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In-Vitro Assessment of The Antidiabetic and Anti-Inflammatory Potential of The Artemisia absinthium, Artemisia vulgaris and Trigonella foenum-graecum Extracts Processed by Membrane Technologies

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In-Vitro Assessment of The Antidiabetic and Anti-Inflammatory Potential of The *Artemisia absinthium, Artemisia vulgaris* and *Trigonella foenum-graecum* Extracts Processed by Membrane Technologies

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Abstract: In recent years there has been increasing interest in the discovery of new natural herbal remedies for the treatment of common conditions such as diabetes and inflammatory diseases. In this context, this paper analyzed the antidiabetic and anti-inflammatory potential of two plants of the genus Artemisia: *Artemisia absinthium* (wormwood) and *Artemisia vulgaris* (mugwort) and *Trigonella foenum-graecum* (fenugreek) plants less studied from this point of view. Thus, hydroalcoholic extracts (50% EtOH v/v, 10% mass) were made, which were processed by membrane technologies, micro- and ultrafiltration, in order to concentrate the biologically active principles. The extracts were then analyzed in terms of content in active principles of interest (polyphenols, flavones). The antidiabetic activity of the extracts was analyzed by testing their ability to inhibit α-amylase and α-glucosidase and the anti-inflammatory activity by testing the ability to inhibit hyaluronidase (HYA) and lipoxygenase (LOX). Thus, the *T. foenum-graecum* extracts showed a high inhibitory activity on α-amylase 3.22 ± 0.3 µg / mL (compared to the standard used - IC_{50} acarbose = 3.5 µg / mL) and a high inhibitory activity on LOX: 19.69 ± 0.52 µg/mL (compared to all standards used). The concentrated extract of *Artemisia vulgaris* showed increased activity of inhibition of α-amylase - IC_{50} = 8.57 ± 2.31 µg/mL and moderate activity of inhibition of HYA - IC_{50} = 17.18 µg/mL compared to ibuprofen, the standard used - IC_{50} = 5.73 µg/mL. The concentrated extract of *Artemisia absinthium* showed a pronounced activity of inhibition of LOX - IC_{50} = 19.71 µg / mL, compared to the standard - rutin - IC_{50} = 22.34 µg/mL).

Keywords: antidiabetic; anti-inflammatory activity; *Artemisia absinthium; Artemisia vulgaris*; *Trigonella foenum-graecum*; membrane technologies; α-amylase; α-glucosidase; hyaluronidase; lipoxygenase

1. Introduction

Known and used for centuries, medicinal plants remain potential sources for finding new remedies useful in treating or ameliorating various diseases including diabetes and inflammatory diseases.

Diabetes is a metabolic disorder that generates severe dysfunctions such as neuropathy, vasculopathy, dyslipidemia, retinopathy, and cardiovascular disease [1]. The incidence of this condition is constantly increasing, from 400 million people in 2016 it is estimated that it will reach 642 million people with type II diabetes in 2040 [2]. Antidiabetic drugs such as acarbose, miglitol, and voglibose reduce the degradation and absorption of sugars by inhibiting the digestive enzymes - α-amylase and α-glucosidase, involved in these processes. However, these drugs also have side effects and that is why alternatives are sought from natural sources [3].

The drugs used to treat inflammation and pain are nonsteroidal drugs that generally have numerous side effects such as ulcers and hemorrhages [4,5]. Their long-term use even leads to cardiovascular, metabolic, endocrine and ophthalmological damage [5].

Medicinal plants have been used in folk medicine to treat many inflammatory diseases and remain important sources of new anti-inflammatory and antioxidant agents [6,7]. Inhibition of enzymes involved in the inflammatory process such as lipoxygenase and hyaluronidase is the basis for finding new treatments for allergies and inflammatory diseases [8,9]. Oxidative stress generated by reactive oxygen species (ROS) has been associated with pathological inflammatory processes and DNA distortions followed by carcinogenesis and tumors [10].

Lipoxygenase (LOX) is involved in inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, allergic rhinitis, atherosclerosis, and certain types of cancer. During LOX activity, peroxy radicals are formed in the reaction medium that can function as sources of free radicals. Thus, antioxidants that have free radical scavenging activity can also act as LOX inhibitors [11].

Hyaluronidase acts on hyaluronic acid which is an important part of the extracellular matrix and is involved in many physiological processes such as embryogenesis, wound healing, and cell migration [7,12]. Studies have led to the discovery in plants of hyaluronidase inhibitors (tannins, cucumbers, flavonoids) that can be used as anti-inflammatory and anti-allergic agents, as antitumor agents, in the treatment of bacterial infections and adjuvant in the treatment of arthritis [13,14].

Membrane technologies - micro- and ultrafiltration - are cheap and effective alternatives to traditional technologies for concentrating biologically active compounds. These processes feature low operating and maintenance costs, easy operation at moderate temperatures and pressure, high permittivity, and selective separations. [15,16]. These technologies can be successfully used for the purification and concentration of plant extracts while maintaining their functional and nutritional properties [17,18].

Artemisia vulgaris and *Artemisia absinthium* are two plants of the Asteraceae family (Compositae) used in traditional medicine due to their numerous therapeutic properties.

Artemisia vulgaris (known as mugwort) is a shrub of temperate zones in Europe, Asia, North Africa, and North America [19]. The plant has been used in folk medicine for the treatment of gastrointestinal and gynecological diseases, alleviation of hypertension and disorders of the nervous system [20,21] as well as for culinary purposes. Studies have revealed various pharmacological properties of *A. vulgaris*, such as anti-inflammatory, antioxidant, antitumor, and immunomodulatory activities [22,23].

Artemisia absinthium L. - also known as wormwood - has been known for centuries and used in folk medicine for gastrointestinal and urinary disorders, fever, and helminthiases [24,25]. *A. absinthium* has been shown to be effective as an antiparasitic and a digestive [25], as well as for reducing symptoms of leukemia, sclerosis, diabetes, malaria, and even some types of cancer in recent years [26].

Trigonella foenum-graecum (also known as fenugreek) is an annual herb from the Fabaceae family known for its medicinal and culinary properties since ancient times. Fenugreek is grown and used as a spice in many countries from Asia, Europe, and Africa and is considered to have a high nutraceutical value [27]. In folk medicine, this plant is used especially for immunity stimulation and for digestive and reproductive disorders [28]. Seed powder lowers blood sugar, and improves symptoms in patients with type 2 diabetes [29]. The seeds and leaves of this herb are rich in flavonoids, alkaloids, and saponins which confer medicinal properties [30]. While fenugreek seeds of fenugreek have been widely investigated for the treatment of inflammation, cancer, and diabetes, little is known about their leaves.

The present paper analyzed the antidiabetic and anti-inflammatory activity of the plants: *Artemisia vulgaris*, *A. absinthium*, and *Trigonella foenum-graecum* by testing the ability to inhibit α-amylase, α-glucosidase activity, respectively, hyaluronidase (HYA) and lipoxygenase (LOX) activity.

2. Results and Discussion

2.1. Phytochemical Analysis and Antioxidant Capacity

The content in total polyphenols and flavones of *Artemisia absinthium*, *Artemisia vulgaris*, and *Trigonella foenum-graecum* extracts as well as the antioxidant activity of the extracts were analyzed by

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spectrophotometric and chromatographic methods presented in the previous chapter and the results are presented in the Tables 1–3.

Table 1. Phytochemical analysis and antioxidant capacity of extracts.

Sample		Polyphenols Content (mg CA/mL)	Flavones Content (mg RE/mL)	Reducing Power %	% DPPH Inhibition	TEAC_ABTS mg/mL
Artemisia absinthium	MF	3232.5±140.32	389.08± 9.56	50.7±1.36	36.06±1.12	204.09±6.36
	concentrate	4777.5±125.52	501.81±26.96	73.5 ± 2.36	65.89±2.31	483.24±3.86
Artemisia vulgaris	MF	7877.5±260.96	505.18±30.12	52.9 ± 1.32	63.64±2.56	316.54±9.56
	concentrate	11440.21±49.96	1020.12±45.12	93.11 ± 3.2	77.57±2.45	541.57±15.37
Trigonella foenum-graecum	MF	1094.37±62.4	395.86±11.6	63.8±2.4	75.43±5.82	420.75± 17.35
	concentrate	2636.87±52.7	777.6 ±2.7	***97.11±5.2***	***82.18±3.7***	***591.23± 22.38***
Ascorbic acid				34.51±1.29	96.97±3.62	

Values represent mean standard deviation of triplicate experiments.

Table 2. Polyphenolic compounds content of the extracts –analysis by HPLC-PDA-MS.

Compound	*A. absinthium* MF µg/mL	*A. absinthium* Concentrate µg/mL	*A. vulgaris* MF µg/mL	*A. vulgaris* Concentrate µg/mL	*T. foenum-graecum* MF µg/mL	*T. foenum-graecum* Concentrate µg/mL
Chlorogenic acid	***259.42***	***297.92***	***301.02***	***350.67***	11.02	68.17
Caffeic acid	2.68	3.26	2.44	3.06	-	-
Rosmarinic acid	***21.10***	***22.65***	3.36	3.89	-	-
Coumaric acid	-	-	-	-	7.29	7.74
Umbelifferone	6.95	7.93	7.12	8.88	-	-
Quercetol	0.62	0.73	0.81	0.91	-	-
Luteolin	4.75	5.57	***12.39***	***14.49***	3.48	3.97
Apigenin	0.72	1.03	2.50	2.66	-	-
Rutin	***12.59***	***15.66***	***18.39***	***19.65***	u.d.l	84.92
Ellagic acid	4.72	5.17	-	-	-	-
Isoquercitrin	1.54	1.96	3.73	3.74	7.30	20.08
Genistin	-	-	-	-	**1286.19**	**2032.98**

u.d.l. – under detection limit.

The *Artemisia vulgaris* extracts have been shown to be richer in polyphenolic compounds with almost double the values of *Artemisia absinthium*. In concentrated extracts, a significant increase in the amount of active ingredients is obtained, especially in *Artemisia vulgaris* concentrate from 7877.5 ± 260.96 µg CA/mL to 11440.21 ± 49.96 µg CA/mL). The amount of flavones was also higher for *Artemisia vulgaris* extracts compared to *Artemisia absinthium*, obtaining even a doubling of the flavones amount by ultrafiltration: from 505.18 ± 30.12 µg QE / mL initially to 1020.12 ± 45.12µg QE / mL in concentrate, thus noting the efficiency of the ultrafiltration process. The analysis of the *Trigonella foenum-graecum* extract by HPLC–MS technique revealed the presence of polyphenolic compounds and showed a high amount of genistin.

The results regarding the antioxidant activity of the obtained and processed *Artemisia* extracts are presented in Table 1. It is unanimously accepted that the antioxidant activity must be verified by several alternative methods because only one method is not significant [31,32}. By all 3 methods used similar results were obtained, the extracts of *T. foenum-graecum* showed the highest antioxidant activity followed by *A. vulgaris and A. absinthium*, the antiradical capacity of the concentrated extracts was higher than microfiltrate. Data from the literature showed that *T. foenum-graecum* seeds extracts were a good antioxidant agent and this was attributed to the total phenolic compounds content [32,22].

There is a direct correlation between the amount of polyphenols and flavones and antioxidant activity, which is consistent with other studies [33,34]. The high content of polyphenols could explain

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the high antioxidant activity of *Artemisia* extracts [35]. Flavonoids also have antioxidant properties as shown by other studies on other *Artemisia* species [36]. In the case of *Trigonella foenum-graecum* extracts the HPLC analysis revealed the presence of a significant amount of genistin, which could explain the high antioxidant capacity.

The antioxidant activity is mainly attributed to phenolic compounds, as they are able to protect living systems from oxidative damage caused by free radicals [37]. Scientific studies have shown that through their antiradical capacity, antioxidants can prevent severe diseases such as cardiovascular diseases, and can also have anti-inflammatory and anti-carcinogenic effects [38].

Previous studies have shown that *Artemisia vulgaris* has increased antiradical activity compared to other species of the genus *Artemisia* [39] and that leaf extracts can be used as an effective antioxidant [40]. Regarding *Artemisia absinthium*, some studies have shown moderate antiradical activity [41] others have shown a high antiradical activity correlated with a large amount of polyphenols and flavonoids [42].

The quantitative analysis determined an increased amount of chlorogenic acid in the two species of *Artemisia*, significantly higher in *Artemisia vulgaris*. In *Artemisia vulgaris*, a higher amount of luteolin and isoquercitrin was also determined. The results are comparable with other data in the literature [39].

Rutin was determined in large quantity in both plants. Rutin has a high antioxidant capacity and has been reported to function for the treatment of several diseases associated with metabolic syndrome, including diabetes and as a treatment for neurodegenerative diseases associated with oxidative stress [43]. Numerous scientific reports claim that regular consumption of flavonoids or polyphenols decreases the risk of cardiovascular disease, diabetes, and cancer [44]. Rosmarinic acid has been identified in greater amount in *Artemisia absinthium*. In most extracts, the concentration of active principles is observed, which shows the efficiency of the ultrafiltration process.

2.2. α-Amylase and α-Glucosidase Inhibition Activity

Several pharmacological approaches are used to control diabetes. One of these refers to the control of postprandial hyperglycemia by inhibiting the absorption of glucose in the intestine [45]. This is achieved by using oral agents that interfere with glucose absorption, respectively inhibitors of pancreatic a-glucosidase and a-amylase.

The plants used in this study showed high inhibitory activity on α-amylase *T. foenum-graecum* concentrated extract, especially (IC_{50} = 3.22±0.3 µg/mL), higher than the acarbose used as a standard, followed by *A. vulgaris* concentrated extract (IC_{50} = 8.57±2.31 µg/mL). The inhibitory activity of the concentrated extracts is significantly higher than that of the microfiltrates, thereby highlighting the efficiency of the ultrafiltration process.

The *T. foenum-graecum* showed also a high inhibitory activity on a-glucosidase (IC_{50} = 11.14 ± 0.9 µg/mL) and the concentrated extract of *A. absinthium* showed moderate inhibitory activity on a-glucosidase (IC_{50} = 31.90 ± 1.89 µg/mL). Concentrated extracts showed higher inhibitory activity than microfiltrate. The results are presented in Table 3.

Table 3. In vitro inhibition of α-amylase and α-glucosidase activity of extracts.

Samples		Inhibition of α-Amylase IC_{50} (µg/mL)	Inhibition of α-Glucosidase IC_{50} (µg/mL)
A. absinthium extracts	MF	22.22 ± 0.89	45.16 ± 1.39
	concentrate	19.42 ± 0.53	***31.90 ± 1.89***
A. vulgaris extracts	MF	17 ± 0.98	96.04 ± 3.21
	concentrate	***8.57 ± 2.31***	77.13 ± 2.36
T. foenum-graecum extracts	MF	24.18 ± 1.4	28.19 ± 1.8
	concentrate	***3.22 ± 0.3***	***11.14 ± 0.9***
Rosmarinic acid		0.89 ± 0.06	0.186 ± 0.008
Chlorogenic acid		1.9 ± 0.07	0.59 ± 0.025
Acarbose		3.5 ± 0.18	5.9 ± 0.38

Values represent mean standard deviation of triplicate experiments.

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Other plants of the genus *Artemisia* such as *Artemisia campestris*, and *Artemisia herba-alba* Asso, are used in traditional medicine to treat diabetes in countries such as Algeria, Morocco, Pakistan, Mexico. The hypoglycemic action of *Artemisia absinthium* extracts by inhibiting α-glucosidase has been confirmed by other studies [46].

In vitro studies have reported the inhibitory activity of α-amylase activity by a 70% ethanolic extract of *Artemisia herba-alba*. This resulted in an 11% reduction in α-amylase activity, which may be a mechanism by which this extract can reduce blood glucose [47]. Studies performed on another species of *Artemisia* - *Artemisia indica* have shown the antihyperglycemic and antihyperlipidemic effect of some methanolic and chloroform extracts comparable to that of glibenclamide [48]. Antidiabetic activity has also been shown in other species of the genus *Artemisia* such as *A. amygdalina* [49], *A. pallens* [50].

The inhibitory effect of *Trigonella foenum-graecum* extracts on α-amylase and α-glucosidase has been scarcely described in the literature. The results obtained are in agreement with previous reports which proved the inhibitory effect of *T. foenum-graecum* on amylase activity [51].

2.3. Lipoxygenase (LOX) and Hyaluronidase (HYA) Inhibition Activity

Synthetic drugs - non-steroidal anti-inflammatory drugs, and anti-rheumatic drugs, reduce the inflammatory process but can also have a number of side effects. Herbal therapies could be beneficial because they contain several medically important chemical constituents, are readily available, the costs are low and the side effects negligible [52].

In the inflammatory process and in pathological conditions in general, enzymes such as lipoxygenase (LOX), cyclooxygenase (COX), and hyaluronidase (HYA) enzymes are activated leading to the synthesis of prostaglandins involved in the inflammatory process and allergic diseases [11].

Studies have shown that plant-derived bioactive compounds such as phenolic acids and flavonoids with antioxidant activity, also possess anti-hyaluronidase activities, showing positive correlations between the amount of these compounds and anti-hyaluronidase activity [7].

The extracts tested in this study showed accentuated anti-inflammatory activity by inhibiting LOX: *T. foenum-graecum* and *A. absinthium* concentrate extracts showed similar inhibitory activities, IC_{50} = 19.69 μg / mL, respectively IC_{50} = 19.71 μg / mL, even higher than the polyphenolic compound used as the reference standard (eg. rutin - IC_{50} = 22.34 μg/mL) (Table 4). The *Artemisia vulgaris* and *T. foenum-graecum* concentrated extracts showed moderate HYA inhibition activity: IC_{50} = 17.18 μg / mL IC_{50} = 17.57 μg / mL compared to ibuprofen, the reference standard used - IC_{50} = 5.73 μg/mL.

Table 4. In vitro inhibition of HYA and LOX activity of extracts.

Samples		Inhibition of HYA IC_{50} (μg/mL)	Inhibition of LOX IC_{50} (μg/mL)
Artemisia absinthium extracts	MF	78.06 ± 2.32	52.89 ± 2.12
	concentrate	34.71 ± 1.26	***19.71*** ± 0.79
Artemisia vulgaris extracts	MF	74.10± 2.63	182.4 ± 10.52
	concentrate	***17.18*** ± 1.19	112.75 ± 8.56
T. foenum-graecum extracts	MF	67.40 ± 2.65	31.07 ± 1.35
	concentrate	***17.57*** ± 1.23	***19.69*** ± 0.52
Ibuprofen		5.73 ± 0.21	-
Rosmarinic acid		-	30.30 ± 1.23
Chlorogenic acid		-	26.12 ± 1.35
Rutin		-	22.34 ± 1.89

Values represent mean standard deviation of triplicate experiments.

The anti-inflammatory activity of *Artemisia vulgaris* extracts has been attributed to the presence of flavonoids [53]. Previous studies have shown that *Artemisia vulgaris* extracts have an anti-inflammatory action by inhibiting cyclooxygenase [54].

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In vitro studies have shown the anti-inflammatory activity of Artemisia campestris extracts by inhibiting lipoxygenase [55]. This species is rich in bioactive compounds such as phenolic acids, flavonoids, and terpenoids, many of the plant's properties such as antioxidant, antidiabetic and anti-inflammatory are attributed to these compounds.

Another species of *Artemisia* - *Artemisia nilagirica*, commonly found in India, has anti-inflammatory potential, as well as antioxidant, antimicrobial, antifungal, and anticancer, properties associated with the presence of phytochemicals such as tannins, flavonoids, alkaloids, saponins, coumarins, steroids and phenols [56,57]. Studies have shown anti-inflammatory properties in other species of the genus *Artemisia*: *Artemisia maritima* L [58] *Artemisia sieversiana* Ehrh. [59].

3. Materials and Methods

3.1. Chemicals

All reagents used for analysis were of analytical purity and were purchased from Sigma Chemical Company (Sigma Aldrich, Germany), Roth (Carl Roth GmbH, Germany), and Fluka (Switzerland).

3.2. The Obtaining of Extracts

The plants were first dried, finely ground with a GRINDOMIX 200GM mill, then hydroalcoholic extracts *Artemisia vulgaris* and *Artemisia absinthium* were obtained in 50% EtOH (10% mass). Extracts were obtained by ultrasound-assisted solvent extraction (UAE) the room temperature for 1 hour, followed by filtration.

The extracts were processed by membrane methods: micro- and ultrafiltration through a KMS Laboratory Cell CF-1 laboratory facility purchased from Koch Membrane (Germany).

Microfiltration was performed by microfiltration membranes with the pore size of 0.45 μm and ultrafiltration by regenerated cellulose membranes with a cut-off of 3,000 Da; the concentration was achieved in the ratio 1: 3 and the pressure used was 6 bar.

3.3. The Bioactive Compounds Determination

Determination of the total polyphenols content was realized using Folin Ciocalteu's technique with slight modification [60]. Spectrophotometric measurements were realized at 760 nm. The experiments were performed in triplicates and the polyphenols concentration was calculated using an etalon curve of chlorogenic acid (CA).

Determination of the total flavonoid content was done using the aluminum chloride colorimetric assay [61]. The flavonoid content was calculated using the rutin calibration curve and was expressed in μg rutin equivalent (RE)/mL of extract.

3.3.1. HPLC Analysis

The chromatographic analysis was performed using a complete HPLC SHIMADZU system, a C18 Nucleosil 3.5, 4.6 × 50mm, Zorbax column. The system was coupled to an MS detector, LCMS-2010 detector (liquid chromatograph mass spectrometer), equipped with an ESI interface. The samples were filtrated before injection using Syringe Driven Filter Unit 0.2 μm (Macherey-Nagel).

All the other reagents, (acetonitrile, formic acid) were analytical pure or of chromatographic grade and were used after filtration. The ultra-pure water was obtained using a system for purification of water, Elix 3 (Millipore).

All standards used: chlorogenic acid, gallic acid, ellagic acid, caffeic acid, rosmarinic acid, coumaric acid, rutin, luteolin, quercetin, quercetin 3-β-D-glucoside, apigenin, umbelliferone, kaempferol, and genistin stock solutions, 1mg/mL, were prepared in ethanol Stock samples were stored at dark and -4°C between the experiments.

The used HPLC method for the analysis of polyphenolic compounds was previously published by Alecu, et al. [62].

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3.4. Antioxidant Assays

The antioxidant activity was measured using 3 methods, ascorbic acid was used as the control in all methods.

- The method was based on decreasing the maximum absorbance of ABTS to 731 nm in the presence of the antioxidant [63]; antioxidant activity was expressed in TEAC equivalents (Trolox Equivalent Antioxidant Capacity) using the formula

$$TEAC_{sample} = C_{Trolox} \cdot f \cdot \frac{A_{sample} - A_{blank}}{A_{Trolox} - A_{blank}} \quad (1)$$

where: A_{blank} = control absorbance; A_{Trolox} = Trolox absorbance. A_{sample} = sample absorbance; f =dilution factor; C_{Trolox} = Trolox concentration

- **DPPH radical scavenging activity**

The scavenging activity on the DPPH radical of samples was determined by measuring the decrease in the DPPH maximum absorbency at 517 nm after 10 min [64] and was calculated:

$$\text{radical scavenging activity (\%)} = [(A_B - A_A)/A_B] \times 100 \quad (2)$$

where: A_B = control absorbance and A_A = sample absorbance.

- **Reducing Power Activity** (Iron (III) to iron (II) reduction)

Reducing power was determined according to a previously described procedure [65]. The absorbance was measured spectrophotometrically at 700 nm and calculation was done using the formula:

$$\text{Reducing power (\%)} = [(A_A - A_B)/A_A] \times 100 \quad (3)$$

where: A_A = sample absorbance, A_B = control absorbance

3.5. Enzyme Inhibitory Activity Assay

3.5.1. Testing the Antidiabetic Capacity of the Extracts

- **α Amylase inhibition assay**

The α-amylase inhibition assay was realized by the Ranilla method slight modification [66]. So, 100 µL of extract were mixed with 250 µL α - amylase from hog pancreas (EC 3.2.1.1) (0.5 mg/mL in 0.02 M sodium phosphate buffer, pH 6.9 with 0.006 M NaCl) and were incubated at 37^0 C, 20 min; then 250 µL of the starch solution (1% in sodium phosphate buffer) were added, and the mixture was re-incubated for 30 min at 370; afterward, 500 µL dinitrosalycilic acid (DNS) was added and the mixture was been boiling for 5 min. Finally, 5 mL of distilled water was added to the reaction mixture. The absorbance measurement was realized at 540 mm using a UV-visible spectrophotometer (Jasco-V630) with acarbose as the positive control. The calculation of the results was realized using the formula:

$$\text{\% Amylase inhibition} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100 \quad (4)$$

The IC_{50} values were calculated by the linear regression analysis. Significant statistical differences were considered $p<0.05$.

- **α-Glucosidase inhibition assay**

The α-glucosidase inhibition assay was realized using the Queiroz et al. method slight modification [67]. Thus, 120 µL α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20) (0.5 U/mL) with 720 µL sodium phosphate buffer (0.1 M, pH 6.9) and 60 µL extract were mixed, then the mixture was incubated at 37°C for 15 min. After the pre-incubation, 120 µL p-nitrophenyl-α-D-glucopyranoside (5mM/L) solution was added and the reaction mixture was incubated at 37^0C for 15

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min. The absorbance measurement was realized at 405 nm using a UV-visible spectrophotometer with acarbose as the positive control. The calculation of the results was realized using the formula:

$$\% \text{ Glucosidase inhibition} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100 \quad (5)$$

The IC_{50} values were calculated by the linear regression analysis. Significant statistical differences were considered $p<0.05$.

3.5.2. Testing the Anti-Inflammatory Capacity of the Extracts

- **Hyaluronidase inhibition assay**

The hyaluronidase activity (Hyaluronidase EC 3.2.1.35 from bovine testicles – Sigma) manifested by the hydrolysis action of hyaluronic acid was tested by the modified Morgan-Elson method (1949) [68] which involves the measurement at 585 nm of the complex formed by enzymatic hydrolysis products by reaction with p-Dimethylaminobenzaldehyde.

Pipette 100 µl of enzyme solution (1 mg/ml) together with 50 µl of plant extract and preincubate for 30 minutes at 37°C to exert the action of inhibiting the centers of hyaluronidase activity. To the reaction mixture is added 100 µl of substrate solution (sodium salt of hyaluronic acid in the vitreous bovine humor - 2.5 mg /ml) is incubated for 60 minutes at 37 ° C - the time required to carry out the enzymatic reaction. The blank is prepared in the same way by replacing the vegetable extract with a buffer solution.

The reaction is stopped by maintaining for 3 minutes at 100°C and the reaction products are highlighted by staining with p-Dimethylaminobenzaldehyde and spectrophotometrically quantification at 585 nm of the pink complex; the calculation of the results was done according to the formula:

$$\% \text{ inhibition} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100 \quad (6)$$

The IC_{50} values were calculated by the linear regression analysis. Significant statistical differences were considered $p<0.05$.

- **Lipoxygenase inhibition assay**

Lipoxidase inhibition testing was performed according to a Sigma-Aldrich protocol [69]: 0.017% (v/v) linoleic acid substrate and a lipoxygenase solution (2200 units/mL) (EC 1.13.11.12); it was determined to increase the absorbance for approximately 5 minutes at 234 nm, using the maximum linear rate for both the sample and the blank, the calculation of the results being made according to the formula:

$$\% \text{ inhibition} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100 \quad (7)$$

The IC_{50} values were calculated by the linear regression analysis. Significant statistical differences were considered $p<0.05$.

4. Conclusions

The phytochemical screening, the antidiabetic and anti-inflammatory potential of *Artemisia vulgaris*, *Artemisia absinthium,* and *Trigonella foenum-graecum* concentrated hydroalcoholic extracts by inhibiting α-amylase and α-glucosidase, respectively lipoxygenase and hyaluronidase were analyzed.

The *T. foenum-graecum* extracts showed a higher genistin content and *Artemisia* species showed a higher polyphenols and flavones content. The T. *foenum-graecum* extracts showed high antioxidant activity by all methods of analysis.

The *T. foenum-graecum* extracts showed a high inhibitory activity on all enzymes studied (α-amylase, α-glucosidase, hyaluronidase, and lipoxygenase). The *Artemisia absinthium* concentrated extract showed high inhibitory activity on LOX, while *Artemisia vulgaris* concentrated extract has a significant inhibitory effect on HYA. Thus, these herbs can be potential sources of phytocompounds useful in the management of type 2 diabetes and inflammatory diseases.

This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.

Author Contributions: E.N. and G.P. obtained, processed, and analyzed the extracts. G.P. conducted research; C.A. was implicated in the determination of phenolic compounds level by HPLC; G.L.R. and G.P. made the final drafting work. All the authors have read and approved the final manuscript. The writing was realized by E.N.

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References

1. IDF (International Diabetes Federation) IDF diabetes atlas, 8th edition, **2017** https://www.idf.org/e-library/epidemiology-research/diabetes-atlas.html
2. Ogurtsova, K.; Da Rocha Fernandes J.D; Huang, Y.; Linnenkamp, U.; Guariguata L.; Cho, N.H.; Makaroff, L.E. IDF diabetes atlas: Global estimates for the prevalence of diabetes for 2015 and 2040, *Diabetes Res. Clin. Pract*. **2017**, *128*, pp. 40-50
3. Vadivelan, R.; Gopala, Krishnan, R.; Kannan, R. Antidiabetic potential of Asparagus racemosus, Willd leaf extracts through inhibition of a-amylase and a-glucosidase. *J. Trad. Complement Med.* **2019**, *9* 1e4.
4. Chester, K.; Zahiruddin, S.; Ahmad, A.; Khan, W.; Paliwal S., Ahmad S., Anti-inflammatory activity of total alkaloids from hypecoum leptocarpum hook f. et Thoms. *Pharmacogn. Mag.* **2017**, *13,* 179-188.
5. Bribi, N;. Algieri, F; Rodriguez-Nogales, A., Garrido-Mesa, J.; Vezza, T.; Maiza, F.;. Utrilla, M.P; Rodriguez-Cabezas, M.E.; Galvez, J. Antinociceptive and anti-inflammatory effects of total alkaloid extract from fumaria capreolata. Evidence-based *Complement Altern. Med.* **2015**, 1-7.
6. Hermanto, F.E., Soewondo, A., Tsubo,i H., et al. The hepatoprotective effect of Cheral as anti-oxidant and anti-inflammation on mice (*Mus musculus*) with breast cancer, *J. Herbmed. Pharmacol*. **2020,** *9*, 153-160.
7. Girish, K.S.; Kemparaju, K.; Nagaraju, S.; Vishwanath, B.S. Hyaluronidase inhibitors: a biological and therapeutic perspective. *Curr. Med. Chem*. **2009**, *16,* 2261–2288.
8. Loncaric, M.; Strelec, I.; Moslavac, T.; Subaric, D.; Pavic, V.; Molnar, M. Lipoxygenase inhibition by plant extracts. *Biomolecules*. **2021**, *11,* p. 152.
9. González-Peña, D.; Colina-Coca, C.; Char, C.D.; Cano, M.P.; Ancos, B.; Sánchez-Moreno, C. Hyaluronidase inhibiting activity and radical scavenging potential of flavonols in processed onion. *J. Agric. Food Chem.* **2013**, *61*, 4862–4872.
10. Sarmiento-Salinas, F. L.; Perez-Gonzalez, A..; Acosta-Casique, A.; Ix-Ballote, A.; Diaz A.; Treviño S.; N.H. Rosas-Murrieta; Millán-Perez-Peña, L. M. Paola, Reactive oxygen species: Role in carcinogenesis, cancer cell signaling and tumor progression, *Life Sciences*, **2021**, *284*, 119942
11. Ribeiro, D.; Freitas, M.; Toméb, Sara, M.; Silva, Artur, M.S.; Porto, Graça; Cabrita, E., J.; Marques, M., Manuel, B. Fernandes, E. .Inhibition of LOX by flavonoids: a structure activity relationship study. *Eur. J. Med. Chem*. **2014**, 72137e145.
12. Sudha, P.N.; Rose, M.H. Beneficial effects of hyaluronic acid. *Adv. Food Nutr. Res.*, **2014**, *72*, 137-176.
13. Ölgen, S.; Kaessler, A.; Zühal, Kiliç-Kurt, Z; Jose, J. Investigation of aminomethyl indole derivatives as hyaluronidase inhibitors, *Zeitschrift für Naturfors C*. **2014**, *65,* 445-450, 10.1515/znc-2010-7-805
14. Michalea, R.; Stathopoulou, K.; Panagiotisa, P.; Dimitrab, B.; Emmanuel, M. Efficient identification of Acetylcholinesterase and Hyaluronidase inhibitors from *Paeonia parnassica* extracts through a Hetero Covariance Approach, Aligiannis Nektariosa, *J. Ethnopharmacol*. **2020**, *257*, 111547
15. Bazinet, L.; Doyen, A. Antioxidants, mechanisms, and recovery by membrane processes Crit. Rev. *Food Sc. Nutr*. **2017**, *57*, 4, 677–700.

16. Conidi, C.; Drioli, E.; Cassano, A. Membrane-based agro-food production processes for polyphenol separation, purification and concentration, *Curr. Opin. Food Sci*. **2018**, *17*, 149–164.
17. Gerke, I.B.; Hamerski, F.; Scheer, A.P.; Silva, V.R. Clarification of crude extract of yerba mate (*Ilex paraguariensis*) by membrane processes: analysis of fouling and loss of bioactive compounds. *Food Bioprod. Process*. **2017,** *102*, 204-212.
18. Dos Santos, L.F.; Vargas, B.K.; Bertol, C.D.; Biduski, B.; Bertolina, T.E.; Dos Santos L.R.; Brião, V.B. Clarification and concentration of yerba mate extract by membrane technology to increase shelf life. *Food Bioprod. Process*. **2020**, *122*, 22–30.
19. Pandey, A.K.; Singh P. The genus artemisia: a 2012–2017 literature review on chemical composition, antimicrobial, insecticidal and antioxidant activities of essential oils, *Medicines*. **2017**, *4*, p. 68, 10.3390/medicines4030068.
20. Ekiert, H.; Pajor., J.; Klin , P.; Rzepiela, A.;. Ślesak, H., Szopa, A. Significance of *Artemisia vulgaris* L. (common Mugwort) in the history of medicine and its possible contemporary applications substantiated by phytochemical and pharmacological studies. *Molecules*. *25* **2020**, p. 4415.
21. Taleghani, A.; Emami, S.A.; Tayarani-Najaran, Z. Artemisia: a promising plant for the treatment of cancer. *Bioorg. Med. Chem*. **2020,** *28,* 10.1016/j.bmc.2019.115180
22. Ben, Nasr, S.; Aazza, S.; Mnif, W.; Miguel, M. In-vitro antioxidant and anti-inflamatory activities of *Pituranthos chloranthus* and *Artemisia vulgaris* from Tunisia. *Int. J. Appl. Pharm. Sci. Res*. **2020**, *11*, 605–614.
23. Tao Liu, Xiangyu Chen, Yuze Hu, Menghe Li, Yanting Wu, Minghui Dai, ZhiLin Huang, Pinghua Sun, Junxia Zheng, Zhe Ren, Yifei Wang, Sesquiterpenoids and triterpenoids with anti-inflammatory effects from *Artemisia vulgaris* L, *Phytochemistry,* 204, **2022,** 113428.
24. Ahamad, J.; Mir, S.R; Amin, S. A pharmacognostic review on *Artemisia absinthium*, *Int. Res. J. Pharm*. 2019, *10*, 25-31.
25. Szopa, A.; Pajor, J.; Klin, P.; Rzepiela, A.; Elansary, H.O,; Al-Mana, F.A.; Mattar, M.A.; Ekiert, H. *Artemisia absinthium* L. - importance in the history of medicine, the latest advances in phytochemistry and therapeutical, cosmetological and culinary uses. *Plants*, **2020**, *9*, pp. 1-33.
26. Al-Malki, A.L. Shikimic acid from *Artemisia absinthium* inhibits protein glycation in diabetic rats. *Int. J. Biol. Macromol*. **2019**, *122*, 1212-1216.
27. Srivastava, R.; Srivastava, P. Hepatotoxicity and the role of some herbal hepatoprotective plants in present scenario. *GJ. Dig. Dis*. **2018**, *3*, p. 2
28. Singh, N.; Yadav, S. S.; Kumar, S.; Narashiman, B. Ethnopharmacological, phytochemical and clinical studies on Fenugreek (*Trigonella foenum-graecum* L.). *Food Bioscience*. **2022**, *46*, 101546.
29. Ruwali, P.; Pandey, N.; Jindal, K.; Singh, R.V. Fenugreek (*Trigonella foenum-graecum*): Nutraceutical values, phytochemical, ethnomedicinal and pharmacological overview, *S. Afr. J. Bot.* **2022**, *151*, 423-431.
30. Singh, P.; Bajpai, V.; Gond, V.; Kumar, A.; Tadigoppula, N.; Kumar, B. Determination of bioactive compounds of fenugreek (*Trigonella foenum-graecum*) seeds using LC-MS techniques. *Methods Mol. Biol.* **2020**, *2107*, 377-393.
31. Chandrasekara, A.; Shahidi, F. Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MSn. *Journal of Functional Foods*. **2011**, *3*, 144–158.
32. Seasotiya, L.; Siwach, P.; Bai, S.; Malik, A.; Bharti, P., Dalal, S. Free radical scavenging activity, phenolic contents and phytochemical analysis of seeds of *Trigonella foenum graecum*, *Asian Pac. J. of Health Sci*. **2014**, *1*, 219-226
33. Mohammad, A. Light-induced fluctuations in biomass accumulation, secondary metabolites production and antioxidant activity in cell suspension cultures of *Artemisia absinthium* L. *J. Photochem. Photobiol B: Biol.* **2014**, *140*, 223-227.
34. Nguyen, H.T.; Radacsi, P.; Gosztola, B.; Nemeth, E. Z. Effects of temperature and light intensity on morphological and phytochemical characters and antioxidant potential of wormwood (*Artemisia absinthium* L.). *Biochem. Sys.Ecol*. **2018**, *79*, 1-7
35. Lee, Y.J.; Thiruvengadam, M.; Chung, I.M; Nagella, P. Polyphenol composition and antioxidant activity from the vegetable plant *Artemisia absinthium* L. *Aust. J. Crop Sci*., **2013**, *7*, 1921-1926.
36. Ferreira, J.F.; Luthria, D.L.; Sasaki, T.; Heyerick, A. Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecules*. **2010,** *15*, 3135-3170.
37. Casazza, A.A; Aliakbarian, B.; De Faveri, D.; Fiori, L.; Perego P. Antioxidants from winemaking wastes: a study on extraction parameters using response surface methodology. *J. Food Biochem*. **2012**, *36*, 28-37.
38. Aliakbarian, B.; Fathi,A.; Perego, P.; Dehghani, F. Extraction of antioxidants from winery wastes using subcritical water. J. Supercrit. Fluids. 2012, 65, 18-24
39. Melguizo-Melguizo, D.; Diaz-de-Cerio, E.; Quirantes-Piné, R.; Švarc-Gajic´, J.; Segura-Carretero, A. The potential of Artemisia vulgaris leaves as a source of antioxidant phenolic compounds. *J. func. foods*. **2014**, *10*, 192–200.

40. Haniya, A.K.;Padma, P. Phytochemical investigation of methanolic extract of *Artemisia vulgaris* L. leaves. IJPBS, **2014** , *5*, 184-195.
41. Orhan, E.I.; Belhattab, R.; Şenol, F. S.; Gülpinar, A.R; .Hoşbaş, S.; Kartal, M. Profiling of cholinesterase inhibitory and antioxidant activities of *Artemisia absinthium, A. herba-alba, A. fragrans, Marrubium vulgare, M. astranicum, Origanum vulgare subsp. glandulossum* and essential oil analysis of two *Artemisia* species. *Ind. Crops Prod*., **2010**, *32*, 566-571.
42. Craciunescu, O.; Constantin, D.; Gaspar, A.; Toma, L.; Utoiu, E.; Moldovan, L. Evaluation of antioxidant and cytoprotective activities of *Arnica montana* L. and *Artemisia absinthium* L. ethanolic extracts. *Chem. Cent. J*. **2012**, *6*.
43. Magalingam, K.B.; Radhakrishnan, A.K.; Haleagrahara, N. Protective mechanisms of flavonoids in Parkinson's disease. *Oxid. Med. Cell. Longev*. **2015**, Article 314560, 10.1155/2015/314560.
44. Bondonno, N.P.; Dalgaard, F.; Kyrø , C.; Murray, K.; Bondonno, C.P. ; Lewis, J.R.; Croft, K.D.; Gislason, G.; Scalbert, A.; Cassidy, A.; Tjønneland, A.; Overvad, K.; Hodgson, J.M. Flavonoid intake is associated with lower mortality in the Danish Diet Cancer and Health Cohort. *Nat. Commun*. **2019,** *10*, 3651.
45. Yao, Y.; Sang, W.; Zhou, M.; Ren, G. Antioxidant and a-glucosidase inhibitory activity of colored grains in China. *J. Agric. Food Chem*. **2010**, 58:770e774.
46. Cruz, E.C.; Andrade-Cetto, A. Ethnopharmacological field study of the plants used to treat type 2 diabetes among the Cakchiquels in Guatemala. *J. Ethnopharmacol*. **2015**, *159*, 238-244.
47. Awad, N.E.; Seida, A.A.; Shaffie, Z.E.N., El-Aziz, A.M, Hypoglycemic activity of *Artemisia herba-alba* (Asso.) used in Egyptian traditional medicine as hypoglycemic remedy. *J. Appl. Pharm. Sci*. **2012**, *02*, 30-39.
48. Ahmad, W.I.; Khan, M.A.; Khan, M.; Ahmad, F. Subhan, Karim, N. Evaluation of antidiabetic and antihyperlipidemic activity of *Artemisia indica linn* (aeriel parts) in Streptozotocin induced diabetic rats. *J. Ethnopharmacol*. **2014**, *151*, 618-623.
49. Ghazanfar, K.; Ganai, B.A.; Akbar, S., Mubashir, K.; Dar, S.A.; Dar, M.Y.; Tantry. M.A. Antidiabetic activity of *Artemisia amygdalina* Decne in streptozotocin induced diabetic rats. *BioMed Res. Int*. **2014**. DOI: 10.1155/2014/185676
50. Nathar, V.N.; Yatoo, G.M. Micropropagation of an antidiabetic medicinal plant, *Artemisia pallens*. *Turk. J. Bot*. **2014**, *38*, 491-498.
51. Herrera, T.; Del Hierro, J.N.; Fornari, T.; Reglero, G.; Martin, D. Inhibitory effect of quinoa and fenugreek extracts on pancreatic lipase and α-amylase under *in vitro* traditional conditions or intestinal simulated conditions. *Food Chem*. **2019**, 509-517
52. Alamgeer Uttra, A.M.; Ahsan, H., Hasan, U.H.; Chaudhary, M.A. Traditional medicines of plant origin used for the treatment of in-flammatory disorders in Pakistan: A review, *J. Tradit. Chin. Med* . **2018**, *38*, 636-656.
53. Afsar S.; Kumar, K.R.; Gopal J.V.; Raveesha P. Assessment of anti-inflammatory activity of *Artemisia vulgaris* leaves by cotton pellet granuloma method in Wistar albino rats, *J. Pharm. Res*. **2013**, *7*, 463-467.
54. Arundina, I.; Budhy, S.T.I.; Nirwanaa I., Indrawatia, R.; Luthfi M. Toxicity Test n-Hexane : Ethyl Acetate (3:7) Fraction of Sudamala (Artemisia vulgaris L.) Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 Conference, MCLS 2015, *Procedia Chem*. **2016**, *18*, 174-178.
55. Dib, I.; Alaoui-Faris, E. F. El. *Artemisia campestris* L.: review on taxonomical aspects, cytogeography, biological activities and bioactive compounds, *Biomed. Pharmacother*. **2019**, *109*, 1884-1906.
56. Parameswari P.; Devika, R. Phytochemical screening of bioactive compounds of Artemisia nilagirica (clarke) pamp. J. *Chem. Pharmaceut. Sci*. **2014**, *7*, 351-353.
57. Farahani, Z.B.; Mirzaie, A.; Ashrafi, F.; Hesari, Alihitgar, M.R.C.; Hassan N. & all. Phytochemical composition and biological activities of *Artemisia quettensis* Podlech ethanolic extract. *Nat. Prod. Res*. **2017**, *31*, 2554-2558.
58. Khan, N.; Ahmed M. Ahmed, A, et al. Important medicinal plants of Chitral Gol National Park (CGNP) *Pakistan Pak. J. Bot.* **2011**, 43, 797-809.
59. Bano, A.; Ahmad, M.; Hadda, T.B.; Saboor, A.; Sultana, S.; Zafar, M.; Khan, M.P.Z., Arshad, M.; Ashraf, M.A. Quantitative ethno-medicinal study of plants used in the Skardu valley at high altitude of Karakoram-Himalayan range, Pakistan J. Ethnobiol. Ethnomed. **2014**, *10*, p. 43.
60. Singleton, V.L; Orthofer, R.; Lamuela-Raventos, R.M. *Methods Enzymol***. 1999**, 152-178.
61. Lin, J-Y.; Tang, C-Y. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem.* **2007**, *101*, 140-147.
62. Alecu, A.; Albu., C.; Litescu, S.C.; Eremia S.A.V., Radu, G.L. Phenolic Anthocyanin Profile of Valea Calugareasca Red Wines by HPLC-PDA-MS, MALDI-TOF Analysis. *Food Analyt. Meth* . **2016**, *9*, 300-310.
63. Rice-Evans, C.; Miller, N.J. *Methods Enzymol*., **1994**, 234, 279.
64. Bondet, V.; Brand-Williams, W.; Berset, C. Kinetics and mechanism of antioxidant activity using the DPPH free radical method. *Lebensm. Wiss Technol*. **1997**, *30*, 609–615.

65. Berker, K.; Guclu, K.; Tor, I.; Apak, R. Comparative evaluation of Fe (III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP) and ferricyanide reagents. *Talanta* **2007**, *72*, 1157–1165.
66. Ranilla, L.G; Kwon, Y.I.; Apostolidis E.; Shetty, K.; Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresour. Technol.*, **2010**, *101*, 4676-4689.
67. Queiroz, D.P.K, Ferreira, A.G., Lima, A.S, Lima, E.S., Lima, M.P., Isolation and identification of α-glucosidase, α-amylase and lipase inhibitors from hortia longifolia. *Int. J. Pharm. Pharm. Sci*. **2013**, *5*, 336-339.
68. Morgan-Elson. *J. Lab.Clin. Med*., **1949,** *34*, 74.
69. Hamberg, M.; Samuelsson, B. On the specificity of the oxygenation of unsaturated fatty acids catalyzed by soybean lipoxidase. *J. Biol. Chem*. **1967**, *242*, 5329.