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Analysis of Trace Elements, Cholinesterase Inhibitory Activity, and ADME/Tox Profiling of some *Achillea* Species

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Abstract: In this study, the anti-cholinesterase activities of different extracts of *Achillea lycaonica, A. vermicularis* and *A. nobilis* L. subsp. neilreichii (Kerner) Formanek species were examined and then trace elements (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, and Pb) of the plants were analyzed by ICP-MS. The pharmacokinetic properties of the phenolic compounds of these plants previously analyzed by us and the ADME-Tox (absorption, distribution, metabolism, excretion, and toxicity) profiles of the trace elements we identified in this study were estimated. According to the data obtained, it was determined that all three plants showed high acetyl-cholinesterase inhibitory activity. The concentration of trace elements was lower than that declared by WHO, except for the *A. lycaonica*. It was determined that V, Cr, Fe, Co, and As metals in *A. lycaonica* exceeded the limit values determined by WHO. According to ADMET estimates, it is thought that the toxic values of all three plants are not high, and therefore the use of *A. nobilis* and *A. vermicularis* plants does not pose any health risk but A *lycaonica* should be used carefully due to the presence of heavy metals.

Keywords: Phenolic compounds, ADMET, cholinesterase inhibitory, ICP-MS.

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INTRODUCTION

The genus *Achillea* is widely found in Europe, temperate areas of Asia, and North America. *Achillea* sp. is one of the most valuable and economic plants of Anatolia. In Turkey, the *Achillea* genus is used in wound healing, abdominal pain, stomachache, symptomatic relief of colds, ulcers, and diarrhea (1).

Herbal teas prepared from aerial parts of different *Achillea* species have traditionally been used as an anti-inflammatory agent for the treatment of spasmolytic and rheumatic pain. The aerial parts of different *Achillea* species are widely used in folk medicine for the preparation of drugs with anti-inflammatory spasmolytic, hemostatic, digestive, and collagic effects. The dried flower heads of the *Achillea nobilis* L. subsp. neilreichii (Kerner) Formanek are used as a diuretic and an emmenagog for wound healing, for abdominal pain, and against diarrhea in Turkey (2). *Achillea lycaonica* Boiss. et Heldr. is a plant native to Turkey and are known to have antioxidant, wound healing, and cytotoxic effects (3). The flowers of *Achillea* vermicularis Trin. are used in the treatment of diarrhea and renal pain. In addition, the plant's capitulum is used in the treatment of asthma (4,5).

Trace elements play an essential role in the formation of chemical components in plants. Some metals such as zinc, iron, copper, chromium, and cobalt are required at certain levels for living things and are toxic in high concentrations. On the other hand, mercury, lead, and cadmium metals are harmful to the body even at low concentrations, and no beneficial properties are known. Medicinal herbs are known to be used in traditional therapy for

certain symptoms due to their low side effects. Plants may pose health risks due to toxic elements that may be contained due to environmental pollution, etc. Therefore, it is very important to know medicinal plants in terms of trace element content (6-9). Inductively coupled plasma-mass spectrometry ICP-MS is a technique frequently used in trace element analysis of medicinal plants due to its high sensitivity and wide linear dynamic range (10-13).

Anticipating the pharmacokinetic properties of pharmaceutical molecules increases the likelihood of reaching the target faster and more guaranteed. The word ADMET is an abbreviation for absorption, distribution, metabolism, excretion, and toxicity. These criteria define the pharmaceutical activities of the drug candidates (14).

In this study, trace metal analysis and anti-cholinesterase activities were determined depending on the medicinal use of plants. In addition, the ADMET profiles of the phenolic compounds we analyzed in our previous studies and the trace elements we detected in this study were estimated.

MATERIAL AND METHODS

Identification of Plant Material

The taxonomic identity of the *A. lycaonica* was confirmed by Prof. Dr. Turan Arabaci. The voucher specimens were deposited in the herbarium of the Faculty of Pharmacy, İnönü University; herbarium number: T. Arabacı 2969. The *A. vermicularis* Trin. was authenticated by Dr. Ahmet Dogan, and a voucher specimen (MARE-18071) was deposited at the Marmara University Pharmacy herbarium for future reference. The taxonomic identity of the *A. nobilis* L. subsp. neilreichii (Kerner) Formanek was confirmed by Assist. Prof. Dr. Ismail Senkardes. The voucher specimens were deposited in the herbarium of the Faculty of Pharmacy, Marmara University; herbarium numbers: MARE-18074.

Preparation of Plants Extracts

The dried aerial parts of plants were extracted chloroform, ethyl acetate, and ethanol by using the soxhlet and maceration methods. All extracts were filtered and evaporated to dryness under reduced pressure at 45°C in a rotary evaporator. The crude extracts were then transferred to vials and kept at +4 °C for future analysis.

Determination of AChE Inhibitory Activities of Different Extracts

Inhibition of cholinesterases was evaluated using a 96-well microplate reader based on the method of Ellman (1961) with some modifications. Spectrophotometric data were recorded by Shimadzu UV-1800 spectrophotometer. Experiments have been carried out at least in triplicate, and galantamine was used as a reference (15).

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) Analysis

An Agilent 7700 ICP-MS and ASX-500 autosampler was used to determine the trace elements in the samples. The internal standard was used for each element to be analyzed. The collision reaction cell was used to eliminate spectral and non-spectral interference, and the required mode for each element was determined by the collision reaction cell. It was noted that the abundance rates were higher in elements with more than one isotope and no interference-effect. Accordingly, the mass of the studied elements is shown in Table 1.

For trace element determination, *Achillea* samples were accurately weighed and placed in teflon containers to be dissolved in a microwave oven (Berghoff). A mixture of HNO_3: H_2O_2, 3:1 (v:v) was prepared, and 10 mL of this mixture was added to the samples and placed in the microwave oven incinerator. The dissolution process was carried out by adjusting the temperature (0- 180 °C) and time (35 minutes) of the microwave oven program. After dissolution, all samples were filtered and completed to 25 mL with ultrapure water and read in Agilent 7700 ICP-MS device.

Table 1. ICP-MS operating conditions.

Element	Mass	ISTD* mass	Tune Mode	Time (sec)
Al	27	45[a]	He	0.30
V	51	45	He	0.10
Cr	52	45	He	1.00
Mn	55	45	He	0.10
Fe	56	45	He	0.10
Co	59	45	He	0.10
Ni	60	45	He	1.00
Cu	63	45	He	0.30
Zn	66	72[b]	He	0.20
As	75	72	He	0.30
Se	78	72	He	5.00
Cd	111	115[c]	No Gas	1.00
Pb	208	209[d]	No Gas	0.30

*Internal Standards: [a]Sc [b]Ge [c]In [d]Bi

ADME/Tox Prediction

Anticipating the pharmacokinetic properties of pharmaceutical molecules increases the likelihood of reaching the target faster and more guaranteed. The ADMET criteria define the pharmaceutical activities of the drug candidates. pkCSM, a free online web server (http://structure.bioc.cam.ac.uk/pkcsm) was used to predict the properties of the previously analyzed compounds and trace elements from *A. lyconica*, *A. nobilis,* and *A. vermicularis*. Toxtree-v3.1.0.1851 software was used to estimate toxicity analysis of substituents of structures according to Cramer's rules. Cramer's rules are divided into three categories. Low (Class I), Substances with simple chemical structures and for which efficient modes of metabolism exist, suggesting a low order of oral toxicity. Intermediate (Class II), Substances which possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III. High (Class III), Substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups.

Molecular polar surface area (PSA) is a very useful parameter for the prediction of drug transport properties. Polar surface area is defined as a sum of surfaces of polar atoms (usually oxygens, nitrogens, and attached hydrogens) in a molecule. This parameter has been shown to correlate very well with the human intestinal absorption, Caco-2 monolayers permeability, and blood-brain barrier penetration. Molecular lipophilicity potential (MLP) is useful property to rationalize various molecular ADME characteristics (like membrane penetration or plasma-protein binding). Analysis of the 3-D distribution of hydrophobicity on the molecular surface is particularly helpful when explaining differences in observed ADME properties of molecules with the same logP, since the 3D parameter contains much more information then logP expressed by just a single value. Molecular polar surface area and molecular lipophilicity potential calculated using Molinspiration cheminformatics (www.molinspiration.com/cgi-bin/properties) (14,16).

In this study, ADMET properties of 18 phenolic compounds and 13 trace metals that we analyzed from these three plants with our previous studies (17-19) were measured by a computer, and the partition coefficients (log P) of the related phenolic compounds were found.

RESULTS AND DISCUSSION

Concentrations of Trace Elements in *Achillea* Species

The accumulation levels of heavy metals resulting from natural or human-induced activities should be regularly measured and monitored in terms of environment and human health. For this, the ICP-MS (Inductively coupled plasma-mass spectrometer) device has been used in recent years, which has advantages such as being economical, fast, and working even at very low concentrations. Accordingly, in this study, trace metal analysis of three different *Achillea* species whose phenolic content we analyzed in previous publications were performed with ICP MS. It is aimed to determine the concentrations of Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, and Pb quantitatively as trace metals, and the results were presented in Table 2. The vanadium content was determined in the range of 0.24–1.58 ng/mg. When compared to each other of *Achillea* species, the most high level of V in AL. The Aluminium and chromium contents were detected in the range of 190.16–638.40 ng/mg and 0.91-2.71 ng/mg, respectively. In Yener's study, the Al and Cr contents were found 1424 and 4.8 mg/kg in *Malvella sherardiana* (20). In another study, heavy metal analysis of 20 medicinal plants was performed, and the highest chromium value was found to be 33.75 µg/g (21). In another study, the heavy metal content of the most used medicinal plants in Sudan was analyzed, and as a result, the highest chromium values were found in *Ammi visnaga* L (2.6 mg/kg) and *Foeniculum vulgare* P. Mill (3.6 mg/kg) (22). In a study by Khan et al., the maximum amount of Mn in *Artemisia vulgaris* L. was 52.94 ppm and the amount of Fe in *Withania somnifera* L., was 206.69 ppm (23). In Karahan et al., that taken 17 medicinal plants collected from Turkey was founded the Fe levels in the range of 78.96- 907.062 mg/kg. And also in that study, they were detected Mn levels in the range of 12.11-362.57 mg/kg (24). In another study, cobalt, nickel, copper, and arsenic were studied in 26 plants, and these heavy metal content ranges were found to be 0.57- 2.86, 3.47-10.83, 5.12- 17.90, and 0.08- 3.03, respectively (25). In another review study, the cadmium content range of medicinal plants analyzed was 0.051- 704.39 µg/g, while the selenium content was 0.3-41.3 µg/g. While the zinc range of medicinal plants analyzed in the same study was found to be 0.14-7695.4 µg/g, the lead range was found to be 0.09-22.95 µg/g (26). The WHO maximum permissible limits of chromium and lead in medicinal herbs are 2 and 10 ppm, respectively (27). In addition, in literature reviews, this value has been reported to be 0.3 ppm in terms of cadmium and 50 ppm for zinc (28,29). The limit values of Fe and Cu accepted by FAO / WHO for edible vegetables are 450 and 40 ppm, respectively (30). Ni limit value accepted by FAO / WHO for plants is 5 ppm (31). Among the analyzed plants, all species are within this limit value While the limit value determined by FAO / WHO for vanadium is 0.03 ppm, it has been reported that these values are 0.50 and 0.01 for arsenic and cobalt, respectively (32).

Table 2. Quantification of trace elements in *Achillea* species by ICP-MS. Concentrations of the analyzed trace elements [mean (ng/mg) ± SD. n=4]

Plant samples	***A. vermicularis***	***A. nobilis subsp. neilreichii***	***A. lyconica***
Al	190.16±12.89	520.39±35.28	638.40±43.28
V	0.24±0.01	0.55±0.02	1.58±0.06
Cr	0.91±0.05	2.54±0.13	2.710±0.001
Mn	18.97±1.09	45.96±2.64	24.73±1.42
Fe	125.57±1.31	319.23±3.32	584.55±6.08
Co	0.095±0.005	0.25±0.01	0.39±0.02
Ni	1.05±0.04	1.83±0.07	2.26±0.08
Cu	4.09±0.32	6.46±0.51	6.04±0.47
Zn	30.17±1.13	40.99±1.54	43.20±1.62
As	0.08±0.01	0.51±0.03	0.565±0.04
Se	0.09±0.01	0.045±0.003	0.052±0.003
Cd	0.06±0.01	0.19±0.01	0.150±0.006
Pb	0.68±0.03	4.43±0.20	3.135±0.140

When the amounts of all metals detected in plants are examined, it is seen that Co and V metals are above the limit values determined in all three plants. In addition, it was determined that the amount of Cr, Fe, and most importantly As, besides V and Co, was above the allowed value in *A. lyconica*. No disease has been detected in humans due to vanadium deficiency. In general, the toxicity of vanadium compounds is also low. In this regard, the possible damage of vanadium excess in plants can be ignored. When all results were examined, it was determined that *A. lyconica* contained higher amounts of heavy metals than the allowed values among the other species. The most important of these heavy metals is As. Considering that arsenic is classified as "Group 1" in terms of being carcinogenic when examining the criteria of the International Cancer Research Agency, it is a natural conclusion that it is necessary to be careful in the use of *A. lyconica*.

Determination of Anticholinesterase Activities of Plants

In the current study, cholinesterase enzyme activities of different extracts obtained from the plants by two extraction methods were compared. It was determined that both the maceration (74.64%) and soxhlet (69.23%) ethanol extracts of *A. lycaonica* showed potent anti-cholinesterase activity compared to other extracts. It was also observed that the chloroform extracts from both extraction methods did not have any enzyme inhibition ability. It was found that both the maceration ethanol (82.11%) and soxhlet ethanol (55.04%) extracts of *A. nobilis L. subsp. neilreichii* exhibited strong anti-cholinesterase activity compared to other extracts. When the results of the *A. vermicularis* were compared, it was found that maceration ethanol (79.49%) and soxhlet ethanol (76.92%) extracts had the highest inhibition of cholinesterase enzyme. As the results, maceration ethanol extracts of all three plants were determined to have the highest enzyme inhibition ability in this study. When all data are compared with the standard compound, galantamine, all extracts were found to have lower activity than the standard compound (95.52%) at 500 µg/mL concentration (Table 3).

Table 3. AChE inhibitory activities of different extracts from plants

Enzyme inhibition (%) (500 µg/mL)

Extracts	***A. lycaonica***	***A. nobilis L. subsp. neilreichii***	***A. vermicularis***
Maceration chloroform	NA*	44.62±1.63	NA
Maceration ethyl acetate	44.62±1.85	30.20±1.69	36.87±1.97
Maceration ethanol	74.64±1.78	82.11±0.84	79.49±0.34
Soxhlet chloroform	NA	26.21±1.64	26.27±0.099
Soxhlet ethyl acetate	54.42±1.04	NA	64.10±0.64
Soxhlet ethanol	69.23±2.05	55.04±2.52	76.92±1.48
Galanthamine		95.52±1.15	

***NA: no activity**

***In silico* ADME/Tox profiling of phenolics and trace elements**

The pharmacokinetics of phenolic compounds and trace metals in plants were predicted by the parameters of absorption, distribution, metabolism, excretion, and toxicity as shown in Table 4-7 and Figure 1. The Caco-2 permeability values (>0.90) of caffeic acid, salicylic acid, 8-hydroxy-salvigenin, naringenin, and apigenin compounds were predicted to be high. Chlorogenic acid, rutin, dicaffeoylquinic acid, apigenin 6,8-di-C-glucoside, and luteolin-3',7-di-O-glucoside were predicted to have poorly intestinal absorption (human) compared to other compounds. Salicylic acid has low skin permeability properties (log Kp>-2.5).

Table 4. The ADMET parameters of the following compounds from plants via pkCSM software.

	Predicted Value									
Property	Caffeic acid	Chlorogenic acid	Quercetin	Salicylic acid	Rutin	8-Hydroxy-salvigenin	Luteolin	Dicaffeoylquinic acid	Naringenin	Apigenin
Absorption										
Water solubility (log mol/L)	-4.30	-2.75	-3.37	-1.66	-2.89	-3.84	-3.09	-2.92	-3.22	-3.33
Caco2 permeability	0.96	-0.62	0.59	1.13	-0.66	1.15	0.10	-1.16	1.03	1.01
Intestinal absorption (% A)	96.14	17.16	74.99	77.71	28.30	68.87	81.13	16.36	91.31	93.25
Skin Permeability (log Kp)	-2.79	-2.74	-2.74	-2.32	-2.74	-2.74	-2.74	-2.74	-2.74	-2.74
P-glycoprotein substrate	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
P-glycoprotein I inhibitor	No	No	No	No	No	No	No	No	No	No
P-glycoprotein II inhibitor	Yes	No	No	No	No	Yes	No	No	No	No
Distribution										
VDss[a]	-0.14	-0.85	0.24	-0.47	-0.07	0.01	1.15	1.96	-0.02	0.82
Fraction unbound	0.08	0.40	0.02	0.53	0.30	0.08	0.17	0.29	0.06	0.15
BBB permeability[b] (log BB)	-0.14	-1.44	-1.36	-0.31	-2.10	-0.40	-0.91	-2.13	-0.58	-0.73
CNS permeability[c] log PS	-2.06	-4.03	-3.43	-2.91	-5.75	-3.20	-2.25	-3.80	-2.22	-2.06
Metabolism										
CYP2D6 substrate	No	No	No	No	No	No	No	No	No	No
CYP3A4 substrate	Yes	No	No	No	No	No	No	Yes	No	No
CYP1A2 inhibitor	Yes	No	Yes	No	No	Yes	Yes	No	Yes	Yes
CYP2C19 inhibitor	Yes	No	No	No	No	Yes	No	No	No	Yes
CYP2C9 inhibitor	Yes	No	No	No	No	Yes	Yes	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	Yes	No	Yes	No	No	Yes	No	No	No	No
Excretion										
Total Clearance (log mL/min/kg)	0.80	0.37	0.58	0.66	-0.27	0.73	0.50	-0.06	0.06	0.57
Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No
Toxicity										
AMES toxicity	No	No	Yes	No	Yes	No	No	No	No	No
Max. tolerated dose (log mg/kg/day)	0.29	1.22	1.06	0.61	0.43	0.85	0.50	0.44	-0.18	0.33
hERG I inhibitor	No	No	No	No	No	No	No	No	No	No
hERG II inhibitor	Yes	No	No	No	Yes	Yes	No	No	No	No
Oral Rat Acute[d] Toxicity	2.57	2.11	2.30	2.05	2.44	2.06	2.46	2.54	1.79	2.45
Oral Rat Chronic[e] Toxicity	1.59	3.37	3.03	2.21	5.46	2.54	2.41	3.89	1.94	2.30
Hepatotoxicity	Yes	Yes	No	No	No	No	No	No	No	No
Skin Sensitization	No	No	No	No	No	No	No	No	No	No
T.Pyriformis toxicity (log µg/L)	0.57	0.29	0.30	-0.23	0.29	0.30	0.33	0.29	0.37	0.38
Minnow toxicity (log mM)	-0.03	3.70	1.41	2.10	2.78	0.06	3.17	3.35	2.14	2.43

[a]Volume of Distribution (log L/kg)
[b]BBB (Blood-brain Barrier)
[c]CNS (Central Nervous System)
[d]Oral Rat Acute Toxicity unit is (mol/kg) and these values are lethal dose, 50% (LD_{50})[e]Oral Rat Chronic Toxicity unit is (log mg/kg bw/day)

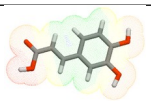
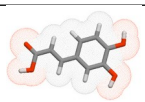
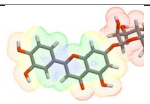
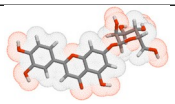
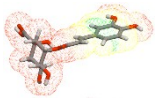
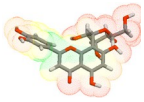
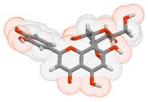
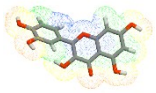
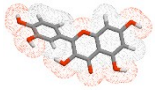
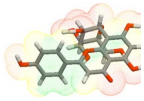
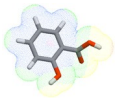
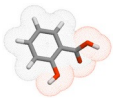
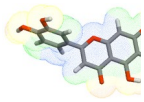
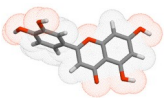
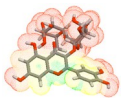
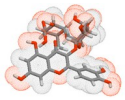
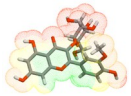
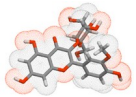
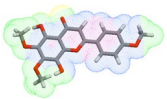
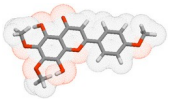
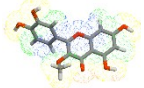
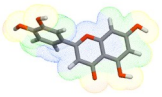
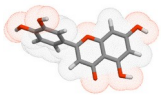
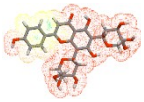
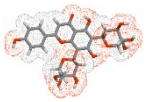
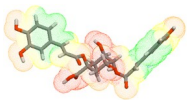
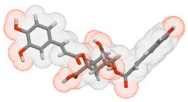
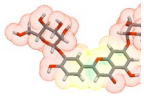
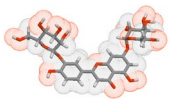
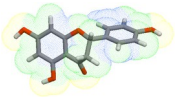
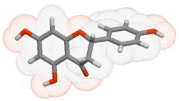
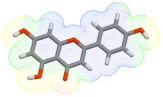
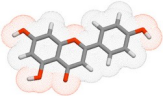
Table 5. The ADMET parameters of the following compounds from plants via pkCSM software.

	Predicted Value							
Property	Luteolin-7-glucoside	Orientin	Vitexin	Isorhamnetin-3-O-glucoside	3-O-Methylquercetin	Apigenin 6,8-di-C-glucoside	Luteolin-3',7-di-O-glucoside	Axillarin
Absorption								
Water solubility	-2.72	-2.91	-2.85	-2.91	-3.16	-2.84	-2.60	-3.17
Caco2 permeability	0.25	-1.25	-0.96	0.33	-0.62	-1.13	-0.77	-0.52
Intestinal absorption	37.56	43.73	46.70	49.71	76.07	14.66	0	82.65
Skin Permeability	-2.74	-2.74	-2.74	-2.74	-2.74	-2.74	-2.74	-2.74
P-glycoprotein substrate	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
P-glycoprotein I inhibitor	No	No	No	No	No	No	No	No
P-glycoprotein II inhibitor	No	No	No	No	No	No	No	Yes
Distribution								
VDss	0.88	1.49	1.07	1.25	0.22	0.57	-0.02	0.78
Fraction unbound	0.22	0.26	0.24	0.11	0.07	0.28	0.29	0.12
BBB permeability	-1.56	-1.64	-1.45	-1.72	-1.16	-1.93	-2.3	-1.52
CNS permeability	-3.93	-4.09	-3.83	-4.22	-3.27	-4.77	-4.94	-3.48
Metabolism								
CYP2D6 substrate	No	No	No	No	No	No	No	No
CYP3A4 substrate	No	No	No	No	No	No	No	No
CYP1A2 inhibitor	No	No	No	No	Yes	No	No	Yes
CYP2C19 inhibitor	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No	No	No
Excretion								
Total Clearance	0.48	0.37	0.44	0.49	0.46	-0.11	-0.13	-0.13
Renal OCT2 substrate	No	No	No	No	No	No	No	No
Toxicity								
AMES toxicity	No	No	No	No	No	No	No	No
Max. tolerated dose	0.58	0.57	0.58	0.64	0.52	0.46	0.27	0.27
hERG I inhibitor	No	No	No	No	No	No	No	No
hERG II inhibitor	No	No	No	Yes	No	Yes	Yes	Yes
Oral Rat Acute Toxicity	2.55	2.57	2.595	2.55	2.53	2.48	2.50	2.50
Oral Rat Chronic Toxicity	4.28	4.39	4.635	4.45	2.26	5.86	6.35	6.35
Hepatotoxicity	No	No	No	No	No	No	No	No
Skin Sensitisation	No	No	No	No	No	No	No	No
T.Pyriformis toxicity	0.29	0.29	0.29	0.29	0.3	0.29	0.29	0.29
Minnow toxicity	6.34	6.13	4.90	6.55	2.37	11.61	8.83	8.83

Table 6. The ADMET parameters of following trace metals in plants via pkCSM software.

	Predicted Value												
Property	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Cd	Pb
Absorption													
Water solubility	0.15	-0.05	-0.03	-0.03	-0.03	-0.03	-0.02	-0.03	-0.07	0.14	0.16	-0.20	-0.01
Caco2 permeability	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.38
Intestinal absorption	100	100	100	100	100	100	100	100	100	100	100	100	100
Skin Permeability	-2.65	-2.59	-2.59	-2.59	-2.59	-2.59	-2.59	-2.59	-2.59	-2.65	-2.65	-2.57	-2.64
P-glycoprotein substrate	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
P-glycoprotein I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No
P-glycoprotein II inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No
Distribution													
VDss	-0.06	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.06	-0.06	-0.04	-0.04
Fraction unbound	0.80	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.80	0.80	0.78	0.80
BBB permeability	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.02
CNS permeability	-2.30	-2.30	-2.30	-2.30	-2.30	-2.30	-2.30	-2.30	-2.30	-2.30	-2.30	-2.30	-2.30
Metabolism													
CYP2D6 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP1A2 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No
Excretion													
Total Clearance	0.52	0.52	0.51	0.51	0.51	0.51	0.51	0.51	0.53	0.52	0.52	0.58	0.58
Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No
Toxicity													
AMES toxicity	No	No	No	No	No	No	No	No	No	No	No	No	No
Max. tolerated dose	1.18	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.18	1.19	1.16	1.16
hERG I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No
hERG II inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No
Oral Rat Acute Toxicity	2.22	2.28	2.29	2.29	2.29	2.30	2.30	2.29	2.28	2.22	2.22	2.22	2.15
Oral Rat Chronic Toxicity	0.96	1.09	1.13	1.15	1.15	1.16	1.17	1.15	1.05	0.94	0.97	0.74	0.57
Hepatotoxicity	No	No	No	No	No	No	No	No	No	No	No	No	No
Skin Sensitisation	No	No	No	No	No	No	No	No	No	No	No	No	No
T.Pyriformis toxicity	-0.68	-0.70	-0.72	-0.73	-0.73	-0.73	-0.74	-0.73	-0.69	-0.67	-0.68	-0.54	-0.49
Minnow toxicity	2.45	2.32	2.36	2.37	2.37	2.38	2.38	2.37	2.29	2.43	2.46	2.04	2.14

Table 7. Molecular Lipophilicity Potential (MLP) and Topological Polar Surface Area (TPSA) 3D structures of compounds from plants based on Molinspiration Cheminformatics.

Compound	MLP	TPSA	Compound	MLP	TPSA
Caffeic acid	 LogP= 0.94	 TPSA= 77.75	Luteolin-7-glucoside	 LogP= 0.19	 TPSA= 190.28
Chlorogenic acid	 LogP= -0.45	 TPSA= 164.74	Orientin	 LogP= 0.03	 TPSA= 201.27
Quercetin	 LogP= 1.68	 TPSA= 131.24	Vitexin	 LogP= 0.52	 TPSA= 181.04
Salicylic acid	 LogP= 1.87	 TPSA= 57.53	Luteolin	 LogP= 1.97	 TPSA= 111.12
Rutin	 LogP= -1.06	 TPSA= 269.43	Isorhamnetin-3-O-glucoside	 LogP= -0.06	 TPSA= 199.51
8-Hydroxy-salvigenin	 LogP= 3.23	 TPSA= 98.39	3-O-Methylquercetin	 LogP= 1.96	 TPSA= 120.36
Luteolin	 LogP= 1.97	 TPSA= 111.12	Apigenin 6,8-di-C-glucoside	 LogP= -2.10	 TPSA= 271.19
Dicaffeoylquinic acid	 LogP= 1.21	 TPSA= 211.28	Luteolin-3',7-di-O-glucoside	 LogP= -1.83	 TPSA= 269.43
Naringenin	 LogP= 2.12	 TPSA= 86.99	Axillarin	 LogP= 1.98	 TPSA= 129.59
Apigenin	 LogP= 2.46	 TPSA= 90.89	Trace Metals	 LogP= n/a*	 TPSA= 0.00

In the surface representation of the molecular lipophilicity potential, the hydrophobic surface is coded with purple and blue colors and the hydrophilic surface is coded with orange and red colors.
A= Al, V, Cr, Mn, Co, Ni, Fe, Cu, Cd, Zn, As; B= Se; C= Pb

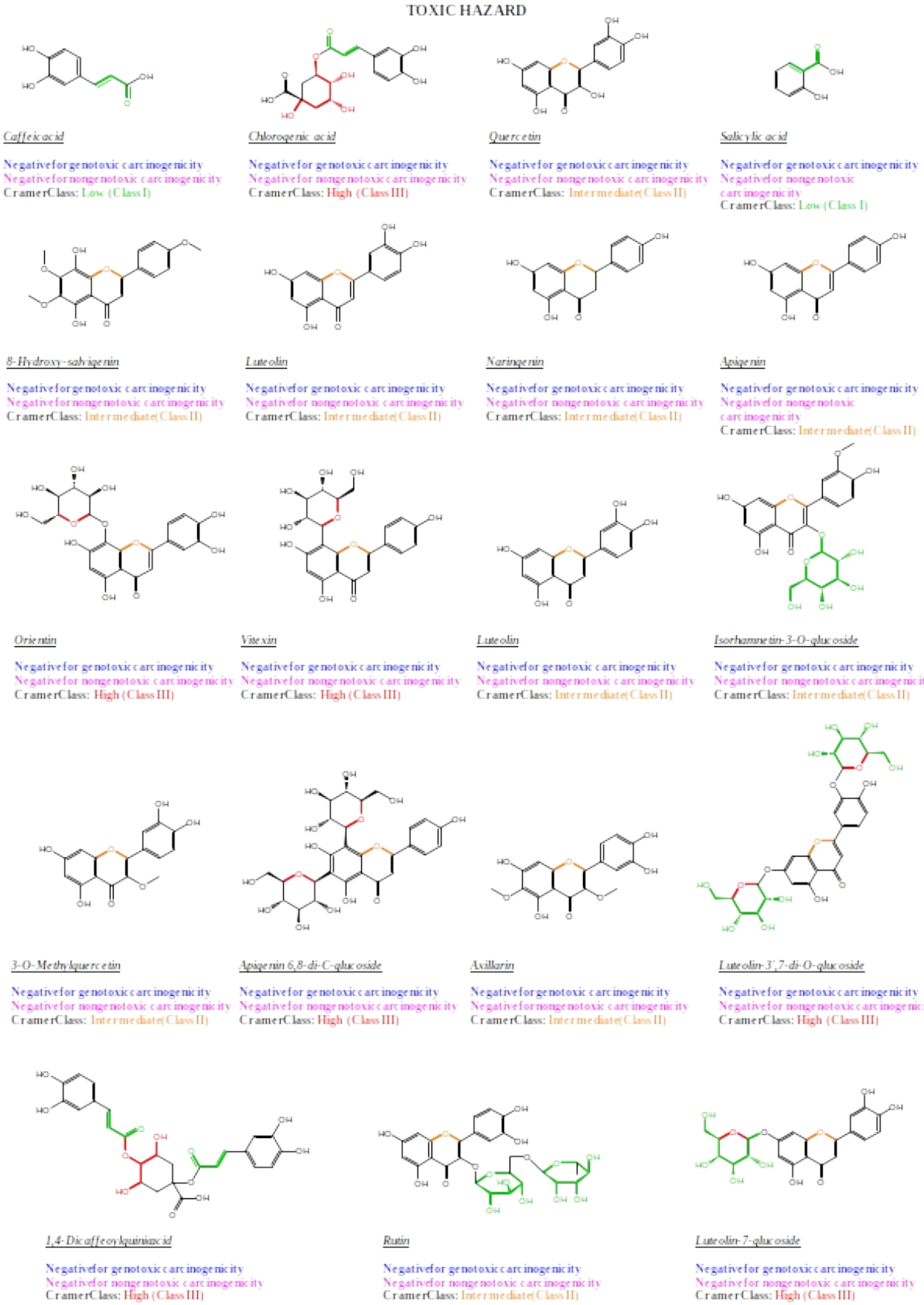


Figure 1. The Carcinogenicity and mutagenicity prediction test of all compounds in this study by Toxtree software.

Chlorogenic acid, quercetin, rutin, 8-Hydroxy-salvigenin, luteolin, dicaffeoylquinic acid, naringenin, apigenin, luteolin-7- glucoside, orientin, vitexin, isorhamnetin-3-O-glucoside, 3-O-methyl quercetin, apigenin 6,8-di-C-glucoside, luteolin-3',7-di-O-glucoside and axillarin compounds have the property of P-glycoprotein substrate. Caffeic acid, 8-hydroxy-salvigenin, and axillarin compounds have only P-glycoprotein II inhibitory properties, while all other compounds do not have P-glycoprotein I inhibitory properties. Chlorogenic acid and salicylic acid had low the volume of distribution (VDss) while luteolin, dicaffeoylquinic acid, apigenin, luteolin-7-glucoside, orientin, vitexin, luteolin, isorhamnetin-3-o-glucoside, apigenin 6,8-di-c-glucoside, and axillarin had high VDss (human) values.

Chlorogenic acid, quercetin, rutin, dicaffeoylquinic acid, luteolin-7-glucoside, orientin, vitexin, isorhamnetin-3-O-glucoside, 3-O-methyl quercetin, apigenin 6,8-di-C-glucoside, luteolin-3',7-di-O-glucoside, and axillarin compounds were predicted to be poorly distributed to the Blood-brain barrier. All compounds were predicted to be unable to penetrate the Central Nervous System (CNS). While caffeic acid and dicaffeoylquinic acid compounds are substrates to the CYP3A4 enzyme, all other compounds do not show the feature of substrates to the enzymes CYP3A4 and CYP2D6. It has been predicted that not all compounds inhibit the CYP2D6 enzyme, but some compounds have an inhibitory effect on CYP1A2 CYP2C19 CYP2C9 and CYP3A4 other enzymes. Not all compounds are predicted to have Renal OCT2 substrate.

Quercetin and rutin compounds were predicted to show AMES toxicity. Caffeic acid chlorogenic acid compounds have a Hepatotoxicity effect. All compounds were predicted to be not Skin Sensitization. All compounds were predicted to be not Minnow toxicity.

Caffeic acid, rutin, 8-Hydroxy-salvigenin, isorhamnetin-3-O-glucoside, apigenin 6,8-di-C-glucoside, luteolin-3',7-di-O-glucoside, and axillarin compounds have hERG II inhibitory effects.

All heavy metals were predicted to have high Caco-2 permeability, Intestinal absorption, and Skin Permeability All heavy metals were predicted to have the property of P-glycoprotein substrate but not P-glycoprotein I and II inhibitory effects. It was predicted that all metals have no effect on metabolic enzymes. All metals were predicted to have no AMES toxicity, Hepatotoxicity, Minnow toxicity, and Skin Sensitization effects. Not all metals were predicted to have hERG I inhibitor and hERG II inhibitory effects.

The parameters selected for estimating the in-silico bioavailability of phenolic compounds and trace metals contained in plants were lipophilicity (LogP) and topological polar surface area. Molecules with a log P value of less than 1 are hydrophilic; 1 to 5 are strongly lipophilic. In QSAR (quantitative structure-activity relationship) studies, LogP (octanol-water partition coefficient), i.e., lipophilicity (fat solubility) and hydrophilicity (water solubility) are important parameters. A negative value for LogP means the compound has a higher affinity for the aqueous phase (it is more hydrophilic); when LogP= 0, the compound is equally partitioned between the lipid and aqueous phases; a positive value for LogP denotes a higher concentration in the lipid phase (i.e., the compound is more lipophilic). Hydrophobicity affects drug absorption, bioavailability, drug-receptor interactions, metabolism of molecules, and their toxicity. Topological polar surface area (TPSA) is a parameter used in the estimation of drug transport properties. The polar surface area is defined as the sum of the surfaces of polar atoms on the molecule (33).

The LogP values of metals are -0.71 for Aluminum (Al), Vanadium (V), Chromium (Cr), Manganese (Mn), Cobalt (Co), Nickel (Ni); -1.39 for Iron (Fe); -1.11 for Copper (Cu) and Cadmium (Cd); 0.39 for Zinc (Zn); -0.61 for Arsenic (As); 0.79 for Selenium (Se) and 2.93 for Lead (Pb), respectively. The most hydrophilic metal is Iron (Fe), the most lipophilic metal is lead.

When Figure 1 is examined, it is estimated that seven compounds will be highly toxic, although the mutagenic and carcinogenic effects of most of the phenolic compounds we have analyzed in relevant publications were predicted at low and medium levels. It has been proven in previous studies that these 7 compounds contain the Achillea nobilis plant. It is understood from Table 7 that most of these toxic compounds are hydrophilic. However, hydrophilic chemicals are easier to destroy by the body than lipophilic toxic substances, and lipophilic toxic substances can accumulate in the body at dangerous levels. In addition to this literature information, it is seen in Table 7 that the log P predicted values of the compounds in this plant except caffeic acid are less than 1. These data show us that these compounds, which are thought to be highly toxic, are hydrophilic. Hence, these results suggested that this plant will not pose a very high risk for human health in the use as food/ medicine.

CONCLUSION

The success rate of drug development from phytochemicals is low due to their poor pharmacokinetics. There is an increasing trend, an imperative for *in vitro* testing and *in silico* prediction before *in vivo* and clinical stages in drug discovery. Therefore, determining the ADMET profile of phytocompounds also provides a preliminary idea for determining their usability as drug substance or raw material. In this study, we investigated computationally the drug candidate

potential of phytocompounds and heavy metals analyzed in some *Achillea* species, using physicochemical and pharmacokinetic approaches.

According to the ADME/Tox profile, caffeic acid, salicylic acid, 8-hydroxy-salvigenin, naringenin, and apigenin are easily absorbable; luteolin, dicaffeoylquinic acid, apigenin, luteolin-7-glucoside, orientin, vitexin, isorhamnetin-3-o-glucoside, apigenin 6,8-di-c-glucoside, and axillarin are dispersible; caffeic acid and dicaffeoylquinic metabolized and excreted and almost all compounds (excluding quercetin, rutin, caffeic and chlorogenic acid) were found to be non-toxic. Therefore, this study reveals that the plants examined have reliable ADME properties in terms of phenolic compounds and trace metals they contain.

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COMPLIANCE WITH ETHICS REQUIREMENTS

Conflict of Interest: No conflict of interest exists in the submission of this manuscript, and all authors approve the manuscript for publication. This work described was an original research that has not been published previously and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is submitted. Duygu Taşkın, Talip Şahin, Mücahit Özdemir, and Bahattin Yalçın have no conflict of interest.

Ethical Approval: This article does not contain any studies with human or animal subjects.

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