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„Pharmacological screening of the therapeutic potential of active ingredients extracted from Morus alba, Angelica archangelica, Valeriana officinalis, and Passiflora incarnata”

SUMMARY OF DOCTORAL THESIS

Conducător de doctorat:
PROF. UNIV. DR. NEGREȘ SIMONA

Student-doctorand:
SUCIU FELICIA

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Contained

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Abstract of the doctoral thesis

The use of medicinal plants has been practiced since ancient times and from this point of view, phytotherapy can be considered the basis of modern medicine. Plant-based compounds have been an important source for the discovery of new semi-synthetic or synthetic drugs. Until the eighteenth century, the therapeutic effects of many medicinal plants were known, but the active ingredients contained in them were unknown.

Subsequently, the development of chemical analyses and the emergence of the microscope made it possible to identify different active ingredients of therapeutic interest.

The objective of the doctoral thesis was to investigate the action of four plant extracts obtained from *Morus alba cortex (MA), Angelica archangelica radix (AA), Passiflora incarnata L herba* (PI) and *Valeriana officinalis radix (VO)* on the activity of the Central Nervous System (CNS).

The research hypothesis started from the selection of plant products, which, through their composition in active ingredients and/or the content of γ-aminobutyric acid (GABA), could modulate the activity of GABA receptors involved in both pain and seizure states.

Chapter 1 summarizes data showing that this inhibitory neuromediator interacts with its receptors influencing pain transduction and modulation (Luhmann, 1991; Furukava, 2023). GABA-A receptors mediate rapid synaptic transmission, while GABA-B receptors mediate slow synaptic transmission (the analgesic effects of GABA are mediated via both receptor categories).

The involvement of GABA in the transduction of the pain signal has also been demonstrated by the in vivo administration of a GABA reuptake inhibitor called NO711. Following this administration, the local concentration of GABA in the sensory ganglia increased, which reduced the intensity of the pain responses induced by the injection of the paw with bradykinin and capsaicin.

In addition to acute pain, similar analgesic effects have been observed for GABA and GABA reuptake inhibitor in neuropathic and inflammatory pain (Du, 2017).

Central GABA-ergic transmission has attracted attention due to its extensive distribution and function in neural circuits, including the generation and development of neuropathic pain. It appears that changes in GABA-ergic inhibitory activity that occur in interneurons along descending and nociceptive modulatory pathways in the central nervous system, generate neuronal plasticity (synaptic plasticity, or functional plasticity of genes or related proteins), which underlies persistent neuropathic pain (Ju, 2024).

Regarding the involvement of GABA in neuropathic pain, it has been shown that the loss of inhibitory tone generated by this neuromediator can be determined (Senba, 2020) by the following mechanisms:

- decrease in the number of GABA neurons due to apoptosis (Dugan, 2020);
- downregulation of glutamic acid decarboxylase (GAD) synthesis (epigenetics);
- decreased GABA release and/or GABA A/B receptor dysfunction;
- the occurrence of abnormal cation-chloride co-transporters (cotransporter-2 K+/Cl-: KCC2) in spinal nociceptive neurons (Kang, 2013; Kang, 2014).

Peripheral nerve damage has been shown to lead to excitotoxic cell death of GABA interneurons in the superficial dorsal horn and contribute to the onset of chronic hyperalgesia (Kami, 2018).

Several experimental and clinical pharmacology evidence has demonstrated that disruption of inhibition mediated by GABA-ergic transmission leads to the initiation and propagation of seizures (Treiman, 2001).

In several studies, on animal models of genetically determined or acquired epilepsy, it is relevant to demonstrate the reduction of GABA-ergic neurotransmission activity by several mechanisms:

- loss of inhibitory interneurons (leads to decreased release of GABA) accompanied by reduced decrease in excitatory cells and the onset of seizures (Liu, 2014);
- increased glutamate levels during seizures, while GABA levels remain relatively constant (Sarlo, 2021); this alteration in the glutamate/GABA ratio results in an imbalance between excitation and inhibition and may contribute to the initiation and propagation of seizure activity.

Chapter 2 presents the phytochemical composition and pharmacological actions for the plant extracts selected in this doctoral thesis.

Studies published in the literature have reported a high content of γ-aminobutyric acid in the leaves of *Morus alba*. The antioxidant properties of the ethanolic extract obtained from the fruit of *Morus alba* have been shown to be different depending on the variety (Bae, 2007). Mature fruits are rich in anthocyanins with a strong antioxidant action against free radicals compared to vitamin C (Wang, 2021). Taking into account the total phenolic content, the action against free radicals, the power to reduce ferric ions and the chelation of ferrous ions, studies have shown that the hydroalcoholic extract obtained from the leaves of *Morus alba* has higher antioxidant efficacy compared to that of fruits (Oliviera, 2016).

For the total extract of *Passiflora incarnata* L it has been shown to induce intense currents that modulate the activity of GABA-A receptors, due to its high content of γ-aminobutyric acid. The pharmacological effects of *Passiflota incarnata* are considered to be mediated by modulation of the GABA system, with the active ingredients having affinity for GABA-A and GABA-B receptors (Appel, 2011).

The antidepressant activity of *Passiflora edulis* extract, conditioned as nanoparticles or as aqueous extract, has been demonstrated in mice, by using the forced swimming test (Ayres, 2015; Alvez, 2020).

In the chemical composition of the *Angelica archangelica* plant, several compounds have been identified: columbianetine, imperatorin, cnidyline, ostol and columbianedine, which, depending on the concentration, potentiated the influx of GABA-induced chlorine at the level of ion channel-dependent receptors (GABA-A and GABA-B). The antioxidant action of aqueous and ethanolic extracts from *Angelica* root was tested using DPPH (2,2-diphenyl-1-picrylhydrazil), ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), hydroxide radical scavenging activity and lipid peroxidation. For aqueous and ethanolic fractions, they were shown to capture DPPH and ABTS radicals and had strong reducing action by inhibiting superoxide dismutase, catalase and preventing DNA damage (Pervin, 2014).

It is also suggested that the pharmacological effects of *Valeriana officinalis* extract and valerenic acid are mediated by modulation of the function of GABA-A receptors, and it is considered that these active ingredients may potentiate the effects of anesthetics and other drugs acting on these receptors. The sedative, tranquilizing, and antihypertensive actions of valeranone have been pharmacologically studied in animal research, but its activity was lower than that of the standard drugs used (Baby, 2005; Yuan, 2004).

In 2015, Torres-Hernández et al., showed that valerenic acid and the total extract obtained from *Valeriana officinalis* (ethanolic aqueous) increased the latency of pentylenetrazole-induced seizures in adult zebrafish in a concentration-dependent manner.

For the four extracts, pharmacological actions were determined both on electroshock-induced seizures and in somatic pain and neuropathic pain induced by cytostatics or alloxanic diabetes.

The topic is topical, because diabetic neuropathic pain usually manifests itself as a symmetrical sensorimotor neuropathy, which includes negative sensory phenomena (numbness, reduction of pain and temperature perception), as well as positive (burning, tingling, sensations similar to electric shocks).

Also, peripheral neuropathy produced by antitumor chemotherapies (Seretny, 2014) affects the somatosensory nervous system and is one of the phenomena frequently encountered in the clinic (70% of patients can develop neuropathy, right from the moment a cytostatic is initiated).

The drugs recommended by treatment guidelines in neuropathic pain do not improve the long-term prognosis, this being the consequence of progressive damage to the peripheral nerves. Current first-line therapies include GABA receptor agonists, tricyclic antidepressants, and serotonin and norepinephrine reuptake inhibitors.

Taking into account the fact that each of the groups of active substances mentioned above, also presents adverse effects specific to the class to which they belong, there is a need to find new therapeutic approaches for the treatment of this pathology.

A particular clinical problem is represented by epilepsy, a neurological pathology that affects approximately 50 million people worldwide. Although research in the field has led to remarkable progress, there are patients in whom pharmacoresistance is installed (about 30%). Also, the low safety index of antiepileptic drugs and the multitude of their side effects make them difficult to handle for many patients.

In order to carry out my doctoral thesis, I set myself the following scientific objectives, developed in *chapters 3*:

The general objectives were:

- Obtaining dry plant extracts from the four selected products;
- Determination of total polyphenol content and determination of antioxidant action in vitro;
- Establishment of the polyphenolic profile of the extracts to be tested by tandem mass spectrometry (MS-MS);
- Conduct in silico studies to establish correlations between phytocompound content and mechanisms of action (other than influencing GABA-ergic transmission) involved in analgesic and anticonvulsant action.
- Determination of analgesic action on a model of somatic pain induced by thermal stimuli and correlation with the antioxidant potential of the active ingredients.
- Investigation of the anti-allodynic and antihyperalgic effect in two neuropathic pain models: paclitaxel-induced neuropathy and alloxan-induced diabetic neuropathy.
- Determination of the anticonvulsant potential for extracts obtained from *Morus alba* cortex (MAE), *Angelica archangelica* radix (AAE), *Passiflora incarnata* L herba (PIE) and *Valeriana officinalis* radix (VOE).

The work has a strong interdisciplinary character, because tandem mass spectrometry (MS-MS) was used to establish the polyphenolic profile of the four extracts, and classical tests were used to determine the pharmacological effects (thermal tests and mechanical tests on animal models of paclitaxel or alloxan-induced neuropathy were used, and to determine the anticonvulsant effect, the seizures were induced by electroshock). The results obtained in the pharmacological tests were correlated with the biochemical tests carried out and with the computational studies to highlight the possible mechanisms of action (the research methods are *described in Chapter 4*).

Given the complex nature of bioactive compounds in plant extracts and their implications on multiple signaling pathways, it was necessary to integrate modern computational methods, such as therapeutic target prediction and molecular docking simulations. These approaches allow the identification of potential target proteins and the evaluation of specific molecular interactions, providing a bridge between in vivo experimental data and the possible molecular mechanisms involved in exerting the pharmacological effect. In the context of epilepsy and diabetic neuropathy, where neuronal and inflammatory processes are disrupted, the use of these methods aimed to propose potential mechanisms of action for the phytocompounds detected in the studied extracts.

We have determined the content of active ingredients and the antioxidant effect in vitro of the four plant extracts: *Morus alba* cortex, *Angelica archangelica* radix, *Passiflora incarnata* herba and *Valeriana officinalis* radix and we have shown that the highest content of total polyphenols and flavonoids is found in the extract of *Morus alba* cortex, which also showed the highest antioxidant activity in vitro (Al Hilfi, 2019; Dudonne, 2009).

We established the polyphenolic profile of the four plant extracts by using the hybrid technique represented by the coupling of ultra high performance liquid chromatography (UHPLC) with high resolution mass spectrometry (HRMS).

The concentration of the major compounds varied substantially between the four extracts, for the one obtained from *Morus alba* the greatest complexity and variety of phytochemical content was recorded: isoramnetin, quercetin, kaempferol, catechin, chlorogenic acid, hyperoside and p-coumaric acid. The extract obtained from *Passiflora incarnata* L had a rich content of flavonoids: quercetin, isoramnetin and p-coumaric acid (Zhang, 2023).

For *Angelica arcangelica* extract, high levels of ferulic acid, chlorogenic acid and p-coumaric acid (1.223 mg/g) have been demonstrated. *Valeriana officinalis* extract was characterized by lower levels of chlorogenic acid and p-coumaric acid, along with rutin and gallic acid (Kaur, 2021; Lim, 2024).

Chlorogenic acid, present in all plant products, includes a family of members that include: 1L-(−)-quinic acid, ferulic acid, caffeoylquinic acids, and feruloylquinic acids (Rana, 2022; Li, 2020). Their involvement in reducing acute inflammatory conditions has been demonstrated in several researches. The modulating action of metabolic homeostasis has also been identified (Xue, 2023; Baghdad, 2020). The proposed mechanisms for the anti-inflammatory properties of chlorogenic acid are related to the decrease in the activity of the signaling pathways of:

- pathogen-activated nuclear factor-κB (NF-κB);
- n-terminal c-Jun kinases (JNK);
- regulated extracellular signal kinases (ERK);
- mitogen-activated protein kinase p38 (MAPK).

In Chapter 7, we performed an analysis of the toxicity data in the literature, which subsequently allowed us to determine the doses we used for the in vivo tests.

Research correlating the antioxidant action in vitro and analgesic effect of the four extracts studied demonstrated that *Morus alba* cortex showed the highest content of flavonoids and polyphenols and the strongest antioxidant activity (*Chapter 8*). In contrast, the most intense analgesic effects were observed for the extracts of *Angelica archangelica* radix and *Valeriana officinalis* radix (the most intense, paracetamol-like effect was obtained in the tail flick test (Suciu, 2024; Suciu, 2025).

The results of research on the action of plant extracts obtained from *Valeriana officinalis* radix and *Passiflora incarnata* herba in paclitaxel-induced neuropathy had a significant effect in reducing tactile hyperalgesia and cytostatic-induced thermal allodynia. Antihyperalgia extracts also had antihyperalgic effects of *Angelica archangelica* radix and *Morus alba* cortex, but after a longer period of treatment.

In this experimental model of cytostatic-induced neuropathy, extracts obtained from *Valeriana officinalis* and *Passiflora incarnata* prevented, but also statistically significantly reduced tactile hyperalgesia (assessed in mechanical tests) and thermal allodynia (assessed by the acetone test).

The extracts obtained from *Morus alba* and *Angelica archangelica* did not prevent the onset of paclitaxel-induced hyperalgesia, but significantly reduced the hyperalgesia assessed post-induction of neuropathy. Furthermore, those who received treatment with gabapentin, as well as all batches treated with plant extracts, experienced an increase in the concentration of total thiols compared to the control group. The most pronounced increase was observed in the case of the batch treated with *Valeriana officinalis* (76.26%), followed by the one treated with

Passiflora incarnata (71.21%), their antioxidant role being presented in the literature (Forouzanfar, 2023).

The integration of molecular target predictions and molecular docking simulations into experimental studies allowed a better understanding of possible pharmacodynamic mechanisms involved in the observed effects. In both the experimental model of epilepsy and diabetic neuropathy, computational methods have shown a potential central role of 5-HT3A and GABA-A receptors and protein kinase AAK1 in the neuroprotective and antihyperalgic effects of the investigated compounds. Thus, these in silico approaches support the translational potential of plant extracts, providing a solid basis for the development of new adjuvant therapies (*chapters 10 and 11*).

We induced diabetic neuropathy in rats by administering a single dose of alloxan (130 mg/kg), administered intraperitoneally. The resulting hyperglycemia, caused by the selective toxicity of β cells, led to increased oxidative stress, a key factor in the development of hypersensitivity to pain. As a result, untreated diabetic rats showed significantly increased responses to thermal and mechanical stimuli over the course of 15 days, confirming the onset of hyperalgesia. By day 15, none of the treatments had restored normoglycemia, although all four extracts significantly lowered blood glucose levels compared to the diabetic group. *Morus alba* extract had the most substantial antihyperglycemic effect (–45.7%).

In tests to assess pain, all extracts obtained from the studied plants and the reference substance, gabapentin (Mehta 2017; Ola, 2019) significantly reduced pain sensitivity in hot plate tests, tail immersion in cold water, von Frey and Randall-Selitto.

The extract obtained from Valeriana officinalis radix showed the most pronounced effect, followed by the extract of *Morus alba* cortex obtained from the thermal hypersensitivity tests and the extract obtained from *Angelica archangelica* radix in the mechanical hypersensitivity tests.

In thermal pain models, extracts of *Morus alba* and *Valeriana officinalis* performed comparable to gabapentin.

The antihyperalgic activity and marked reduction of TNFα of *Morus alba* cortex extract in diabetic neuropathic pain can be attributed to the high content of bioactive flavonoids, previously characterized in phytochemical analysis, but also to the fact that it reduces oxidative stress by improving glycemic status (Suciu, 2025b).

Biochemical analysis supports the anti-inflammatory potential of the tested extracts. In this study, administration of alloxan significantly increased levels of TNF-α and IL-6 in brain and liver tissues. The treatment with the extracts taken in the work and especially the extracts

obtained from *Angelica archangelia* radix and *Morus alba* cortex, led to substantial reductions of both cytokines (*Angelica* extract produced the most significant reduction of brain TNF-α, –64.25%, and *Morus* extract reduced the level of this cytokine in the liver, –71.4%. Similar reductions for the two extracts have also occurred for IL6.

In silico studies suggest that the active ingredients identified in extracts have antihyperalgic effects can interact with multiple targets, AAK1 being one of them (Baier, 2020; Alhadrami, 2024).

In the evaluation of anticonvulsant action, *Morus alba* cortex extract has been shown to have a significant anticonvulsant effect (probably due to its high flavonoid content and their interactions with GABA-A and 5-HT3A receptors). Extracts of *Angelica archangelica* radix and *Passiflora incarnata* herba showed moderate, insignificant effects, while *Valeriana officinalis* radix showed outstanding antioxidant and anti-inflammatory properties but limited protection against seizures (Manavi, 2024; Mulyawan, 2020).

In the electroshock-induced seizure test (Suciu, 2025a), of the extracts tested, Morus alba had the most intense anticonvulsant effect (reduced the duration of seizures: −92.86% and their incidence to 50%). The effect could be attributed to the high content of flavones (in particular: quercetin, kaempferol and isoramnetin).

The results obtained correlate with a reduction in TNF-α levels in the brain and an increase in the concentration of total thiols, especially for batches treated with plant extracts of *Morus alba*, *Passiflora incarnata* and *Valeriana officinalis* (Rana, 2022; Li, 2020).

The main limitations of pharmacological studies are related to: the use of animal models that do not accurately reflect the pathology encountered in humans, evaluation by using fixed doses that do not allow dose-effect relationships to be achieved, and the relatively short duration of the treatments administered.

Personal contributions

We have demonstrated that *Morus alba* cortex extract has the most intense antioxidant activity in vitro, expressed by the lowest IC_{50} value: 0.0695 mg/mL.

We also highlighted the fact that the extract obtained from *Morus alba* had the highest content of total polyphenols, polyphenolic acids and flavonoids, which correlates with intense antioxidant activity.

We demonstrated, using the hybrid UHPLC-HRMS/MS technique, that chlorogenic acid is present in all the extracts tested, which contributed to the reduction of the concentration of proinflammatory cytokines: IL6 and TNFα in the animal model of alloxanic diabetes-induced neuropathy and in mice-induced seizures (both models being characterized by increased oxidative stress).

A major contribution was the performance of therapeutic target prediction and molecular docking studies for the compounds identified by UHPLC-HRMS/MS, in order to correlate with the pharmacological results obtained in vivo.

In the diabetic neuropathy model, computational analysis revealed AAK1 as a promising target for modulating hyperalgesia, with several compounds in the extracts exhibiting similar affinities with a known inhibitor of this kinase. These results contributed to the understanding of the potential molecular mechanisms by which plant extracts can exert neuroprotective and antihyperalgic effects.

In studies targeting antiepileptic action, we predicted promising interactions between flavonoids and the 5-HT3A receptor, both murine and human, supporting the anticonvulsant effect observed for *Morus alba* extract.

In the evaluation of the analgesic effect in mice, we demonstrated that although flavones and polyphenols reduce oxidative stress, the analgesic action is dependent on the intrinsic mechanisms of action of the various active substances and their interaction with certain receptor subtypes.

We have highlighted the intense antihyperalgic and antiallodynic effect for extracts obtained from Valeriana officinalis and *Passiflora incarnata*, in cytostatic-induced neuropathic pain (experimental model with paclitaxel). The intense analgesic action of these two extracts is correlated with a marked increase (over 70%) in the concentration of total thiols in brain homogenates.

We demonstrated the antihyperalgic action of extracts obtained from *Morus alba* cortex, *Angelica archangelica* radix, *Valeriana officinalis* radix and *Passiflora incarnata* herba, using an experimental model of alloxanic diabetes.

The study showed an intense antihyperalgic effect for the extract obtained from *Morus alba* cortex, correlated with the high flavone content, but also with the improvement of glycemic status.

The antihyperalgic action of this extract could be attributed to quercetin which activates the AMPK/PGC-1α pathway and improves mitochondrial functions, increases nerve conduction speed, and preserves the integrity of the myelin sheath in rats. The experimental pharmacological results correlate with the biochemical determinations, which showed that the extract of *Morus alba* marked the reduction of IL6 levels, and that of *Angelica archangelica* decreased the levels of TNF-α the most intensely.

In the experimental model of electroshock-induced seizures, we demonstrated that *Morus alba* extract (100 mg/kg) has significant anticonvulsant effects, reducing both the duration and incidence of seizures, likely mediated by flavonoid interactions with GABA-A and 5-HT3A receptors, as suggested by target prediction and molecular docking analyses.

The extracts of *Angelica archangelica* (100 mg/kg) and *Passiflora incarnata* (50 mg/kg) showed moderate, insignificant anticonvulsant activities. At the same time, *Valeriana officinalis* (50 mg/kg) demonstrated considerable antioxidant and anti-inflammatory properties, but limited protection against seizures.

All extracts significantly reduced markers of brain inflammation (TNF-α) and increased antioxidant capacity, expressed by increased total thiols. Molecular docking further supported the interaction of key active ingredients, including naringenin and chlorogenic acid, with human and mouse 5-HT3A receptors.

Overall, *Morus alba* extract has shown promising therapeutic potential for the management of epilepsy, warranting further investigations into chronic seizure patterns and optimized dosing strategies.

The research brings current therapeutic perspectives for the development of new pharmaceutical products with antihyperalgic potential in neuropathic pain and with anticonvulsant activity.

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