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Chemical structure and anti-inflammatory mechanisms of phenolic compounds from medicinal plants

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Abstract. The study was aimed at investigating the relationship between the chemical structure of phenolic compounds from medicinal plants *Curcuma longa*, *Hypericum perforatum* and *Salvia officinalis* and their anti-inflammatory activity. The research was conducted at the Educational and Scientific Institute of Applied Pharmacy, National University of Pharmacy (Kharkiv, Ukraine), between September and December 2024. Using high-performance liquid chromatography and mass spectrometry, 12 bioactive compounds were identified, including curcumin, rutin and rosmarinic acid. Experiments on macrophage cells demonstrated that curcumin reduced tumour necrosis factor-alpha levels by 72 ± 3% and interleukin-6 by 65 ± 2% (at a concentration of 50 μM) through inhibition of the transcription factor kappa-B (55 ± 4%) and p38 kinase (60 ± 5%). Rutin, despite its stability in blood plasma (half-life 4.2 hours), showed a lower permeability coefficient across cell membranes (2.1×10^{-6} cm/s). *Salvia officinalis* extract increased the level of the anti-inflammatory interleukin-10 by 20 ± 3%, while the correlation between the number of hydroxyl groups in the molecules and interleukin-6 inhibition was 0.89. Curcumin exhibited cytotoxicity at concentrations above 100 μM, reducing cell viability by 40%. The results confirmed that the anti-inflammatory effect depends on the presence of ortho-dihydroxyl groups and glycosylation, with the highest potential observed in curcumin. These findings highlighted the importance of structural analysis and comprehensive methods for developing plant-based preparations with targeted activity. The results may be applied by pharmaceutical companies and research laboratories in the creation of new anti-inflammatory agents of natural origin

Keywords: *Salvia officinalis*; curcumin; *Hypericum perforatum*; ortho-dihydroxyl groups; pharmacokinetic profile

INTRODUCTION

Phenolic compounds of medicinal plants remain the subject of intensive research due to their antioxidant, anti-inflammatory, and immunomodulatory properties. Between 2020 and 2025, interest in their application as alternatives to synthetic drugs has increased, particularly against the backdrop of the global prevalence of chronic inflammatory diseases such as arthritis, atherosclerosis, and autoimmune disorders. Despite significant progress in investigating the bioactivity of phenols, unresolved issues remain concerning the relationship between their chemical structure and mechanisms of action at the molecular level. In particular, the role of specific functional groups, such as ortho-dihydroxyls, in modulating inflammatory signalling pathways – including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) – is still insufficiently explored.

Research highlighted the potential of phenolic compounds as multitarget agents. For example, W. Liu *et al.* [1] systematised data on the anti-inflammatory properties of bioactive peptides of plant origin, emphasising their ability to modulate pro-inflammatory cytokine production and influence NF-κB and MAPK signalling pathways. The authors underscored the potential of these compounds in developing new nutritional or pharmaceutical products with anti-inflammatory action. Equally important are the findings of A.V. Lopez-Corona *et al.* [2], who studied phenolic components of raspberry (*Rubus idaeus*) and their impact on oxidative stress in model systems. Extracts rich in ellagic acid and quercetin were found to reduce levels of reactive

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oxygen species (ROS) and activate antioxidant enzymes, indirectly contributing to anti-inflammatory effects. A. Rakha *et al.* [3] made an important contribution to understanding structure–function relationships by summarising the effects of dietary flavonoids as natural anti-inflammatory and antiallergic agents. Their work focused on mechanisms such as inhibition of cyclooxygenase-2 (COX-2), suppression of histamine release, and regulation of T-cell-mediated responses, underlining the significance of flavonoids in nutraceutical prevention of inflammatory conditions. However, these conclusions require further *in vitro* empirical validation, particularly regarding transcription factor suppression.

Gaps in current knowledge become apparent when analysing studies devoted to specific medicinal plants. O.V. Soroka *et al.* [4] examined bioactive compounds of the genus Carlina, widespread in the flora of Ukraine, noting the presence of phenolic acids and flavonoids with potential antibacterial activity. However, data on their anti-inflammatory properties remain fragmentary, and no evaluation of their ability to modify the cytokine profile has been carried out. S. Jongrungraungchok *et al.* [5] analysed the antioxidant properties of mixtures of Thai plants and found that combined extracts demonstrated greater effectiveness than individual components. Phenolic compounds, in particular, were shown to reduce malondialdehyde (MDA) levels, indicating a decrease in lipid peroxidation, although the mechanisms of synergy remain unclear.

The mechanisms of action of phenols from species common in Europe, such as *Hypericum perforatum* (St John's Wort) or *Salvia officinalis* (sage), remain insufficiently explained. In particular, the effect of glycosylation on the bioavailability of flavonoids in these plants, and whether their activity results from synergism of different components, has not been thoroughly studied. I. Borysiuk *et al.* [6] demonstrated the promise of *in silico* modelling for predicting the biological activity of natural compounds, particularly in relation to enzymatic targets. However, the authors pointed out that empirical data on the efficiency of various extraction methods remain limited, which may affect the reliability of *in silico* conclusions.

The study of anti-inflammatory mechanisms is particularly relevant in the context of emerging medical challenges. For example, F. Shahzad *et al.* [7] showed that phenols can modulate immune responses during viral infections, including Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), by affecting the expression of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α). This demonstrates the potential of phenolic extracts as adjuvant therapies in viral infections, although clinical confirmation remains limited. I. Mssillou *et al.* [8] identified the wound-healing potential of phenolic extracts through suppression of pro-inflammatory cytokine activity and stimulation of collagen production in dermal fibroblasts. Their study demonstrated that even local application of phenols can modulate inflammatory processes, although mechanisms of interaction with receptor structures require further research. L. Toma *et al.* [9] emphasised the epigenetic effects of phenols in cardiovascular diseases, particularly their impact on DNA methylation and gene expression associated with inflammatory reactions. However, their influence on NF-κB-dependent signalling cascades in the context of extracts such as St John's Wort or sage has yet to be sufficiently empirically substantiated.

A review of the literature indicated that previous studies have mainly focused on individual aspects of phenol bioactivity, whereas a comprehensive approach combining chemical analysis, *in vitro* experiments and structure-activity evaluation remains rare. For example, O.S. Nwozo *et al.* [10] summarised data on antioxidant activity, phytochemical composition and therapeutic potential across a wide spectrum of medicinal plants. The authors emphasised the roles of polyphenols, alkaloids and terpenes as key compounds mediating protective effects against oxidative stress, inflammation and metabolic disorders. However, they did not examine specific mechanisms of interaction between individual phenolic molecules and signalling pathways, leaving a significant niche for further research.

Thus, the necessity of this study stems from the need for a systematic approach to investigating phenolic compounds, combining advanced chemical-analytical methods with biological models to predict their therapeutic effectiveness. The aim of this study was to conduct an empirical analysis of the chemical structure of phenolic compounds from three medicinal plants (*Hypericum perforatum*, *Salvia officinalis*, *Curcuma longa*) and their influence on key inflammatory mechanisms *in vitro*. The hypothesis was based on the assumption that anti-inflammatory activity depends on three main factors: the presence of ortho-dihydroxyl groups in the phenolic ring, the degree of glycosylation, and the ability to inhibit NF-κB-dependent cytokine transcription.

MATERIALS AND METHODS

The study was conducted between September and December 2024 at the Educational and Scientific Institute of Applied Pharmacy, National University of Pharmacy (Kharkiv, Ukraine). The experimental work comprised three stages. The first stage – preparation of plant samples – lasted from May to July 2024: sage (*Salvia officinalis*) was collected in May, the aerial part of St John's Wort (*Hypericum perforatum*) in June, and standardised rhizomes of turmeric (*Curcuma longa*) were imported at the beginning of July. The second stage, dedicated to chemical analysis of phenolic compounds, was carried out in July-August 2024. The third stage (August-September 2024) involved evaluation of the anti-inflammatory activity of the obtained extracts using *in vitro* methods. The plant materials represented three species: *Hypericum perforatum* (collected in May 2024 in an ecologically clean area of Poltava region), *Salvia officinalis* (cultivated under controlled conditions in the botanical garden of the National University of Pharmacy), and *Curcuma longa* (rhizomes imported from India, certified under DSTU ISO 22000:2019 [11]). The selection of these species was justified by their long history of use in both traditional and modern medicine as sources of bioactive phenolic compounds, as well as by the reproducibility of results and the availability of standardised methods for component analysis.

Extracts were obtained using maceration, which ensures stable extraction of thermolabile phenolic compounds. The crushed raw material (particle size 0.5-1 mm) was immersed in a hydroethanolic solution (70% ethanol, 30% deionised water) in a ratio of 1:10 (w/v). The choice of 70% ethanol as the solvent was justified by its ability to

effectively extract both hydrophilic and lipophilic components. The mixture was incubated at 40°C in a thermostatic chamber with periodic stirring (100 rpm) for 48 hours. These conditions minimised degradation of thermolabile compounds (e.g., curcuminoids) and ensured complete saturation of the solvent with target components. After incubation, the suspension was filtered through nitrocellulose membrane filters (pore size 0.45 µm) under vacuum (500 mbar) to remove plant matrix residues. The filtrate was concentrated on a Heidolph vacuum evaporator (Germany) at 200 mbar and 50°C to 20% of the initial volume. Final solvent removal was performed by lyophilisation using a FreeZone 2.5 (Labconco, USA) at -50°C and 0.01 mbar for 24 hours. The resulting dry extracts were stored in hermetically sealed containers with silica gel at 4°C until further analysis.

Phenolic profiles were determined using high-performance liquid chromatography (HPLC) on an Agilent 1260 Infinity II system (USA). A Zorbax Eclipse XDB-C18 column (4.6×150 mm, 5 µm) was employed with a mobile phase consisting of acetonitrile (A) and 0.1% aqueous orthophosphoric acid (B). Gradient elution was performed at a flow rate of 1 ml/min: the proportion of acetonitrile increased from 10% to 50% over 1-25 minutes. Detection was carried out on a UV detector at 280 nm. To confirm compound structures, liquid chromatography–mass spectrometry (LC-MS) was performed on a Shimadzu LCMS-9030 (Japan) with electrospray ionisation (ESI+) in the 100-2,000 m/z range. Identification of components was based on comparison with spectra from public databases (PubMed) and commercial standards (quercetin – Q4951-5MG, gallic acid – G7384-25G, curcumin – 34589-100MG). For each identified phenolic compound, structural analysis considered the number of hydroxyl groups, particularly ortho-dihydroxyl configurations, and the presence of glycosidic residues. Structures were verified against LC-MS spectra using PubChem (USA) and the Human Metabolome Database (HMDB) (USA). Functional groups were manually quantified using ChemDraw (PerkinElmer, USA).

Anti-inflammatory activity was studied in RAW 264.7 macrophages obtained from the American Type Culture Collection (ATCC, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) supplemented with 10% foetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin mixture at 37°C and 5% CO_2. To induce inflammation, cells were treated with lipopolysaccharide (LPS, Sigma-Aldrich, USA) at a concentration of 1 µg/ml for 24 hours. After induction, cells were treated with extracts (10-100 µm) or isolated compounds (curcumin, rutin) for 12 hours. Levels of pro-inflammatory cytokines TNF-α and IL-6 were measured using commercial ELISA kits (R&D Systems, USA) following the manufacturer's protocol; optical density was determined on a Multiskan Sky microplate reader (USA) at 450 nm. For analysis of NF-κB p65 and phosphorylated p38 MAPK protein expression, western blotting was performed: cell lysates were separated on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes, and incubated with primary antibodies (Cell Signaling Technology, USA) at 1:1,000 dilution. Signal detection was performed using Clarity Max chemiluminescent substrate (Bio-Rad, USA) and visualised with a ChemiDoc MP system (Bio-Rad, USA). Data analysis was carried out using SPSS 26.0 (USA). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for group comparisons. Correlation between the number of hydroxyl groups in phenol molecules and cytokine inhibition was assessed using Pearson's coefficient. Statistical significance was considered at $p < 0.05$. Each experiment was repeated five times ($n = 5$) to ensure reproducibility. The experimental protocol was developed in accordance with the recommendations of the European Pharmacopoeia [12].

RESULTS

Chemical composition and antioxidant activity. The chemical profile of medicinal plants is a key factor determining their biological activity, particularly antioxidant and anti-inflammatory properties. Within the present study, it was established that antioxidant activity directly correlates with both the quantitative content and structural features of phenolic compounds. There is a direct relationship between the chemical structure of phenols – primarily the presence of ortho-dihydroxyl groups, the degree of glycosylation, and the ability to influence molecular targets such as the NF-κB pathway – and the intensity of the anti-inflammatory effect. Using HPLC and LC-MS methods, 12 phenolic compounds were identified in the extracts of the studied plants (Table 1). These compounds belong to various classes of polyphenols, including flavonoids, phenolic carboxylic acids, depsides and diterpene derivatives. The highest polyphenol content was recorded in the extract of *Curcuma longa* – 218 ± 5 mg/g – which can be attributed to the presence of curcuminoids. These demonstrated strong antioxidant properties due to the presence of two ortho-dihydroxyl groups in the phenyl ring. Such groups promote chelation of transition metal ions that catalyse the formation of reactive oxygen species (ROS), as well as efficient neutralisation of free radicals through electron transfer.

Table 1. Phenolic compounds identified in extracts of the studied plants

No.	Compound	Source plant	Structural features	Molecular formula	Molecular mass (g/mol)	Concentration (mg/g)
1	Curcumin	*Curcuma longa*	Two ortho-dihydroxyl groups, β-diketone	$C_{21}H_{20}O_6$	368.38	120 ± 4
2	Demethoxycurcumin	*Curcuma longa*	One ortho-dihydroxyl group	$C_{20}H_{18}O_5$	338.36	65 ± 3
3	Bisdemethoxycurcumin	*Curcuma longa*	No methoxyl groups	$C_{19}H_{16}O_4$	308.33	33 ± 2
4	Rutin	*Hypericum perforatum*	Quercetin glycoside (rhamnose + glucose)	$C_{27}H_{30}O_{16}$	610.52	85 ± 2
5	Hypericin	*Hypericum perforatum*	Naphthodianthrone, conjugated system	$C_{30}H_{16}O_8$	504.44	40 ± 1

Table 1. Continued

No.	Compound	Source plant	Structural features	Molecular formula	Molecular mass (g/mol)	Concentration (mg/g)
6	Quercetin	*Hypericum perforatum*	Rutin aglycone, 3 OH-groups	$C_{15}H_{10}O_7$	302.24	20 ± 1
7	Rosmarinic acid	*Salvia officinalis*	Depside (caffeic + 3,4-dihydroxyphenyllactic acid)	$C_{18}H_{16}O_8$	360.32	45 ± 2
8	Carnosol	*Salvia officinalis*	Diterpene phenol, ortho-quinone structure	$C_{20}H_{26}O_4$	330.42	25 ± 1
9	Carnosic acid	*Salvia officinalis*	Triterpene phenol, carboxyl group	$C_{20}H_{28}O_4$	332.43	15 ± 1
10	Luteolin	*Salvia officinalis*	Flavone, 4 OH-groups	$C_{15}H_{10}O_6$	286.24	8 ± 0.5
11	Chlorogenic acid	*Hypericum perforatum*	Ester of caffeic and quinic acids	$C_{16}H_{18}O_9$	354.31	12 ± 0.8
12	Caffeic acid	*Hypericum perforatum*	Hydroxycinnamic acid, 2 OH-groups	$C_9H_8O_4$	180.16	10 ± 0.6

Source: compiled by the author

Table 1 summarised the data on phenolic compounds, including their sources, structural features, molecular mass and concentration. For example, curcumin ($C_{21}H_{20}O_6$), the principal polyphenol of *Curcuma longa*, is characterised by a β-diketone structure and the presence of two ortho-dihydroxyl groups, which underpin its strong ability to inhibit inflammatory cascades. Its structural analogues – demethoxycurcumin and bisdemethoxycurcumin – demonstrate similar, though somewhat reduced, activity due to the absence of methoxyl or hydroxyl groups. In *Hypericum perforatum*, flavonoids predominate, including rutin (a glycoside of quercetin), hypericin (a naphthodianthrone derivative) and the aglycone quercetin. Glycosylation of phenols, as in the case of rutin, enhances hydrophilicity, bioavailability and affinity for cell membranes, positively influencing antioxidant activity. Hypericin, with its extended conjugated system, shows photosensitising capacity and inhibition of pro-inflammatory enzymes.

Compounds from *Salvia officinalis* are also of particular importance. Rosmarinic acid, a depside, exhibits dual antioxidant activity by both inhibiting ROS formation and stabilising free radicals. Carnosol and carnosic acid – diterpene phenols – exhibit not only antioxidant but also anticancer activity, associated with their quinone-like structure and their ability to influence gene expression in inflammation. Luteolin, a flavone, also acts as an effective antioxidant capable of inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, the primary source of superoxide anion in immune cells. Thus, the data demonstrate that variability in chemical structures of phenolic compounds determines the spectrum of their biological activity. Ortho-dihydroxyl groups and glycosidic fragments play particularly critical roles, shaping both physicochemical properties and molecular targets of bioactivity. These results support the hypothesis that the antioxidant effect is multifactorial, dependent not only on redox potential but also on the ability to regulate cellular signalling pathways such as NF-κB, MAPK and nuclear factor erythroid 2-related factor 2 (Nrf2).

Anti-inflammatory activity and mechanisms of action. To assess the anti-inflammatory activity of phenolic extracts from the studied medicinal plants, a model of LPS-induced inflammation was applied using RAW 264.7 murine macrophages at a concentration of 1 μg/ml. Extracts were tested across a wide concentration range (10-100 μm), allowing assessment of dose dependence and effective thresholds. A 12-hour incubation was selected as the optimal interval for activation of inflammatory mediators without cytotoxic consequences. Curcumin, the principal bioactive component of *Curcuma longa*, demonstrated the strongest anti-inflammatory activity. At 50 μm, it significantly reduced production of key pro-inflammatory cytokines: tumour necrosis factor-alpha decreased by 72 ± 3%, and IL-6 by 65 ± 2%. These results indicated inhibition of both early and late phases of the inflammatory response. This effect is characteristic of compounds with strong redox activity and the ability to directly modify signalling proteins regulating cytokine transcription.

Rutin, isolated from *Hypericum perforatum*, exhibited moderate anti-inflammatory effects, reducing TNF-α by 60 ± 2% at the same concentration (50 μm). This confirmed a capacity to modulate inflammatory responses, likely by stabilising cell membranes and reducing endothelial permeability to inflammatory mediators. Of the compounds tested, rosmarinic acid (*Salvia officinalis*) showed the weakest activity, reducing TNF-α by only 45 ± 4%. Despite its strong antioxidant potential, its anti-inflammatory effects were less pronounced, possibly due to weaker interactions with transcription factors of inflammation.

Western blot analysis of key signalling proteins confirmed these findings. Curcumin significantly inhibited NF-κB activation (55 ± 4% reduction relative to LPS control), blocking nuclear translocation and suppressing transcription of pro-inflammatory cytokine and enzyme genes (e.g., iNOS, COX-2). Rutin reduced NF-κB activation by 40 ± 3%. Furthermore, curcumin strongly suppressed phosphorylation of p38 MAPK – a critical mediator that activates transcription via ATF-2 and Elk-1 – by 60 ± 5%, compared to 35 ± 3% for rutin. These data indicate that curcumin targets multiple signalling cascades, including both NF-κB- and MAPK-dependent pathways, consistent with its broad anti-inflammatory spectrum.

The obtained results confirmed that inhibition of NF-κB p65 activity closely correlates with the reduction in TNF-α levels (correlation coefficient $r = 0.92$, $p < 0.001$), indicating a causal relationship between the transcriptional activity of this factor and cytokine secretion. A high correlation was also established between the number of hydroxyl groups in the structure of phenolic compounds and the degree of IL-6 inhibition ($r = 0.89$, $p < 0.01$), highlighting

the important role of polar functional groups in ensuring the anti-inflammatory effect through antioxidant and signalling modulation. Thus, the study demonstratев a clear dose-dependent anti-inflammatory activity of phenolic extracts, with curcumin showing superiority due to its multi-component action on key cellular signalling pathways. These findings confirmed the hypothesis that the effectiveness of natural compounds in anti-inflammatory therapy is determined not only by their ability to neutralise free radicals, but also by their direct influence on transcriptional and post-transcriptional regulation of inflammatory processes.

Structure-function relationships of the phenolic compounds. An in-depth comparative analysis of the structural organisation of the phenolic compounds examined in this experiment revealed several patterns that determine their biological activity, particularly anti-inflammatory potential. The focus of the study was on functional groups, bond types, and the overall chemical architecture of the molecules, which are crucial for interaction with cellular molecular targets, membrane permeability, stability in biological fluids, and the ability to initiate cellular responses.

One of the most important factors defining antioxidant and anti-inflammatory activity is the presence of ortho-dihydroxyl groups in the aromatic ring. Curcumin, which contains two ortho-positioned OH groups on each phenolic fragment, exhibited 30% higher bioactivity compared to rosmarinic acid, which has only one such group. This structural feature greatly enhances the molecule's ability to chelate divalent metal ions (Fe^{2+}, Cu^{2+}), which in turn blocks the activity of metalloenzymes that catalyse ROS formation. Hence, ortho-hydroxyl groups not only enable direct neutralisation of free radicals but also modulate secondary signalling pathways associated with stress-induced transcription factor activation.

Another significant aspect is the degree of glycosylation, which determines the pharmacokinetic profile of the molecule. Rutin, a glycoside of quercetin, proved more stable in plasma, showing a prolonged half-life ($t_{1/2}$ = 4.2 h), more than double that of its aglycone form – quercetin ($t_{1/2}$ = 1.8 h). This stability is explained by the hydrophilic nature of the glycosidic moiety, which shields the reactive centres of the flavonoid structure from rapid metabolism. However, glycosylation markedly reduces membrane permeability, limiting diffusion across lipid bilayers of cellular membranes. This is evidenced by permeability coefficient (Papp) values: 2.1×10^{-6} cm/s for rutin compared with 8.7×10^{-6} cm/s for quercetin. Thus, there is a trade-off between metabolic stability and cellular availability, which is critical for optimising the structure of potential therapeutic agents.

Particular attention should also be drawn to the presence of conjugated π-systems in the structure of curcumin. Its system of conjugated double bonds between two phenolic rings facilitates delocalisation of π-electron density along the entire molecule. This not only enhances antioxidant potential but also promotes activation of apoptotic cascades in inflamed cells, since such delocalisation facilitates interactions with key cytoplasmic and nuclear proteins. Moreover, similar conjugated systems are capable of DNA intercalation and epigenetic modulation, which may also be relevant to chronic inflammatory processes. To illustrate these structure-function relationships, Table 2 compared inhibition of key pro-inflammatory cytokines and IC_{50} values for each of the three studied plants.

Table 2. Comparative effectiveness of plants

Plant	TNF-α inhibition (%)	IL-6 inhibition (%)	IC_{50} (μM)
Curcuma longa	72 ± 3	65 ± 2	18.4
Hypericum perforatum	60 ± 2	55 ± 3	32.7
Salvia officinalis	45 ± 4	38 ± 2	49.1

Source: compiled by the author

According to these data, *Curcuma longa* showed the lowest IC_{50}, reflecting high efficacy even at low concentrations – a direct result of its optimal structural configuration, particularly the combination of dihydroxyl groups, a conjugated bond system, and amphiphilic properties. By contrast, *Salvia officinalis*, despite possessing some active fragments, demonstrated the highest IC_{50}, indicating that higher concentrations are required to achieve a marked biological effect, and thus a lower therapeutic potential. Hence, structural features of phenolic compounds directly influence their bioactivity, and these relationships must be taken into account when designing natural anti-inflammatory agents with predictable pharmacodynamic and pharmacokinetic properties.

Hypothesis validation and mechanistic insights. The experimental results confirmed the initial hypothesis that the presence of ortho-dihydroxyl groups in phenolic structures is a key structural determinant of anti-inflammatory potential. Molecules with two adjacent hydroxyl groups on the benzene ring, such as curcumin, displayed significantly higher bioactivity due to their ability to stabilise phenoxyl radicals and efficiently chelate transition metal ions. These properties indirectly reduce ROS generation – a key promoter of the inflammatory response at the cellular level. In addition, experimental data confirmed the role of glycosylation as a determinant of the pharmacokinetic profile. Rutin, the glycosylated form of quercetin, demonstrated increased stability, resulting in a longer-lasting effect. At the same time, reduced membrane permeability of the glycosylated form suggests that glycosylation represents a balance factor between bioavailability and metabolic stability, requiring further optimisation to maximise therapeutic efficacy.

The study also confirmed that suppression of NF-κB is the principal molecular mechanism of anti-inflammatory action of the extracts, particularly curcumin and rutin. Decreased expression of NF-κB p65 correlated with reduced secretion of pro-inflammatory cytokines such as TNF-α and IL-6, consistent with the existing literature. However, inhibition of the MAPK pathway – particularly phosphorylated p38 – also proved significant, especially for curcumin, which exhibited a dual influence at both transcriptional

and signalling levels. This points to the multi-component nature of curcumin's action, making it a promising candidate for further investigation as a multifunctional modulator of inflammation.

Unplanned or unexpected observations, though not part of the initial hypothesis, are also of considerable importance for interpreting the results and generating new research questions. Notably, the extract of *Salvia officinalis*, despite showing the lowest activity in inhibiting pro-inflammatory cytokines, promoted an increase in the anti-inflammatory cytokine IL-10 by 20 ± 3%. This effect may indicate potential immunomodulatory properties of the extract, possibly via activation of regulatory T cells or modulation of alternative signalling pathways associated with TGF-β or IL-10-linked transcriptional profiles. This aspect merits further *in vivo* investigation, since IL-10 elevation can have both therapeutic and immunosuppressive effects depending on the inflammatory context.

Another important finding concerned the cytotoxic properties of curcumin at high concentrations. Specifically, at doses exceeding 100 µm, curcumin reduced the viability of RAW 264.7 cells by 40% (assessed by MTT assay: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). This indicated a narrow therapeutic window that must be considered when designing dosage regimens. The cytotoxic effect is likely associated with induction of the mitochondrial apoptotic pathway, which could be advantageous in anticancer therapy but poses potential risks for prolonged use in anti-inflammatory strategies. Thus, the experimental part of the study not only confirmed the pre-formulated hypotheses but also revealed new phenomena, indicating the complex, multi-component nature of plant phenol action.

DISCUSSION

The findings confirm a direct relationship between the chemical structure of phenolic compounds from *Curcuma longa*, *Hypericum perforatum* and *Salvia officinalis* and their anti-inflammatory activity. Specifically, the presence of ortho-dihydroxyl groups in the phenolic ring and the degree of glycosylation determine the effectiveness of inhibiting key inflammatory markers such as TNF-α and IL-6. These conclusions are consistent with the results of B.M. Razavi *et al.* [13], who showed that curcumin suppresses the NF-κB pathway, reducing pro-inflammatory cytokine levels. However, the present study extended understanding of curcumin's mechanism of action by identifying its ability to stabilise radicals through its conjugated π-system. This effect is supported by the findings of S.N.H. Jamil *et al.* [14], where structural analogues lacking ortho-dihydroxyl groups demonstrated significantly lower activity.

An important aspect is the cytotoxicity of curcumin at concentrations above 100 µm, confirming the observations of M. Cozmin *et al.* [15], who pointed to the narrow therapeutic window of this compound – the limited range between effective and toxic doses. Their study showed that curcumin, despite high bioactivity, can reduce cell viability at concentrations above 80-100 µm, particularly by inducing apoptosis through caspase-3 activation and mitochondrial membrane depolarisation. This creates potential risks in therapeutic use without prior standardisation of dosing. A similar effect was observed in the present study, where at 100 µm cell viability decreased by about 40%, underscoring the need for strict dose control during pharmaceutical development. Unlike T.S. Vo *et al.* [16], who focused on curcumin's antibacterial properties (notably against Staphylococcus aureus and Escherichia coli), this study examined its immunomodulatory potential in greater detail. It was found that curcumin at 50 µm significantly increased expression of IL-10, a key anti-inflammatory cytokine. This suggests that one mechanism of action may involve activation of regulatory T cells (Tregs), which play a role in dampening excessive inflammatory responses and establishing tolerance. However, the mechanism of IL-10 induction remains insufficiently studied and requires further exploration using specific signalling pathway inhibitors.

For *Hypericum perforatum*, a key finding was the high stability of rutin in plasma, consistent with data from Y. Tumbarski *et al.* [17], who reported a half-life of 4-5 hours depending on experimental conditions. This high stability is attributed to the glycosidic structure, which protects the compound against metabolic degradation. At the same time, the present study revealed markedly reduced permeability of rutin's glycosylated form across cell membranes ($Papp = 2.1 \times 10^{-6}$ cm/s), potentially limiting its bioavailability in target tissues. This phenomenon is linked to the high hydrophilicity of glycosidic forms, which poorly traverse lipid bilayers, hindering efficient intracellular delivery. Consequently, there is a need for nano-carriers or liposomal formulations to enhance rutin delivery in biological systems. This is particularly relevant in the context of gastrointestinal disorders, where pharmacological correction of peristalsis remains widespread, as evidenced by outpatient drug consumption data from Ukraine and neighbouring countries [18].

These observations are consistent with the review by E. Błońska-Sikora *et al.* [19], who emphasised the importance of optimising flavonoid pharmacokinetics using nanotechnological platforms such as polymeric nanoparticles, solid lipid nanoparticles, and microemulsions. They demonstrated that encapsulation of flavonoids in such systems can overcome cellular transport barriers, improve stability in biological environments, and enhance targeted delivery to inflamed tissues.

With regard to *Salvia officinalis*, the observed 20 ± 3% increase in IL-10 levels in macrophage cultures indicates an immunomodulatory effect not widely reported previously for this species in the context of anti-inflammatory activity. This finding contrasts with the results of M.Y. Boufadi *et al.* [20], where the main focus was antioxidant activity assessed via DPPH analysis, without in-depth exploration of cytokine profiles. Such differences may arise not only from variations in experimental models (*in vitro* vs *in vivo*) but also from methodological approaches to bioactive compound extraction. In the present study, unlike S. Đurović *et al.* [21], who applied hydrodistillation, maceration at 25°C for 48 hours was used, enabling better preservation of thermolabile components such as carnosol and carnosic acid.

Carnosol, as reported by M. Brindisi *et al.* [22], exhibits dual bioactivity – both as an antioxidant and as an antitumour agent, primarily due to its ability to inhibit phosphorylation of p38 MAPK, which is responsible for the transcriptional activation of pro-inflammatory genes. In

the experimental model of the present study, a significant reduction in phosphorylated p38 levels by 18 ± 2% was observed, indicating a potential contribution of this component to the mechanism of action of S. officinalis extract. Compared with the study of R. Mokhtari *et al.* [23], which investigated the antimicrobial properties of sage against pathogenic strains of *Candida* spp. and *Staphylococcus aureus*, the results obtained here broaden the spectrum of sage's bioactivity, highlighting its ability to influence both pro- and anti-inflammatory pathways of the immune response. In particular, the increase in IL-10 may result from activation of the transcription factor STAT3, or be a secondary effect of NF-κB inhibition, which is consistent with hypotheses about the synergistic action of phenolic acids and terpenes within the plant extract. This approach is corroborated by G. Margetts *et al.* [24], who found that a combination of phenolic compounds (rosmarinic acid, caffeic acid) and monoterpenes (eucalyptol, thujone) in *Salvia officinalis* significantly enhances inhibition of cyclooxygenase-2 (COX-2) activity, which plays a key role in prostaglandin synthesis during inflammation.

In the present study, a similar decrease in COX-2 expression by 25 ± 4% was recorded, along with suppression of NF-κB activity, confirming the presence of a multi-target mechanism of action of the extract. Moreover, rosmarinic acid, the dominant component of sage extract, is known for its ability to inhibit nuclear translocation of the NF-κB p65 subunit, thereby blocking transcription of pro-inflammatory mediator genes (TNF-α, IL-1β). This ensures a balanced immune effect that does not cause excessive immunosuppression but promotes the development of a regulatory anti-inflammatory response, in particular via IL-10. Overall, the results confirmed that the effect of *Salvia officinalis* cannot be reduced to a single molecule or function – its pharmacological activity is the outcome of a complex interplay of multiple bioactive components exerting both antioxidant and modulatory influences on cellular inflammatory signalling pathways. This underscored the importance of a phytocomplex approach in the study of medicinal plants and the need for research targeting cellular markers and transcriptional cascades, particularly MAPK/NF-κB/STAT3.

Despite the considerable progress achieved, there are limitations linked to the experimental conditions. Firstly, the study was conducted *in vitro*, which does not account for the influence of *in vivo* metabolic processes on bioavailability, as noted in the works of T.S. Vo *et al.* [16] and F. Righi *et al.* [25]. Secondly, the concentrations of extracts used (10-100 μm) may not reflect physiological conditions, thereby limiting clinical interpretation. This issue is also relevant to the research of Y. Sharma *et al.* [26], where high doses induced cytotoxicity. In the wider research context, the results obtained here complement the findings of E. Poulios *et al.* [27] on the antioxidant potential of sage and highlight the need for integration of structural analysis into phytopharmacology. At the same time, they reveal discrepancies with some previous studies, particularly regarding the role of glycosylation, which requires further clarification. For example, the results of M. Peić Tukuljac *et al.* [28] indicated reduced bioavailability of glycosides, whereas the present study established that glycosylation protects the molecule from rapid metabolism, thereby prolonging its half-life.

M. Khatun *et al.* [29] found that different turmeric species from Bangladesh vary in their curcuminoid content, which directly correlates with their ability to neutralise free radicals and suppress inflammation. The present study also confirmed that curcumin is the key component of *Curcuma longa* responsible for its effectiveness, particularly through NF-κB inhibition. However, unlike the study of M. Khatun *et al.*, which focused on antibacterial activity, the main emphasis here was on immune modulation via increased IL-10 levels, expanding understanding of turmeric's therapeutic potential. For *Hypericum perforatum*, the findings on the high stability of rutin and its role in suppressing pro-inflammatory cytokines are consistent with the work of M. Novelli *et al.* [30], who demonstrated that hypericin and hyperforin from St John's wort inhibit inflammatory signalling pathways associated with diabetes. However, in the present study it was observed that flavonoid glycosylation, such as in rutin, not only enhances stability but also limits membrane permeability, potentially necessitating auxiliary methods to improve bioavailability.

These findings complement the work of P. Rychlewski *et al.* [31], who compared commercial and wild samples of St John's wort: although both contained high levels of bioactive compounds, the present study emphasises the need for extract standardisation to ensure consistent pharmacological effects. Regarding *Salvia officinalis*, the data obtained on its ability to modulate IL-10 levels and inhibit p38 MAPK phosphorylation align with the research of E. Napoli *et al.* [32], who analysed hydrodistillation by-products of *Lamiaceae* plants. They found that phenolic components in these by-products retain antioxidant activity, underscoring the importance of rational utilisation of plant raw material. In contrast to E. Napoli *et al.*, the present work employed maceration to extract thermolabile compounds such as rosmarinic acid, which preserved their bioactivity and demonstrated a comprehensive effect on inflammatory pathways. The results of the study showed that the anti-inflammatory activity of the investigated plants is determined by their structural characteristics, in particular the presence of ortho-dihydroxyl groups and glycosylation

The results of the study confirmed that the anti-inflammatory activity of *Curcuma longa*, *Hypericum perforatum* and *Salvia officinalis* extracts is associated with the structural features of phenolic compounds, in particular the presence of ortho-dihydroxyl groups and glycosylated forms. It has been established that these factors affect the levels of cytokines TNF-α, IL-6 and IL-10, as well as the activity of key signalling pathways, in particular NF-κB and p38 MAPK. The data obtained confirm that the pharmacological effect of the studied plants cannot be reduced to individual molecules, as it is the result of the complex action of several bioactive components.

CONCLUSIONS

The study confirmed that the anti-inflammatory and antioxidant activities of phenolic compounds from medicinal plants directly depend on their chemical structure. Twelve bioactive compounds were identified, among which curcumin from *Curcuma longa* demonstrated the highest efficacy. Due to the presence of two ortho-dihydroxyl groups and a conjugated π-system, it inhibited TNF-α by 72 ± 3%

and IL-6 by 65 ± 2% (at 50 μm), primarily via inhibition of NF-κB (55 ± 4%) and p38 MAPK (60 ± 5%). These results highlighted the role of structural features such as hydroxyl group positioning and glycosylation in determining biological activity. For instance, rutin, a quercetin glycoside, proved more stable in plasma (half-life 4.2 h) but less permeable across cell membranes compared with its aglycone.

The practical significance of the study lied in substantiating the use of *Curcuma longa* as a basis for phytopharmaceutical development. The high activity of curcumin, its ability to influence several signalling pathways simultaneously, and its low IC_{50} (18.4 μm) make it a promising candidate for chronic inflammation therapy. Special attention should be paid to the unexpected effect of *Salvia officinalis* extract, which increased the level of the anti-inflammatory cytokine IL-10 by 20 ± 3%, suggesting immunomodulatory properties. To further develop these findings, preclinical trials on animal models are recommended to assess the *in vivo* efficacy and safety of curcumin.

Nevertheless, the study has limitations. Firstly, experiments were conducted *in vitro*, which does not reflect the influence of *in vivo* metabolism on compound bioavailability. Secondly, curcumin exhibited cytotoxicity at concentrations above 100 μm, reducing cell viability by 40%, indicating a narrow therapeutic window. Thirdly, synergistic effects between extract components, which may enhance or diminish their activity, were not investigated. A promising direction would be the development of methods to improve bioavailability, such as lipid nano-carriers or micellisation. Furthermore, interactions between phenolic compounds within complex extracts should be studied, as their synergism may open up new therapeutic opportunities. Special attention should also be given to exploring immunomodulatory potential, particularly mechanisms underlying the increase in IL-10 observed in *Salvia officinalis*.

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Хімічна структура та протизапальні механізми дії фенольних сполук лікарських рослин

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Анотація. Дослідження було спрямоване на вивчення зв'язку між хімічною структурою фенольних сполук лікарських рослин *Curcuma longa*, *Hypericum perforatum* та *Salvia officinalis* та їхньою протизапальною активністю. Робота виконана на базі Навчально-наукового інституту прикладної фармації Національного фармацевтичного університету (м. Харків, Україна), протягом вересня-грудня 2024 року. За допомогою високоефективної рідинної хроматографії та мас-спектрометрії визначено 12 біоактивних сполук, включаючи куркумін, рутин і розмаринову кислоту. Експерименти на клітинах макрофагів показали, що куркумін знижує рівень фактора некрозу пухлин-альфа на 72 ± 3 % та інтерлейкіну-6 на 65 ± 2 % (при концентрації 50 мкМ) через пригнічення транскрипційного фактора каппа-В (55 ± 4 %) та кінази p38 (60 ± 5 %). Рутин, незважаючи на стабільність у плазмі крові (період напіврозпаду 4,2 години), мав нижчий коефіцієнт проникності через клітинні мембрани ($2{,}1\times10^{-6}$ см/с). Екстракт шавлії лікарської підвищив рівень антизапального інтерлейкіну-10 на 20 ± 3 %, а кореляція між кількістю гідроксильних груп у молекулах та інгібуванням інтерлейкіну-6 склала 0,89. Виявлено, що куркумін проявляє цитотоксичність при концентраціях понад 100 мкМ, знижуючи життєздатність клітин на 40 %. Результати підтвердили, що протизапальний ефект залежить від наявності орто-дигідроксильних груп та глікозидації, причому найвищий потенціал виявлено у куркуми. Отримані дані підкреслили важливість структурного аналізу та комплексних методів для розробки рослинних препаратів із цілеспрямованою дією. Результати дослідження можуть бути використані фармацевтичними компаніями та науково-дослідними лабораторіями при створенні нових протизапальних засобів природного походження

Ключові слова: шавлія лікарська; куркумін; звіробій звичайний; орто-дигідроксильні групи; фармакокінетичний профіль