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RESEARCH ON THE PHENOLIC PROFILE, ANTIRADICAL AND ANTI-INFLAMMATORY ACTIVITY OF A THICK HYDROALCOHOLIC FEVERFEW (*Tanacetum parthenium* L.) HERB EXTRACT

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The aim *– to study the phenolic complex of a thick hydroalcoholic extract of the feverfew (Tanacetum parthenium (L.) herb (FTHAE), its antiradical activity and anti-inflammatory properties in a model of carrageenan and histamine oedema.*

Materials and methods. *The studied extract was obtained from the Tanacetum parthenium herb, collected in Sumy and Poltava regions of Ukraine during the period of mass flowering (June-August): degree of grinding of raw materials 2.0–3.0 mm, extraction temperature – 25 °C, extractant – 70 % ethanol, raw material/extractant ratio – 1:12, infusion time – 12 hours, multiplicity of extractions – 3. HPLC and spectrophotometric methods were used to determine the composition and amount of phenolic compounds of FTHAE. HPLC analysis was performed using a "Waters e2695 Alliance system" (Waters, Milford, MA, USA) with a photodiode array detector "Waters 2998" according to the HPLC-PDA method for phenolic compounds. The scavenging of ABTSA radical cation evaluated the radical scavenging activity. In addition, the anti-inflammatory properties of FTHAE were studied on carrageenan and histamine paw oedema in rats. Anti-inflammatory activity (AIA) was evaluated as the ability to reduce oedema compared to the control pathology. FTHAE was used at a dose of 50 mg/kg.*

The results. *The content of the sum of hydroxycinnamic acids in the obtained extract was determined by spectrophotometry, which was 13.92±0.02 % and the content of the sum of flavonoids – 5.16 ± 0.03 %. The content of 12 compounds with a total amount of 72432.09μg/g was identified and determined by HPLC. The dominant compounds were hydroxycinnamic acids, namely 3,4-dicaffeoylquinic, 4,5-dicaffeoylquinic and chlorogenic acids. The antiradical activity of the extract was 620.19±4.53μmol/g. On the model of carrageenan oedema, the maximum effect of oedema suppression was 71.0–73.2 %. In the model of histamine oedema, the anti-inflammatory effect of the extract was 57.8; 51.8; and 49.1 % for 30 minutes, 1 and 1.5 hours of oedema, respectively. The severity of the anti-inflammatory activity of the extract during the first hour is not inferior to the diclofenac sodium, quercetin and loratadine.*

Conclusions. *Due to the HPLC method, 12 compounds were determined to cause antiradical activity, among which chlorogenic acid and rutin were identified.*

The studied extract has a pronounced anti-inflammatory effect, which is due to the antiradical properties of the extract and its inhibitory effect on inflammatory mediators

Keywords: *Tanacetum parthenium, extract, phenolic profile, hydroxycinnamic acids, antiradical activity, anti-inflammatory activity*

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1. Introduction

Inflammation is a typical pathological process that develops in organs and tissues in response to damage. This process is necessary for the isolation, neutralization and removal of pathological factors and necrotized cells and tissues with subsequent replacement of connective tissue formed defect [1, 2]. An invariable symptom of the inflammatory process is pain resulting from the algogenic action of inflammatory mediators (biogenic amines, kinins, prostaglandins) on the nociceptors of organs and systems of the body [3, 4].

Non-steroidal anti-inflammatory drugs (NSAIDs) are the basic drugs for the pharmacotherapy of inflammatory processes [5, 6]. Today, the group of NSAIDs, along with widespread damage to the stomach and intestines [7], is characterized by hepatotoxic reactions [8] and the ability to increase the risk of cardiovascular and renal disorders [9]. Gastro- and hepatotoxic effects of NSAIDs limit their use in some cases, especially in comorbid pathologies of the gastrointestinal tract and hepatobiliary system [10].

Since many NSAIDs on the market have significant undesirable effects, the need for new anti-inflammatory drugs contributes to the advancement of research for newer, safer, effective molecules with fewer side effects and from plant sources. Therefore, it can be observed that many substances of plant origin form part of the therapeutic arsenal of modern medicine [11, 12]. Furthermore, due to the production of secondary metabolites with clinically curative effects, medicinal plants play an important role in developing new and potent drugs [13, 14].

Feverfew (*Tanacetum parthenium* (L.) Schultz Bip) is a perennial herbaceous plant-heliophyte of the Tansy (*Tanacetum*) genus of the Aster family (*Asteraceae*) with anti-inflammatory and analgesic properties. The plant is native to the Balkan Peninsula but widely cultivated in Europe and Ukraine. Externally extracts from the feverfew herb are recommended for the treatment of psoriasis, dermatitis accompanied by itching, the treatment of open skin lesions, and rinsing the mouth after dental surgery [15]. Feverfew herb has anti-inflammatory, cardiotonic, antipyretic, antispasmodic and antioxidant effects [16]. Studies confirm the anticancer effect of the feverfew herb [16]. However, the feverfew attracts the interest of scientists worldwide due to its antimigraine and anti-inflammatory activity owing to sesquiterpene lactones and phenolic compounds [17].

In previous studies, we studied the phenolic profile of the feverfew herb collected in 7 regions of Ukraine. The total content of phenolic compounds ranged from 12184.79 to 23701.62 μg/g of DW. The highest content of phenolic compounds was determined in the samples of the feverfew herb collected in Sumy and Poltava regions [18]. Given the high content of phenolic components in Ukrainian raw materials, it was decided to obtain a thick hydroalcoholic extract for studying its chemical composition and pharmacological activity. Using a thick hydroalcoholic extract from the feverfew herb (extractant 70 % ethyl alcohol) (FTHAE) as an anti-inflammatory agent will increase the effectiveness and safety of anti-inflammatory pharmacotherapy.

The aim of the research was to study the phenolic complex of a thick hydroalcoholic extract of the feverfew (*Tanacetum parthenium* (L.) herb, its antiradical activity and anti-inflammatory properties in a model of carrageenan and histamine oedema.

2. Research planning (methodology)

The basic principles of the concept of Quality by design were used for research planning [19]. Fig. 1 shows a graphical representation of the re-search planning process.

In addition, carrageenan and histamine paw oedema in rats is a classic model of acute inflammation [20, 21]. Taking this into account, these experimental models were used in the study.

3. Materials and methods

The studied extract was obtained from the Tanacetum parthenium herb, collected in Sumy and Poltava regions of Ukraine during the period of mass flowering (June-August): degree of grinding of raw materials 2.0–3.0 mm, extraction temperature – 25 °C, extractant – 70 % ethanol, raw material/extractant ratio – 1:12, infusion time – 12 hours, multiplicity of extractions – 3, followed by combining the extracts, filtering from the raw material, settling for 10–12 hours, filtering from the sediment and removing the solvent according to with the help of a rotary vacuum-evaporator up to 24 g (humidity 7 %).

The resulting FTHAE is a dark brown viscous mass with a pronounced specific aroma [22].

Chemicals and Solvents. Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). Ethanol (96 %) was obtained from Vilniaus degtine (Vilnius, Lithuania). Anhydrous acetic acid (99.8 %) and hydrochloric acid (37 %) were purchased from Sigma-Aldrich (Buchs, Switzerland). The following reagents were used: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). In addition, the following standards were used: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), quercetin, chlorogenic acid, apigenin, 4-o-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, neochlorogenic acid, kaempferol-3-rutinoside, ellagic acid from Sigma-Aldrich (Buchs, Switzerland); santin from PlantMetaChem (Giesen, Germany). All the reagents and standards were of analytical grade.

The stock solutions of phenolic compounds were prepared in 96 % ethanol.

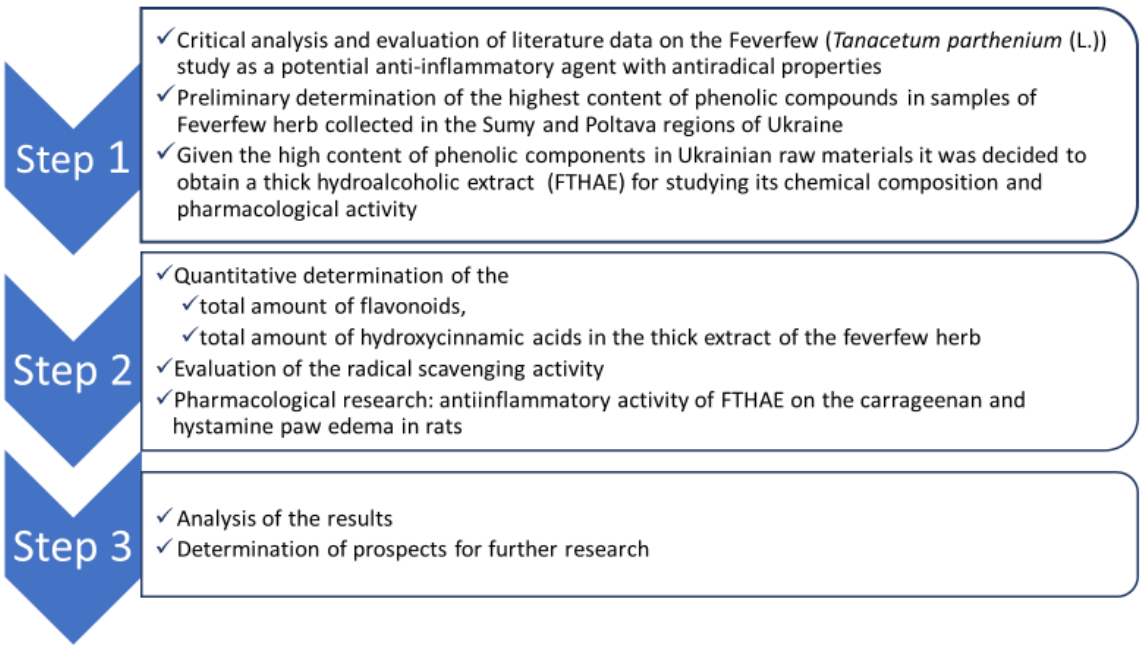

Fig. 1. Planning of the research

Quantitative determination of the total amount of flavonoids and the number of hydroxycinnamic acids in the thick extract of the feverfew herb was carried out by spectrophotometry using unified methods described [23, 24].

Quantitative determination of the sum of hydroxycinnamic acids in the sample was performed by a unified pharmacopoeial spectrophotometric method based on the determination of hydroxycinnamic acids after reaction with sodium nitrite P and sodium molybdate following the requirements of NPhU 2.0 monograph 2.2.25 [23].

Quantitative determination of the content of the number of flavonoids in the sample was performed by a unified pharmacopoeial spectrophotometric method based on the determination of flavonoids after reaction with a mixture of boric acid P and oxalic acid P in a mixture of formic acid P and acetic acid P [25, 26].

The radical scavenging activity was evaluated by the scavenging of ABTSA (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation. To determine the antiradical activity of the feverfew herb, a standard calibration curve obtained using a standard sample of trolox was constructed. ABTS radical cation scavenging activity of extracts was obtained from the regression equation: y=0.0002x + 0.0181 (R=0.9976) and expressed as antioxidant Trolox equivalents (TE) per gram of material [26].

HPLC analysis was performed using a "Waters e2695 Alliance system" (Waters, Milford, MA, USA) with a photodiode array detector "Waters 2998" according to the HPLC–PDA method for phenolic compounds. Briefly, the "ACE" (ACT, UK) column (C18, 150 mm × 4.6 mm, particle size 3 μm) column was used. The gradient consisted of eluent A (0.05 % trifluoroacetic acid) and B (acetonitrile) and followed: 0–5 min – 12 % B, 5–50 min – 12–30 % B, 50–51 min – 30–90 % B, 51–56 min – 90 % B, and 57 min – 12 % B with the flow rate – 0.5 mL/min and injection volume – 10μL. In addition, the analyte, reference compound retention time, and UV absorption spectra were used for peak identification [27].

Pharmacological research. Male non-linear white laboratory rats weighing 190-220 g were used. Experiments were conducted following the "Directive 2010/63/ EU of the European Parliament of the Council of September 22 2010, on the protection of animals used for scientific purposes". The draft research plan was approved by the bioethics commission of the National University of Pharmacy (protocol No. 6, dated June 25, 2021).

The rats were housed in standard polypropylene cages and kept at 20–26 °C and 50 % humidity in a well-ventilated room with a 12 h light/dark cycle with free access to food and water.

A freshly prepared aqueous suspension of FTHAE was stabilized with Tween-80 and a freshly prepared aqueous. Diclofenac sodium – NSAID, COX-1 and COX-2 inhibitor (50 mg tablets produced by pharmaceutical plant "Chervona Zirka") and quercetin (2 g granules produced by PJSC SIC "Borshchahivskiy CPP") – lipoxygenase inhibitor, they are used as comparison drugs in the models of carrageenan and histamine oedema. The antihistamine loratadine (tab. 10 mg produced by PJSC "KMP") was also used in the model of histamine oedema. All comparison drugs except quercetin were stabilized with Tween-80 and a freshly prepared aqueous. Quercetin was administered in the form of a jelly formed after contact with water at a temperature of 45–50 °C due to the content of pectin in the composition of the drug.

Inflammation caused by carrageenan. Animals were divided into groups of 6 rats: 1 – control pathology (animals with carrageenan oedema, which received distilled water); 2 – animals with carrageenan oedema, which received FTHAE at a dose of 50 mg/kg; 3 – animals with carrageenan oedema, which received a comparison drug diclofenac sodium in its effective dose of 8 mg/kg [28], 4 – animals with carrageenan oedema, which received a comparison drug quercetin at a dose of 11 mg/kg. FTHAE and comparison drugs were administered intragastrically in the treatment-and-prophylactic mode 1 time per day for 5 days and 6 days 1 hour before carrageenan injection (1 % solution was injected subplantarly into the hind foot of a rat in a volume of 0.1 ml) [25]. The development of oedema was observed in the dynamics after 30 min, 1; 1.5; 2; 4 and 24 hours, for which the volume of paws in cm^3 was measured using a Panlab V29/10/2014 plethysmometer (Spain).

Anti-inflammatory activity (AIA) was evaluated as the ability to reduce the amount of oedema of the affected limb compared with that in the control pathology by the formula [28]:

$$AIA=(V_{CP} – V_{EG}) / V_{CP}×100, (\%)$$

where AIA – anti-inflammatory activity, %; V_{CP} (cm^3) – the volume of the foot in animals from the group of control pathology; V_{EG} (cm^3) – the volume of the foot in animals from the experimental group.

Inflammation caused by histamine. Animals were divided into groups of 6 rats: 1 – control pathology (animals with histamine oedema (HE), which received distilled water); 2 – animals with HE who received FTHAE at a dose of 50 mg/kg; 3 – animals with HE who received the comparison drug diclofenac sodium at a dose of 8 mg/kg, 4 – animals with HE who received the comparison drug quercetin at a dose of 11 mg/kg, 5 – animals with HE who received the comparison drug loratadine at a dose 1 mg/kg [28]. The test extract and reference drugs were administered in treatment-and-prophylactic mode once a day for 5 days and 6 days 1 hour before histamine injection (0.1 % solution was injected subplantarly into the hind foot of rats in a volume of 0.1 ml) [28]. The development of oedema was observed in dynamics after 30 min, 1 and 1.5 h, for which the volume of paws in cm^3 was measured using a plethysmometer Panlab V29/10/2014 (Spain). Anti-inflammatory activity was evaluated in the same way as in the model of carrageenan oedema.

Statistical analysis was performed using SPSS 26.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Office Excel 365 (Microsoft, Redmond, WA, USA). All measurements were made in triplicate, and results were expressed

as mean ± standard deviation (SD). Linear regression analysis was performed to calculate the concentration-response relationship of each investigated compound by ABTS assay. Correlations were tested by using the Pearson correlation test. One-way analysis of variance was performed by ANOVA test. Significant differences between means were determined by Tukey HSD multiple comparison test. The p-values less than 0.05 were considered statistically significant. Statistical processing of the obtained pharmacological results using the program "Statistica 8.0" was performed. The non-parametric Mann–Whitney U-test was used, and the level of significance $P < 0.05$ was adopted [29].

3. Research results

Obtaining a thick hydroalcoholic extract from the feverfew herb (70 %) (FTHAE): 100 g of air-dried raw materials – feverfew herb crushed to the size of particles, which passed through a sieve with a hole diameter of 2000–3000, was loaded into the extractor, poured 400 ml of 70 % ethanol. The raw material was left to infuse at room temperature (25 °C) for 3 hours. In the end, the extract was drained, and the raw material was extracted twice more under the same conditions with new portions of the extractant with a total use of 1200 ml (1:12). The extracts were combined, the raw materials were squeezed, and the extract was also added to the total extract. The combined extracts were allowed to stand for 10–12 h to remove fine particles of raw materials and possible macromolecular compounds, filtered from the precipitate and concentrated mainly by rotary evaporation at 55 °C and reduced pressure to a soft consistency. Received 24 g of a thick extract. The yield of the finished product was 24 % by weight of air-dry raw materials. The finished product was obtained in the form of a dark brown substance with a specific odour.

The quantitative content of the total amount of flavonoids and hydroxycinnamic acids in the thick extract of the feverfew herb was calculated in terms of hyperoside and chlorogenic acid, respectively. Therefore, the quantitative content of flavonoids was 5.16 ± 0.03 %, and the quantitative content of hydroxycinnamic acids was 13.92 ± 0.02 %.

According to the study results, the phenolic profile of FTHAE by HPLC 12 compounds was detected, and their quantitative content was determined. The total amount of identified compounds were 72432.09 μg/g. Furthermore, according to the results of the study of the thick extract of the feverfew herb, Ellagic acid, 5 flavonoids (Quercetin, Kaempferol-3-rutinoside, Apigenin, Isoquercitrine, Rutin) and 6 hydroxycinnamic acids (Chlorogenic acid, 4-o-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid та Neochlorogenic acid) were identified. The results of the study are presented in Table 1.

The results of the study indicate that the total content of hydroxycinnamic acid is much higher than the content of flavonoids in the extract of the feverfew herb. Furthermore, the obtained results confirm our previous studies of the identification of phenolic compounds in the obtained extract by TLC, where the most intense zones were the zones of hydroxycinnamic acids [30].

Table 1

Quantitative composition (μg/g of DW) of identified phenolic compounds in the thick extract of the feverfew herb (FTHAE)

Compounds	Quantitative composition (μg/g of DW) in the FTHAE
Apigenin	2021.60 ± 43.55
Quercetin	579.62 ± 5.74
Chlorogenic acid	9593.99 ± 32.95
4-o-caffeoyl-quinic acid	2072.92 ± 4.60
3,4-dicaffeoyl-quinic acid	27304.75 ± 113.37
3,5-dicaffeoyl-quinic acid	9341.57 ± 53.29
4,5-dicaffeoyl-quinic acid	14631.30 ± 116.28
Neochlorogenic acid	2545.30 ± 6.54
Kaempferol-3-rutinoside	1193.07 ± 4.60
Ellagic acid	739.10 ± 1.70
Rutin	1901.08 ± 54.94
Isoquercitrine	507.79 ± 19.85
Total of all quantitated compounds	72432.09

Note: values are means±SD (n=3)

As a result of determining the antiradical activity of the thick hydroalcoholic extract from the feverfew herb by spectrophotometry, the value of free radical binding was 620.19 ± 4.53 μmol/g. In addition, individual substances of phenolic nature with antiradical properties were determined by HPLC (Fig. 2).

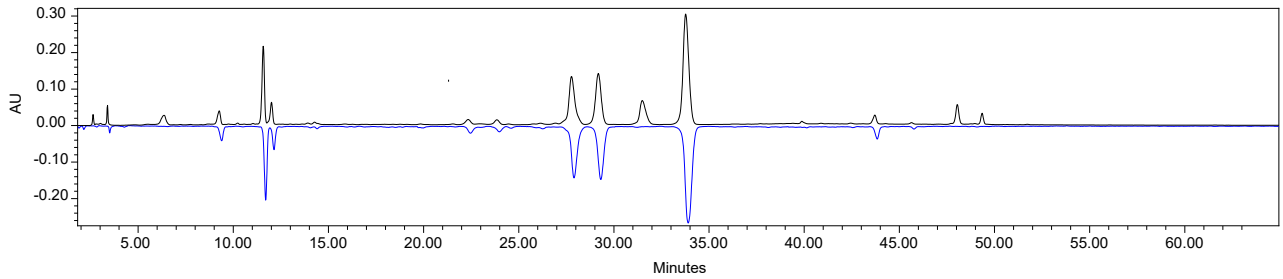

Fig. 2. Chromatogram of FTHAE phenolic compounds having antiradical properties

According to the results of the study, 11 compounds with antiradical activity were found in the feverfew thick hydroalcoholic extract, of which chlorogenic acid and rutin were identified. The total amount of Trolox equivalent of the antiradical capacity of the test extract was 27285.52 μg/g. The largest contribution to total antiradical activity was made by unidentified compounds with a retention time of 33.9 min (about 39 % of all identified compounds), 27.90 min (18.10 %) and 29.31 min (18.08 %), more likely than all, which are derivatives of caffeic acid. The chlorogenic acid content was 11.43 % among all identified compounds and rutin – 1.75 %, respectively.

Pharmacological Activity

On carrageenan paw oedema in rats, the tissue volume increased and reached its maximum by 3 hours (Table 2). In the first hours after the introduction of carrageenan increases the permeability of blood vessels – as a result of the action of biogenic amines: histamine and serotonin [31], in the second hour – kinins due to activation of the kallikrein-kinin system [32]. The latter promotes the local release of hydrolytic enzymes of lysosomes, which stimulate the formation of prostaglandins (PG), which mediate the late phase of inflammation, which develops after 3 hours. Nitric oxide is also released in the third hour [33]. PGE2 and nitric oxide are formed by the induction of cyclooxygenase (COX2) and inducible NO synthase, respectively. PGE2, synergistically with histamine and bradykinin, causes increased inflammation, oedema, exudate, erythema, redness, pain and fever. Some scientists distinguish only two phases of carrageenan edema [31, 34]: the first is caused by histamine, serotonin and bradykinin, while the second (3–5 h) is mediated by PG. Quite a high swelling of the paw 24 hours after the introduction of carrageenan due to the peak concentration of PGE2, which is observed exactly 12–24 hours after manipulation.

Prophylactic administration of FTHAE, quercetin and diclofenac sodium to animals prevented the development of oedema caused by carrageenan. In the early stages under the influence of FTHAE, the maximum effect of suppression of oedema was 71.0–73.2 %, while remaining consistently high and at the peak of inflammation (3 hours after administration of carrageenan) – 78.3 %. (Table 2) and was practically not inferior to NSAIDs of diclofenac sodium, the anti-inflammatory effect of which was – 80.9 %. The anti-inflammatory activity of the studied extract had the same dynamics as in the comparison drug quercetin (Table 2).

In the model of histamine oedema, the anti-inflammatory effect of FTHAE was 57.8; 51.8; and 49.1 % for 30 minutes, 1 and 1.5 hours of oedema, respectively (Table 3).

Table 2

Anti-inflammatory activity of the studied agents on the model of carrageenan oedema (Median (Q_{25}; Q_{75}))

Experimental conditions	30 min		1 h		2 h		3 h		4 h	
	V, cm^3	AIA, %	V, cm^3	AIA, %	V, cm^3	AIA, %	Volume, cm^3	AIA, %	V, cm^3	AIA, %
Control pathology (carrageenan edema, CE)	0.38 (0.35; 0.42)	–	0.60 (0.55; 0.61)	–	0.97 (0,74; 1,15)	–	1.57 (1.49; 1.75)	–	0.97 (0.84; 1.46)	–
FTHAE, 50 mg/kg	0.11* (0.10; 0.13)	71.0	0.20* (0.10; 0.34)	66.7	0.26* (0.16; 0.33)	73.2	0.34* (0.25; 0.42)	78.3	0.86 (0.79; 0.94)	11.3
Sodium diclofenac, 8 mg/kg	0.19* (0.15; 0.27)	50.0	0.31* (0.17; 0.42)	48.3	0.22* (0.17; 0.29)	77.3	0.30* (0.27; 0.47)	80.9	0.47* (0.29; 0.56)	51.5
Quercetin, 11 mg/kg	0.12* (0.09; 0.14)	68,4	0,20* (0.15; 0.28)	66.7	0.24* (0.20; 0.35)	75.3	0.38* (0.14; 0.90)	75.8	0.83 (0.40; 1.03)	14.4

*Note: V, cm3 – the amount of foot swelling; AIA – anti-inflammatory activity (%); * – reliable in relation to control pathology, p<0,05.*

Table 3

Anti-inflammatory activity of the studied agents in a model of histamine oedema (Median (Q_{25}; Q_{75}))

Experimental conditions	30 min		1 h		1.5 h	
	V, cm^3	AIA, %	V, cm^3	AIA, %	V, cm^3	AIA, %
Control pathology (histamine edema, HE)	1.09 (1.07; 1.18)	–	0.80 (0.66; 0.81)	–	0.57 (0.49; 0.61)	–
Sodium diclofenac, 8 mg/kg	0.50* (0.50; 0.51)	51.4	0.28* (0.24; 0.35)	65.4	0.19* (0.17; 0.23)	66.7
Quercetin, 11 mg/kg	0.39* (0.37; 0.49)	64.2	0,40* (0.32; 0.48)	50.6	0.20* (0.15; 0.25)	64.9
Loratadine, 1 mg/kg	0.39* (0.31; 0.46)	64.2	0.37* (0.36; 0.40)	54.3	0.17* (0.13; 0.24)	70.2
FTHAE, 50 mg/kg	0.46* (0.33; 0.48)	57.8	0.39* (0.34; 0.45)	51.8	0.29*/** (0.23; 0.36)	49.1

*Note: V, cm3 – the amount of swelling of the foot; AIA – anti-inflammatory activity (%); * – reliable in relation to control pathology, p < 0.05; ** – reliable in relation to sodium diclofenac, p < 0.05.*

The severity of anti-edematous activity during the first hour of FTHAE is not inferior to the drug comparing diclofenac sodium, quercetin and H1-histamine blocker loratadine.

4. Discussion of research result

According to the results of the study among hydroxycinnamic acids, 3,4-dicaffeoylquinic acid (27304.75 ± 113.37 μg/g), 4,5-dicaffeoylquinic acid (14631.30 ± 116.28 μg/g), chlorogenic acid (9593.99± ± 32.95 μg/g) were the dominant components in the thick hydroalcoholic extract from the feverfew herb. Thus, 3,4-dicaffeoylquinic acid accounts for 37.8 % of all substances, a significant advantage of the obtained extract. It is known that 3,4-dicaffeoylquinic acid has pronounced cytoprotective and antioxidant activity [35]. Compared with the data obtained by other researchers, the content of 3,4-dicaffeoylquinic acid is significantly higher in the obtained extract and amounted to 56.12 μg/ml and than in the obtained extract from the feverfew herb collected in Brazil – 1.85 μg/ml [36]. Regarding percentage, 4.5 dicaffeoylquinic acids accounted for almost 20.2 % of all identified compounds. 4,5-dicaffeoylquinic acid was present in FTHAE in 30.00 μg/ml, which was almost 2 times more than in the alcohol extract from raw material cultivated in Brazil [36]. However, the content of 3,5-dicaffeoylquinic acid was 2.4 times lower in our extract (19.00 μg/ml) compared to the data of other researchers – 44.72 μg/ml [36].

Chlorogenic acid as a secondary metabolite is formed by the esterification of one or more trans-cinnamic acid derivatives with quinic acid. This compound has several pharmacological effects, mainly antioxidant, anti-inflammatory [36, 37], and antiviral [38, 39]. The content of chlorogenic acid (5-caffeoylquinic acid) was significantly higher according to the results of our study, namely 20.00 μg/ml, in contrast to the extract from raw material collected in Brazil, where its content was 7.91 μg/ml [38]. According to the data of the analyzed extract from raw material collected in China, the content of chlorogenic acid was 12 % of all identified compounds. However, our results showed a higher percentage – 13 %.

Among flavonoids, apigenin accumulates in the most quantity, and its content was 2.8 % of all calculated substances.

The established composition of biologically active substances (BAS) from FTHAE allowed us to predict the anti-inflammatory activity of this extract, which was experimentally established in histamine and carrageenan oedema models. Comparison of the described dynamics of the release of various mediators of inflammation on the model of carrageenan edema [28] with the severity of the anti-edematous activity of the studied agents suggests their mechanism of action. In this oedema model, the COX1 and COX2 inhibitor diclofenac sodium shows the greatest activity at the third hour of oedema (prostaglandin phase). The flavonoid quercetin, whose anti-inflammatory mechanism is multi-vector (scavenging free radicals, stabilization of cell membranes) showed a pronounced anti-edematous effect during the first three hours with depression for 4 hours. FTHAE showed anti-inflammatory activity, which in dynamics was similar to quercetin, which indicates the involvement of various mechanisms in the anti-inflammatory action of FTHAE.

Oxidative stress has a main role in the development of many pathological processes, in particular, inflammatory [41]. One of the components of the anti-inflammatory action of FTHAE is the antioxidant effect of BAS – their ability to capture free radicals and inhibit free radical processes. To a greater extent, the antioxidant activity of FTHAE is provided by hydroxycinnamic acids (3,4-dicaffeoyl-quinic acid; 4,5-dicaffeoyl-quinic acid; 3,5-dicaffeoyl-quinic acid [42]; chlorogenic acid) [43, 44] and flavonoids (mainly rutin) [43], which were identified in the extract. 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid inhibit PGE2 release and IL-6 secretion [46], in combination with chlorogenic acid, which inhibits inducible NO synthase, NO synthesis and proinflammatory cytokines (IL-1β and TNF-α), NF-κB, IL-6 and COX1 expression [36, 37], which provide anti-inflammatory activity of the extract. The anti-inflammatory effect is also characteristic of neochlorogenic acid [47], which is a significant part of FTHAE. The pronounced anti-edematous effect of FTHAE on the model of histamine-induced inflammation, in which the extract was not inferior to the comparison drugs diclofenac sodium, quercetin and H1-histamine blocker loratadine, is probably due to the above mechanisms of action.

Thus, the results of the study showed that the thick hydroalcoholic extract from the feverfew herb (*Tanacetum parthenium* (L.) Schultz Bip.) has a pronounced anti-inflammatory effect with many mechanisms, which determines its effectiveness in the model of arthritis [48].

Study limitations. The effect of the extract on the level of inflammatory mediators was not investigated in the experiment due to financial difficulties.

Prospects for further research. In the future, it is planned to study the level of inflammatory mediators.

5. Conclusions

1. The phenolic profile of the thick hydroalcoholic extract from the feverfew herb collected in Ukraine was studied. The content of the sum of hydroxycinnamic acids in the obtained extract was determined by spectrophotometry, which was 13.92 ± 0.02 %, and the content of the sum of flavonoids was 5.16 ± 0.03 %.

2. The content of 12 compounds with a total amount of 72432.09 μg/g was identified and determined by HPLC. The dominant compounds were hydroxycinnamic acids, namely 3,4-dicaffeoylquinic acid (27304.75 ± 113.37 μg/g), 4,5-dicaffeoylquinic acid (14631.30±116.28 μg/g) and chlorogenic acid (9593.99 ± 32.95 μg/g). The antiradical activity of the thick hydroalcoholic extract from the feverfew herb was 620.19 ± 4.53 μmol/g.

3. Due to the HPLC method, 12 compounds were determined that caused antiradical activity, among which chlorogenic acid and rutin were identified. In addition, the anti-inflammatory activity of the thick hydroalcoholic extract from the feverfew herb on the models of carrageenan and histamine oedema was studied.

4. The studied extract has a pronounced anti-inflammatory effect, which is due to the antioxidant properties of the extract and its inhibitory effect on inflammatory mediators. Therefore, this extract is of interest for further preclinical and clinical studies to create new effective herbal medicines for the prevention and pharmaco-correction of inflammatory processes.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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