Anti-inflammatory evaluation of Ukrainian herbal extracts

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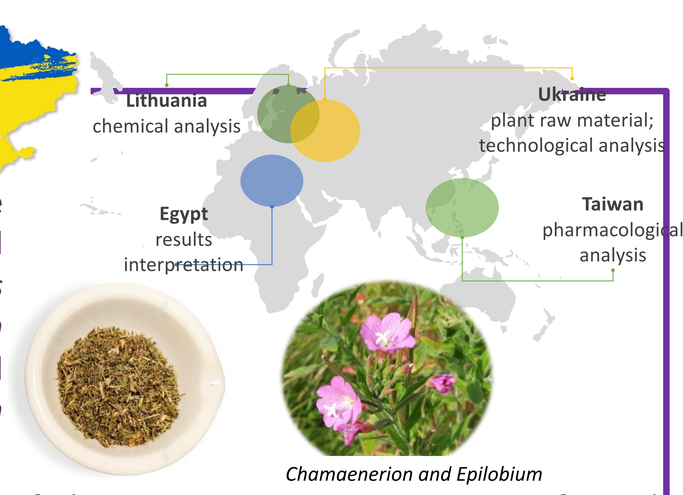
DEDICATED TO PEOPLE OF UKRAINE

Background

- Neutrophils play a crucial role in protection against intracellular pathogens such as viruses and mycobacteria but also in regulating systemic anaphylaxis or allergic skin reactions. **Neutrophils intimately shape the adaptive immune response** at various levels, including B cells, dendritic cells, and T cell populations.
- Significant attention in pharmacy is given to the search for **natural substances** that can affect the immune system and neutrophil function, with less adverse side effects.
- The current study further extends the analysis of various groups of biologically active substances in extracts from Ukrainian plants.

Materials & Methods

Plant samples: polysaccharide complexes of *Crocus* flowers and corms, *Juno* leaves and corms, *Iris* leaves and rhizomes, *Chamaenerion* and *Epilobium* leaves; water and ethanolic extracts *Chamaenerion* and *Epilobium* leaves.

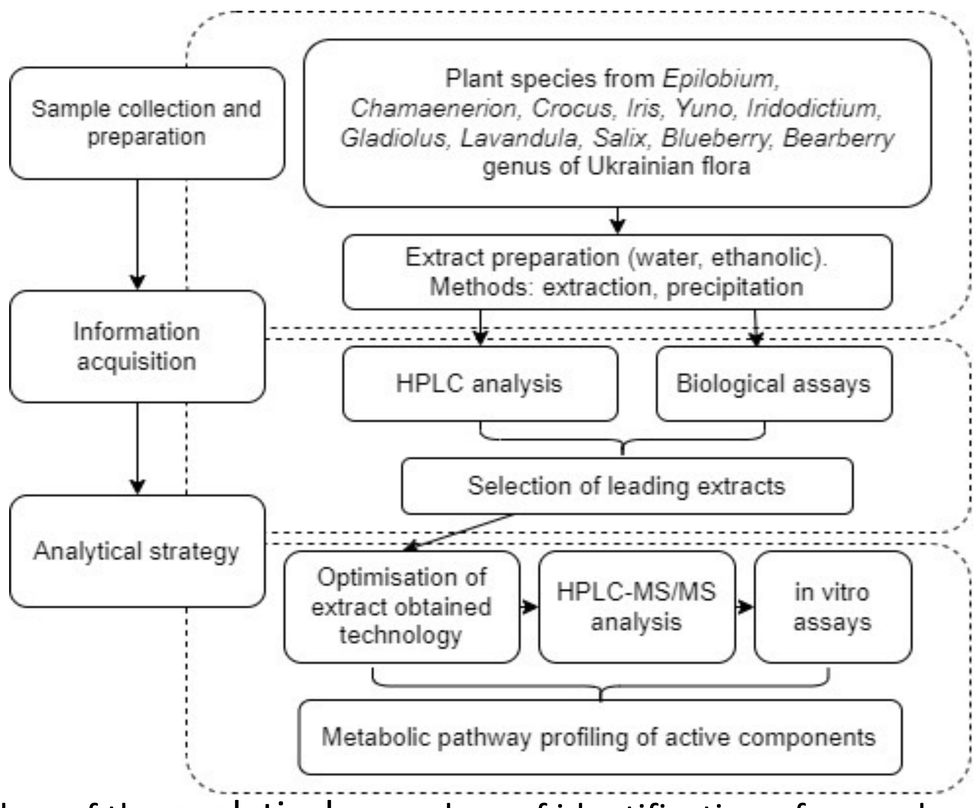


Chamaenerion and Epilobium

HPLC Analysis: The chemical analysis of *Chamaenerion* composition was performed by using Waters e2695 Alliance HPLC system coupled with a 2998 PDA detector (Waters, Milford, MA, USA). Phenolic compounds were separated on an ACE Super C18 (250 mm × 4.6 mm, 3 µm) column (ACT, Aberdeen, UK) with a full run time 81 min at 25 °C. The gradient elution mode of 0.1% (v/v) trifluoroacetic acid in pure water (A) and acetonitrile (B) was as follows: 0 min, 5% B; 8–30 min, 20% B; 30–48 min, 40% B; 48–58 min, 50% B; 58–65 min, 50%; 65–66 min, 95% B, 66–70 min, 95% B, 70–81 min, 5% B. Flow rate 1 mL/min, injection volume 10 µL.

Pharmacology tested: *Anti-inflammatory*: Superoxide anion generation and elastase release assay induced by fMLF/CB in human neutrophils (respiratory burst and degranulation). *Antivirus*: Coronavirus strain Omicron pseudotyped lentivirus assay in hACE-2 overexpressed cells (virus entry).

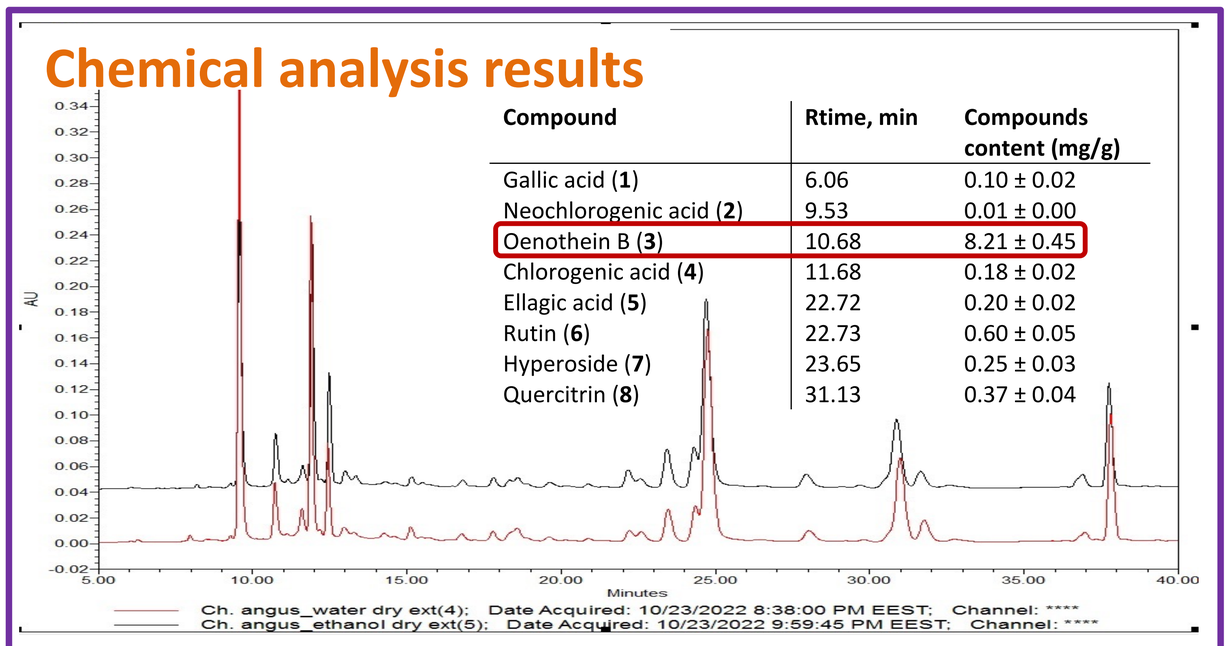
Design of Experiment



Work flow of the analytical procedure of identification of secondary metabolites and the bioactive principles

Results & Discussions

Chemical analysis results



Compound	Rtime, min	Compounds content (mg/g)
Gallic acid (**1**)	6.06	0.10 ± 0.02
Neochlorogenic acid (**2**)	9.53	0.01 ± 0.00
Oenothein B (**3**)	10.68	8.21 ± 0.45
Chlorogenic acid (**4**)	11.68	0.18 ± 0.02
Ellagic acid (**5**)	22.72	0.20 ± 0.02
Rutin (**6**)	22.73	0.60 ± 0.05
Hyperoside (**7**)	23.65	0.25 ± 0.03
Quercitrin (**8**)	31.13	0.37 ± 0.04

The HPLC-DAD chromatograms of *Ch. angustifolium* extracts. The results of the content of substances in raw materials are given.

Pharmacological analysis results

Effects of crude extracts on SARS-CoV-2 pseudovirus infection.

	Sample	Inh%[a]	Superoxide Inh%		Elastase release Inh%	
1	Dry polysaccharide extract of Crocus sativus flowers[bd]	9.15 ± 4.01	17.91 ± 1.66	***	2.04 ± 1.50	
2	Dry polysaccharide extract of Crocus sativus corms[cd]	37.07 ± 1.21	3.56 ± 0.54	**	0.64 ± 1.02	
3	Dry polysaccharide extract of Juno bucharia leaves[bd]	43.00 ± 2.58	8.38 ± 0.96	***	2.09 ± 2.82	
4	Dry polysaccharide extract of Juno bucharia corms[bd]	43.08 ± 2.17	3.55 ± 1.58		2.46 ± 3.38	
5	Dry polysaccharide extract of Iris hungarica leaves[b]	43.40 ± 0.57	7.84 ± 2.76	*	**39.02 ± 2.98**	***
6	Dry polysaccharides extract of Iris hungarica rhizomes[bd]	36.88 ± 1.47	1.04 ± 0.80		1.43 ± 3.34	
7	Dry Iridodictium Dight leaves extract[b]	19.87 ± 1.80	17.54 ± 0.79	***	18.79 ± 4.86	*
8	Dry Iridodictium Cantab leaves extract[cd]	-27.29 ± 1.08	18.39 ± 1.85	***	25.84 ± 6.26	*
10	Dry Crocus stigma extract 80% ethanol[b]	6.55 ± 0.93	17.80 ± 6.76		**44.70 ± 7.17**	**
11	Water extract of Lavandula angustifolia[b]	39.98 ± 1.75	**49.02 ± 1.31**	***	23.71 ± 2.50	***
12	Polysaccharides extract of Chamaenerion angustifolium[bd]	46.72 ± 0.32	**45.49 ± 0.60**	***	17.24 ± 4.82	*
13	Water extract of Chamaenerion angustifolium[b]	55.18 ± 1.60	NT		89.61 ± 6.82	***
14	Ethanolic (50%, vol/vol) extract of Chamaenerion angustifolium[b]	53.84 ± 0.97	NT		93.81 ± 5.94	***
15	Polysaccharides extract of Epilobium hirsutum[bd]	38.39 ± 1.54	43.96 ± 1.93	***	14.98 ± 3.51	*
16	Water extract of Epilobium hirsutum[b]	46.13 ± 0.68	NT		85.76 ± 3.55	***
17	Ethanolic (50%, vol/vol) extract of Epilobium hirsutum[c]	2.92 ± 3.61	NT		102.81 ± 3.70	***

Percentage of inhibition at 10 µg/ml. Results are presented as mean±S.E.M. (n = 3). *** $P < 0.001$ compared with control (DMSO).

[a] SARS-CoV-2 pseudovirus infection assay, inhibition %; cepharantine served as a positive control, IC_{50} 0.95 µM.

[b,c,d] different solvent was used due to solubility issues

Conclusion

- ***C. angustifolium* and *E. hirsutum* ethanolic (50%, vol/vol) extracts, both rich in oenothein B, almost completely inhibited fMLF/CB-induced elastase release at 10 µg/mL (IC_{50} 2.79 and 2.44 µg/mL, resp.), while *C. angustifolium* extracts exhibited the best anti-Omicron pseudovirus effect**
- Interestingly, *Iris* leaf polysaccharides inhibited elastase release by 39.0%, and *C. angustifolium* polysaccharides superoxide by 45.5% at 10 µg/mL
- This suggests that their components - **phenolic compounds** may possess better activity in comparison to polysaccharides
- The present study provided primary pharmacological evidence for **anti-inflammatory agents from *C. angustifolium* and *E. hirsutum***

"Neutrophils in ARDS (COVID)"