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EXPERIMENTAL PAPER

Permeation-enhancing properties of *Nepeta cataria var. citriodora* dry extract

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Summary

This paper presents the research on permeation enhancing properties of *Nepeta cataria* var. *citriodora* (catnip) dry extract in comparison to oleanolic acid and ursolic acid. Progesterone was chosen as a model substance for permeation test. The hydrogels made of hydroxypropylmethylcellulose with progesterone, enhancers and ethanol were applied in the study. The *in vitro* progesterone penetration test was based on the method proposed by Fürst, using artificial lipophilic membranes which were made of colloxylin and dodecanol. Statistical analyses showed an increase in penetration of progesterone caused by catnip dry extract in comparison to ursolic acid and blank sample. HPLC assay was applied to study the effect of enhancers on progesterone physicochemical properties. The solubility of progesterone was tested in solvent systems corresponding to liquid phases of gels. The statistical increase in progesterone solubility was observed in the presence of dry extract in comparison to the result from ursolic acid-containing sample. The partition coefficient of progesterone was evaluated by standard procedures.

The statistically significant reduction of log P values for progesterone was determined in the presence of catnip dry extract.

Key words: *progesterone, catnip, lipophilic membranes, solubility, partition coefficient, ursolic acid, oleanolic acid*

INTRODUCTION

Catnip (*Nepeta cataria* L. var. *citriodora* Balb.) is a perennial herb native to Asia Minor and South-Eastern Europe, cultivated in many countries in Europe and North America. *N. cataria* var. *citriodora* is an aromatic and traditional medicinal plant, known as a sedative, antispasmodic and tonic remedy. The essential oil is the best known fraction of biological active components probably responsible for sedative and antispasmodic effects. It contains mainly monoterpene alcohols (geraniol, nerol, citronellol etc.) and monoterpene aldehydes (citral mostly) [1]. Beta-caryophyllene, the representative of sesquiterpenes, is also a component of the catnip essential oil. Polyphenols (flavone glycosides – apigenin and luteolin derivatives) and phenolic acids (rosmarinic, caffeic and chlorogenic acids) are the most important non-volatile constituents of catnip [2].

Triterpenoids (α-amyrin, ursolic acid and oleanolic acid) and sterols (stigmasterol and β-sitosterol glycoside) are also found in aerial parts of catnip [3].

The leading triterpenoid of catnip is ursolic acid, occurring in this source in substantial amounts (0.95–1.30% of dry weight) [3]. This compound is of a great interest because of its anti-inflammatory, anti-angiogenic and anticancer activities [4]. According to recently published studies, ursolic acid may improve epidermal permeability barrier function of skin and stimulate the differentiation of epidermal keratinocytes [5]. The steroid compounds may enhance some active substances (e.g. verapamil) absorption through nasal cavity [6]. Oleanolic acid is an isomeric form to ursolic acid. These compounds are often present together, in *N. cataria* var. *citriodora* herb as well. The effect of ursolic and oleanolic acids on human skin fibroblasts was compared by Wójciak-Kosior et al. and the presented data point at ursolic acid as more cytotoxic agent [7]. Some data concerning pharmacological activities of ursolic and oleanolic acids sugges that both compounds are relatively non-toxic antitumor-promotion agents [8].

In this study, we have presented the influence of *N. cataria* var. *citriodora* dry extract on penetration of progesterone from hydrogel preparations containing 40% of ethanol into artificial lipophilic membranes witch imitate *stratum corneum*, in comparison with activities of oleanolic and ursolic acids.

MATERIALS AND METHODS

Preparation of *N. cataria* var. *citriodora* methanolic extract

100 g of plant dry herb and 300 ml of pure methanol (POCH Gliwice, Poland) were placed into a round flask and maintained in boiling point for 1 h. The obtained extract was filtered through paper. A filtered paper with plant material was once placed again into flask and extracted with 200 ml of pure methanol. The procedure was repeated twice. Three portions of methanolic extract were evaporated in rotavapor (Büchi Labortechnik A.G., Switzerland) to dry mass.

Isolation of ursolic acid

A portion of dry methanolic extract of *N. cataria var. citriodora* herb was solved in 2.5 ml of pure methanol, mixed with 5 g of silica gel Kieselgel 60 (Merck, Germany) and chromatographed at silica gel column with *n*-hexane:acetone gradient as eluent (from 9:1 to 8:2 *n*-hexane/acetone). A presence of ursolic acid in acquired fractions was confirmed by Thin Layer Chromatography. About 100 ml of the obtained fractions were placed at a silica gel plates (Merck, Germany) and runned in mobile phase (toluene:chloroform:methanol 3:2:1). A violet (VIS) and orange (UV) spots of ursolic acid appeared after spraying chromatograms with Liebermann-Burchard reagent (acetic anhydride with concentrated sulphuric acid in methanol). The merged fractions with ursolic acid were condensed and chromatographed in a column filled with Liphophilic Sephadex (Sigma-Aldrich, Germany) with pure methanol as an eluent. Detection of ursolic acid in obtained fractions was performed as previously described. All the fractions rich in ursolic acid were condensed and dried in rotavapor. Ursolic acid as white fine powder was assigned for further experiments.

Preparation of progesterone gels

Into tared beaker, 27.0 g distilled water (DE8/80 TELMED, Poland) was weighed. A beaker with water was placed on a hob and heated to 80°C. Two grams of hydroxypropylmethylcellulose – HPMC (Colorcon®, Flagship House, England) was added slowly to the hot water with continuous stirring. The solution of HPMC was gradually cooled after complete dispersion of polymer. After system gelation, the evaporated water was made up and mixed.

In another beaker, 0.5 g of progesterone (Acros Organics, USA) was dissolved in 10.0 g of ethanol (POCH Gliwice, Poland). The ethanolic solution of progesterone was transferred to the beaker with cooled gel and mixed with a glass rod. The obtained 39.5 g of gel-concentrate were transferred into a lacquer aluminum tube.

In a mortar, 0.02 g of enhancer (ursolic acid or *N. cataria* var. *citriodora* dry extract) was dissolved in 0.4 g of cold ethanol. Subsequently, 1.58 g of gel-concentrate was added and mixed using a pestle. The gel was made up with ethanol to 2.0 g and transferred into a lacquer aluminum tube.

Oleanolic acid (Sigma-Aldrich Co., Germany), as an enhancer, was dissolved in hot ethanol to be incorporated into the gel.

Table 1 shows the qualitative and quantitative composition of gels.

Table 1.

Composition of gels containing triterpenes as enhancers (per 100 g of gel)

Type of gel	Progesterone	HPMC	Ethanol	Distilled water	*N. cataria* dry extract	Oleanolic acid	Ursolic acid
Blank	1	4	40	55	—	—	—
Gel with *N. cataria* dry extract	1	4	40	54	1	—	—
Gel with olenolic acid	1	4	40	54	—	1	—
Gel with ursolic acid	1	4	40	54	—	—	1

Preparation of lipophilic membranes

Artificial lipophilic membranes containing dodecanol (Fluka Sigma-Aldrich Chemie, Germany) and colloxylin were used to study the release and penetration of progesterone from gels. At the bottom of the glass cylinder with diameter of 5 cm, 1.5 ml solution of dodecanol, diethyl ether (POCH SA Gliwice, Poland) and collodion (POCH SA Gliwice, Poland) was applied with an automatic pipette. After 6 hours, the membranes were removed and stored for 24 hours in a desiccator. The membranes thickness was in the range 8–10 μm.

Penetration of progesterone from gels

A multilayer of 5 lipophilic membranes (labeled No. 1-5) was placed on a plexiglass plate and covered with a plate-template. Onto the surface of the first membrane (4 cm^2), a portion of 0.0100±0.0002 g of gel was applied and incubated at 37±0.1°C for 60 minutes. Then, the membranes were separated and dissolved in 3.000 ml of methanol, which gave methanolic solutions of absorbed progesterone in the respective membranes.

Progesterone was quantified spectrophotometrically (UVmini-1240 Shimadzu, Kyoto, Japan) after color reaction with isonicotinylhydrazine (Sigma-Chemical Co, Germany) in acidic conditions, at a wavelength of λ_{max} = 370.5 nm, $a_{1cm}^{1\%}$ = 404.08 [9].

HPLC analyses

HPLC assay was used for the assessment of the effects of *N. cataria* var. *citriodora* dry extract, oleanolic and ursolic acids, on the *n*-octanol/water partition coefficient and the solubility of progesterone in the gels.

The analysis was done using Shimadzu equipment (Shimadzu Corporation, Japan): a LC-20AD parallel-type double plunger pump, a SPD-M20A UV-Vis detector, a SIL-20A/20AC autosampler with cooling, a CTO-20A/20AC column oven, a DGU-20A5 degassing unit and a LC-Solution software. A LiChrospher® 100 RP-18 + pre-column (250 mm + 5 mm) × 4.6 mm column (Merck, Germany) with 5 μm particle size and 110 Å pore size was applied. The mobile phase was a mixture of water (System Synergy® UV, Millipore Corporation, France) and acetonitrile (POCH SA Gliwice, Poland) (40 : 60 *v/v*); both solvents were of chromatography grade. Aliquots of 10 μl were injected and separated at 1.0 ml/min rate flow eluent at a 30°C oven temperature. Progesterone was detected at 242 nm.

Measurement of progesterone solubility in the presence of enhancers

The solubility of progesterone in the presence of the sorption promoters was carried out in following solvent systems:

ethanol : water	40 : 60	parts by weight;
ethanol : water : oleanolic acid	40 : 59 : 1.0	parts by weight;
ethanol : water : ursolic acid	40 : 59 : 1.0	parts by weight;
ethanol : water : *N. cataria* dry extract	40 : 59 : 1.0	parts by weight.

The solubility of progesterone was determined after saturation of the solution, by agitation of an excess of progesterone with an appropriate amount of each of the solvent systems. Into glass vials, 20.00 ± 0.01 mg of enhancers and 30.0 mg of progesterone were weighed; added 2.0 ml of the solvent systems – ethanol and water. The samples were capped and placed in a thermostatted shaker bath (GLS400 Grant, England) and shaken for 24 hours at 25ºC ± 1ºC. The content of vials was transferred to plastic centrifuge tubes and placed in the centrifuge with thermostat (MPW-350R, Poland) (10 min, 25ºC, 5000 rpm). After centrifugation, 0.15 ml of the supernatant was transferred to test tubes and made up to 5.0 ml with methanol. The samples were filtered through a 0.45 μm filter and analyzed by a HPLC method.

Measurement of the *n*-octanol/water partition coefficient of progesterone in the presence of enhancers

N-octanol (Sigma-Aldrich Co., Germany) saturated with water and water saturated with n-octanol were applied in the study. 100 ml of n-octanol were shaken

with 160 ml of a HPLC-grade water at 25±1ºC for 1 h. The phases were separated after 24 hour standing. Subsequently, 0.1% solution of progesterone was prepared by dissolving 0.05000±0.00001 g of progesterone in 50 ml of n-octanol saturated with water. The exact content of progesterone was determined by HPLC.

To determine the partition coefficient, the solution of progesterone in *n*-octanol and water saturated with *n*-octanol were heated on the water bath to 25ºC. 3.000±0.001 ml of n-octanolic progesterone solution and 3.000 ± 0.001 ml of water saturated with *n*-octanol were added into centrifuge tubes. The tubes were capped and rotated through 180° for 5 minutes (20 rpm) around their transverse axis, so that air was not passing through the two phases. The samples were placed in the centrifuge with thermostat (2 min, 25ºC, 5000 rpm). A syringe with removable needle was used to collect the water phase. The syringe was filled with air. Air was pushed out while the needle passed through the *n*-octanol phase. The aqueous phase was collected into the syringe. The samples were filtered through a 0.45 μm filter and analyzed by HPLC after the removal of the needle.

In the same way, the partition coefficient of progesterone measurement was carried out, in the presence of enhancers (0.0200 g/3ml *n*-octanol).

For the calculation of the *n*-octanol/water partition coefficient, the following formula was applied:

$$logP = \log \frac{C_0 - C_W}{C_W}$$

where:
P – the n-octanol/water partition coefficient of progesterone;
C_0 – the initial concentration of progesterone in the *n*-octanolic phase;
C_w – the concentration of progesterone in the aqueous phase after partition.

RESULTS AND DISCUSSION

The permeation of progesterone was evaluated into a model system used to examine penetration of drug from the ointment [10]. The multiple layer of 5 lipophilic membranes was applied in the test. The amount of progesterone absorbed in each membrane was considered as a permeation level of active ingredient. Table 2 shows results of progesterone penetration into lipophilic membranes. The largest amount of progesterone was absorbed from gel containing *N. cataria* var. *citriodora* dry extract – 76.22%. Total amount of absorbed progesterone, using oleanolic acid and ursolic acid as enhancers, was 72.51% and 70.41%, respectively.

Table 2.

Amount of absorbed progesterone in the various membranes

Number of membrane	% absorbed progesterone			
	Blank	*N. cataria* dry extract	Oleanolic acid	Ursolic acid
5	6.86±0.71	9.43±0.87	8.20±0.96	8.72±0.97
4	8.78±0.82	11.94±1.09	10.84±1.02	10.82±0.66
3	11.32±0.92	14.12±1.46	13.94±1.35	13.43±1.49
2	13.75±1.21	18.07±0.63	17.97±0.86	17.42±2.09
1	18.76±0.99	22.66±0.83	21.56±0.96	20.02±0.71
Total	59.48±1.97	76.22±1.71	72.51±2.69	70.41±3.97

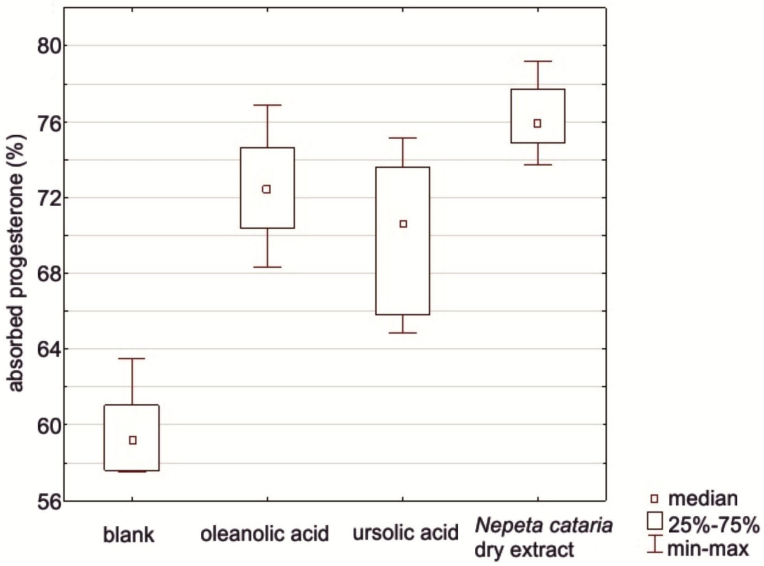

Figure 1.

The difference of total amount of absorbed progesterone influenced enhancers

Table 3 shows the effect of various enhancers on progesterone solubility in the solvent system used in the gels. The solubility of progesterone in the presence of catnip dry extract was 3.787 mg/ml, oleanolic acid 3.335 mg/ml, ursolic acid 3.054 mg/ml and without enhancer 3.493 mg/ml.

Table 3.

The solubility of progesterone in the presence of the enhancers

	Blank	*N. cataria* dry extract	Oleanolic acid	Ursolic acid
Solubility [mg/ml]	3.493±0.096	3.787±0.005	3.335±0.003	3.054±0.008

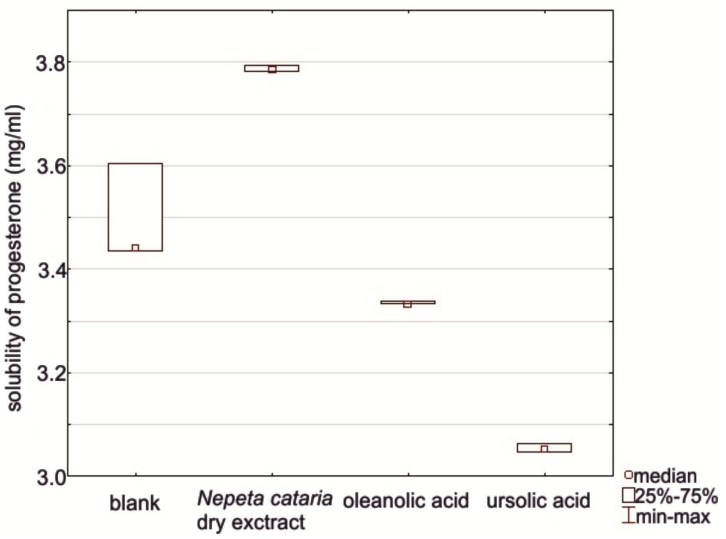

Figure 2.

The difference of solubility of progesterone influenced enhancers

Table 4 presents the mean value of progesterone partition coefficient (log P) in the presence of enhancers. Partition coefficient of progesterone was 3.909 (3.87 according to data presented by Cronin et al. [11]), and in the presence of enhancers – 3.855 (catnip dry extract), 3.901 (oleanolic acid) and 3.881 (ursolic acid).

The statistical significance of the influence of the enhancers on penetration, solubility and coefficient partition of progesterone was carried out by Kruskal-Wallis multiple comparisons test (α=0.05).

Table 4.

The log P of progesterone in the presence of the enhancers

	Blank	*N. cataria* dry extract	Oleanolic acid	Ursolic acid
Log P	3.909±0.038	3.855±0.009	3.901±0.010	3.881±0.024

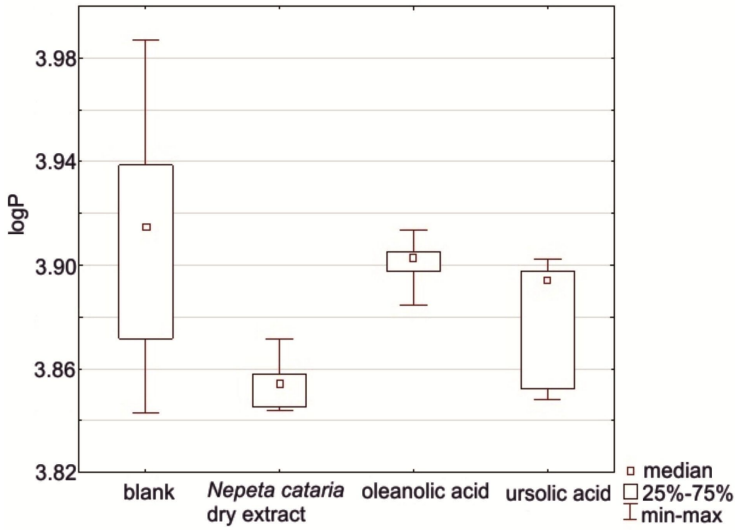

Figure 3.

The difference of log P of progesterone influenced enhancers

The n-octanol/water partition coefficient is one of the main parameters determining the ability of drug penetration through the stratum corneum. This value characterizes the lipophilicity and affinity of drug to the stratum corneum. The influence of enhancers and catnip dry extract was examined in this study. The high log P value of progesterone (3.908) indicates a substantially lipophilic nature of the compound. Statistical analysis showed a significant decrease of progesterone log P in the present of the plant extract in comparison to the blank sample ($p=0.00641$).

Non-significant decrease in the partition coefficient value suggests that the catnip extract is capable of rising progesterone solubility in hydrophilic conditions which is corroborated in our current study with hydrophilic solvents (tab. 3).

The solubility of progesterone and the influence of enhancers on solubility were determined in the solvent system corresponding to qualitative and quantitative composition of gels. Solubility studies showed that the plant extract increased solubility of progesterone in comparison to a system solvent containing ursolic acid only ($p=0.01340$). The solubility of progesterone does not ensure complete dissolution of the active substance used in the gel. Progesterone is partly suspended and partly dissolved in the hydrogel which is an optimal arrangement for the penetration of the active substance from gel, due to an adequate gradient concentrations.

Significant difference of absorbed progesterone in collodion membranes was observed between blank probe and *N. cataria* var. *citriodora* dry extract gel ($p=0.00000$), oleanolic acid gel ($p=0.00524$), ursolic acid gel ($p=0.03528$).

The permeation of progesterone as a result of the application of plant extract increased by 16.94%. Plant extract showed the greatest ability to increase penetration. Statistically significant difference occurred only when compared extract penetration level of progesterone with ursolic acid ($p=0.04980$).

It should be noted that the same amount of extract as the individual enhancers was used in the study. Therefore, total content of oleanolic and ursolic acids in the extract is smaller because the plant extract contains additionally a complex of other biologically active compounds. The influence of catnip dry extract on physicochemical properties and increased penetration of progesterone is probably the result of the synergistic action of oleanolic and uroslic acids with other substances present in the dry extract, such as β-sitosterol 3-O- β-D-glucopyranoside. The effect of the latter on the penetration of active compounds was reported by Hiruta et al. [12].

CONCLUSIONS

1. *N. cataria* dry extract has shown enhancing properties of progesterone into artificial lipophilic membranes.
2. *N. cataria* dry extract has increased solubility of progesterone.
3. *N. cataria* dry extract has decreased partition coefficient of progesterone.

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WŁAŚCIWOŚCI SUCHEGO WYCIĄGU Z *NEPETA CATARIA* VAR. *CITRIODORA* ZWIĘKSZAJĄCE ZDOLNOŚĆ PRZENIKANIA

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S t r e s z c z e n i e

W pracy oceniono wpływ wyciągu suchego z *Nepeta cataria* var. *citriodora* (kocimiętki cytrynowej) oraz kwasu oleanolowego i ursolowego (promotory wchłaniania) na penetrację substancji czynnej. W badaniach wykorzystano hydrożele wykonane z hydroksypropylmetylcelulozy, progesteronu (substancja modelowa), promotorów wchłaniania i etanolu. Badania *in vitro* penetracji progesteronu były oparte o metodę zaproponowaną przez Fürsta, wykorzystującą sztuczne lipofilowe błony wykonane z koloksyliny i dodekanolu. Analiza statystyczna wykazała zwiększoną penetrację progesteronu spowodowaną obecnością wyciągu suchego w porównaniu z kwasem ursolowym i próbą odniesienia. Metodę HPLC

wykorzystano do zbadania wpływu promotorów na właściwości fizyko-chemiczne progesteronu. Rozpuszczalność progesteronu była badana w układzie rozpuszczalników odpowiadającej fazie płynnej żeli. Statystycznie istotny wzrost rozpuszczalności progesteronu zaobserwowano w obecności wyciągu suchego w porównaniu z próbą zawierającą kwas ursolowy. Współczynnik podziału określono za pomocą standartowych procedur. Statystycznie istotny spadek wartości log P progesteronu zaobserwowano w obecności wyciągu suchego z kocimiętki.

Słowa kluczowe: *progesteron, kocimiętka, błony lipofilowe, rozpuszczalność, współczynnik podziału, kwas ursolowy, kwas oleanolowy*