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RESEARCH

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Bioactivity assays and phytochemical analysis upon *Achillea filipendulina*, focusing on xanthine oxidase inhibitory and antimalarial properties

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Abstract

Background *Achillea filipendulina* (L.) is an important medicinal plant and an Asian species of the Asteraceae family. *A. filipendulina* that grows in Central and Southwest Asia. It was chosen for study with the aim of screening biologically active compounds in the essential oil of the aerial parts and the antioxidant, antimalarial and xanthine oxidase inhibitory activities of its extract. Essential oil was extracted by using Clevenger apparatus. Extraction was done by Soxhlet apparatus with solvents of n-hexane, DCM (dichloromethane) and MeOH (methanol), and then, fractionation of extracts was accomplished through various chromatographic techniques including VLC (vacuum liquid chromatography) approach and SPE (solid phase extraction) technique.

Results Approximately 79% of *A. filipendulina* essential oil components were recognized by the gas chromatography mass spectrometry (GC–MS), of which monoterpenes were 63.2% the most abundant components, except for terpenes, other compounds such as fatty acids, phenols, aromatic compounds and ketones were identified, as well antimalarial activity test was done by β-hematin formation assay and xanthine oxidase inhibitory activity was accomplished by Ransod kit, then antioxidant activity was performed by DPPH (2,2-diphenyl 1-picrylhydrzyl) free radical scavenging technique. Preliminary phytochemical screening of MeOH and DCM extracts was done by TLC (thin layer chromatography) and total phenol assay. Monoterpenoids were the main component of the aerial part's essential oil. Among different extracts, MeOH extract had significant antioxidant and xanthine oxidase inhibitory activities with RC_{50} (mg/mL) = 0.0707 and IC_{50} = 12.8741 µg/mL, respectively. Moreover DCM extract had significant antimalarial activities with IC_{50} (mg/mL) = 0.2511. Among the fractions, 40% and 60% Sep-Pak fraction of MeOH extract had stronger xanthine oxidase inhibitory and antioxidant activities. The 40% fraction of MeOH extract showed the strongest antioxidant and xanthine oxidase inhibitory activities, which might be related to the presence of phenolic compounds. The 80% VLC fraction of DCM extract also indicated the strong antimalarial activities.

Conclusion As this plant revealed a high content of phenols along with high antioxidant, antimalarial and xanthine oxidase inhibitory activities, it could have great medicinal value. Our results suggested the possible use of *A. filipendulina* in development of highly potent new pure medicinal compounds.

Keywords *Achillea filipendulina*, DPPH, Antimalarial, Xanthine oxidase, GC–MS

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1 Background

Achillea filipendulina (commonly known as milfoil, yarrow, nosebleed and Fernleaf yarrow) is an Asian species of the Asteraceae family, native to Central and Southwest Asia (Afghanistan, Iraq, Pakistan, Iran, Turkey, Kazakhstan and the Caucasus) [1]. It has also been grown in some parts of North America and Europe [2]. Loamy and sandy soils are the best soil for the growth of this plant no matter the pH of the soil is normal, acidic and alkaline. It is best grown in full sun and drought tolerant [3]. According to a number of studies, the best condition for propagation is a saline environment that contains sugar and indole-3-acetic acid and 6-benzyl adenine and is exposed to UV light for about 16 h [4]. There are various scientific reports in the literature upon the genus *Achillea spp.* reporting different activities of those plants such as antibacterial [5], anti-inflammatory [6], antioxidant [7], antispasmodic, wound healing, pain killer [8]. *A. filipendulina* has a good reputation as an anti-inflammatory and antiseptic plant and is widely used as a medicinal plant in southwest of Asia [5].

Malaria, also known as tropical fever, nausea, Wellers fever, intermittent fever and forest fever, is an infectious blood disease caused by a *Plasmodium protozoa* and transmitted by *Anopheles mosquitoes* [9]. There are studies reporting the usage of local herbs in treating malaria and evaluating their antimalarial activity against *P. falciparum* [10, 11]. The ineffectiveness of chloroquine (the main drug for the treatment of malaria) created an emergency that led to further studies by scientists and the discovery of artemisinin. They believed that the usefulness of this drug is the key to discovering other herbal antimalarial compounds [12]. As regards, *A. filipendulina* was assessed for its antimalarial activity based on the stated mechanism by which the conversion of heme to hemozoin was inhibited.

Another disease that was investigated in this study was Gout which is called the rich people's disease that is caused by a constant increase in the uric acid level and causes symptoms such as swelling, inflammation attacks, acute arthritis, heat and redness of the joint or tofu and kidney stones [13]. The initiator of this disease is an increase in the amount of uric acid in the blood. Uric acid causes mono amino urate crystals to form, and settle in the joints, tendons and surrounding tissues [14]. Allopurinol inhibits an enzyme called xanthine oxidase that produces uric acid in the human body, thus reducing uric acid levels in the body by reducing production [15]. Xanthine oxidase inhibitors such as allopurinol and febuxostat are currently part of the anti-gout therapy protocol [16]. The intention of this study was to *assess* in vitro antimalarial activities and xanthine oxidase inhibitory activity of different extracts and the essential oil of *A. filipendulina.*

2 Methods

2.1 Essential oil extraction

Essential oil of *A. filipendulina* aerial parts was extracted by Clevenger apparatus. The extraction of the essential oil from 50 g dried plant sample was achieved by hydrodistillation for 3 h. The isolated essential oil was stored in tightly closed dark vials at 4 °C until analysis [17].

2.1.1 Gas chromatography and mass spectroscopy (GC–MS)

The yielded essential oil after dilution in n-hexane was injected into the GC–MS (Shimadzu, Japan, DB1). The temperature of the injector was 200 °C, and the detector's temperature was 260 °C. The linear rate of the carrier gas (helium) was 1.3 mL min^{-1} at a split ratio of 1:33. The primary temperature was held at 50 °C for 3 min, and then, the temperature was increased to 300 °C at a rate of 5 °C/min and then was continued at 300 °C for 9 min [18]. Based on the fragmentation pattern of mass, compounds were identified; then, for each of them, the KI index (Kovats retention) was calculated with the $\left(I = 100 \times \left[n + (N - n) \cdot \frac{t'r(\text{unknown}) - t'r(n)}{t'r(N) - t'r(n)}\right]\right)$ formula based on the standard n-alkane series, C_8–C_{20}.

2.2 Solvent extraction

Hundred grams of the powdered dried plant material was placed in a suitable cartridge paper in Soxhlet apparatus and then extracted with solvents of n-hexane, dichloromethane (Duksan, South Korea) and MeOH (Merck, Germany), respectively, each for about 8 h. After the extraction process, the system was cooled and extracts were concentrated and dried in a vacuum at the lowest possible temperature (45 °C) via rotary evaporator (Buchi, Switzerland). The dried extracts were kept at − 20 °C until next analysis [19].

2.3 Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS)

LC–MS/MS analysis was accomplished using a MS/MS detector. Experiments were completed with an Alliance HT system coupled to a micro-mass Quattro micro-LC–MS/MS instrument equipped with an ESI source working in negative ion mode. A waters Symmetry C_{18} 150×4.6 mm, 5 µm particle size, analytical column operating at 40 °C was used for chromatographic separation. The flow rate of the solvent was kept at 0.5 mL/min. The elution gradient consisted of mobile phases (A) acetonitrile/water/formic acid (10:89:1) and (B) acetonitrile/water/formic acid (89:10:1) which were used. The

gradient system was used, and mobile phase B increased from 10 to 100% in run time of 40 min. LC ESI–MS/MS data were collected, and then, it was analyzed [20].

2.4 Fractionation of MeOH extract

Solid phase extraction (SPE) method was used for fractionation of the MeOH extract. First 2 g of MeOH extract was weighed and dissolved in 10:90 MeOH in water. The extract solution was transferred to Sep-Pak ODS cartridge and washed with 10%, 20%, 40%, 60%, 80% and 100% MeOH in water (each 200 ml) using vacuum. It should be noticed that prior to loading the sample, the activation and washing steps of Sep-Pak ODS cartridge were accomplished with 150 ml of pure MeOH, 150 ml of water and 150 ml of a mix of 10% MeOH in water, respectively. As a result, 6 fractions were acquired, and all of them were fully dried by rotary evaporator at 45 °C in vacuum [21].

2.5 Fractionation of DCM extract

Vacuum liquid chromatography (VLC) method was excreted for fractionations of DCM extract. Before loading the sample, the washing and activation steps of the 60 silica gel (GF_{254}, Merck, Germany) column were executed with 200 ml of MeOH, 200 ml of ethyl acetate and 200 ml of a mixture of 10% ethyl acetate in hexane, respectively. Then, 130 mg of DCM extract was weighed and dissolved in a mix of hexane and ethyl acetate with the ratio of 10:90, and then, it was transferred to a smooth silica gel and washed with mixtures of 10%, 20%, 40%, 60%, 80% and 100% ethyl acetate in hexane finally with MeOH solvent of 200 ml each using a vacuum pump. As a result, 6 fractions were obtained, all of which were fully dried by a rotary evaporator at 45 °C [22].

2.6 Assay for antimalarial activity

The malaria parasite provides its food source by breaking down the hemoglobin of the host's red blood cells. Excessive breakdown of hemoglobin produces a toxic byproduct that is toxic to both the host cell and the malaria parasite. The malaria parasite uses several metabolic reactions for detoxification, which is an interesting and accepted mechanism of heme to hemozoin conversion [23, 24]. The potential antimalarial activity of the extracts was first investigated by Fitch et al. In later years, Tripathi et al. made changes to this method. In the method used in this study, different concentrations of the extracts and the fractions obtained from VLC of DCM extract were incubated with 300 µM hematin (Sigma-Aldrich, UK) solution (fresh in 0.1% NaOH solution), 10 mM oleic acid (Fluka, India) And 10 µM hydrochloric acid (Merck, Germany). The reaction volume was increased to 1000 µl used 500 mM sodium acetate (Sigma-Aldrich, UK) buffer at pH = 5. Chloroquine disulfate was used as the positive control. The samples were incubated for 24 h at 37 °C with constant and gentle shaking. After incubation, the samples were centrifuged (11,000 rpm for 5 min) and then the hemozoin pellets were repeatedly washed and centrifuged by sonication. The solvent detergent was SDS (Sigma-Aldrich, UK) 5.2% w/v in phosphate buffer salt with pH = 4.7. The final wash was then performed in 0.1 M sodium bicarbonate (Sigma-Aldrich, UK) solution at pH = 9 until the sample was completely clear (about 3–5 times). The hemozoin pellets were redissolved with 1 ml of 0.1 M sodium hydroxide (Sigma-Aldrich, UK), and then, the hemozoin content was assessed by determining the absorption at 400 nm. The concentration of the desired substance that inhibits 50% was calculated and the results were presented as IC_{50} with the following formula [25].

$$\%\text{Inhibition} = \frac{\text{Absorbance(control)} - \text{Absorbance(sample)}}{\text{Absorbance(control)}} \times 100$$

2.7 Assay for xanthine oxidase inhibitory activity

In this study, superoxidase dismutase kits (Ransod, UK) were used to evaluate xanthine oxidase inhibitory activity of the essential oil and various extracts of *A. filipendulina*. A new method using this kit was used to facilitate the test and get more accurate results.

This method employs xanthine and xanthine oxidase (XOD) to generate superoxidase radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. The xanthine oxidase inhibitory activity is then measured by the degree of inhibition of this reaction.

The reaction in the first step of the test in this kit is as follows:

$$\text{Xanthine} \xrightarrow{\text{XOD}} \text{Uric acid} + O_2^{\circ}$$

$$\text{I.N.T.} O_2^{\circ} \rightarrow \text{formazan dye}$$

The kit includes buffer, mixed substrate and the enzyme (Xanthine oxidase).

Allopurinol (Sigma-Aldrich, UK) was the positive control. Different concentrations of allopurinol (0.08–3 µg/mL) were prepared by serial dilution to plot the calibration curve. Then, the two relevant kit reagents were prepared according to the kit instructions. 10 µl of the sample was added to the microtube, and then, 350 µl of reagent R_2 was added and then placed in incubator at 37 °C for 5 min. After 5 min, 600 µl of reagent R_1a was added and this time it was placed in the incubator for 15 min. After leaving the incubator,

the absorbance of solutions was measured with a spectrophotometer at 505 nm. Then, in order to obtain the inhibitory level of each of the extracts, different concentrations of them were prepared by serial dilution and the above steps were repeated for them. Then, based on the graph, the concentration was plotted against the percentage of inhibition, and the concentration that had inhibition equal to 50% was reported as IC_{50} with the following formula [26].

$$\%\text{Inhibition} = \frac{\text{Absorbance(control)} - \text{Absorbance(sample)}}{\text{Absorbance(control)}} \times 100$$

2.8 Assay for in-vitro antioxidant activity

The 2,2-diphenyl 1-picrylhydrazyl (DPPH) (Sigma, Germany) free radical scavenging technique was performed to determine the *in-vitro* antioxidant activity of *A. filipendulina*. In this study, the antioxidant activity of MeOH, n-hexane and DCM extracts and Sep-Pak fractions of MeOH extract as well as essential oil of the plant were assessed. For this purpose, DPPH was used as an oxidant. The solution of this substance in the oxidized state is purple, which is reduced in the presence of the antioxidants, and depending on the intensity of the antioxidant activity, color of the solution turns bright yellow. This color change is the basis for measuring antioxidant activity. In this method, DPPH solution with a concentration of 0.08 mg/ml was mixed one by one with different concentrations of samples in the range of 1000–15.625 µg/ml. After 30 min of incubation at 25 °C, the absorbance of the solutions was determined at a wavelength of 517 nm. The concentration of the sample that scavenged 50% of the DPPH free radicals in the solution was calculated and the results were presented as RC_{50}. Quercetin was the positive control. The inhibition rate of free radicals in percent (I %) was measured as follows: $I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$, where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the sample [27].

2.9 Assay for total phenols content

In order to measure the amount of total phenol compounds of the MeOH extract, the Folin–Ciocalteu (Merck, Germany) method was applied [28]. Two groups of solutions were prepared for this assay: The first group was standard solutions of gallic acid with a concentration of 1–6 mg/ml, and the second group was solutions of MeOH extract with a concentration of 0.8–3 mg/ml. The standard curve was drawn applying the concentration and absorbance of gallic acid solutions using Excel software, and according to the standard curve the amount of phenols was calculated as a percentage in 100 g of extract [29].

2.10 Preliminary phytochemical analysis

Thin layer chromatography (TLC) was used to evaluate and qualitatively identify the groupings of the phytochemical present in the plant extracts. As regards, TLC plates with a thickness of 0.25 mm with silica gel 60 GF_{254} Merck adsorbent were used. Different solvent systems were tested in order to search for the best components separation in the MeOH extract and Sep-Pak fractions. The best solvent system was ethyl acetate (Duksan, South Korea), formic acid (Merck, Germany) and water (8:1:1). Eventually, after spraying $AlCl_3$ reagent, the results were examined under a UV lamp at the wavelength of 254 nm and 366 nm [30]. Additionally, presence or absence of various plant secondary components was determined through qualitative phytochemical assessments. Different biochemical tests such as test for alkaloids, tannins, flavonoids and saponins were done to achieve this aim according to the reference book phytochemical methods, Harborne [31].

3 Results

3.1 Analysis of essential oil

The percentage of light yellow color essential oil of *A. filipendulina* was obtained, and after injection into the GC–MS, approximately 79% of its compounds were identified, which included 41 compounds that are shown in Table 1, and the GC–MS chromatogram is shown in Fig. 1.

3.2 LC–MS/MS analysis

In this study, it was determined that the most active extract among the all was methanol extract, so the results of its activity were investigated. The phytochemical pattern of the methanol extract was assessed via LC–MS/MS analysis (Fig. 2). Results of the LC–MS/MS data established its chief phenolic compounds that are shown in detail within Table 2. Methoxyflavonoid hexoside, feruloylquinic acid, isorhamnetin, amcinonide and kaempferol were detected at signals 460 m/z, 367 m/z, 300 m/z, 359 m/z and 287 m/z, respectively.

3.3 Analysis of antimalarial activity

After performing antimalarial test on the extracts, the results were noted as IC_{50} which is the concentration of sample that inhibits the conversion of 50% of each into hemozoin [25].

Table 1 Components of the essential oil of *A. filipendulina*

No.	Compounds	Retention time	Percentage	Mw*	Formula	KI**
1	2,5,5 Trimethyl-3,6-heptadien-2-ol	15.93	0.5	154	$C_{10}H_{18}O$	876
2	Para cymene	16.62	0.6	134	$C_{10}H_{14}$	968
3	Eucalyptol	17.05	7.5	154	$C_{10}H_{18}O$	1006
4	Santolina alcohol	17.37	17.9	154	$C_{10}H_{18}O$	1018
5	Butyric acid	17.9	0.6	170	$C_{10}H_{18}O_2$	1039
6	Gamma-terpinene	18.01	0.3	136	$C_{10}H_{16}$	1043
7	Butanoic acid	18.22	1.4	170	$C_{10}H_{18}O_2$	1051
8	Artemisia alcohol	18.68	0.7	154	$C_{10}H_{18}O$	1068
9	Alpha-campholene aldehyde	19.92	0.6	154	$C_{10}H_{16}O$	1113
10	Alpha-campholenic aldehyde	20.03	0.6	154	$C_{10}H_{18}O$	1116
11	Camphor	20.54	1.6	152	$C_{10}H_{16}O$	1131
12	Pinocarveol	20.62	1.5	152	$C_{10}H_{16}O$	1135
13	Pinocarvone	21.11	1.4	150	$C_{10}H_{14}O$	1149
14	Isoborneol	21.51	4.8	154	$C_{10}H_{18}O$	1161
15	Borneol	21.69	5.5	154	$C_{10}H_{18}O$	1166
16	Terpinen-4-ol	21.94	2.5	154	$C_{10}H_{18}O$	1174
17	α-Terpinyl propionate	22.22	1.3	154	$C_{10}H_{18}O$	1183
18	Alpha-terpineol	22.45	0.6	152	$C_{10}H_{16}O$	1189
19	Piperitol	22.77	0.2	152	$C_{10}H_{18}O$	1199
20	Carveol	23.04	0.4	152	$C_{10}H_{16}O$	1208
21	Nerol	23.36	0.9	154	$C_{10}H_{18}O$	1218
22	Isobornyl formate	23.45	1.3	182	$C_{11}H_{18}O_2$	1221
23	Alpha-terpinyl acetate	23.63	2.4	182	$C_{12}H_{20}O_2$	1227
24	Ascaridole	24.03	0.3	168	$C_{10}H_{16}O_2$	1240
25	Chrysanthenyl acetate	24.47	3.6	192	$C_{12}H_{16}O_2$	1255
26	Isobornyl acetate	25.28	4.3	196	$C_{12}H_{20}O_2$	1281
27	Isoascaridole	25.51	0.3	168	$C_{10}H_{16}O_2$	1288
28	Eugenol	26.93	0.2	164	$C_{10}H_{12}O_2$	1336
29	Methoxyallylbenzene	27.24	1.9	196	C12H20O2	1347
30	β-Elemene	28.69	0.2	204	$C_{15}H_{24}$	1398
31	Trans-β-Caryophyllene	29.65	0.4	204	$C_{15}H_{24}$	1432
32	Germacrene	31.28	2.9	204	$C_{15}H_{24}$	1492
33	Geranyl isovalerate	33.3	1.73	238	$C_{15}H_{26}O_2$	1570
34	Spathulenol	33.68	2.8	220	$C_{15}H_{24}O$	1585
35	Mint ketone	33.99	0.4	999	$C_{15}H_{24}O$	1597
36	Torreyol	35.03	2.7	222	$C_{15}H_{26}O$	1640
37	Tridecanal	36.5	0.3	198	$C_{13}H_{26}O$	1707
38	Alloaromadendrene	37.37	0.9	204	$C_{15}H_{24}$	1739
39	γ-costol	37.51	0.4	220	$C_{15}H_{24}O$	1744
40	Hexahydrofarnesyl acetone	39.57	0.3	268	$C_{18}H_{36}O$	1836
41	Palmitic acid	41.93	0.2	254	$C_{16}H_{32}O_2$	1946
Total		78.93				
Monoterpene hydrocarbons		59.3				
Oxygenated Monoterpenes		3.9				
Sesquiterpenes		10.6				
Non-terpenoids		3.73				
Unidentified		1.4				

M_W*: Molecular weight

KI**: Kovats retention Index

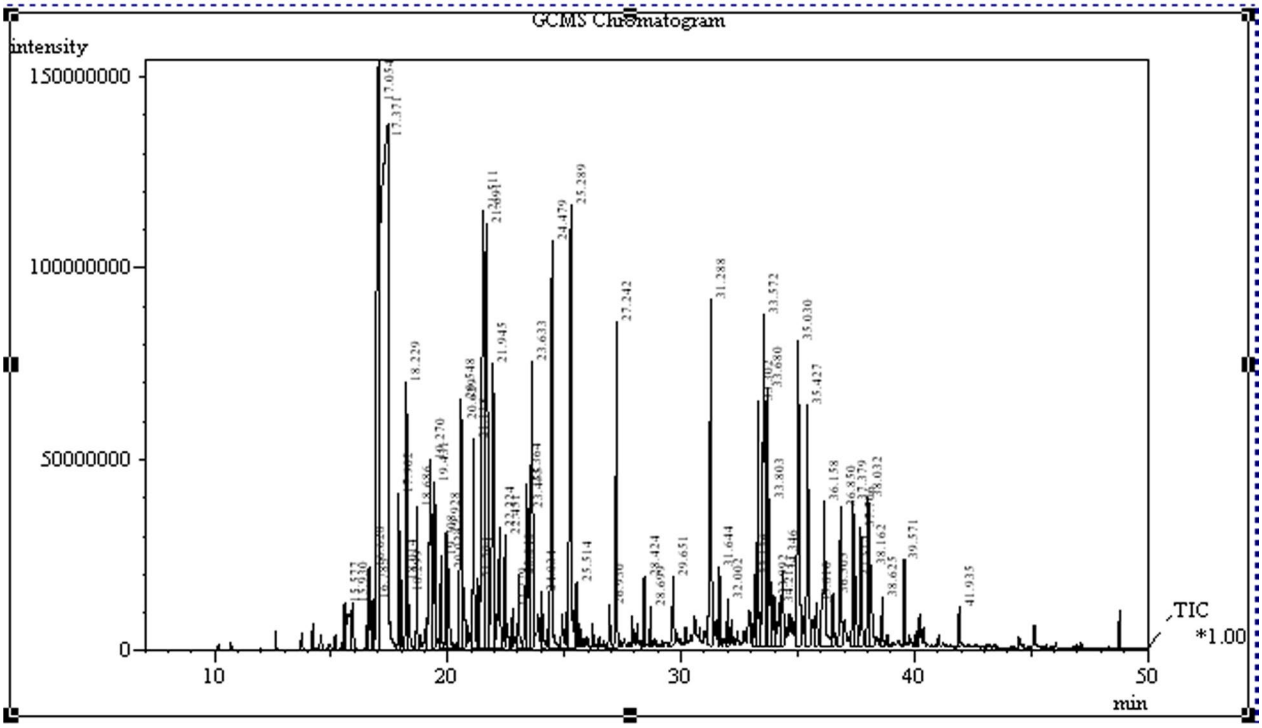

Fig. 1 GC–MS chromatogram of the essential oil of *A. filipendulina*

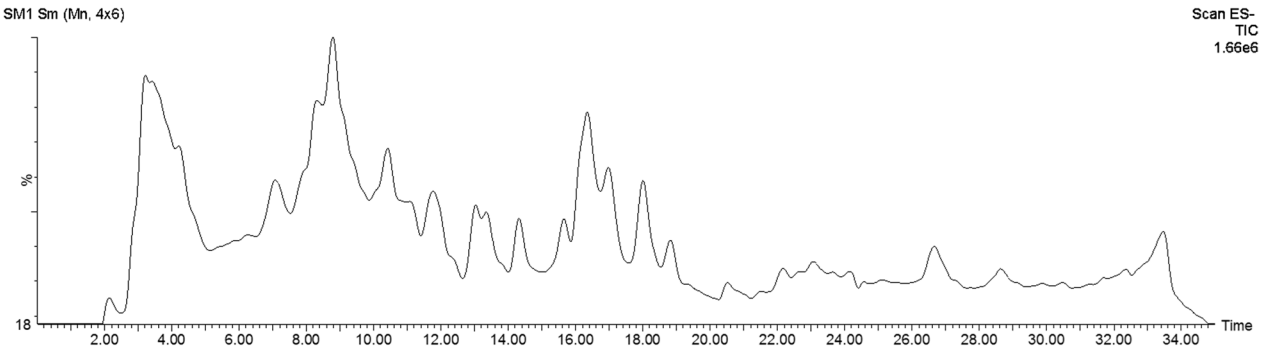

Fig. 2 LC–MS/MS chromatogram of methanol extract of *A. filipendulina*

The IC_{50} obtained for each of the compounds are shown in Tables 3 and 4, and Fig. 3 shows the IC_{50} related to the antimalarial activity of chloroquine, various extracts, VLC fractions of DCM extract and the essential oil.

3.4 Evaluation of xanthine oxidase inhibitory activity

The inhibitory activities of xanthine oxidase on plant aerial parts essential oil, MeOH, DCM, n-hexane extracts and various Sep-Pak fractions of MeOH extract had been determined, and the IC_{50} obtained for each are shown in Tables 3 and 4.

In Fig. 4, the comparison diagram of xanthine oxidase inhibitory activity of aerial parts essential oil, different extracts and Sep-Pak fractions of MeOH extract of *A. filipendulina* had been depicted.

3.5 Analysis of antioxidant activity

Antioxidant activity of aerial parts essential oil, MeOH, DCM, n-hexane extracts and Sep-Pak fractions of

Table 2 Characterization of the chief phenolics in the methanol extract of *A. filipendulina*

Retention time	[M-H]-m/z	Fragments	Identification	References
7.81	460	285,298	Methoxyflavonoid hexoside	[32]
10.3	367	173,191	Feruloylquinic acid	[33] [34] [35]
17.76	300	315	Isorhamnetin	[32, 36]
18.79	359	297,161	Amcinonide	https://massbank.eu/MassBank/Search
26.74	287	231	Kaempferol	https://massbank.eu/MassBank/Search

Table 3 Antioxidant, antimalarial and xanthine oxidase inhibitory activity of the different extracts of *A. filipendulina*

	Assay	MeOH extract S.D. (n = 3)	DCM extract S.D. (n = 3)	N-hexane extract S.D. (n = 3)	Essential oil S.D. (n = 3)	Positive control
RC50 (µg/ml)	DPPH free radical scavenging	70.7 ± 0.0017	2039 ± 0.42	1033 ± 0.05	1163 ± 1.35	Quercetin: 0.278
IC50 (µg/ml)	β-hematin formation	> 100,000	250 ± 0.01	2920 ± 0.22	–	Chloroquine disulfate: 14 ± 0.003
IC50(µg/ml)	Ransod Kit	12.78 ± 0.60	44.27 ± 18.82	63.25 ± 131.24	26.61 ± 2.21	Allopurinol: 1.631

Table 4 The activities of fractions of MeOH and DCM extracts of *A. filipendulina*

Fractions of MeOH extract	RC50 (µg/ml) Antioxidant activity	IC50 (µg/ml) Inhibitory of xanthine oxidase	Fractions of DCM extract	IC50 (µg/ml) Antimalarial activity
Sep-Pak 10%	60	21.6	VLC 10%	13,797
Sep-Pak 20%	60	11.16	VLC 20%	3310
Sep-Pak 40%	10	4.17	VLC 40%	1520
Sep-Pak 60%	30	11.08	VLC 60%	3170
Sep-Pak 80%	120	98.29	VLC 80%	910
Sep-Pak 100%	360	77.88	VLC 100%	67,057

MeOH extract was determined, and the RC_{50} obtained for each of the compounds are shown in Tables 3 and 4. Comparison of the results obtained from this assay is shown in Fig. 5.

3.6 Total phenol assay

The total phenol content of MeOH extract and Sep-Pak fractions of MeOH extract was determined, and the amount of phenol in 1 g of extracts was obtained based on gallic acid calibration curve via the equation below (Sigma-Aldrich, UK) and is shown in Table 5.

$$\text{Absorbance} = 3.3486 \times \text{gallic acid}(\text{mg}) + 0.7023 \left(R^2 = 0.98\right)$$

3.7 Phytochemical analysis

The data obtained for solvent extraction and fractionation of different extracts from *A. filipendulina* aerial parts are as follows: percentages of n-hexane, DCM and MeOH extracts were calculated as 5.64, 0.42 and 1.61 g in 100 g of dried plant material, respectively. The amounts of SPE and VLC fractions are summarized in Table 6.

3.8 TLC results

According to the results of TLC, the compounds of the fractions were well separated based on the polarity of the components. As seen, the main components of the extract were distributed in the fractions of 10%, 20% and 40% of the extract. Sprayed reagents had shown that the main compounds were mostly phenols.

The results of presence or absence of various plant secondary components are shown in Table 7.

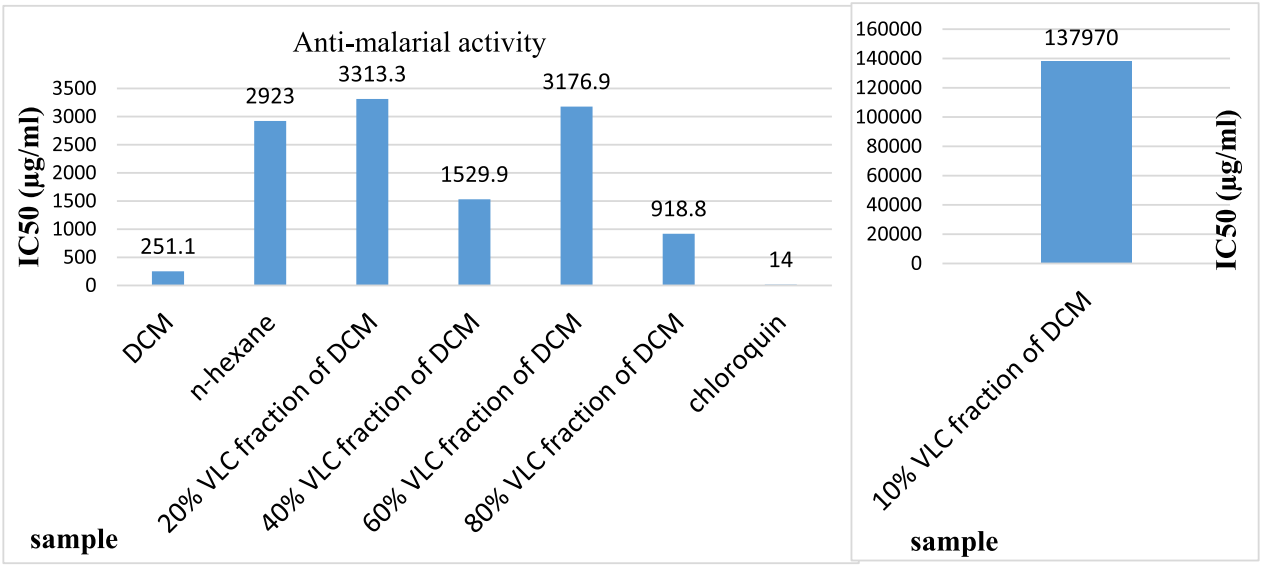

Fig. 3 Comparison diagram of antimalarial activity of different samples of *A. filipendulina*

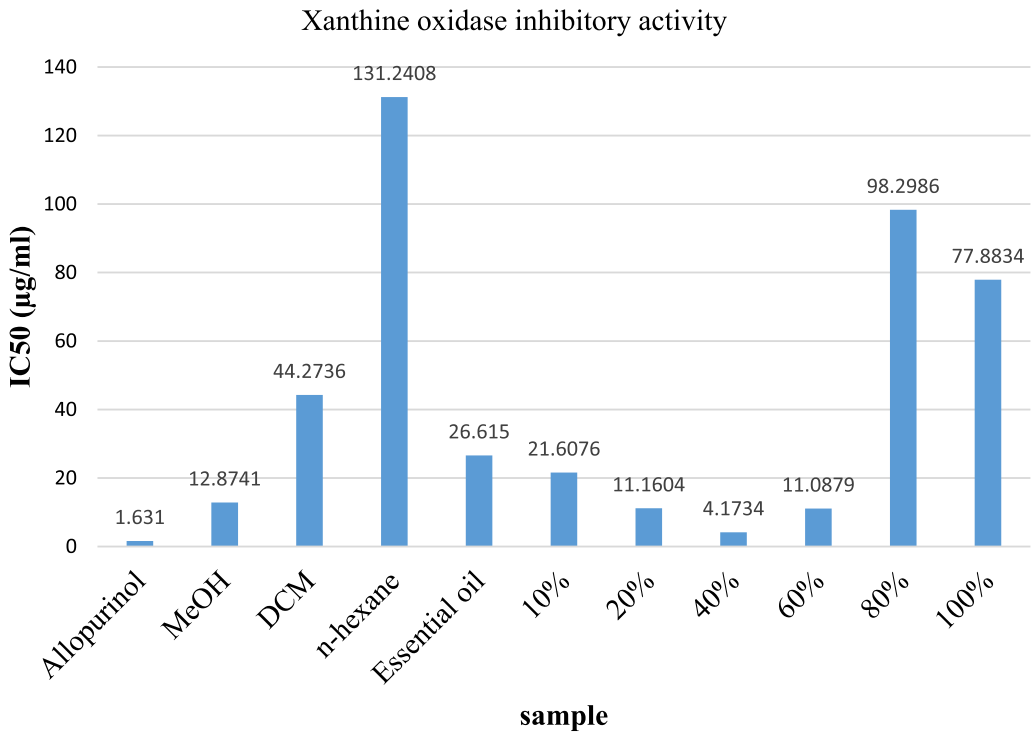

Fig. 4 Comparison diagram of xanthine oxidase inhibitory activity of different samples of *A. filipendulina*

4 Discussion

In this study, the antimalarial activity of essential oil, MeOH, DCM and n-hexane extracts of *A. filipendulina* was studied comparing with chloroquine as a positive control. DCM extract showed significant activity compared to other extracts. In order to evaluate DCM extract as much as possible, this extract was fractionated and among these fractions, 80% VLC fraction showed the highest antimalarial activity with IC_{50} = value (0.910 mg/mL), which suggested that the more non-polar structures of DCM extract were responsible for antimalarial properties. IC_{50} is inversely related to the antimalarial activity of

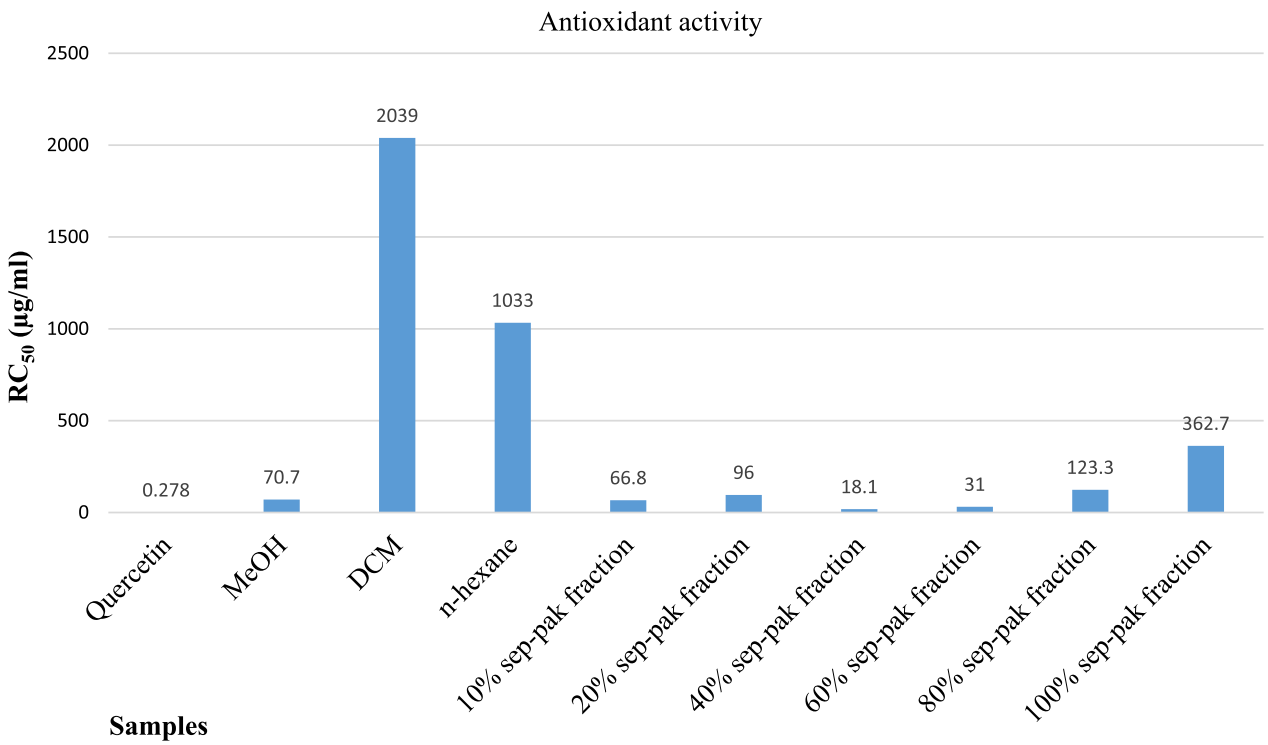

Fig. 5 Comparison diagram of antioxidant activity of different samples of *A. filipendulina*

Table 5 Total phenols content of MeOH extract and Sep-Pak fractions of MeOH extract of *A. filipendulina*

Sample	Total phenol content in 100 g of extract (%)
MeOH extract	46.57
10% Sep-Pak MeOH extract	24.41
20% Sep-Pak MeOH extract	53.97
40% Sep-Pak MeOH extract	71.51
60% Sep-Pak MeOH extract	56.17
80% Sep-Pak MeOH extract	66.22
100% Sep-Pak MeOH extract	10.35

Table 6 Yield of fractions of MeOH and DCM extracts of *A. filipendulina*

Sample	Sep-Pak fractionation of MeOH extract (mg)	VLC fractionation of DCM extract (mg)
Fraction 10%	344	15.8
Fraction 20%	284	20.1
Fraction 40%	269	30
Fraction 60%	317	19.8
Fraction 80%	307	19.1
Fraction 100%	264	9.4

Table 7 Presence or absence of various secondary components of MeOH, DCM and n-hexane extracts of *A. filipendulina*

Sample	Tannins	Anthraquinones	Flavonoids	Alkaloids
MeOH	+	+	+	+
N-hexane	+	−	−	−
DCM	+	−	−	−

the samples, and apparently the lower the IC_{50}, the more the antimalarial activity. Earlier studies also confirmed that the extracts of *A. filipendulina* [37] and other species of genus *Achillea* like *A. millefolium* showed good enough antimalarial activities [38, 39].

Based on the prior studies, other species of the *Achilla* genus like *A. millefolium* and *A. biebersteinii* had shown anti-inflammatory and anti-gout activities [40, 41], and it was decided to investigate the anti-gout effects of this plant for the first time. Based on the results, MeOH extract had the strongest inhibitory activity of xanthine oxidase. All Sep-Pak fractions of MeOH extract showed xanthine oxidase inhibitory activities. The strongest activity was related to the 40% Sep-Pak fraction. The analysis of the essential oil of *A. filipendulina* had the following results: 79% of volatile oil components were identified, of which monoterpenes were the most abundant in 63.2% of essential oils. Other components included:

sesquiterpenes, fatty acids, phenols, ketones, etc. The 5 major compounds that had the highest frequencies were: santolina alcohol, eucalyptol, borneol, isoborneol and isobornyl acetate, respectively. According to previous studies conducted in Turkey in several years, the essential oil of *A. filipendulina* of the Diyarbakir region of Turkey contained the major compounds 1,8-cineol or eucalyptol (23%) and Trans-2,7-dimethyl-4,6-octadien-2.-ol (21.9%) and borneol (8.1%) [42] and the essential oil of the plant gathered from Eastern Turkey, included large amounts of Santolina alcohol (43.6%), eucalyptol (14.5%) and cristanthenyl acetate (12.5%) [37]. Another study conducted in 2021 showed that the essential oil had the lowest composition of sesquiterpenes and the highest monoterpenes at the time of 100% flowering [43].

Compared to the standard compound, MeOH extracts as well as Sep-Pak fractions had acceptable antioxidant activity, which are relevant with the total phenolic content of them. Many previous studies also indicated a direct relationship between antioxidant activity and phenolic content of extracts [44].

To determine the category of compounds in the 40% fraction of MeOH extract (the strongest fraction in terms of antioxidant activity and inhibitor of xanthine oxidase), TLC was performed to qualitatively identify non-volatile compounds. TLC results also indicate that the extract and fractions are rich in phenolic compounds that may include other compounds such as flavonoids and phenylethanoids with a phenolic ring. During past studies, it had been found that flavonoid compounds can also show good anti-inflammatory and anti-gout effects [45].

Another study performed in India on MeOH extract of *A. filipendulina* leaves confirmed the existence of alkaloid, flavonoid, terpenoid, glycosidic, steroidal, saponin and tannins, and GC–MS evaluation of this extract showed the existence of 13-docosamine and 9-octadecanamide [46].

5 Conclusion

According to the results, it seems that the strongest antimalarial activity was related to DCM extract and the strongest antioxidant and xanthine oxidase inhibitory was associated with MeOH extract of *A. filipendulina*. Through the analysis of Sep-Pak fractions of MeOH extract, it was found that 40% fraction of this extract had the most antioxidant and inhibitory activities of xanthine oxidase, which could be ascribed to the presence of flavonoid compounds which was confirmed by TLC. Assessment of the essential oil compositions showed that monoterpenoids were the main component of the essential oil of *A. filipendulina*. The results suggested the possible use of *A. filipendulina* to produce a very strong, safe and new antioxidant, antimalarial and anti-gout drugs. In addition, *A. filipendulina* is valuable for investigating the pharmaceutical potential for use as a development of herbal medicines.

Abbreviations

DCM	Dichloromethane
MeOH	Methanol
VLC	Vacuum liquid chromatography
SPE	Solid phase extraction
GC–MS	Gas chromatography mass spectrometry
DPPH	2,2-Diphenyl 1-picrylhydrzyl
TLC	Thin layer chromatography

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Author contributions

SA provided the plant and compiled the initial rules for the experiments, SM did the experiments and tests and wrote the initial text, and AV and SB helped in performing the tests and analyzing them. AD provided laboratory facilities for conducting tests. SH supervised all the work and proofread and corrected the initial text. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Rajbhandari M, Mentel R, Jha P, Chaudhary R, Bhattarai S, Gewali M, Karmacharya N, Hipper M, Lindequist U (2009) Antiviral activity of some plants used in Nepalese traditional medicine. Evid-Based Complement Altern Medicine 6(4):517–522
2. Pala NA, Negi A, Todaria N (2010) Traditional uses of medicinal plants of Pauri Garhwal, Uttrakhand. Nat Sci 8(6):57–61
3. Dorling K (2008) RHS A-Z encyclopedia of garden plants
4. Evenor D, Reuveni M (2004) Micropropagation of *Achillea filipendulina* cv. 'Parker.' Plant Cell Tissue Organ Culture 79(1):91–93
5. Sepasgozar SME, Mohseni S, Feizyzadeh B, Morsali A (2021) Green synthesis of zinc oxide and copper oxide nanoparticles using *Achillea Nobilis* extract and evaluating their antioxidant and antibacterial properties. Bull Mater Sci 44(2):1–13
6. Strzępek-Gomółka M, Gaweł-Bęben K, Kukula-Koch W (2021) Achillea species as sources of active phytochemicals for dermatological and cosmetic applications. Oxid Med Cell Longev. https://doi.org/10.1155/2021/6643827
7. Şabanoğlu S, Gökbulut A, Altun ML (2019) Characterization of phenolic compounds, total phenolic content and antioxidant activity of three *Achillea* species. J Res Pharm 23:567–576
8. Saeidnia S, Gohari A, Mokhber-Dezfuli N, Kiuchi F (2011) A review on phytochemistry and medicinal properties of the genus *Achillea*. DARU J Fac Pharm Tehran Univ Med Sci 19(3):173
9. Neva F, Brown H (1994) Basic clinical parasitology, 3rd edn. Meredith Corporation, Des Moines
10. O'neill M, Bray D, Boardman P, Phillipson J, Warhurst D (1985) Plants as sources of antimalarial drugs part. 1. In vitro test method for the evaluation of crude extracts from plants. Planta Med 51(05):394–398
11. Simonsen HT, Nordskjold JB, Smitt UW, Nyman U, Palpu P, Joshi P, Varughese G (2001) In vitro screening of Indian medicinal plants for antiplasmodial activity. J Ethnopharmacol 74(2):195–204
12. Odugbemi TO, Akinsulire OR, Aibinu IE, Fabeku PO (2007) Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria. Afr J Tradit Complement Altern Med 4(2):191–198
13. Merriman TR, Stamp L (2016) Gout Lancet (Review) 388:2039–2052
14. Prevention CFDCA. Gout.
15. Pacher P, Nivorozhkin A, Szabo C (2006) Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. Pharmacol Rev 58(1):87–114
16. Abd El-Rahman HS, Abd-Elhak NA (2015) Xanthine oxidase inhibitory activity and antigout of celery leek parsley and molokhia. Adv Biochem 3(4):40–50
17. Sarikurkcu C, Ozer MS, Eskici M, Tepe B, Can Ş, Mete E (2010) Essential oil composition and antioxidant activity of *Thymus longicaulis* C. *Presl* subsp. *longicaulis* var. *longicaulis*. Food Chem Toxicol 48(7):1801–1805
18. Cebi N, Arici M, Sagdic O (2021) The famous Turkish rose essential oil: characterization and authenticity monitoring by FTIR, Raman and GC–MS techniques combined with chemometrics. Food Chem 354:129495
19. Fagbemi KO, Aina DA, Olajuyigbe OO (2021) Soxhlet extraction versus hydrodistillation using the Clevenger apparatus: a comparative study on the extraction of a volatile compound from *Tamarindus indica* seeds. Sci World J. https://doi.org/10.1155/2021/5961586
20. Dİkpinar T, Bİtlş L (2022) LC-MS/MS analysis and biological activities of endemic *Achillea sieheana* Stapf from Türkiye. J Res Pharm 26(6).
21. Fritz JS, Dumont PJ, Schmidt LW (1995) Methods and materials for solid-phase extraction. J Chromatogr A 691(1–2):133–140
22. Delazar A, Sarker SD, Nahar L, Jalali SB, Modaresi M, Hamedeyazdan S, Babaei H, Javadzadeh Y, Asnaashari S, Moghadam SB (2013) Rhizomes of *Eremostachys laciniata*: isolation and structure elucidation of chemical constituents and a clinical trial on inflammatory diseases. Adv Pharm Bull 3(2):385
23. Gluzman IY, Francis SE, Oksman A, Smith CE, Duffin KL, Goldberg DE (1994) Order and specificity of the *Plasmodium falciparum* hemoglobin degradation pathway. J Clin Investig 93(4):1602–1608
24. Egan TJ (2002) Physico-chemical aspects of hemozoin (malaria pigment) structure and formation. J Inorg Biochem 91(1):19–26
25. Tripathi AK, Gupta A, Garg SK, Tekwani BL (2001) In vitro β-hematin formation assays with plasma of mice infected with *Plasmodium yoelii* and other parasite preparations: comparative inhibition with quinoline and endoperoxide antimalarials. Life Sci 69(23):2725–2733
26. Umamaheswari M, Asokkumar K, Sivashanmugam A, Remyaraju A, Subhadradevi V, Ravi T (2009) In vitro xanthine oxidase inhibitory activity of the fractions of *Erythrina stricta* Roxb. J Ethnopharmacol 124(3):646–648
27. Ghazizadeh J, Hamedeyazdan S, Torbati M, Farajdokht F, Fakhari A, Mahmoudi J, Araj-Khodaei M, Sadigh-Eteghad S (2020) *Melissa officinalis* L. hydro-alcoholic extract inhibits anxiety and depression through prevention of central oxidative stress and apoptosis. Exp Physiol 105(4):707–720
28. Hamedeyazdan S, Fathiazad F, Sharifi S, Nazemiyeh H (2012) Antiproliferative activity of *Marrubium persicum* extract in the MCF-7 human breast cancer cell line. Asian Pac J Cancer Prev 13(11):5843–5848
29. Zargoosh Z, Ghavam M, Bacchetta G, Tavili A (2019) Effects of ecological factors on the antioxidant potential and total phenol content of *Scrophularia striata* Boiss. Sci Rep 9(1):1–15
30. Vogel H, González M, Faini F, Razmilic I, Rodríguez J, San Martín J, Urbina F (2005) Antioxidant properties and TLC characterization of four Chilean *Haplopappus*-species known as bailahuén. J Ethnopharmacol 97(1):97–100
31. Javed B, Nawaz K, Munazir M (2020) Phytochemical analysis and antibacterial activity of tannins extracted from *Salix alba* L. against different gram-positive and gram-negative bacterial strains. Iran J Sci Technol Trans A Sci 44(5):1303–1314
32. Olennikov DN, Chirikova NK, Kashchenko NI, Nikolaev VM, Kim S-W, Vennos C (2018) Bioactive phenolics of the genus *Artemisia* (Asteraceae): HPLC-DAD-ESI-TQ-MS/MS profile of the Siberian species and their inhibitory potential against α-amylase and α-glucosidase. Front Pharmacol 9:756
33. Dias MI, Barros L, Dueñas M, Pereira E, Carvalho AM, Alves RC, Oliveira MBP, Santos-Buelga C, Ferreira IC (2013) Chemical composition of wild and commercial *Achillea millefolium* L. and bioactivity of the methanolic extract, infusion and decoction. Food Chem 141(4):4152–4160
34. Bravo L, Goya L, Lecumberri E (2007) LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis*, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. Food Res Int 40(3):393–405
35. Kiselova-Kaneva Y, Galunska B, Nikolova M, Dincheva I, Badjakov I (2022) High resolution LC-MS/MS characterization of polyphenolic composition and evaluation of antioxidant activity of *Sambucus ebulus* fruit tea traditionally used in Bulgaria as a functional food. Food Chem 367:130759
36. Toplan GG, Taşkın T, İşcan G, Göger F, Kürkçüoğlu M, Civaş A, Ecevit-Genç G, Mat A, Başer KHC (2022) comparative studies on essential oil and phenolic content with in vitro antioxidant, anticholinesterase, antimicrobial activities of *Achillea biebersteinii* Afan. and *A. millefolium* subsp. *millefolium* Afan. L. growing in Eastern Turkey. Molecules 27(6):1956
37. Demirci B, Başer K, Aytaç Z, Khan SI, Jacob MR, Tabanca N (2018) Comparative study of three Achillea essential oils from eastern part of Turkey and their biological activities. Records Nat Prod 12(2):195–200
38. Murnigsih T, Matsuura H, Takahashi K, Yamasaki M, Yamato O, Maede Y, Katakura K, Suzuki M, Kobayashi S, Yoshihara T (2005) Evaluation of the inhibitory activities of the extracts of Indonesian traditional medicinal plants against *Plasmodium falciparum* and *Babesia gibsoni*. J Vet Med Sci 67(8):829–831
39. Rücker G, Walter R, Manns D, Mayer R (1991) Antimalarial activity of some natural peroxides[1]. Planta Med 57(03):295–296
40. Akram M (2013) Minireview on *Achillea millefolium* Linn. J Membr Biol 246(9):661–663
41. Jaffal SM, Abbas MA (2019) Antinociceptive action of Achillea biebersteinii methanolic flower extract is mediated by interaction with cholinergic receptor in mouse pain models. Inflammopharmacology 27(5):961–968
42. Hasimi N, Kizil S, Tolan V (2015) Essential oil components, microelement contents and antioxidant effects of *Nepeta italica* L. and *Achillea filipendulina* Lam. J Essent Oil Bear Plants 18(3):678–686
43. Afshari M, Rahimmalek M (2021) Variation in essential oil composition, anatomical, and antioxidant characteristics of *Achillea filipendulina* Lam. as affected by different phenological stages. J Essent Oil Res 33(3):283–298
44. Silva S, Gomes L, Leitao F, Coelho A, Boas LV (2006) Phenolic compounds and antioxidant activity of *Olea europaea* L. fruits and leaves. Food Sci Technol Int 12(5):385–395

45. Ling X, Bochu W (2014) A review of phytotherapy of gout: perspective of new pharmacological treatments. Die Pharm Int J Pharm Sci 69(4):243–256
46. Khan S, Richa KH, Jhamta R (2019) Evaluation of antioxidant potential and phytochemical characterization using GCMS analysis of bioactive compounds of *Achillea filipendulina* (L.) Leaves. J Pharmacogn Phytochem 8(3):258–265

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