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# Epilobium Hirsutum L and Malva Neglecta L. Propylene Glycol Extracts for Cosmetic Use; Chemical Composition, Microbiological Stability and Diffusion Aspects

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**Abstract** **Original Research Article**

Plant derived products provide numerous beneficial effects on the human skin; for example, the moisturizing effect, the capacity of stimulation of collagen and elastin synthesis, strengthening of micro-circulation and skin immunity, anti-inflammatory and anti-microbial effect, as well as certain antioxidant effect due to the capacity to counteract the free radicals produced by aging, pollution and solar radiation. However, bio-availability and the ability of the plant derived products to penetrate the epidermis layers are two aspects that need to be better studied. Diffusion studies and data regarding microbiological stability of the plant derived products of cosmetic use also are of great utility, as these can result in more effective formulations, and can reduce the number of preservatives, surfactants and other potential allergenic ingredients, thus obtaining in more safe cosmetic products. The present work provides chemical composition, microbiological and diffusion data for two standardized (50%,v/v) propylene glycol extracts for cosmetic use isolated from *Epilobium hirsutum* L. and, respectively, *Malva neglecta* L. plant species, and offer recommendation on their use in the design and development of more active dermato-cosmetic purpose products.

**Keywords:** Great willowherb, common mallow, polyphenols fingerprint, microbiological stability, diffusion aspects.



## INTRODUCTION

Along with increasing numbers of allergies and side effects to chemical cosmetic ingredients, vegetal extracts and specific phytocompounds have become of high interest in the dermato-cosmetic and hygiene product industry.

For example, polysaccharides compounds from specific plant species are frequently used as skin hydration ingredients (especially oligosaccharides type), due to their capacity to retain high quantities of water, by dipole-dipole attraction. Proving these, *Aloe vera* (aloe) polar extracts, reach in polysaccharides compounds with high compliance with the human skin and mucous tissues are frequently used as cosmetic ingredient with hydrating effect [1, 2]; *Glycyrrhiza glabra* (licorice) extracts, also act as hydrating and tonic cosmetic use ingredient due to the synergistic effect of oligosaccharides, phytoestrogens and flavonosides compounds, leading to a final rejuvenating effect and porcelain skin appearance, too [3]; *Malus domestica* (apple fruit) extracts are used for the moisturizing effect of the pectin type polysaccharides contained, which also have high hygroscopicity that favor water retention in skin and mucous tissues [4]; the same hydrating effect for pectin compounds from *Rosa damascena* (Damask rose) flower extracts has been proved [5, 6]. In the specific case of *Cucumis sativus* (cucumber) and *Vaccinium oxycoccos* (small cranberry) extracts, the moisturizing effect is provided by the content of phytosterols compounds acting as surfactants, which are able to facilitate the passage of water and of other co-administered components through the impermeable *stratum corneum*, as well as to facilitate the rapid spread of the components in the applied area [7, 8]. Oils fraction from *Arachis hypogaea* (peanut) [9], *Argania spinosa* (argan) [10], *Cocos nucifera* (coconut) [11] and *Olea europaea* (olive) [12] are known for hydrating effect which owes to the proper fatty acid components ratio, too.

Furthermore, plant extracts with anti-inflammatory and antimicrobial properties are of great utility in cosmetic products for oily, acne prone skin.

Studies in the recent years support the effectiveness of plant extracts with modulator potential on the immune system in the prevention and treatment of acne skin of various etiologies [13, 14]. According to the recent statistic study [15], the incidence of acne in adolescents is 83%, the prevalence in males being higher than in women. Since the numerous side effects and teratogenicy potency of current, allopath treatment are proved [16, 17], the use of the knowledge in the field of phytotherapy is of great interest, especially given the success of past studies; for example, the vegetal extracts from *Echinacea purpurea* (eastern purple coneflower) [13] and *Leonuri herba* (motherwort) [18], both proved the capacity to reduce the bacterial induced inflammation in acne by decreasing splenic T lymphocytes activity and the interleukin IL-6 and IL-8 serum level, i.e. the mechanism of development of skin acne lesions respectively; the extracts from *Hedyotis diffusa* (snake-tongue grass), *Scutellaria baicalensis* (wolf's mouth), *Salvia milliorrhiza* (salvia) and *Prunella vulgaris* (common self-heal) also were shown to improve the cellular and humoral immunity in humans, which has led to significantly modified levels of immunoglobulin IgG and interleukin IL-2 in peripheral blood of acne patients [19].

Another aspect to be mentioned is the microbial infection and resistance to antimicrobials; therefore, the vegetal extracts can often be more effective than usual antibiotics due to the microbial failure to develop resistance against mixtures of antimicrobials found in plant derived products. Studies in the last years have shown augmented antimicrobial potency, and also anti-inflammatory effects, of *Epilobium hirsutum* (great willowherb) [20-22], *Salvia officinalis* (salvia) [23] and *Rosmarinus officinalis* (rosemary) [24] plant extracts; the rosemary extracts proved the ability to suppress *Propionibacterium acnes* - induced inflammatory responses, too. The extracts from *Camelia sinensis* (green tea), *Glycyrrhiza glabra* and *Calendula officinalis* (marigold), also were proved to act against *Staphylococcus aureus*, *Staphylococcus epidermi*s and *Propionibacterium acnes*, all known as involved in the initiation and development of the acne process [25].

Above all, plant extracts contain numerous antioxidant compounds (e.g., gallic acid, rosmarinic acid, miricetin and quercetin derivates), very useful in combating free radicals principally caused by pollution and solar radiation, the cause of (premature) skin aging, but also promoters of skin cancer [26-28]; *Lonicera caerulea* (blue honeysuckle) [29, 30], *Prunella vulgaris* (self-heal) [31], *Scutelaria baicalensis* (Baikal skullcap) [32] and *Vaccinium myrtilus* (bilberry) [29, 33, 34] extracts were proved with high antioxidant potency, thus being found in numerous cosmetic formulations.

The present study has aimed to study chemical composition, microbiological stability and diffusion aspects of two standardized (50%, v/v) propylene glycol extracts isolated from *Epilobium hirsutum* L. and, respectively, *Malva neglecta* L. plant species. Plant material selection has been done on basis of their high value as cosmetic ingredients, due to the augmented anti-inflammatory and anti-microbial properties and the capacity to improve skin hydration, respectively.

## MATERIAL AND METHODS

### Plant material description

The aerial part (*herba et flores*) of *Epilobium hirsutum* L. (*Onagraceae* family) plant species was harvested in August from the Romanian Carpathians Mountains (Sinaia), at about 1000 m altitude; the leaves (*folium*) of *Malva neglecta* L. (*Malvaceae* family) plant species were harvested in August from the Romanian sub-Carpathian region (Prahova), at about 150 m altitude. The two plant species were authenticated by the biologist's team of National Institute of Chemical-Pharmaceutical R&D (ICCF), Bucharest, Romania; voucher specimens (codified Eh-POCD38 and Mn-POCD38) are deposited in ICCF Plant Material Storing Room. Plant materials were shade dried, ground to medium-size plant powders, and then used in the technological studies.

### Chemicals, reagents and reference substances used

Chemicals (namely sodium acetate, sodium carbonate and aluminum chloride), reagents (*Folin-Ciocalteau*, *Natural Product*/NP and *Polyethylene Glycol* 4000/PEG), solvents (ethanol, formic acid, glacial acetic acid, ethyl acetate, propylene glycol) and the *reference products* used in (HP)TLC studies ((rutin (min. 95%), chlorogenic acid (>95%) and caffeic acid (99%)) were purchased from *Merck Co* (Bucharest, Romania).

### Experimental setup and procedure

Two charges of 100 grams of each plant powder were separately extracted with 1000 mL of 70% (v/v) ethanol solution, one hour at 82°C. The two extracts were separately filtered on the paper filter and the resulted 70% ethanolic extracts (codified Eh-D38 and, respectively, Mn-D38) measuring 650 mL and, respectively, 800 mL, both were analyzed as concerning polyphenols composition, qualitative and quantitative aspects. Further, five hundred (500) mL of each Eh-D38 and Mn-D38 vegetal extracts were concentrated at low pressure (*Büchi* Rotary Evaporator) and the resulted *spiss* products were (separately) solved into 50% (v/v) propylene glycol solution to assure the final concentration of 1 mg total flavones content (expressed as rutin equivalents [GAE]) *per 1* mL sample. Two standardized, 50% (v/v) propylene glycol extracts for cosmetic use have resulted: EhPG-D38 extract (measuring 630 mL) and, respectively, MnPG-D38 extract (170 mL). The two standardized propylene glycol extracts were kept in glass bottle in the dark and tested as concerning chemical quantitative and chemical qualitative aspects (polyphenols fingerprint),

microbiological stability and diffusion of the active compounds (polyphenols) by their passage into 20%, 50% and 70% ethanol solution, respectively.

**Chemical qualitative analysis - Polyphenols fingerprint**

Polyphenols assessment have been done by (HP)TLC method, according to Wagner *et al.* [35] and Reich *et al.* [36] recommendations: solvent system - ethyl acetate:glacial acetic acid:formic acid:water/100:12:12:26; plates - Silica gel 60F254 (10x10), (*Merck*, Darmstadt, Germany*)*; reference compounds - mixtures of polyphenols compounds in 70% ethanol; test vegetal samples - ethanolic extracts (Eh-D38 and Mn-D38) and corresponding propylene glycol extracts (EhPG-D38 and MnPG-D38). Test vegetal samples were loaded as 8 mm band length by using the Hamilton syringe device and Linomat 5 instrument (CAMAG, Muttentz, Switzerland), then analyzed as described in the authors studies [20, 21].

**Chemical quantitative analysis - Estimation of total flavones content**

The total flavones content in test vegetal extracts were appraised by standard *Romanian Pharmacopoeias* method [37], as previously described in the authors studies [20, 21]. The total flavones content was computed compared to Rutin [R] reference compound calibration curve ($R^2$ =0.9987), and the results were expressed as mg [R] equivalents, *per* 100 mL test extract.

**Microbiological assay**

The purpose of these study was to determine the degree of contamination of the two standardized (50%, v/v) propylene glycol extracts for cosmetic purpose, EhPG-D38 and MnPG-D38 extracts respectively. Tests were done by using diffusion method in plates [37], as previously described in the author's studies [20, 21].

**Diffusion experiments on Franz cell**

Studies have been done on the two standardized propylene glycol extracts (EhPG-D38 and MnPG-D38) with a content of 1 mg total flavones, Rutin equivalents, *per* 1 mL test sample, using an adapted Franz cell device. The purpose of the study was to determine the solubility of the active compounds (polyphenols species from *E. hirsutum* and *M. neglecta* phytomedicines) in ethanol solutions of increasing concentration: 20%, 50% and 70% ethanol, respectively (v/v). Accordingly, two series of three diffusion experiments were designed, one for each test vegetal extract: *experiment a* - diffusion experiments on the 50% propylene glycol extract EhPG-D38 passed into 20%, 50% and 70% ethanol solution and, respectively, *experiment b* - diffusion experiments on the 50% propylene glycol extract MnPG-D38 passed into 20%, 50% and 70% ethanol solution. In brief, 0.70 g test vegetal extract was applied on a hydrophile cotton pad then covered with a cellulose acetate membrane and displayed in the donor compartment of the Franz cell. The donor compartment was introduced into the acceptor compartment filled with the diffusion medium (100 mL of each 20%, 50% and 70% ethanol solution respectively) and kept on a magnetic stir bar at 37 ± 1°C and 100 rpm stirring, for 180 minutes. During the diffusion process, a sample of 5 mL was removed while adding the same volume of fresh medium at each point time (10, 15, 20, 25, 30, 35, 45, 60, 90, 120, 150 and 180 minutes). The released polyphenols compounds in each time point were determined spectrophotometrically in UV at 213nm (EhPG-D38) and 206nm (MnPG-D38), using the *Helios Gamma UV-Vis Spectrophotometer* device. The active compounds content (polyphenols) in the acceptor compartment of each test sample was determined using linear regression and the results were compared to a calibration curve made of *nine* concentrations series of each test sample; EhPG-D38 ($R^2$>0.95) and, respectively, MnPG-D38 ($R^2$>0.95).

The kinetics of the active compounds, diffusion profile of polyphenols compounds from EhPG-D38 and MnPG-D38, into 20%, 50% and 70% ethanol solution, i.e. *experiment a* and *experiment b*, were computationally modeled by the statistical comparison of seven mathematical models (see table 1). The selected mathematical models [38] for the study were the following: two empirical models (first order and Higuchi), one semi-empirical model (Korsemeyer-Peppas) and four statistical models (modified Gompertz and modified logistic, Richards and Ratkowsky).

**Table-1: The mathematical models used for the study**

| Name | Equation | Parameters | *Ref.* |
|---|---|---|---|
| **Empirical Models** | | | |
| 1. First order | $Q_t = Q_0(1-\exp(-K_1 t))$ | $Q_t$: the quantity delivered during t; $Q_0$: the initial quantity from solution; $K_1$: first order diffusion constant | 39 |
| 2. Higuchi | $Q_t = K_H\sqrt{t} + C_H$ | $Q_t$: the quantity delivered during t; $K_H$, $C_H$: Higuchi constants | 40 |
| **Semiempirical Models** | | | |
| 3. Korsmeyer - Pepas | $Q_t = C_{KP} + K_{KP}t^n$ | Qt: the quantity delivered during t; KKP, CKP: Korsmeyer - Pepas constants | 41 |
| **Statistical Models** | | | |

| | | | |
|---|---|---|---|
| 4. Modified Gompertz | $Q_t = A_{GP}\exp\left\{-\exp\left[\frac{\mu_{GP}e}{A_{GP}}(\lambda_{GP}-t)+1\right]\right\}$ | $Q_t$: the quantity delivered during t; $A_{GP}$, $\mu_{GP}$, $\lambda_{GP}$: specific parameters to the modified Gompertz model | 42, 43 |
| 5. Modified Logistic | $Q_t = \frac{A_{LG}}{\left\{1+\exp\left[\frac{4\mu_{LG}}{A_{LG}}(\lambda_{LG}-t)+2\right]\right\}}$ | $Q_t$: the quantity delivered during t; $A_{LG}$, $\mu_{LG}$, $\lambda_{LG}$: specific parameters to the modified logistic model | 42, 44 |
| 6. Richards | $Q_t = A_{Rch}\left\{1+\nu_{Rch}\cdot\exp(1+\nu_{Rch})\exp\left[\frac{\mu_{Rch}}{A_{Rch}}(1+\nu_{Rch})^{\left(1+\frac{1}{\nu_{Rch}}\right)}(\lambda_{Rch}-t)\right]\right\}^{\left(-\frac{1}{\nu_{Rch}}\right)}$ | $A_{Rch}$, $\mu_{Rch}$, $\lambda_{Rch}$: specific parameters to the Richards model, $\nu_{Rch}$ shape factor | 42 |
| 7. Ratkowsky | $Q_t = \frac{A_{Rkw}}{1+\exp(B_{Rkw}-C_{Rkw}.t)}$ | $A_{Rkw}$, $B_{Rkw}$ and $C_{RKW}$ specific parameters | 45 |

The model estimation was carried out by non-linear parameter optimization by the use of the Marquardt - Levenberg method [46], the initial values being obtained by linearizing the models. The nonlinear optimization method has been preferred since, although most models have linearized expressions, the truncation of the results and the transformation errors can damage the final result. Used has been made by the CurveExpert Professional 2.5.6. Software from Hyams Development. The software computes a score ranking for the model discrimination taking account of the maximum of the determination ($R^2$) and the adjusted ($R^2_{adj}$) coefficient [38], minimum of the root mean square error (RMSE), mean square error of the regression (MSE) [40] and minimum of the corrected Akaike information criteria (AICC) [47].

When comparing several models for a given set of data, the model associated with the smallest value of AICC is regarded as giving the best fit out of that set of models according to (I), where ***n*** is the number of the data points and k the number of the parameters plus 1.

$$AICC(k) = n.\ln\left(\frac{SSE(k)}{n}\right) + 2.k + \frac{2k.(k+1)}{n-k-1} \qquad (I)$$

## STATISTICAL ANALYSIS

All tests have been done as three (n=3) consecutive measurements and the results were expressed as *means*±(SD).

## RESULT AND DISSCUTION

**Polyphenols composition of the two tests vegetal extracts**

Figure 1 - chromatogram A shows (HP)TLC aspects of the ethanolic extract from *Epilobium hirsutum* (Eh-D38): there were evidenced [20, 21] several flavonol derivates, myricetin(galloyl) and quercetin derivates (the red-orange fluorescent/fl. spots s2, s3, s5, s6, s7 and the indigo fl. spots s1 and s4), and two major phenolic acids, chlorogenic acid (blue fl. zone s2') and gallic acid (dark blue fl. zone s8); even if identical as concerning chemical qualitative and chemical quantitative aspects, 50% propylene glycol extract EhPG-D38 (chromatogram B) has highlighted a smaller number and amounts of polyphenols compounds, which indicates a much lower accuracy of HPTLC analysis in testing plant derived products prepared in propylene glycol solvent.

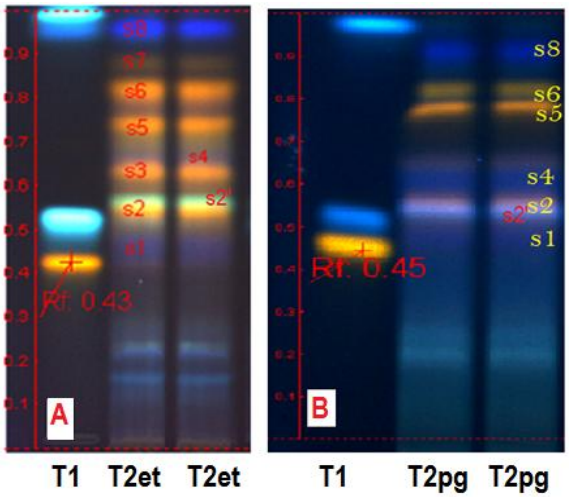


**Fig-1: (HP) TLC aspects of 70% ethanolic and 50% propylene glycol extracts from *Epilobium hirsutum* plant species in comparison with reference compounds (ref.). Chromatogram A - Track 1, rutin, chlorogenic acid and caffeic acid (ref.); Tracks T2et, *Epilobium hirsutum* 70% ethanolic extracts (Eh-D38 - duplicate sample); Chromatogram B - Track 1, rutin, chlorogenic acid and caffeic acid (ref.); Tracks T2pg, *Epilobium hirsutum* 50% propylene glycol extracts (EhPG-D38 - duplicate sample)**

Figure 2 - chromatogram A present polyphenol fingerprint of 70% ethanolic extract from *Malva neglecta* (Mn-D38); there were revealed numerous quercetin (poly)glycosides (the yellow-orange fluorescent/fl. spots s1, s2, s4, s5, s6 and s8) and several caffeic acid derivates, chlorogenic acid and its isomers (blue fl. spots s3, s7, s9, s10 and s11) respectively; 50% propylene glycol extract MnPG-D38 (chromatogram B) highlighted traces of the above quercetin and caffeic acid derivates, also confirming the lower accuracy of (HP)TLC analysis in testing propylene glycol based plant extracts.

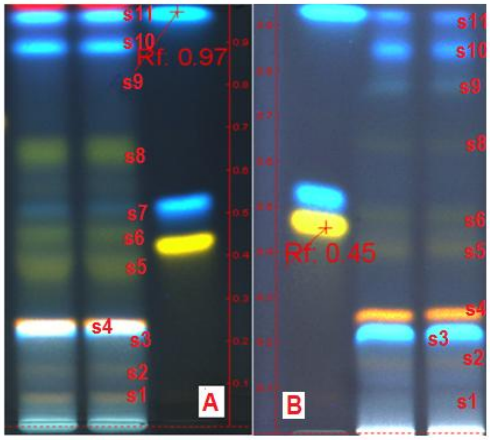


**Fig-2: (HP) TLC aspects of 70% ethanolic and 50% propylene glycol extracts from Malva *neglecta plant* species in comparison with reference compounds (ref.). Chromatogram A - Tracks T2et, *Malva neglecta* 70% ethanolic extracts (Mn-D38 - duplicate sample); Track 1, rutin, chlorogenic acid and caffeic acid (ref.); Chromatogram B - Track 1, rutin, chlorogenic acid and caffeic acid (ref.); Tracks T2pg, *Malva neglecta* 50% propylene glycol extracts (MnPG-D38 - duplicate sample)**

**Microbiological stability of the two tests vegetal extracts**

Studies were done by *Microbial Contamination Test* (MCT), casually done on the non-sterile products in order to verify 1)the level of microbial (bacterial and fungal) contamination and 2)the presence or absence of specific pathogenic microorganism. MCT has been done on basis of the number of total viable microorganisms, aerobic bacteria, yeasts and fungi, as recommended by standard *Romanian Pharmacopoeia* method [37].

It must be reminded that the plant material extraction has been done using 70% (v/v) ethanol solution (in order to sterilize the plant material during the technological extraction), followed by the passage of the corresponding aqueous concentrate extract (*spiss*) into 50% (v/v) propylene glycol solution in a manner to assure the final concentration level of 1 mg total flavones [R] *per* 1 mL vegetal extract. The two final, standardized propylene glycol extracts were placed in two glass bottles in the dark, without using any conservation or preservative compounds. The technological approach used conducted for two different situations: *Epilobium hirsutum* propylene glycol extract emphasized microbiological conformity and stability after six month of preparation (see Table 2); *Malva neglecta* propylene glycol extract emphasized microbiological conformity at the initial testing time, after that indicated the susceptibility to microbial contamination, suggesting the necessity of using preservative compounds (see Table 3).

**Table-2: Microbial Contamination Test on the 50% propylene glycol extracts EhPG-D38**

| Test | Time | Test condition | Test results | Microbiological conformity/stability |
|---|---|---|---|---|
| Total Aerobic Microbial Count (cfu/g) | 0 months | $\leq 10^3$ cfu/g at 30 - 35ºC, $\leq$ 3 days for bacteria, $\leq$ 5 days for fungi | $\leq 10^3$ CFU/g | YES |
| Total Aerobic Microbial Count (cfu/g) | 3 months | $\leq 10^3$ cfu/g at 30 - 35ºC, $\leq$ 3 days for bacteria, $\leq$ 5 days for fungi | $\leq 10^3$ CFU/g | YES |
| Total Aerobic Microbial Count (cfu/g) | 6 months | $\leq 10^3$ cfu/g at 30 - 35ºC, $\leq$ 3 days for bacteria, $\leq$ 5 days for fungi | $\leq 10^3$ CFU/g | YES |

**Table-3: Microbial Contamination Test on the 50% propylene glycol extracts MnPG-D38**

| Test | Time | Test condition | Test results | Microbiological conformity/stability |
|---|---|---|---|---|
| Total Aerobic Microbial Count (cfu/g) | 0 months | $\leq 10^3$ cfu/g at 30 - 35ºC, $\leq$ 3 days for bacteria, $\leq$ 5 days for fungi | 657 CFU/g | YES |
| Total Aerobic Microbial Count (cfu/g) | 3 months | $\leq 10^3$ cfu/g at 30 - 35ºC, $\leq$ 3 days for bacteria, $\leq$ 5 days for fungi | 1450 CFU/g | NO |
| Total Aerobic Microbial Count (cfu/g) | 6 months | $\leq 10^3$ cfu/g at 30 - 35ºC, $\leq$ 3 days for bacteria, $\leq$ 5 days for fungi | 4557 CFU/g | NO |

**Diffusion aspects of the test vegetal extracts**

Studies have as the main purpose the establishment of the diffusion profile of polyphenols compounds found in the two standardized, propylene glycol extracts, EhPG-D38 and MnPG-D38, by using the models presented in table 1. The models were compared according the score ranking previously described including the use of statistical coefficients shown above [38- 41], and the corrected Akaike information criteria (AICC) [47].

The complete values of the ranking scores, statistical coefficients together with the AICC values obtained by the mathematical models described above are presented in the table 4.

**Table-4: Statistical coefficients of the studied models, *experiment a* and *experiment b***

| Model | First Order | Higuchi | Korsmayer Peppas | Modified Gompertz | Modified Logistic | Richards | Ratkovski |
|---|---|---|---|---|---|---|---|
| **Experiment a. EhPG-D38 extract** | | | | | | | |
| *20% ethanol* | | | | | | | |
| Score | 851 | 951 | 993 | 955 | 934 | 953 | 934 |
| $R^2$ | 0.993 | 0.978 | 0.999 | 0.981 | 0.967 | 0.981 | 0.967 |
| $R^2_{adj}$ | 0.992 | 0.975 | 0.999 | 0.976 | 0.960 | 0.973 | 0.960 |
| RMSE | 1.174 | 2.068 | 0.417 | 1.923 | 2.507 | 1.923 | 2.507 |
| MSE | 1.286 | 2.266 | 0.482 | 2.221 | 2.895 | 2.356 | 2.895 |
| AICC | 12.851 | 19.842 | -15.633 | 21.033 | 27.394 | 24.705 | 27.394 |
| *50% ethanol* | | | | | | | |
| Score | 840 | 882.000 | 972.000 | 982.000 | 974.000 | 981.000 | 974.000 |
| $R^2$ | 0.980 | 0.928 | 0.990 | 0.995 | 0.992 | 0.995 | 0.992 |
| $R^2_{adj}$ | 0.978 | 0.920 | 0.988 | 0.994 | 0.990 | 0.994 | 0.990 |
| RMSE | 3.633 | 3.583 | 1.302 | 0.911 | 1.207 | 0.912 | 1.207 |
| MSE | 158.410 | 3.925 | 1.504 | 1.052 | 1.394 | 1.117 | 1.394 |
| AICC | 48.963 | 33.029 | 11.671 | 3.100 | 9.857 | 6.799 | 9.857 |
| *70% ethanol* | | | | | | | |
| Score | 860 | 848.000 | 918.000 | 982.000 | 991.000 | 992.000 | 991.000 |
| $R^2$ | 0.988 | 0.906 | 0.960 | 0.996 | 0.999 | 0.999 | 0.999 |
| $R^2_{adj}$ | 0.987 | 0.897 | 0.951 | 0.995 | 0.999 | 0.999 | 0.999 |
| RMSE | 2.857 | 7.934 | 5.182 | 1.605 | 0.874 | 0.765 | 0.874 |
| MSE | 3.130 | 8.692 | 5.984 | 1.853 | 1.010 | 0.937 | 1.010 |
| AICC | 43.196 | 52.109 | 44.820 | 16.689 | 2.113 | 2.572 | 2.113 |
| **Experiment b. MnPG-D38 extract** | | | | | | | |
| *20% ethanol* | | | | | | | |
| Score | 750 | 816.000 | 852.000 | 903.000 | 905.000 | 956.000 | 905.000 |
| $R^2$ | 0.278 | 0.876 | 0.909 | 0.948 | 0.949 | 0.984 | 0.949 |
| $R^2_{adj}$ | 0.205 | 0.864 | 0.889 | 0.936 | 0.938 | 0.978 | 0.938 |
| RMSE | 2.249 | 0.932 | 0.797 | 0.604 | 0.595 | 0.338 | 0.595 |
| MSE | 2.463 | 1.021 | 0.921 | 0.698 | 0.687 | 0.413 | 0.687 |
| AICC | 37.449 | 0.706 | -0.104 | -6.764 | -7.120 | -17.065 | -7.120 |
| *50% ethanol* | | | | | | | |
| Score | 860.000 | 816.000 | 852.000 | 903.000 | 905.000 | 956.000 | 905.000 |
| $R^2$ | 0.887 | 0.784 | 0.897 | 0.980 | 0.984 | 0.994 | 0.984 |
| $R^2_{adj}$ | 0.876 | 0.762 | 0.875 | 0.976 | 0.981 | 0.991 | 0.981 |
| RMSE | 2.601 | 3.607 | 2.483 | 1.088 | 0.971 | 0.616 | 0.971 |
| MSE | 2.849 | 3.951 | 2.867 | 1.257 | 1.122 | 0.754 | 1.122 |
| AICC | 40.941 | 33.190 | 27.163 | 7.363 | 4.638 | -2.634 | 4.638 |
| *70% ethanol* | | | | | | | |
| Score | 840.000 | 467.000 | 576.000 | 966.000 | 980.000 | 987.000 | 980.000 |
| $R^2$ | 0.883 | 0.444 | 0.618 | 0.987 | 0.994 | 0.997 | 0.994 |
| $R^2_{adj}$ | 0.871 | 0.388 | 0.534 | 0.984 | 0.993 | 0.996 | 0.993 |
| RMSE | 5.709 | 12.436 | 10.301 | 1.885 | 1.247 | 0.861 | 1.247 |
| MSE | 6.254 | 13.623 | 11.895 | 2.176 | 1.440 | 1.055 | 1.440 |
| AICC | 59.811 | 62.895 | 61.308 | 20.542 | 10.639 | 5.417 | 10.639 |

Figures 3 to 8 show the results of the non-linear optimization performed by the models revealed with the best ranking scores, statistical coefficients and AICC values in the test conditions, namely the modified Logistic, modified Gompertz and Richards models respectively, the allure of the diffusion process of polyphenol compounds from the two propylene glycol extracts for cosmetic use (EhPG-D38 and MnPG-D38), *experiement a* and *experiment b* respectively.

*Epilobium hirsutum* propylene glycol extract EhPG-D38 (Figures 3, 4, 5) present a better diffusion process as the polarity of the release medium resembles that of 70% ethanol solution (up to 98% diffusion

efficacy); the active compounds in great willowherb are myricetin, quercetin, and gallic and caffeic acid derivates known with both, augmented anti-inflammatory and antimicrobial properties. Concerning numerical values, if 20% ethanol indicated a maximum diffusion percentage of 34% at 125 minutes, 50% ethanol achieved maximum 53% diffusion percentage, while 70% ethanol reached up to 98% diffusion percentage and the best release efficacy at 125 minutes tested. The comparison of the three models indicates an upper plateau (A) ranging from 44.681 for modified logistic to 45.914 for Richards (for ethanol 20%), 74.784% for modified logistic to 75.280 for modified Gompertz (for ethanol 50%) and 95.471 % for Richards to 97.052 for modified Gompertz (for ethanol 70%); the slope $\mu_m$ values were ranging from 0.414 %/h for modified Gompertz to 0.472 %/h for modified logistic (for 20%), 0.739 %/h for modified logistic to 0.985 %/h for modified Gompertz (for 50%), 1.237 %/h for modified logistic to 1.324 for modified Gompertz (for 70%).

In the specific case of *Malva neglecta* propylene glycol extract MnPG-D38 (Figures 6, 7, 8), the diffusion intensity also increased along with ethanol concentration in the medium (up to 51% diffusion efficacy at 70% ethanol), but an instant release of polyphenols compounds at 20% ethanol concentration has been noticed too; as resulted from HPTLC study, the active compounds in common mallow are quercetin polyglycosides and caffeic acid high molecular derivates with numerous hydroxyl (-OH) groups, so that with high moisturizing potency. Punctually, 20% ethanol experiment indicated a maximum diffusion percentage of 41%, 50% ethanol indicated maximum 35% diffusion percentage, while 70% ethanol reached up to 51% diffusion percentage and the best release efficacy at 125 minutes tested. The comparison of the three models indicates an upper plateau (A) ranging from 60.088 % for Richards to 60.648 for modified Gompertz (for ethanol 20%), 50.977% for Richards to 51.542 for modified Gompertz (for ethanol 50%) and 72.848 % for Richards to 73.418 for modified Gompertz (for ethanol 70%). Similarly, $\mu_m$ values were from 0.334 %/h for modified logistic to 0.467 %/h for modified Gompertz (for 20%), 0.654 %/h for modified logistic to 0.820 %/h for modified Gompertz (for 50%), 3.233 %/h for modified logistic to 3.621 %/h for modified Gompertz (for 70%).

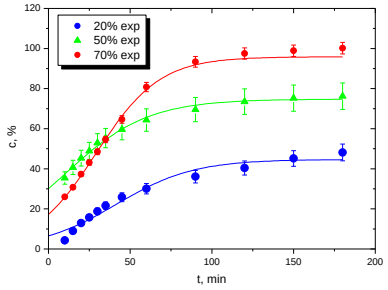


**Fig-3: EhPG-D38 extract diffusion profile, modified Logistic model**

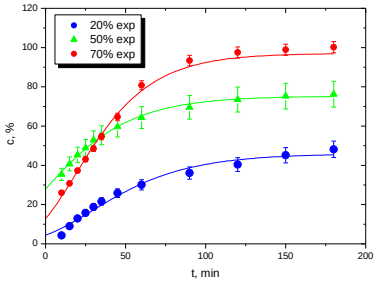


**Fig-4: EhPG-D38 extract diffusion profile, modified Gompertz model**

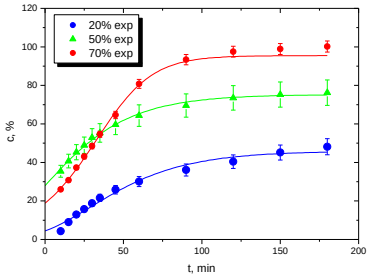


**Fig-5: EhPG-D38 extract diffusion profile, Richards model**

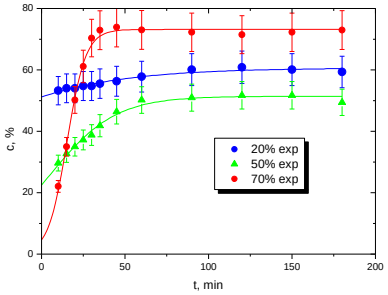


**Fig-6: MnPG-D38 extract diffusion profile, modified Logistic model**

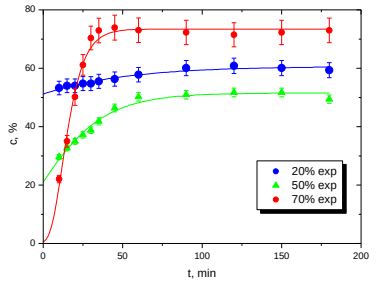


**Fig-7: MnPG-D38 extract diffusion profile, modified Gompertz model**

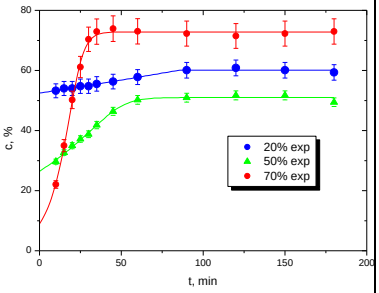


**Fig-8: MnPG-D38 extract diffusion profile, Richards model**

## CONCLUSION

Plant derived products are of major interest not only in chemical-pharmaceutical domain, but also in dermato-cosmetic and hygiene products industry. Even if the current advances in the formulation techniques (for example the use of liposomes and micro-encapsulation techniques) help to incorporate cosmetic ingredients with very different solubility requirements, the diffusion aspects of a specific plant extract and separate compounds are of real utility since they can predict the most properly environment polarity to produce the maximum skin benefits. Proving these, the present study on two standardized (50%, v/v) propylene glycol extracts from *Epilobium hirsutum* L. and *Malva*

*neglecta* L. plant species suggested increased availability of *Epilobium hirsutum* active compounds (meaning myricetin, quercetin and gallic acid derivates with augmented anti-inflammatory and antimicrobial properties) in the release medium resembling with 70% ethanol solution. *Malva neglecta* active compounds (meaning high molecular quercetin and caffeic acid derivates with high moisturizing potency) indicated an instant release of polyphenols compounds at 20% ethanol solution. Furthermore, *Epilobium hirsutum* propylene glycol extract demonstrated stability and microbiological conformity after six month of preparation, while *Malva neglecta* propylene glycol extract indicated susceptibility to microbial contamination, suggesting the necessity of using preservative compounds.

Therefore, if *Epilobium hirsutum* propylene glycol extract is better justified in the case of oily skin formulations, the more so as the active compounds contained are proved with anti-inflammatory and antimicrobial properties, *Malva neglecta* propylene glycol extract may be better developed in the case of dry and normal skin formulations.

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## REFERENCES

1. Fox LT, Du Plessis J, Gerberc M, Van Zyl S, Boneschans B, Hamman JH. In Vivo skin hydration and anti-erythema effects of Aloe vera, Aloe ferox and Aloe marlothii gel materials after single and multiple applications. Pharmacogn Mag. 2014; 10(2): 392-403.
2. Shelch R, Princeton C, Narayan B. Aloe Vera for Tissue Engineering Applications. J Funct Biomater. 2017; 8(1): 6.
3. Yamashita T, Asano Y, Taniguchi T, Nakamura K, Saigusa R, Miura S, Toyama T, Takahashi T, Ichimura Y, Yoshizaki A, Trojanowska M, Sato S. Glycyrrhizin Ameliorates Fibrosis, Vasculopathy, and Inflammation in Animal Models of Systemic Sclerosis. J Invest Dermatol. 2017; 137(3): 631-640.
4. www.ulprospector.com/en/eu/PersonalCare/Detail/2443/71067/Apple-Extract-G.
5. Mohammad HB, Mohammad NS, Zahra S, Somayeh A. Pharmacological Effects of Rosa Damascena. Iran J Basic Med Sci. 2011; 14(4): 95–307.
6. Mahboubi M. Rosa damascena as holy ancient herb with novel applications. J Tradit Complement Med. 2016; 6(1): 10–16.
7. Fiume MM, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, Marks JG Jr, Shank RC, Slaga TJ, Snyder PW, Andersen FA. Safety Assessment of Cucumis sativus (Cucumber) - Derived Ingredients as Used in Cosmetics. Int J Toxicol. 2014; 33(2 Suppl): 47S-64S.
8. Chatelain K, Phippen S, McCabe J, Teeters CA, O'Malley S, Kingsley K. Cranberry and Grape Seed Extracts Inhibit the Proliferative Phenotype of Oral Squamous Cell Carcinomas. Evid Based Complement Alternat Med. 2011; Article ID 467691.
9. Zhai H, Ramirez RG, Maibach HI. Hydrating effects of a corticoid oil formulation and its vehicle on human skin. Skin Pharmacol Appl Skin Physiol. 2003; 16(6):3 67–371.
10. Manca ML, Matricardi P, Cencetti C, Peris JE, Melis V, Carbone C, Escribano E, Zaru M, Fadda AM, Manconi M. Combination of argan oil and phospholipids for the development of an effective liposome-like formulation able to improve skin hydration and allantoin dermal delivery. Int. J. Pharm. 2016; 505(1-2): 204–211.
11. Evangelista MT, Abad-Casintahan F, Lopez Villafuerte, L. The effect of topical virgin coconut oil on SCORAD index, transepidermal water loss, and skin capacitance in mild to moderate pediatric atopic dermatitis: A randomized, double-blind, clinical trial. Int. J. Dermatol. 2014; 53(1): 100–108.
12. Lin TK, Zhong L, Santiago JL. Anti-Inflammatory and Skin Barrier Repair Effects of Topical Application of Some Plant Oils. Int. J. Mol. Sci. 2017; 19(1): 70.
13. Sharma M, Schoop R, Suter A, Hudson JB. Thepotential use of Echinacea in acne: control of Propionibacterium acnes growth and inflammation. Phytotherapy Research. 2011; 25: 517–521.
14. Sinha P, Srivastava S, Mishra N, Yadav NP. Review of New Perspectives on Antiacne Plant Drugs: Contribution to Modern Therapeutics. BioMed Research International. 2014; Article ID 301304, 19 pages.
15. Shen Y, Wang T, Zhou C, Wang X, Ding X, Tian S, Liu Y, Peng G, Xue S, Zhou J, Wang R, Meng X, Pei G, Bai Y, Liu Q, Li H, Zhang J. Prevalence of acne vulgaris in Chinese adolescents and adults: a community-based study of 17,345 subjects in six cities. Acta Derm Venereol. 2012; 92(1): 40-4.
16. Xu WL. Adverse effects of antibiotics. Chin. J. Clin. Ration. Drug Use. 2011; 4(10): 75–76.
17. Yan F, Chen LJ. Application of retinoids in the treatment of skin diseases. Shanghai Med. Pharm. J. 2010; 21(4): 156–159.
18. Li Y, Xi P, Wang T, Miao MS. Effect of Total Alkali in Leonuri Herba on rat ear acne model of serum IL-6 level, Thymus and Spleen Tissue Morphology. Saudi J Biol Sci. 2017; 4(3): 718–723.
19. Wu XH, Liu WL. The clinical study of She-dan Herbal Decoction in treatment of acne vulgaris and its effects on the levels of IgG and IL-2 in

peripheral blood of the patients. Chin. J. Dermatovenereol. 2006; 20: 23–25.
20. Pirvu L, Nicorescu V, Hlevca C, Udeanu DI, Nicorescu I. Antimicrobial and synergistic activity of some whole and selective Epilobium hirsutum L. (great willowherb) extracts tested on standard and wild Staphylococcus aureus strains. Farmacia. 2015; 63: 690-695.
21. Pirvu L, Nicorescu I, Hlevca C, Albu B, Nicorescu V. Epilobi Hirsuti Herba Extracts Influence the In Vitro Activity of Usual Antibiotics on Standard Bacteria. Open Chem. 2016; 14: 1-11.
22. Ruszova E, Cheel J, Paves S, Moravcova M, Hermannova M, Matejkova I, Spilkova J, Velebny V, Kubala L. Epilobium angustifolium extract demonstrates multiple effects on dermal fibroblasts in vitro and skin photo-protection in vivo. Gen Physiol Biophys. 2013; 32(3): 347-59.
23. Abu-Darwish MS, Cabral C, Ferreira IV, Goncalves MJ, Cavaleiro C, Cruz MT, Al-Bdour TH, Salgueiro L. Essential Oil of Common Sage (Salvia officinalis L.) from Jordan: Assessment of Safety in Mammalian Cells and Its Antifungal and Anti-Inflammatory Potential. Biomed Res Int. 538940 (2013).
24. Tsai TH, Chuang LT, Lien TJ, Liing YR, Chen WY, Tsai PJ. Rosmarinus officinalis extract suppresses Propionibacterium acnes-induced inflammatory responses. J Med Food. 2013; 16(4): 324-33.
25. Nand P, Drabu S, Gupta RK. Phytochemical and antimicrobial screening of medicinal plants for the treatment of acne. Indian Journal of Natural Products and Resources. 2012; 3: 28–32.
26. Datta HS, Mitra SK, Paramesh R, Patwardhan B. Theories and Management of Aging: Modern and Ayurveda Perspectives. Evid Based Complement Alternat Med. 2011; 528527.
27. Medellin-Luna MF, Castaneda-Delgado JE, Martinez-Balderas VY, Cervantes-Villagrana AR. Medicinal Plant Extracts and Their Use As Wound Closure Inducing Agents. J Med Food. 2019; Apr 3.
28. Ribeiro AS, Estanqueiro M, Oliveira MB, Sousa Lobo JM. Main Benefits and Applicability of Plant Extracts in Skin Care Products. Review. Cosmetics. 2015; 2: 48-65.
29. Svobodová A, Zdarilová A, Vostálová J. Lonicera caerulea and Vaccinium myrtillus fruit polyphenols protect HaCaT keratinocytes against UVB-induced phototoxic stress and DNA damage. J Dermatol Sci. 2009; 56(3): 196-204.
30. Shusong W, Satoshi Y, Jihua C, Ayami H, Kozue S, Xi H, Jianhua H, De-Xing H. Polyphenols from Lonicera caerulea L. Berry Inhibit LPS-Induced Inflammation through Dual Modulation of Inflammatory and Antioxidant Mediators. J. Agric. Food Chem. 2017; 65(25): 5133–5141.
31. Vostálová J, Zdarilová A, Svobodová A. Prunella vulgaris extract and rosmarinic acid prevent UVB-induced DNA damage and oxidative stress in HaCaT keratinocytes. Arch Dermatol Res. 2010; 302(3): 171-181.
32. Kim TW, Song IB, Lee HK, Kim MS, Ham SH, Cho JH, Lim JH, Yun HI. Assessment of dermal safety of Scutellaria baicalensis aqueous extract topical application on skin hypersensitivity. Planta Med. 2013; 79(11): 959-62.
33. Bucci P, Prieto MJ, Milla L, Calienni MN, Martinez L, Rivarola V, Alonso S, Montanari J. Skin penetration and UV-damage prevention by nanoberries. J Cosmet Dermatol. 2018; 17(5): 889-899.
34. Calò R, Marabini L. Protective effect of Vaccinium myrtillus extract against UVA- and UVB-induced damage in a human keratinocyte cell line (HaCaT cells). J Photochem Photobiol B. 2014; 5(132): 27-35.
35. Wagner H, Bladt S. Plant Drug Analysis. Berlin Heidelberg:2nd Ed.,Springer; 1996.
36. Reich E, Schibli A. HPTLC for the Analysis of Medicinal Plants. N.Y-Stuttgart: Thieme. 2008.
37. Romanian Farmacopoeaia. Bucureşti-Romania: Xth Ed.,Medicală (in Romanian). 1993.
38. Ghosal K, Chandra A, Rajabalaya R, Chakraborty S, Nanda A., Mathematical modeling of drug release profiles for modified hydrophobic HPMC based gels. Pharmazie. 2012; 67:147-55.
39. Shah MV, De Gennaro MD, Suryakasuma H. An evaluation of albumin microcapsules prepared using a multiple emulsion technique, J. Microencapsul. 1987; 4: 223-238.
40. Higuchi T. Rate of release of medicaments from ointment bases containing drugs in suspension, J. Pharm. Sci., 1961; 50: 874-875.
41. Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers, Pharm. Acta Helv. 1985; 60: 110-111.
42. Zwietering MH, Jongenburger I, Rombouts FM, Van'T Rie TK. Modeling of the bacterial growth Curve. Appl. Env. Microbiol. 1990; 56: 1975–81.
43. Ramteke KH, Dighe PA, Kharat A, Patil SV. Mathematical Models of Drug Dissolution: A Review. Sch. Acad. J. Pharm. 2014; 3: 388-396.
44. Verhulst PF. Notice sur la loi que la population suit dans son accroissement. Correspondance mathematique et physique. 1983; 10: 113–21.
45. Ratkowski DA. Handbook of Nonlinear Regression Models. Marcel Dekker, New York. 1990.
46. Marquardt DW. An Algorithm for Least-Squares Estimation of Nonlinear Parameters, Journal of the Society for Industrial and Applied Mathematics. 1963; 11(2): 431–441.
47. Burnham KP, Anderson DR. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York. 2002.