Skin sensitization evaluation of a flavanol from processed rose wastewater for cosmetic application

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Abstract. Flavanols are extremely relevant for application and study due to the beneficial effect they have for humans when used internally or externally. They are identified in almost all plant cultures, as their metabolic products. Their pronounced benefit for protecting and restoring the skin in case of mechanical damage, radiation, allergens, chemicals and infections has been proven. Due to their natural origin, their dermatological application is also preferred. In such a case, the action of the chemical compounds and the generated skin metabolites have different mechanisms of action with respect to DNA and binding to proteins. The preliminary assessment of skin sensitization is a complex and lengthy task. Depending on the results, it is necessary to have an efficient technological approach for the isolation of flavanols with skin sensitization. Evaluation of the probable skin metabolic activity of cosmetic products containing flavanol (Luteolin) from processed rose wastewater is the aim of the conducted research, as well as prediction of protein and DNA binding of its metabolites by *in silico* methods (OECD QSAR Toolbox). The parent structure of the compound, as well as some of its generated metabolites, exhibit reactivity, i.e. they have different mechanisms of action in terms of DNA and protein binding.

1 Introduction

The main sources of bioactive substances necessary for the viability of humans and animals are the different agricultural crops. Natural chemical compounds are increasingly preferred by consumers as ingredients in pharmaceutical, cosmetic, food products. The agricultural sector has appreciated the opportunity and is actively developing the cultivation and processing of plants as a source of natural biomolecules. Bulgarian agriculture is a leader in terms of quality of raw materials grown and their processing [1]. Various crops such as rose, lavender, thyme, etc. are of marked economic and cultural importance. They are a source of

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biologically active plant extracts containing a rich chemical composition. It has been proven that polyphenolic compounds, vitamins and minerals predominate in the composition. Plant polyphenols are synthesized by almost all phytoorganisms. More than 10 000 compounds have been identified, which are generally defined as flavonoids, nonflavonoids, and tannins. The bioactivity of natural polyphenols is realized through their participation in antioxidant processes, which is due to their natural chemical structures [2]. It has been proven that anti-inflammatory, immunomodulatory, antimutagenic and many other benefits for the human body are due to them, combined with their ability to modulate enzyme functions in animal organisms [3,4]. In the cosmetics industry, not only natural extracts of aromatic oils are used, but also other natural extracts including polyphenols. In the cosmetics industry, various natural extracts and essential oils are used in skin care products. These products, even containing ingredients of natural origin, can be skin allergens for some people [5]. The detection of skin sensitization is a complex task given the large number of users and compounds. Methods validated to identify the causative agents of a skin reaction are unable to rapidly predict and assess risk [6]. The results will also direct the industry to the implementation of innovative engineering solutions for obtaining valorized products [7]. The selective separation of components from complex mixtures according to the current technology is difficult, expensive and not very efficient. Membrane technology is an alternative for this purpose [8-10].

Evaluation of the probable skin metabolic activity of cosmetic products containing flavanol (Luteolin) from processed rose wastewater is the aim of the conducted research, as well as prediction of protein and DNA binding of its metabolites by *in silico* methods (OECD QSAR Toolbox). The parent structure of the compound, as well as some of its generated metabolites, exhibit reactivity, i.e. they have different mechanisms of action in terms of DNA and protein binding.

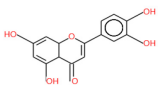
2 Materials and methods

2.1 Compound data

A flavanol (Luteolin) is presented in Table 1 [11]. Isolation of phytocomponents causing skin irritation will improve the tolerability of plant-based cosmetic and dermatological products. The separation of flavonoids and in particular flavanol (Luteolin) can be achieved by a baromembrane process using a polyacrylonitrile membrane.

Name and chemical structure of the Luteolin is presented in Table 1.

Table 1. Name and chemical structure of the compound (Luteolin)

Chemical name	Chemical structure
Luteolin	

2.2 Organisation for economic co-operation and development (OECD) QSAR Toolbox (version 4.3).

The OECD QSAR Toolbox is a freely available alternative software that is applied to assess the likely hazard of chemical compounds as well as their mechanistic and other knowledge based on the use of toxicological assessment methods and minimizes unnecessary animal testing. They are successfully applied by governments, the chemical industry and others [12].

2.2.1 Skin metabolism simulator

The skin metabolic simulator mimics the metabolic behavior of chemicals in the skin area. The metabolic skin simulator contains a list of master transformations arranged in a hierarchy [12].

2.2.2 DNA binding by OASIS

DNA binding by OASIS is a profiler that is based on the Ames mutagenicity model and is part of the OASIS TIMES system. It consists of 85 structural alerts responsible for the interaction with DNA, which examine the presence of signals in target molecules that can interact with DNA [12].

2.2.3 Protein binding by OASIS

Protein binding by OASIS is a profiler that examines the presence of caveats in target molecules responsible for interacting with proteins. It consists of 112 structural warnings that are divided into 11 mechanistic domains [12].

The polyacrylonitrile (PAN) membrane was obtained by the phase inversion method from a 16 wt.% solution of PAN (produced by LUKOIL Neftochim Bourgas Co., Bulgaria) in N,N - dimethylformamide (DMF) purchased from Fluka (Switzerland) [13]. The membrane has an asymmetric structure with a nominal separation limit relative to the molecular weight of the components in the retentate of 25 kDa. The object of separation in the membrane process is waste water from the hydrodistillation hydrodistillation of a flower from an oil-bearing rose variety of the Rosa damascena Mill. The permeability of the flow from the membrane (J, l/m2.h) and rejection (R, %) of the membrane were studied as function of the pressure (J,R=f(P) on an laboratory module SM-165-26 ("Sartorius", England).

Total flavonoids were estimated according to the proposed method in the literature [14] and the values are reported as quercetin equivalents. The analysis was performed by adding 10% $Al(NO_3)_3.9H_2O$, 1 M $C_2H_3KO_2$ and H_2O to the sample. The processed sample is kept in the dark for 50 minutes and then the absorption is measured spectrophotometrically at λ = 410 nm. The concentration of flavonoids is calculated according to the equation of the constructed standard curve y=2.8575.x+0.21476, R^2 = 0.9737.

3 Results and discussion

The flavanol (Luteolin) was predicted and studied for skin metabolic activity and binding (DNA and protein) by the software (QSAR Toolbox).

The parent structure of the flavanol (Luteolin) is DNA reactive but is not protein reactive. The Luteolin can bind to DNA in the domains with mechanistic action (AN2, non-covalent interaction and radical mechanism). The mechanistic alerts are Michael-type addition, quinoid structures, DNA intercalation and radical mechanism via ROS formation (indirect))

and a structural alert (quinones and trihydroxybenzenes). The flavanol (Luteolin) was metabolically activated through the software OECD (Q)SAR Toolbox.

Seven metabolites were generated using the skin metabolite simulator. Results of metabolic activation (metabolites) of the flavanol (Luteolin) in the skin are presented in Table 2.

Table 2. Number and chemical structure of the generated skin metabolites of the flavanol (Luteolin)

1	2	3	4
			
5	**6**	**7**	
			

The general mechanistic profiler (DNA binding by OASIS) consists of a list of structural signals (the TIMES mutagenicity AMES model). The purpose of DNA binding is to examine the presence of signals in target molecules that can interact with DNA [15].

The possible mechanism of DNA binding (mechanism of reaction) of the skin metabolites for the flavanol (Luteolin) is predicted by QSAR Toolbox software. DNA binding results of generated skin metabolites for the Luteolin are presented in Table 3.

Table 3. The probable mechanisms of DNA binding of the skin metabolites for the Luteolin by the OECD QSAR Toolbox (skin metabolism simulator)

Number (metabolite)	Reaction mechanisms of DNA binding by OASIS		
	structural alert (SA)	mechanistic alert (MA)	mechanistic domain (MD)
1-7	quinones and trihydroxybenzenes	Michael-type addition, quinoid structures	A_N^2
1-7	quinones and trihydroxybenzenes	DNA intercalation	Non-covalent interaction
1-7	quinones and trihydroxybenzenes	Radical mechanism via ROS formation (indirect)	Radical mechanism

The seven metabolites are reactive, as a structural alert regarding DNA binding was found. The mechanistic domains (MDs) of the DNA binding for the seven metabolites are A_N^2, non-covalent interaction, radical mechanism. The possible mechanistic alerts (MAs) of metabolites are Michael-type addition, quinoid structures, DNA intercalation and radical mechanism via ROS formation (indirect) with the following structural alert (quinones and trihydroxybenzenes).

The protein binding is based on the OASIS TIMES skin sensitivity model. It consists of structural signals that are divided into eleven mechanistic domains, and these are divided into more than two mechanistic caveats, but the absence of a structural signal should not be taken as a lack of toxicity [15].

The possible skin sensitivity protein binding results for Luteolin are presented in Table 4.

Table 4. The probable protein binding of the skin metabolites for the Luteolin by the OECD QSAR Toolbox (skin metabolism simulator)

Number (metabolite)	Reaction mechanisms of protein binding by OASIS		
	structural alert (SA)	**mechanistic alert (MA)**	**mechanistic domain (MD)**
3	No alert found		
1,2,4,5	Conjugated systems with electron withdrawing group	Michael addition on conjugated system with electron withdrawing group	Michael addition
1,2,4,5	Quinone methide(s)/imines; quinoide oxime structure; nitroquinones; naphthoquinone(s)/imines.	Michael addition on quinoid type compounds	Michael addition
1,2,4,5,6,7	1,2-dicarbonyls and 1,3-dicarbonyls	Direct acting Schiff base formers	Schiff base formation

For the seven metabolites regarding protein binding, one metabolite with number 3 was found to be non-reactive and the rest were reactive. The mechanistic domains (MDs) of the generated skin metabolites are Michael addition and Schiff base formation as the mechanistic alerts (MAs) are Michael addition on conjugated system with electron withdrawing group, Michael addition on quinoid type compounds and direct acting/Schiff base formers with the following structure alerts - conjugated systems with electron withdrawing group, quinone methide(s)/imines; quinoide oxime structure; nitroquinones; naphthoquinone(s)/ imines and 1,2-dicarbonyls and 1,3-dicarbonyls.

The spectrophotometrically determined concentration of total flavonoids, of which flavanols are a part, in the wastewater from the hydrodistillation of pink flower is 7.8 mgQeE/ml. The results for the permeability and percentage retention of this group of polyphenolic compounds are presented in Fig. 1. The baromembrane process was investigated at increasing pressure from 0 to 0.5 MPa with a step of 0.1 MPa. Membrane permeability is growing as the driving force of the process increases, by activating the pore potential. This behavior corresponds to a very high degree of retention not only of flavanols but also of the flavonoids present. The results showed a retention of up to 97 %, corresponding to a reduced amount of flavonoids in the filtrate to 0.38 mgQeE/ml. It should be noted that during the process, the mechanical stability of the membrane remains high and stable, proven by the hysteresis curve.

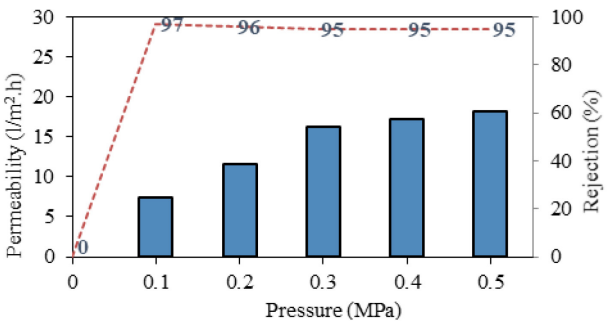


Fig. 1. Membrane permeability and retention of flavanols and flavonoids from hydrodistillation water in a baromembrane process

Electrophiles can bind to nucleophilic centers of intracellular macromolecules (DNA and proteins). The parent structure of Luteolin as well as its generated metabolites are electrophilic in nature and can bind to DNA and proteins. Electrophiles can react with DNA to form DNA-electrophile adducts that cause depurination of purine bases. When electrophiles interact with proteins, their structure and functional domain are affected, which sometimes leads to unpredictable activation of cell signaling pathways and apoptosis. Possible skin sensitizing electrophiles (parent structure and metabolites) for DNA are Luteolin, quinones and trihydroxybenzene, and for proteins are conjugated systems with electron withdrawing group, quinone methide(s)/imines (quinoide oxime structure, nitroquinones, naphthoquinone(s)/imines), 1,2-dicarbonyls and 1,3-dicarbonyls. The DNA binding of the skin metabolites and parent structure (Luteolin) is in the mechanistic domains (A_N^2, non-covalent interaction and radical mechanism) with mechanistic alerts (Michael-type addition, quinoid structures, DNA intercalation and radical mechanism via ROS formation (indirect)) and the protein binding of the skin metabolites is in the mechanistic domains (Michael addition and Schiff base formation) with mechanistic alerts (Michael addition on conjugated system with electron withdrawing group, Michael addition on quinoid type compounds and direct acting Schiff base formers).

4 Conclusions

Flavanols are of important biological importance and are used in the cosmetic industry, so it is important to check their possible effects on the skin. Theoretical studies show that it is possible to observe metabolic activation of the Luteolin in the skin under certain conditions. The skin sensitivity can be caused by the parent structure of the Luteolin, as well as its metabolites, which are electrophilic in nature and can interact with DNA and proteins in different mechanistic domains (DNA binding - A_N^2, non-covalent interaction and radical mechanism and protein binding - Michael addition and Schiff base formation). Retention, up to 97% of flavanols and flavonoids, some of which is and Luteolin, shows the ability to effectively isolate in a baromembrane process with the polyacrylonitrile membrane. The process applied to rose wastewater, will allow the treated water to be used in products intended for skin sensitive to the luteolin metabolites.

Burgas State University "Prof. Dr. Asen Zlatarov" funded the research work in connection with scientific project 478/2023.

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