Pharmacological Effects of Natural Compounds Extracted from *Urtica dioica* Evaluated by *in Silico* and Experimental Methods

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Urtica dioica is a perennial plant of the family of Urticaceae, originating in Eurasia. Nettle lives in temperate regions, prefers shady places, moist soil and rich in nutrients. Worldwide nettle is considered weed. Nettle was used in the past for its medicinal properties on the body and to cure diseases such as arthritis, rheumatism, urinary tract infections, kidney stones, gingivitis, hair loss, or ulcer. It is a plant rich in nutrients, with a high content of minerals and vitamins, especially vitamin A and C. The nettle has uses in both pharmaceutical and cosmetic and nutrition processes. Nettle extract contains ursolic acid and quercetin, giving the plant antioxidant and anti-aging effects. Studies on the nettle have demonstrated a multitude of pharmacological uses including: pharmacological, antiviral, antibacterial, hepato-protective, hypotensive, diuretic and anti-inflammatory effects. The methods of extraction of the compounds used in the plant were maceration and distillation using ethanol-water-soluble solvent, used because of its extraction capacity. At the same time, the metals in the nettle were determined taking 2 samples of the root, the stalk and the nettle leaves, but also the soil from which the plant grew and were analyzed by Spectroscopy Elemental Isotope Analysis using an X-ray Fluorescence (XRF) analyzer. By cheminformatics and bioinformatics tools we elucidate for first time: (i) the drug like features and bioavailability of ursolic acid and quercetin (ii) by predictive ADMET a set of pharmacokinetic features like: absorption, distribution, metabolized, blood brain barrier and nervous system barrier crossed and toxicology features (AMES, carcinogens, hepatotoxicity, Maximum tolerated dose (human)). Our results showed that quercetin is a good candidate as antioxidant and anti-aging effects.

Keywords: Nettle, extraction, metal absorption, bioinformatics, pharmacological profiles

Urtica dioica is from botanical family *Urticaceae* and is a perennial plant that grows in temperate and tropical areas around the world [1]. Originally, the nettle is a Eurasian plant [2]. In the Himalayas, the nettle is found at altitudes of 2100-3200 meters.

The plant is known as the stinging nettle [3]. The nettle name comes from the Latin *uro* which means burning, the name refers to the burning sensation caused by the plant and the *dioica* comes from two Greek words: *dis* which means *two* and *oikos* which translates into *house* [4].

Urtica dioica is known for its medicinal properties and the benefits it brings to the human body. Studies have shown that nettle has antioxidant, antimicrobial, anti-inflammatory, analgesic and other biological effects [5]. Nettle has been used since ancient times in popular medicine to cure many diseases including arthritis, rheumatism, ulcer, eczema and other affections [6].

Nettle is a highly nutritious and easily digestible plant, rich in vitamin A and C and minerals especially iron. The medicinal components in the nettle are very commonly used in pharmaceuticals, as well in cosmetics and food [7, 8].

Nettle extracts are used in cosmetics not only for their anti-inflammatory effect but also for their antioxidant and anti-aging properties. Nettle preparations have immediate effects and are beneficial to skin health. The most common cosmetic based on nettle is shampoo. Among other chemical compounds, ursic acid and quercetin molecules responsible for anti-aging action are found in nettle. Ursolic acid accumulates in the plant's roots and is known as an elastase inhibitor. Quercetin usually accumulates in the leaves. This is one of the most important antioxidants.

These compounds confirm the antioxidant capacity of nettle [6].

Nettle extract has been shown to have anti-diabetic activity, with significant blood glucose lowering effects [9]. Hepato-protective activity was proven with nettle seed extract that has liver protective effect by increasing paraoxonase, arylesterase activity and liver catalase activity [10]. Leaf extract showed hepato-protective activity by decreasing alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) [11]. It also turned out antiviral activity [3]. The microbial activity of the plant was tested on various gram positive and vegan bacteria, inhibiting bacteria: Streptococcus aureus, Enterococcus faecalis, Bacillus subtilis, E-coli [12,13]. The nettle also has a hypotensive effect, the aqueous nettle extract marked a decrease in heart rate without changing the cardiac pressure [14]. The diuretic effect was tested on the rabbits. It has been shown that the plant has diuretic activity by increasing diuresis in the animal [15,16].

Anti-inflammatory effects were tested in mice and rats, resulting in the nettle extract used in painful and inflammatory conditions having attenuating effects [17].

New strategy in treatment of various disorders is to use the natural compounds identification of natural compounds as possible new pharmacological targets, with

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reduce side effects but with still now doubtfully molecular mechanism, as main effect, is presently necessary. Here, based on our expertise in silica tools applied to small and very large synthetic and natural compounds [18, 19], we quantified the pharmacological features of ursolic acid and quercetin. Also, ADMET features of these natural compounds were analyzed.

Experimental part

The main chemical compounds of nettle are: flavonoids, tannins, volatile compounds and fatty acids, polysaccharides, isolectins, sterols, terpenes, proteins, vitamins and minerals [3]. Among the most important flavonoids in the nettle are: kaempferol, isorhamnetin, quercetin, isoquercetin, astragalin, routine and 3-rutinoside and 3-glycosides [20-24].

Quercetin is a very beneficial flavonoid for health, but also for the appearance of the skin. It has many biological actions such as: anticancer, antiviral, antiallergic, antitumoral, anti-inflammatory [25] (fig.1).

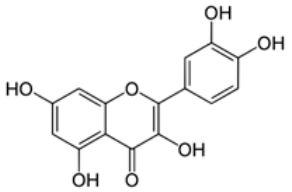

Fig.1. The structural formula of Quercetin

Carotenoids such as β-carotene, hydroxy-β-carotene, lutoxanthin, lutein epoxide and viraxanthin are also present [21, 23, 24]. Carotene is the most known carotenoid and is a powerful antioxidant. β-carotene was among the main factors of growth (fig.2) [24].

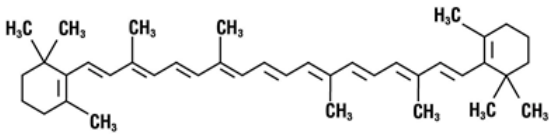

Fig. 2. The structural formula of β–carotene

The leaves are rich in vitamins B, C, K and minerals like: Ca, Fe, Mg, P, K, Na. Other main compounds present: essential amino acids, glucocinins and chlorophyll [3].

The extraction of the active principles from *Urtica dioica* was carried out by maceration processes in the first stage and distillation in the second. Thus, we extracted chemical constituents from the fresh vegetable material by means of chemical solvents. In this experiment we used 96% ethanol. Ethanol 96% is a hydrophilic solvent that can extract a wide range of chemical compounds [26].

Was made a pharmacologically finished product with a soap. The recipe through which the soap was made is the classic one, to which was added the previously obtained extract. The soap contains distilled water, sodium hydroxide, animal fat, coconut oil, olive oil and nettle extract previously obtained.

Steps leading to soap: - dissolve sodium hydroxide in distilled water. In a separate pan melt the butter and mix with the olive oil, after the mixture has reached room temperature, add the nettle extract. Over the entire mixture, pour the sodium hydroxide dissolved in the distilled water and mix until homogeneous. The entire composition begins to thicken, then poured into a container of the desired shape and left to solidify for about 48 h. It is preferable that the soap be left to mature for 2-3 weeks prior to use.

In parallel, samples of *Urtica dioica* from two different areas of Timisoara were taken. Samples contained the nettle root, nettle stalk, nettle leaves, and a monster in the soil where the nettle grew. The goal was to see what metals the plant contains, in what proportions and which part of the plant body retains more metal.

The plants were separated and dried at room temperature (20°C). Dried plants were separated into root, stalk and leaf and heavy metal content have been analyzed by Spectroscopy Elemental Isotope Analysis using an X-ray Fluorescence (XRF) analyzer. The soil was dried and analyzed using the same method of analysis.

Computational strategy

Molecular modeling and minimum energy calculation of natural compounds

In this study we used 2D format of ursolic acid and quercetin extracted from ChEMBL database [27] ursolic acid compound (ID=CHEMBL169; quercetin compound ID= CHEMBL50).

The spatial structure 3D of each compounds was obtained in Discovery Studio software [28] and save as mol2 file. After molecular modeling, minimum potential energy calculation was performed using Forcefield MMFF94x at a 0.05 gradient. After energy minimization, Gasteiger partial charges were used.

Drug-Like Feature of the Compounds Isolated from natural compounds evaluated by Lipinski's

Rule of Five Lipinski's *rule of five* is an important criterion to predict if chemical compounds are likely to exhibit pharmaceutical activity in biological systems. According to this rule, a chemical compound is expected to present drug-like features if the following criteria are respected: molecular weight (Weight) less than 500, hydrophobicity (logP(o/w)) no more than 5, hydrogen bond donors (lip don) no more than 5, and hydrogen bond acceptors (lip acc) no more than10 [29].

Bioavailability evaluated by Veber rules

A compound presents a good bioavailability if molecular descriptors respected the criteria: number of rotatable bonds (RBN) no more 10, and b) polar surface area (TPSA) is no more than 140 Å2 or the sum of H-bond acceptors and H-bond donors no more than 12 [30].

ADMET Predicted Profile

For ADMET (Absorption Distribution Metabolism Excretion Toxicity) predictions was used pkCSM - pharmacokinetics database [31] and Discovery Studio software [28]. The ADMET prediction was performed for ursolic acid and quercetin. We selected from predictive ADMET parameters follow: (i) Absorption- Intestinal absorption (human) (% Absorbed) [31].

The Intestine is normally the primary site for absorption of a drug from an orally administered solution. For a given compound it predicts the percentage that will be absorbed through the human intestine. A molecule with an absorbance of less than 30% is considered to be poorly absorbed; Caco-2 Permeability (LogPapp, cm/s)-A compound is considered to have a high Caco-2 permeability if it has a Papp > 8 x 10-6 cm/s. For the pkCSM predictive model, high Caco-2 permeability would translate in predicted values > 0.90 [31]; (ii) Distribution - BBB permeability (Numeric (log BB))-was used as model 320 compounds, log BBB more than 0.3 good BBB permeability, less than -1 low BBB permeability; CNS permeability (Numeric (log PS))-log Ps more than -0.2, penetrate CNS, less than -3 no penetrate CNS; Fraction unbound (human)(Numeric (Fu))-predictive model was used for 562 compounds in human blood (Fu);(iii**)** Metabolisation - CYP3A4 substrate (3A4 is one of the isoform of cytochrome

P450 responsible for metabolism of a drug); (iv) Excretion-Total Clearance (renal and hepatic clearance) and (v) **Toxicity**- AMES toxicity (mutagenic potential of a drug), Max. tolerated dose (human)(Numeric (log mg/kg/day)))-MTD = 0.477 = low; more than 0.477 = high; Oral Rat Acute Toxicity (LD50) (lethal dose for 50% of rats used in test) [31].

Results and discussions

According to the nettle extract properties, the product can have antimicrobial, anti-inflammatory, antiseptic, anti-seborrheic and even cicatrizing effects. The product may be a replacement shampoo because it has anti-hair loss effects. It also regulates the excess of sebum on the scalp. The pharmacological product has no negative effects even if it is used repeatedly because it does not contain toxic organic substances.

The content of metals present in plants and soil is presented in table 1.

Computational strategy

Lipinski and Veber rules for natural compounds isolated from Urtica dioica

For Lipinski rule validation we calculate the descriptors in Discovery Studio software (table 2).

Parameter	Experimental concentration, [mg/Kg d.w.]				Maximum permitted concentration of legislation [mg/Kg d.w.]
	Soil	Root	Stalk	Leaf	
Sample 1					
Pb	61	<0.01	<0.01	<0,01	20
Zn	160	26	<0.01	<0.01	100
Cu	46	<0.01	<0.01	<0.01	20
Ni	53	<0.01	<0.01	<0.01	20
Co	112	<0.01	<0.01	<0.01	15
Fe	$21 \cdot 10^3$	$3.7 \cdot 10^3$	<0.01	960	-
Mn	422	<0.01	<0.01	<0.01	900
Ca	$25 \cdot 10^3$	$12 \cdot 10^3$	$2.8 \cdot 10^3$	<0.01	-
K	$6.3 \cdot 10^3$	20.5	$2.5 \cdot 10^3$	<0.01	-
Sample 2					
Pb	27	<0.01	<0.01	<0.01	20
Zn	164	13	20	<0.01	100
Cu	44	<0.01	<0.01	<0.01	20
Ni	56	<0.01	<0.01	<0.01	20
Co	162	<0.01	<0.01	<0.01	15
Fe	$26 \cdot 10^3$	663	781	111	-
Mn	639	<0.01	<0.01	<0.01	900
Ca	$20 \cdot 10^3$	$6.5 \cdot 10^3$	$18 \cdot 10^3$	<0.01	-
K	$63 \cdot 10^3$	$33 \cdot 10^3$	$42 \cdot 10^3$	<0.01	-

Table 1.
THE CONTENT OF METALS PRESENTS IN PLANTS AND SOIL USING X-RAY FLUORESCENCE (XRF) ANALYZER

Compound	Lipinski's Validation	Veber Validation
quercetin	yes	yes
ursolic acid	yes	yes

Table 2
LIPINSKI'S AND VEBER RULE VALIDATION FOR NATURAL COMPOUNDS FROM URTICA DIOICA

Table 3
IN SILICA ADMET PREDICTED VALUES FOR NATURAL COMPOUNDS URSOLIC ACID AND QUERCETIN

Property	Model Name	Ursolic acid predicted value	Quercetin predicted value	Unit	Interpretation
Absorption	Intestinal absorption (human)	100	74.535	% Absorbed	>0.90 high permeability
Absorption	Caco2 permeability	1.287	0.162	log Papp in 10-6 cm/s	
Distribution	BBB permeability	-0.174	-1.461	log BB	poorly cross -1 >logBBB >0.3 crosses
Distribution	CNS permeability	-1.118	-3.374	logPS	logPS>-2 penetrate CNS logPS<-3 don't penetrate CNS
Distribution	Fraction unbound (human)	0	0.107	Numeric (Fu)	
Metabolism	CYP3A4 substrate	Yes	No	Yes/No	
Excretion	Total Clearance	0.083	0.522	log ml/min/kg	
Toxicity	AMES toxicity	No	Yes	Yes/No	
Toxicity	Max. tolerated dose (human)	0.371	0.983	log mg/kg/day	
Toxicity	Oral Rat Acute Toxicity (LD50)	2.683	2.675	mol/kg	
Toxicity	Hepatotoxicity	Yes	No	Yes/No	

Our results showed that quercetin and ursolic acid are in accord with Lipinski rules and Veber rules. This lead to observation that both compounds can be considered as drug like.

Also, the bioavailability of compounds is very good. The bioavailability of ursolic acid presents a higher bio-availability in comparison with quercetin.

ADMET profile evaluation for ursolic acid and quercetin

Caco-2 cell line is used in *in vitro* studies to predict the absorption of orally administrated medication. In this model a Caco-2 permeability with a value above 0.90 is considerate high. Ursolic acid present a predicted value of 1.287, this indicate a good Caco-2 permeability in the other side the Caco-2 permeability of quercetin is low (-0.162). Intestinal absorption indicates if a molecule is absorbed or not by human intestine, a value less than 30% means that the molecule is unwell absorbed by human intestine. Both molecules present a high intestinal absorption: Ursolic acid 100% and quercetin 74.535%. Fraction unbound of a drug is referred at the fraction of a drug that is not bound with the protein, a high fraction unbound indicate that the molecule traverse or diffuse more efficiently the cellular membrane. In this case the unbound fraction for ursolic acid is 0 and for quercetin is 0.107.

The BBB permeability indicate if a molecule penetrates the BBB, in this model a logBB lower than -1 indicate that the molecule is poorly crosses the BBB and higher than 0.3 indicate a very good permeability, quercetin (-1.461) indicate a low BBB permeability and ursolic acid (-0.174) indicate a medium BBB permeability.

CNS permeability indicate if a molecule can penetrate the CNS ursolic acid has a CNS permeability value of -1.118, this indicate that this molecule penetrates CNS in the other hand quercetin (-3.374) don't have CNS permeability.

Ursolic acid is substrate for CYP3A4 that means that ursolic acid is metabolized by this enzyme; in the other side quercetin is not. Total Clearance of ursolic acid is 0.083 log mL/min/kg and for quercetin is 0.522 log mL/min/kg.

AMES toxicity test indicates if a compound is mutagenic or not ursolic acid present no AMES toxicity but quercetin presents.

Maximum tolerated dose (human) is lower in case of ursolic acid (0.371) than quercetin 0.983 this indicate that quercetin can be administrated in a higher dose than ursolic acid without a toxic effect.

LD50 represent the dose of a drug that is lethal for 50% of the group of rats tested with the drug. In this case the dose is similar for both compounds quercetin (2.675) and ursolic acid (2.683). quercetin presents no hepatotoxicity but ursolic acid presents [31] (table 3).

Conclusions

In conclusion we can state that nettle and its extracts have curative, detoxifying, liver regeneration and

immunization properties due to the high amount of vitamin C and mineralization of the body. Due to the compounds in the nettle we can say that nettle also has anti-inflammatory, anti-seborrheic, but antioxidant and anti-aging role.

Tests on the nettle showed overtaking of normal amounts of metal admitted. Although Zn, Fe and Cu are some of the metals that exceed the permitted concentration of the nettle extract included in dermato-cosmetic products, it does not seem to have any harmful effects on the skin or hair, and on the contrary, it does not affect its properties.

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