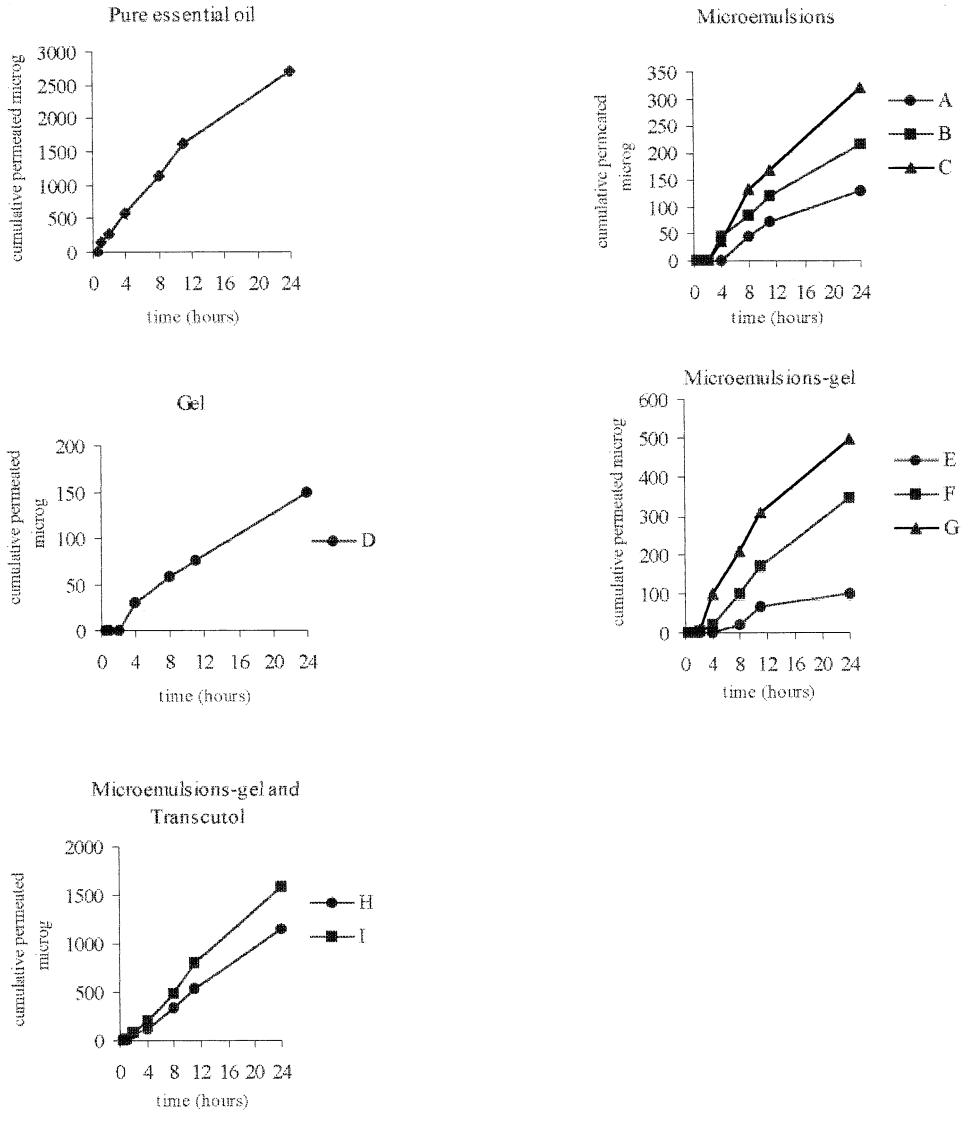


Fig. 1. Permeation profiles of the sums of the *S. desoleana* essential oil components through porcine buccal mucosa from the pure oil and from the different formulations.

concentration, while it crosses the membrane from the microemulsions at 10 and 15% of concentration with a constant $K_p$. This component doesn't cross the membrane from the microemulsion–gel at 5% of concentration while it crosses the membrane from the microemulsions–gel at 10 and 15% of concentration with a constant $K_p$.

Comparing the $K_p$ of the single components of the essential oil, the terpenic components (β-pinene, cineole, or-terpineol and linalool) have a higher $K_p$ when compared with the other components (linalyl acetate and α-terpinil acetate). The terpenic components are usually used as enhancers in the percutaneous absorption (Obata et al., 1991; Williams and Barry, 1991) but they are also good permeant of the buccal mucosa.

The relative enhancer ratio ER of the microemulsions–gel with diethylene glycol monoethyl

Table 3
Flux and $K_p$ of the *S. desoleana* essential oil components in the pure oil and in the different formulations (Nd, not detectable)

| Components in the different formulations | $J_s$ (μg cm$^{-2}$ h$^{-1}$) | $K_p$ ($\times 10^{-3}$ cm h$^{-1}$) |
|---|---|---|
| *Pure essential oil* | | |
| β-Pinene | 11.652 SD 1.233 | 0.584 SD 0.021 |
| Cineole | 90.281 SD 2.564 | 0.885 SD 0.041 |
| α-Terpineol | 32.062 SD 0.526 | 1.781 SD 0.021 |
| Linalool | 53.531 SD 1.423 | 0.370 SD 0.068 |
| Linalyl acetate | 17.9062 SD 0.841 | 0.067 SD 0.004 |
| α-Terpinil acetate | 14.53125 SD 0.451 | 0.086 SD 0.009 |
| *Formulation A* | | |
| β-Pinene | 3.844 SD 0.215 | 3.863 SD 0.211 |
| Cineole | 5.664 SD 0.144 | 1.111 SD 0.021 |
| α-Temineol | 1.914 SD 0.094 | 2.127 SD 0.253 |
| Linalool | ND | ND |
| Linalyl acetate | ND | ND |
| α-Terpinil acetate | ND | ND |
| *Formulation B* | | |
| β-Pinene | 4.395 SD 0.457 | 2.208 SD 0.013 |
| Cineole | 11.719 SD 1.258 | 1.149 SD 0.041 |
| α-Terpineol | 3.906 SD 0.851 | 2.170 SD 0.025 |
| Linalool | ND | ND |
| Linalyl acetate | ND | ND |
| α-Terpinil acetate | 1.687 SD 0.252 | 0.099 SD 0.005 |
| *Formulation C* | | |
| β-Pinene | 5.062 SD 0.254 | 1.693 SD 0.053 |
| Cineole | 17.187 SD 0.851 | 1.123 SD 0.058 |
| α-Terpineol | 6.121 SD 1.893 | 2.222 SD 0.015 |
| Linalool | ND | ND |
| Linalyl acetate | 1.781 SD 0.121 | 0.044 SD 0.004 |
| α-Teminil acetate | 2.812 SD 0.541 | 0.110 SD 0.007 |
| *Formulation D* | | |
| β-Pinene | ND | ND |
| Cineole | 5.812 SD 0.212 | 1.140 SD 0.015 |
| α-Terpineol | 5.437 SD 0.546 | 6.041 SD 0.017 |
| Linalool | ND | ND |
| Linalyl acetate | ND | ND |
| α-Terpinil acetate | ND | ND |
| *Formulation E* | | |
| β-Pinene | 1.969 SD 0.235 | 6.595 SD 0.029 |
| Cineole | 1.660 SD 0.459 | 1.085 SD 0.087 |
| α-terpineol | 2.343 SD 0.867 | 8.681 SD 0.098 |
| Linalool | 3.281 SD 0.218 | 1.513 SD 0.049 |
| Linalyl acetate | 1.031 SD 0.144 | 0.260 SD 0.006 |
| α-Terpinil acetate | ND | ND |
| *Formulation F* | | |
| β-Pinene | 2.734 SD 0.211 | 4.580 SD 0.032 |
| Cineole | 3.515 SD 0.155 | 1.149 SD 0.022 |
| α-Terpineol | 4.297 SD 0.266 | 7.957 SD 0.089 |
| Linalool | 4.883 SD 0.568 | 1.126 SD 0.056 |
| Linalyl acetate | 2.930 SD 0.113 | 0.365 SD 0.092 |
| α-Terpinil acetate | 1.191 SD 0.203 | 0.234 SD 0.015 |
| *Formulation G* | | |
| β-Pinene | 3.656 SD 0.524 | 4.083 SD 0.015 |
| Cineole | 5.437 SD 0.101 | 1.184 SD 0.026 |
| α-Terpineol | 6.563 SD 0.615 | 8.102 SD 0.064 |
| Linalool | 6.375 SD 0.897 | 0.980 SD 0.005 |
| Linalyl acetate | 5.437 SD 0.414 | 0.451 SD 0.026 |
| α-Terpinil acetate | 5.719 SD 0.558 | 0.747 SD 0.062 |
| *Formulation H* | | |
| β-Pinene | 6.75 SD 0.194 | 11.306 SD 0.066 |
| Cineole | 10.312 SD 0.228 | 3.371 SD 0.065 |
| α-Terpineol | 7.875 SD 0.414 | 14.583 SD 0.065 |
| Linalool | 12.752 SD 1.217 | 2.939 SD 0.095 |
| Linalyl acetate | 11.253 SD 1.126 | 1.401 SD 0.087 |
| α-Terpinil acetate | 15.754 SD 1.522 | 3.088 SD 0.054 |
| *Formulation I* | | |
| β-Pinene | 12.753 SD 0.933 | 21.356 SD 0.064 |
| Cineole | 11.252 SD 1.113 | 3.676 SD 0.095 |
| α-Terpineol | 13.218 SD 2.055 | 24.479 SD 0.101 |
| Linalool | 24.468 SD 2.092 | 5.640 SD 0.051 |
| Linalyl acetate | 14.254 SD 1.228 | 1.775 SD 0.065 |
| α-Terpinil acetate | 19.312 SD 1.112 | 3.787 SD 0.051 |

ether at 10 and 20% of concentration with respect to the microemulsion–gel at the same concentration, in the essential oil, are represented in Table 4. In Table 4, the ratio of formulation H to formulation I is also represented.

The $K_p$ of the microemulsions–gel with diethylene glycol monoethyl ether is largely improved and this increase is directly proportional to the diethylene glycol monoethyl ether concen-

Table 4
ER of the microemulsions–gel with diethylene glycol monoethyl ether at 10 and 20% of concentration with respect to the microemulsion–gel at the same concentration in the essential and ration of formulation H to formulation I

| | Components | | | | | |
|---|---|---|---|---|---|---|
| | β-Pinene | Cineole | α-Terpineol | Linalool | Linalyl acetate | α-Terpinil acetate |
| ER $K_{pH}/K_{pF}$ | 2.958 | 3.519 | 0.943 | 3.137 | 6.440 | 16,052 |
| ER $K_{pI}/K_{pF}$ | 5.129 | 6.546 | 1.424 | 5.527 | 8.791 | 22.336 |
| $K_{pI}/K_{pH}$ | 1.734 | 1.860 | 1.510 | 1.762 | 1.365 | 1.391 |

tration with a ratio included between 1.36 and 1.7.

## 4. Conclusion

The *S. desoleana* essential oil components are able to permeate the in vitro porcine buccal mucosa in Franz cells.

For this reason the *S. desoleana* essential oil may be used in the stomatological field for its antimicrobic properties, and for its anti-inflammatory properties as well as for its analgesic qualities. In particular, the best formulation is the microemulsion–gel with diethylene glycol monoethyl ether at 20% of concentration which allows the highest $K_p$ across the buccal mucosa for all of the essential oil components.

## References

Babar, A., Solanki, U.D., Cutie, A.J., Plakogiannis, F., 1990. Piroxicam release from dermatological bases: In vitro studies using cellulose membrane and hairless mouse skin. Drug Dev. Ind. Pharm. 16, 523–540.

De Vries, M.E., Junginger, H.E., Bodde, H.E., Verhoef, J.C., Ponec, M., Craane, W.I., 1991. Localization of the permeability barrier inside porcine oral mucosa: a combination in vitro study of drug permeabilty, electrical resistance and tissue morphology. Int. J. Pharm. 49, 25–29.

Franz, T.J., 1975. Percutaneous absorption. On the relevance of in vitro data. J. Invest. Dermatol. 64, 190–196.

Friend, D.R., 1992. In vitro skin permeation techniques. J. Cont. Rel. 18, 235–248.

Moretti, M.D.L., Peana, A.T., Satta, M., 1997. A study on anti-inflammatory and peripheral analgesic action of *Salvia sclarea* L. essential oil and its main components. J. Essent. Oil. Res. 9, 199–204.

Obata, Y., Takayama, K., Machida, Y., Nagai, T., 1991. Combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of diclofenac sodium. Drug Des. Discov. 8, 137–144.

Peana, A.T., Satta, M., 1993. Preliminary research on essential oils of *Salvia sclarea* L. and *Salvia desoleana* Atzei & Picci. Pharmacol. Res. 27, 25–26.

Peana, A.T., Moretti, M.D.L, Satta, M., 1996. Olio essenziale di *Salvia desoleana* Atzei & Picci: studio dell'attività antiinfiammatoria ed analgesica periferica. Acta of 8th Meeting of the Italian Society of Phytochemistry, Arzana, 22–25 May.

Peana, A.T., Moretti, M.D.L., Juliano, C., 1999. Chemical composition and antimicrobial action of the essential oils of *Salvia desoleana* Atzei & Picci and *Salvia Sclarea* L. Planta Medica 65, 779–780.

Squier, C.A., Rooney, L., 1976. The permeability of keranitized and nonkeranitized oral epithelium to lanthanum in vivo. J. Ultrastruct. Res. 54, 286–290.

Squier, C.A., Philip, W.W., 1996. Structure and function of the oral mucosa and implications for drug delivery. In: Rathbone, M.J. (Ed.), Oral Mucosal Drug Delivery. Marcel Dekker, New York, pp. 1–26.

Williams, A.C., Barry, B.W., 1991. The enhancement index concept applied to terpene penetration enhancers for human skin and model lipophilic (estradiol) and hydrophilic (5-fluorouracile) drugs. Int. J. Pharm. 74, 157–168.

Zhang, H., Robinson, J.R., 1996. In vitro methods for measuring permeability of the oral mucosa. In: Rathbone, M.J. (Ed.), Oral Mucosal Drug Delivery. Marcel Dekker, New York, pp. 90–93.