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Comparative Pharmacognostical Study between Flowers and Arial parts of *Anthemis tinctoria* L. Cultivated in Egypt along with Different Biological activities :Therapeutic and Protective Implications

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Abstract

The current research aims to investigate the bioactivities of both petroleum ether and methanolic extracts of the flowers and aerial parts of *Anthemis tinctoria* L., along with characterizing its metabolomic profile. The acetylcholinesterase inhibitory, antidiabetic, and anti-inflammatory effects of the plant extracts were measured through quantitative colorimetric assays were evaluated, revealing that the methanol extract of flowers had higher activity than that of extract of aerial parts. Additionally, the antioxidant activity was determined using the DPPH assay. The effectiveness of both petroleum ether and methanolic extracts from the aerial part and flowers of *Anthemis tinctoria* was investigated for their protective and therapeutic anti-inflammatory and antioxidant properties (500 mg/kg.b.wt/day for 7 days, orally) in monosodium urate (MSU)-induced arthritis in Wistar albino male rats joints. Serum uric acid, pro-inflammatory markers including tumor necrosis factor–α and interleukin 1β (TNF-α and IL-1β, respectively), oxidative stress biomarkers such as lipid peroxide, reduced glutathione and super oxide dismutase (MDA, GSH and SOD respectively) levels were all measured. According to our findings, the four extracts of *Anthemis tinctoria*markedly decreased uric acid levels and ankle swelling in both the protective and therapeutic groups, attributable to their anti-inflammatory properties that involve the downregulation of inflammatory cytokines. This effect is primarily mediated by a reduction in oxidative stress and an enhancement of antioxidant status. The improvements in all the aforementioned lesions were significantly observed in both the protected and treated rats receiving the extracts of *Anthemis tinctoria*, which was confirmed by our histopathological findings. Comparatively GC/MS analysis identified nineteen compounds in the flowers, representing 95.81% of the total composition. Heptacosane was the major identified compound (10.72%). Moreover, α-amyrin was noted as the principal sterol, with a concentration of 3.06%. While, the aerial parts contained twenty-seven compounds, accounting for 94.53%, with methyl isostearate being the most abundant at 9.84%. Moreover, quantitative evaluation of total phenolics and flavonoids in the methanol extract of the flowers showed greater concentrations compared to the aerial parts. HPLC analysis indicated the presence of 17 phenolics and flavonoids out of 22 standards for each extract, with predominance of diodzein in the flowers, with a concentration of 8585.311µg/g, while naringin was the most significant flavonoid in the aerial parts, measured at 3835.559 µg/g. Five flavonoids were isolated and identified from the flowers' methanolic extract as apigenin, 7-*O*-β-D-glucopyranoside, kaempferol 3, 7-*O*-dirhamnopyranoside, chrysoeriol 7-*O*-glucopyranoside, and rutin. In conclusion, both petroleum ether and methanolic extracts from *Anthemis tinctoria* hold significant potential as protective and therapeutic agents for arthritis, assisting in the prevention and management of this chronic inflammatory disorder.

Keywords: *Anthemis tinctoria*; flavonoids; antioxidant; anti-inflammatory; arthritis

1. Introduction

The Asteraceae family, also known as Compositae, ranks as one of the most extensive families of flowering plants, encompassing over 1,600 genera and approximately 2,500 species globally. Members of this family have historically been utilized for medicinal purposes and as food sources [1]. Within the Asteraceae, the genus *Anthemis* stands as the second largest, consisting of around 210 species primarily found in western Eurasia, the Mediterranean region, and a limited area of eastern Africa. Plants belonging to the Anthemis genus are characterized as annual and perennial herbs, notable for their striking and appealing flowers [2] **.**The fundamental characteristic of the genus *Anthemis* is the presence of sesquiterpene lactones, flavonoids. It has been demonstrated that components of the *Anthemis* species have antioxidant, hepatoprotective, and anti-inflammatory properties. In Turkish traditional medicine, *Anthemis* species are frequently used to cure stomachaches, hemorrhoids, dysmenorrhea, and other gastrointestinal issues. In Europe, aerial and root components are used as tinctures, salves, tisanes, and antibacterial/antispasmodic agents for the treatment of inflammatory illnesses [3]**.** Various bioactive compounds were isolated from different parts of *A. tinctoria* L. [4]**.** Raal et al. [5]isolated new cyclitol glucoside, conduritol F-1-O-(6'-O-E-p-caffeoyl)-beta-D-glucopyranoside from methanol extract of *A. tinctoria* aerial parts together with four

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flavonoids, nicotiflorin , isoquercitrin , rutin and patulitrin and examining their free radical scavenging activity using (DPPH) free stable radical and also studied their inhibitory activity toward soybean lipoxygenase using linoleic acid as substrate so it can be used in pharmaceutical preparations and cosmetic formulations**.** According toRaal et al.[5] aerial parts of *A. tinctoria* L. has been used as traditional anticancer remedies as they **investigated** the content of essential oilsby **gas** chromatography and estimated that palmitic acid, *p*-cymene, and *α*-muurolene were the principal compounds of the essential oil from *A. tinctoria* and the major polyphenols were caffeoylquinic and dicaffeoylquinic acids in addition to various flavonoidal glycosides. The essential oil of *A. tinctoria* showed a strong effect on carcinoma in the mouth (KB cells) and prostate carcinoma (LNCaP cells). The antibacterial activity of the methanolic extract and its fractions of aerial parts of *A. tinctoria*, was investigated against various gram-positive and gram-negative bacteria [6]**.** It is believed that gout is an inflammatory reaction brought on by the accumulation of crystals of monosodium urate (MSU) around the joints [7]**.**The inflammatory reaction is the initial step in the development of gout. The fundamental mechanism is that MSU crystals induce complement organization, which in turn increases macrophages and neutrophils and speeds up the breakdown of synovial and cartilage tissue, potentially leading to joint injury [8]**.** Nucleotide-binding oligomerization domain-like receptor protein-3 (NLRP3) is drawn to MSU crystals and facilitates the release of inflammatory mediators, including interleukin-6 (IL-6) and interleukin-1β (IL-1β) [9]. It is believed that the initial sign of gout is an elevated release of (IL-1β) [10]**.** Redox signaling particles can inhibit NLRP3-mediated inflammatory responses by mediating the generation of NLRP3 inflammasomes, such as reactive oxygen species (ROS) [11]**.** In a number of inflammatory conditions, the NLRP3 inflammasome becomes important. Moreover, the pathophysiology of inflammatory illnesses such gout, obesity, multiple sclerosis, Alzheimer's disease, and diabetic complications has been connected to its atypical activation [12] **.**The innate immune system contains a particular kind of recognition receptor called toll-like receptors (TLRs). Recent data suggests that TLRs might play a role in the identification and activation of MSU-crystals [13]**.** All of this has been found by inducing growth factor (TGF-β) kinase activity transformation, which triggers transcription factor nuclear factor kappa-B (NF-B) and starts the transcription and expression of the IL-1β-specialized messenger molecule. The two main clinical treatments for gouty arthritis are colchicine and nonsteroidal anti-inflammatory medications (NSAIDs). However, thier therapeutic applicability is limited by the adverse effects [14]. Elderly patients may experience kidney damage from *indomethacin*. Additionally, it is anticipated that long-term colchicine medication will compromise bone marrow and hematological function [7]**.** Thus, this study aimed to analyze the chemical composition of petroleum ether extract from the aerial parts of *A. tinctoria*, as well as the methanolic extract from its flowers. Although, the methanol extract for arial part for different species and their anti-inflammatory activity were studied by Zengin et al. and Yıldırım et al., [15,16],as there are no comprehensive detailed comparison study on biological capacities and phytochemical composition of flowers and aerial parts. The objective was to assess their efficacy as antioxidant and anti-inflammatory agents in male Wistar rats with inflammation induced by MSU crystals. Furthermore, the study investigated the lipoidal profile of the petroleum ether extract from both the flowers and aerial parts of *A. tinctoria*. It also provided a quantitative analysis of total phenolic and flavonoid contents, complemented by HPLC analysis of these compounds in both extracts. The results were subsequently linked to notable free radical scavenging effects, as well as anti-diabetic, anti-Alzheimer, and anti-inflammatory activities.

2. Experimental (Materials and Methods)

*2.1.*Chemicals and drugs

All reagents and chemicals used in the research were purchased from Merck of high analytical grades. Ascorbic acid (Nova Nutritions), acarbose (Bayer Pharma), donepezil (Pfizer), and diclofenac sodium (NOVARTIS) were used in the biological assessment.

*2.2.*Phytochemical Investigation

*2.2.1.*Plant material

The fresh *Anthemis tinctoria* L. flowers and aerial parts were collected in February 2022, from Hurghada governorate, Egypt. Specimen from the plant was authenticated by Mrs. Theresa Labib, former head of El-Orman botanical garden and taxonomy consultant at the Ministry of Agriculture.

*2.2.2.*Plant extraction

The air-dried powdered flowers and aerial parts of *A. tinctoria* (500 g) underwent a defatting process using 6 liters of petroleum ether (40-60 ºC) across five iterations. Following this, the defatted powders of both the flowers and aerial parts were subjected to multiple extractions with 8 liters of absolutemethanol untilthe full extraction was achieved. The resulting four extracts were then concentrated to dryness under vacuum at 40º C and stored in a refrigerator for subsequent comparative phytochemical and biological analyses.

*2.2.3.*Phytochemical investigation of petroleum ether extracts

The petroleum ether extracts of both flowers and aerial parts of *A. tinctoria* were subjected to gas chromatography-mass spectrometry (GC/MS) analysis at the Department of Medicinal and Aromatic Plants Research, National Research Centre in

accordance with the specifications provided by El-Feky et al. [17]**.** The identification of most compounds was achieved through comparing the mass spectra against authentics, as well as utilizing the Wiley spectral library collection and the NSIT library.

*2.2.4.***Phytochemical investigation of methanolic extracts**

*2.2.4.1.***Phenolics and flavonoids quantification**

Determination of total phenolic content of the methanolic extracts of both flowers and aerial parts of *A. tinctoria* was performed using Folin-Ciocalteu procedure according to Žilićet al.[18]**.**The total phenolic content was expressed as mg gallic acid (GAE) per g of sample. While the total flavonoid content was determined using thecolorimetric assay with aluminum chloride ($AlCl_3$) [18]**.** The total flavonoid content was determined as mg catechin (CE) per g of sample.

*2.2.4.2.***Phenolics and flavonoids identification**

High-performance liquid chromatography (HPLC) analysis ofthe methanolic extracts of both flowers and aerial parts of *A. tinctoria*was carried out separately according to Seikel [19]using agilent technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector in alignment with the criteria established byHamed et al. [20]**.** Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

*2.2.4.3.***Flavonoids isolation**

Absolute methanolic extract of *A. tinctoria* flowers (5 g) was applied to a glass column (25× 4) packed with silica gel G60 (BDH, England). Elution was carried out with progressive ratios of chloroform and methanol and the obtained fractions were observed on TLC plates (aluminum sheets 20x20, Merck) using chloroform:ethyl acetate (4 :1) as a developing system**.** The similar fractions were collected together, and the spots that turned to yellow after spraying with NH_3 and $ALCL_3$ [21]were separately isolated and purified by preparative TLC. Identification was performed using melting point and different spectroscopic analyses (UV, MS, ^{1}H-NMR, ^{13}C-NMR), as well as reviewing the literature. The flavonoidal compound which gave positive Molisch test [22]were subjected to complete hydrolysis as illustrated by Harborne et al. [23] **.** The aglycone was obtained by extracting the reaction solution with ethyl acetate, and the sugar found in the aqueous residues was detected using paper chromatography (Whatmann No. 1 paper sheets, Maidstone, Kent, England) against the standard sugars. The n-butanol, acetic acid, water (4:2:1) lower layer solvent solution was used by descending approach. Detecting the sugars was carried out after spraying aniline-phthalate reagent and heating at 110 ^oC for 5 minutes [24]**.**
Additionally, the methanolic extract of *A. tinctoria* aerial parts (0.5g) was successively applied on TLC **Silica gel** 60 F_{254} plates (plastic **sheets,** 20 x 20 cm, 0.2 mm thick, **Merck,** Germany) using chloroform: methanol (9:1) as developing system alongside the isolated flavonoids from the flowers.

2.3. In vitro **Biological activities**

All the *in vitro* biological activities were assayed in both petroleum ether and methanolic extracts of *A. tinctoria* flowers and aerial parts, the analyses were carried out in three replicates.

2.3.1. Antioxidant Activity

The evaluation of scavenging activity involved measuring the potential of each plant extract to eliminate free radicals. The assessment of activity against 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) radicals was conducted by calculating the inhibition percentage, in accordance with the procedure outlined by Rahman et al. [25]**,** with ascorbic acid serving as the standard reference.

2.3.2. Anti-Diabetic activity

The evaluation of anti-diabetic activity was conducted by determining the percentage of inhibition of the enzymes α-amylase and α-glucosidase, following the methodologies outlined by Wickramaratne *et al.*[26] ,Pistia-Brueggeman and Hollingsworth et al. [27], respectively, with acarbose serving as the reference drug.

2.3.3. Anti-Alzheimer's activity

The inhibition percentage (%) of the acetyl cholinesterase (AChE) enzyme was established by employing donepezil as the standard drug, in accordance with the methodology outlined by Ellman et al. [28]

2.3.4. Anti-inflammatory Activity

The assay was conducted by determining the percentage of protein denaturation [29]and assessing proteinase inhibition [30] utilizing diclofenac sodium, which was prepared according to the methodology previously proposed [31], as the standard drug.

2.4.*In vivo* biological activities

2.4.1. Animal Preparation

In our study, 72 male Wistar rats weighing 200 ± 20 g were acquired from the National Research Centres' Animal House in Egypt. The animals were housed in cages with *ad libitum* eating, 12 hours of light/dark cycles, 22 ± 1 °C temperatures, and 40–60% humidity. The-National Research Centre's Ethics Committee approved all animal treatments and experimental-procedures with ethical approval no: 04420125

2.4.2. MSU-Crystal Synthesis

After dissolving 0.8 g of uric acid in 155 mL of Aquabidest, which also contained 5 mL of NaOH (1 M), the pH was brought down to 7.2 using HCl. After cooling and stirring at room temperature, the gout solution was kept overnight at 4 °C to crystallize. The precipitate was filtered out of the solution, dried for four hours at 70 °C, made into a fine powder, sieved through a 200-mesh metal filter, sterilized by heating it to 180 °C for two hours, and then stored in a sterile environment. Before being administered, MSU crystals were suspended at a concentration of 20 mg/mL in phosphate-buffered saline (pH 7.2) [32].

2.4.3. Acute toxicity study

To determine the acute toxicity of *A. tinctoria* petroleum ether extract of aerial part and methanolic extract of flower, serial concentrations of 500-3000 mg/kg b.wt. Four rats per group (24 rats in all, across all groups) were utilized [33].

2.4.4. Gouty-Arthritis-Animal Model

An MK-550 volume meter was used to measure the joint's size just prior to the injection in order to gauge the progression of arthritis. After seven days, measurements were made of the same ankle joint. Following anaesthesia with 10% intraperitoneal (ip) chloral hydrate (3.5 mL/kg), 50 µL of MSU solution (20 mg/mL) was injected into the left ankle joint cavity of each rat in experimental groups (2–9) [10].

In our experimental protocol, we used two only extracts, the petroleum ether extract of aerial part and the methanol extract of flower of *A. tinctoria* for their most potent and effective activities. 72 rats were split into nine groups of eight rats each: Group 1: As a negative control, each animal's left ankle joint cavity was injected with 50 µL of saline. Groups (2, 3): Control groups received both petroleum ether and methanolic extracts in a dose 500 mg / kg body weight / day for 14 days according to acute toxicity dose. 50 µL of MSU solution (20 mg/mL) was injected into the left ankle joint cavity of the remaining 48 rats for seven days in order to cause arthritis until the uric acid level (as measured by a blood sample collected from the retro-orbital plexus) reached 10 mg/dL and after seven days, ankle edema was measured [34]. After then, the groups were divided into: Group 4: MSU-induced arthritic rats (positive control), Groups 5-6: MSU + petroleum ether extract and methanolic extract (500 mg/kg.b.wt/day) protective groups, where rats in these groups received FE (500 mg/kg.b.wt/day) according to acute toxicity dose, combination therapy, administered daily for seven days concurrently with MSU injection). Groups 7-8 rats with MSU-induced arthritis for seven days, following an MSU injection, rats were treated with both extracts (500 mg/kg.b.wt/day) for another 7 days (Therapeutic group). Group 9: After seven days of MSU-induced arthritis, the rats were given the reference medication indomethacin at a dose of 5 mg/kg.b.wt/day [32]for another 7 days (Therapeutic reference group). The joint's synovial tissue was removed at the conclusion of the experiment, partially homogenized, and centrifuged to produce a supernatant that was kept for analysis at 20 °C.

2.4.5. Blood Sampling

To measure the levels of uric acid in various groups, blood samples were extracted from the retro-orbital plexus and centrifuged for 15 minutes at 3000 rpm.

2.4.6. Biochemical Assays

Using an ELISA kit and the manufacturer's instructions, the levels of TNF-α and IL-1β in serum were measured.The Rat NF kappaB p65 ELISA Kit (ab176648), which was acquired from Abcam in the United States, was utilized. The Rat IL-1 beta ELISA Kit (ab255730) is a single-wash, 90-minute sandwich ELISA intended for the quantitative measurement of IL-1 beta protein in serum, cell culture, and plasma. The Rat TNF alpha ELISA Kit (ab181421) is created for the quantitative measurement of TNF alpha protein (Abcam, United States).A technique for quantifying lipid peroxide (Malondialdehyde; MDA) was presented by Ohkawa et al. [35] Reduced glutathione (GSH) concentration was measured using Ellman [36] . A technique for determining Superoxide Dismutase (SOD) activity was presented by Fridovich [37].

2.4.7. Histopathological Examination

Samples of ankle joint tissue from different experimental groups were gathered, preserved in 10% neutral buffered formalin, cleaned, decalcified using EDTA, dried, and then embedded in paraffin for histopathological examination,

Hematoxylin and Eosin were used to stain the paraffin-embedded blocks after they were sectioned at a thickness of 5 microns [38]**.** To analyze stained sections, a light microscope (Olympus BX50, Ina, Japan) was utilized.

2.4.8. Histopathological Lesion Scoring

Changes in histopathologyin paw were noted and assigned the following percentage-based grading: no changes (0), mild (1), moderate (2), and severe (3) changes <30% changes are considered mild, <30% to 50% changes are considered moderate, and >50% changes are considered severe [39]**.**

2.4.9. Statistical Analyses

Statistical analysis is conducted utilizing the SPSS software, specifically employing One Way Analysis of Variance (ANOVA), alongside the co-state software. Significance is determined at $p \leq 0.05$, with different letters indicating significant differences. The calculation for percentage change relative to the control group is expressed as: (mean of treated group − mean of negative control/mean of negative control) × 100%. The formula for percentage of improvement is: (mean of positive control − mean of treated group/mean of positive control) × 100%.

3. Results

3.1. Phytochemical investigation of petroleum ether extracts

Identification of the fatty constituents in the petroleum ether extract of *A. tinctoria* flowers and aerial parts were carried using GC/MS analysis. Results are illustrated in Tables 1&2. Nineteen compounds representing 95.81% were identified in the flowers consisted of 5 oxygenated compounds amounted of 26.31%, and 14 hydrocarbons calculated as 73.68%. Heptacosane was the major identified compound (10.72%). Moreover, α-amyrin was the main identified sterol with concentration of 3.06%. On the other hand, twenty-seven compounds were recognized in the aerial parts signifying 94.53%, comprised of 17 oxygenated compounds amounted of 62.96%, and 10 hydrocarbons calculated as 58.82%. Methyl isostearate was the major (9.84%). GC-MS chromatogram of the petroleum ether extract of*A. tinctoria* flowers and aerial parts is shown in Figure (1, A &B), respectively.

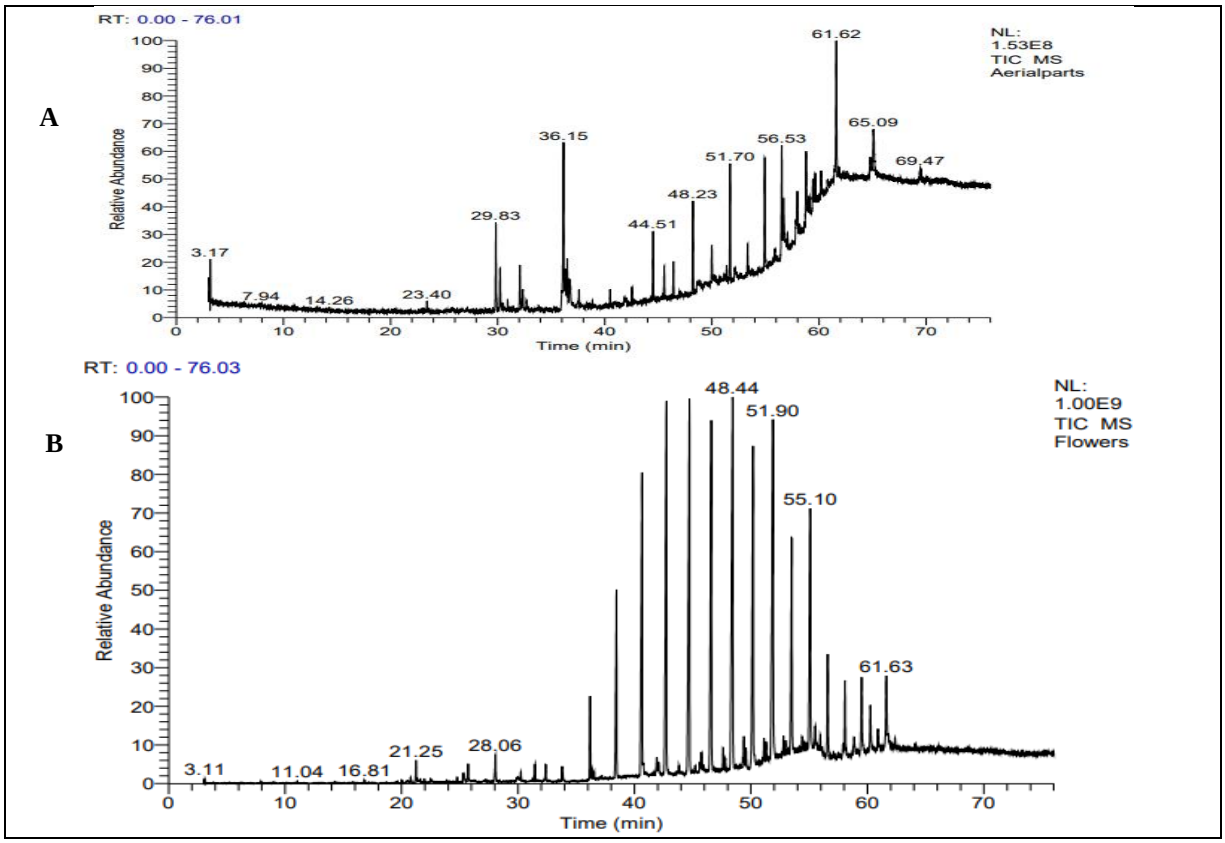


Figure 1: GC-MS chromatograms of the petroleum ether extract of*A. tinctoria* flowers (A) and aerial parts (B)

Table 1: GC/MS identification of petroleum ether extract of *A. tinctoria* flowers.

Peak No.	**Rt.** (min.)	**RRt.**	**Peak area%**	**M.Wt.***m/z*	**M.F.**	**Identified Compound**
1	21.25	0.44	0.40	204	$C_{15}H_{24}$	Cadinene
2	25.71	0.53	0.42	222	$C_{15}H_{26}O$	Cadinol
3	36.19	0.75	1.75	296	$C_{21}H_{44}$	Heneicosane
4	38.46	0.79	4.32	310	$C_{22}H_{46}$	Docosane
5	40.66	0.84	8.45	324	$C_{23}H_{48}$	Tricosane
6	42.75	0.88	10.49	338	$C_{24}H_{50}$	Tetracosane
7	44.72	0.92	10.33	352	$C_{25}H_{52}$	Pentacosane
8	46.61	0.96	9.57	366	$C_{26}H_{54}$	Hexacosane
9	48.44	1	10.72	380	$C_{27}H_{56}$	Heptacosane
10	50.18	1.04	8.72	394	$C_{28}H_{58}$	Octacosane
11	51.89	1.07	9.64	408	$C_{29}H_{60}$	Nonacosane
12	53.5	1.1	5.06	422	$C_{30}H_{62}$	Triacontane
13	55.1	1.13	6.13	436	$C_{32}H_{64}$	Tetracontane
14	55.95	1.16	0.42	430	$C_{29}H_{50}O_2$	Vitamin E
15	56.59	1.17	2.12	450	$C_{32}H_{66}$	Dotriacontane
16	58.07	1.2	1.69	464	$C_{33}H_{68}$	Tritracontane
17	58.86	1.21	0.55	414	$C_{29}H_{50}O$	β-sitosterol
18	60.25	1.24	3.06	426	$C_{30}H_{50}O$	α-Amyrin
19	61.63	1.27	1.97	426	$C_{30}H_{50}O$	Lupeol

RRt.: Relative to heptacosane, **M.Wt.: Molecular weight, M.F.: Molecular formula**

Table 2: GC/MS identification of petroleum ether extract of *A. tinctoria* aerial parts.

Peak No.	**Rt.** (min.)	**RRt.**	**Peak area%**	**M.Wt.** *m/z*	**M.F.**	**Identified compounds**
1	29.83	0.83	4.61	278	$C_{20}H_{38}$	Neophytadiene
2	32.08	0.89	2.73	282	$C_{20}H_{42}$	2,6,10,14-tetramethylhexadecane
3	32.35	0.89	1.27	270	$C_{17}H_{34}O_2$	Hexadecanoic acid methyl ester
4	36.15	1	9.84	298	$C_{19}H_{38}O_2$	Methylisostearate
5	36.32	1.01	1.36	294	$C_{19}H_{34}O_2$	9,12-Octadienoic acid methyl eter
6	36.49	1.01	2.07	292	$C_{19}H_{32}O_2$	9,12,15-Octatrenoic acid methyl ester
7	36.71	1.02	2.02	296	$C_{20}H_{40}O$	Phytol
8	44.51	1.23	3.78	352	$C_{25}H_{52}$	Pentacosane
9	45.55	1.26	1.93	390	$C_{24}H_{38}O_4$	bis(2-methylhexyl)phthalate

10	46.4	1.28	2.02	366	$C_{26}H_{54}$	Hexacosane
11	48.23	1.33	4.88	380	$C_{27}H_{56}$	Heptacosane
12	50.00	1.38	2.21	394	$C_{28}H_{58}$	Octacosane
13	51.7	1.43	6.19	408	$C_{29}H_{60}$	Nonacosane
14	53.34	1.48	1.63	422	$C_{30}H_{62}$	Triacontane
15	54.94	1.52	6.41	436	$C_{32}H_{64}$	Tetracontane
16	55.86	1.55	1.21	430	$C_{29}H_{50}O_2$	Vitamin E
17	56.53	1.56	6.91	408	$C_{27}H_{52}O_2$	Methyl 17-hexacosanoate
18	56.69	1.57	4.07	352	$C_{23}H_{44}O_2$	Tricosane 2,4-dione
19	57.86	1.6	1.17	412	$C_{29}H_{48}O$	Stigmasterol
20	57.98	1.61	2.90	464	$C_{33}H_{68}$	Tritracontane
21	58.79	1.63	4.58	426	$C_{30}H_{50}O$	Lanosterol
22	59.43	1.64	2.28	426	$C_{30}H_{50}O$	α-Amyrin
23	59.65	1.65	2.05	404	$C_{27}H_{48}O_2$	Cholestane-5,6-diol
24	60.2	1.67	2.09	426	$C_{30}H_{50}O$	Lupeol
25	61.62	1.7	9.34	414	$C_{29}H_{50}O$	β-Sitosterol
26	64.79	1.79	1.36	322	$C_{21}H_{38}O_2$	Isopropyl linoleate
27	65.09	1.8	3.62	320	$C_{21}H_{36}O_3$	Isopropyl alphalinolenate

RRt.: relative to methyl isostearate, **M.Wt.: Molecular weight, M.F.: Molecular formula**

3.2. Phytochemical investigation of methanolic extracts

Quantitativeestimation of total phenolics and flavonoids in the methanolic extracts of both *A. tinctoria* flowers and aerial parts were performed using Folin-Ciocalteu and aluminum chloride reagents, respectively. Results in table(3)revealed that methanolextract of *Anthemis tinctoria*flowers had greater amount of phenolics and flavonoids than that in the aerial parts which finger post to predict beneficial activities of the flowers.

Additionally, identification of the phenolics and flavonoidal compounds in the methanolic extracts of both *A. tinctoria*flowers and aerial parts were carried out by HPLC analysis using different standard phenolics and flavonoids (Figure 2, A; for flowers & B; for aerial parts), which led to detection of 17 compounds out of 22 standards for each one as illustrated in Table (4). It is to be noted that daidzein was the major identified flavonoid in the flowers (8585.311µg /g), while naringin was the chief one in the aerial parts with concentration of 3835.559 µg/g. Furthermore, chlorogenic acid was the main detected phenolic compound in the two parts of the plant having the value of 722.065 µg/g in the flowers, and 633.503 µg/g in the aerial parts.

Table 3: Total phenolics and flavonoids contentsin *A. tinctoria* flowers and aerial parts.

Measured parameters	***Anthemis tinctoria***	
	Flowers	**Aerial parts**
Total phenolics **(**mg gallic acid/gm**)**	25.91±0.156	20.95±0.475
Total flavonoids **(**mg catechin/gm**)**	18.28± 0.109	17.56± 0.220

Data were calculated from three replicates and expressed as mean ± S.D.

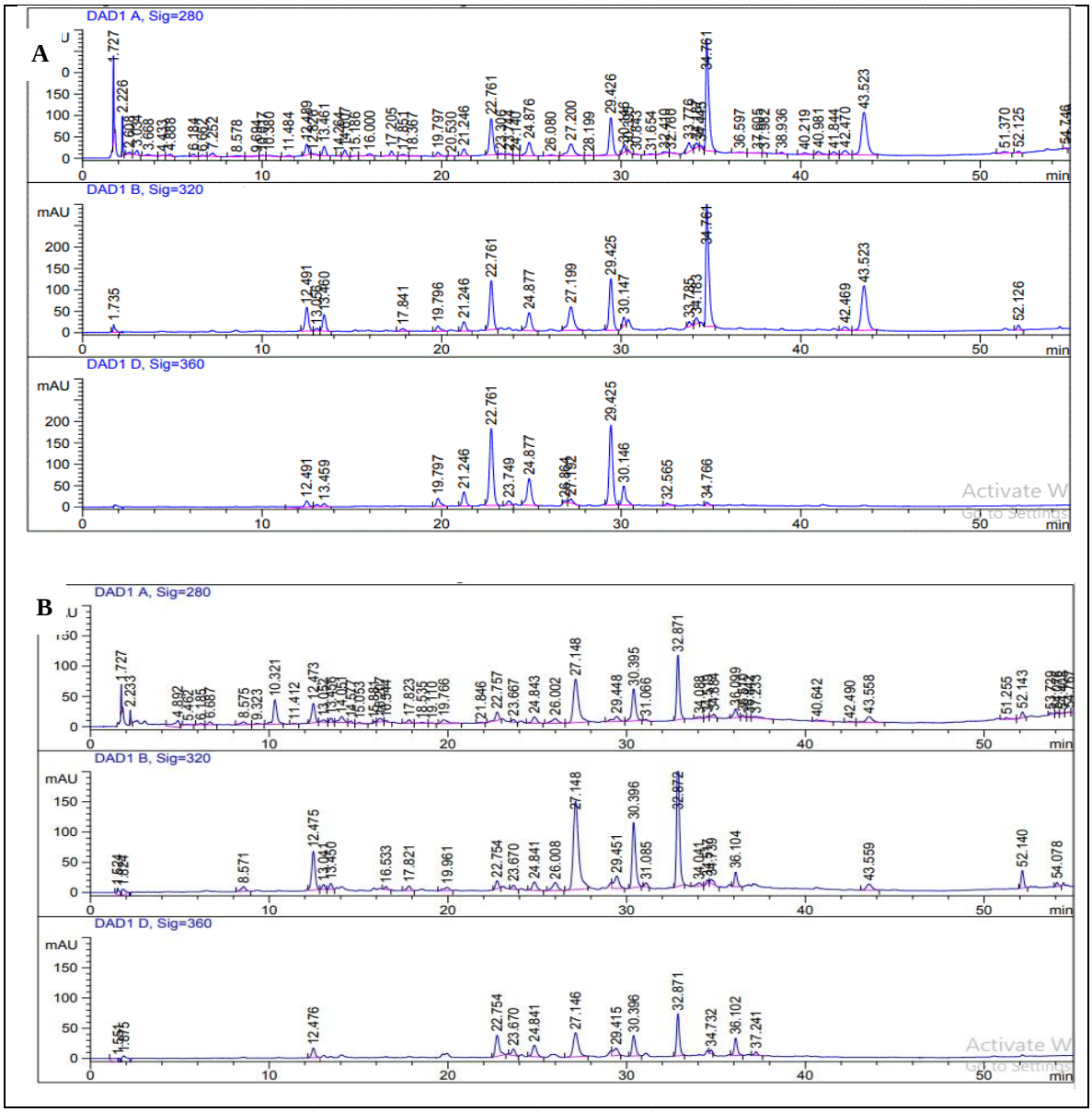


Figure 2: HPLC chromatograms of the methanolic extract of*A. tinctoria* flowers (A) and aerial parts (B)

Table 4: HPLC analysis of phenolics and flavonoids in *A. tinctoria* flowers and aerial parts.

No.	Rt.	RRt.	Standard compounds	Concentration (µg/g DW)	
				Flowers	Aerial parts
1	4.09	0.27	Gallic acid	ND	ND
2	6.9	0.46	Protocatechuic acid	54.595	70.285
3	10.4	0.70	*p*-Hydroxybenzoic acid	27.687	561.435
4	12.76	0.86	Gentisic acid	ND	ND
5	13.91	0.93	Cateachin	143.025	47.134
6	14.9	1	Chlorogenic acid	722.065	633.503

7	16.8	1.12	Caffeic acid	225.900	40.437
8	20.45	1.37	Syringic acid	133.659	21.426
9	21.16	1.42	Vanillic acid	43.815	31.681
10	21.88	1.47	Ferulic acid	86.919	39.711
11	24.01	1.61	Sinapic acid	154.878	ND
12	27.51	1.85	p-coumaric acid	57.489	153.341
13	28.78	1.93	Rutin	233.561	188.226
14	29.42	1.97	Hesperidin	ND	ND
15	30.13	2.02	Naringin	631.060	3835.559
16	35.81	2.40	Apegnin-7-glycoside	198.524	176.930
17	36.01	2.41	Daidzein	8585.311	87.421
18	36.20	2.43	Genistin	ND	ND
19	36.36	2.44	Cinnamic acid	ND	33.387
20	39.9	2.68	Quercetin	298.430	205.585
21	53.31	3.58	Kaempferol	152.791	109.342
22	59.77	4.01	Chrysin	85.913	72.156

R_t: retention time, RR_t: Relative retention time to chlorogenic acid, ND: not determined.

3.3. Structural elucidation of the isolated flavonoids

Flavonoids are currently attracting a lot of interest due to their oxidative characteristics and positive impacts on health [40]**.** Column chromatography technique was used to isolate 5 flavonoids from the methanolic extract of *A. tinctoria* flowers as they hold the heigher phenolic and flavonoids contents than aerial parts. Structure elucidation of the isolated compounds was performed using the spectroscopic analyses (UV, MS, ^{1}H-NMR, ^{13}C-NMR) and reviewing the literature.

Compound 1 was eluted as yellow powder from mixture of chloroform: methanol (95:5) giving purple color with UV light 254 nm, and R_f 0.78 in chloroform: methanol (9:1), melting point (m.p.) 345ºC, UV-λmax nm; MeOH (254, 340); MeOH+NaOMe (261,304sh,373); MeOH+$AlCl_3$ (269, 308, 382); MeOH+$AlCl_3$/HCl (268,311, 384); MeOH+NaOAc (265, 376); MeOH+NaOAc/ H_3BO_3 (266,378). EI-MS showed molecular weight at *m/z* 270 for molecular formula $C_{15}H_{10}O_5$**,** in addition to other significant fragments at m/z 242, 213, 153, and 121 which are in agreement with Bhatti et al.[41]**.** ^{1}H-NMR (500 MHz, CD_3OD): δ 6.81 (1H, s, H-3), 6.23 (1H,d, J= 2.4 Hz, H-5), 6.52 (1H, d, J= 2.4 Hz, H-8), 7.89 (2H, d, J= 8.2 Hz, H-2',H-6'), 6.76 (2H, d, J= 8.2 Hz, H-3', H-5'), 12.34 (-OH). ^{13}C-NMR spectrum (250 MHz, CD_3OD,ppm): 164.23 (C-2), 101.52 (C3), 179.46 (C-4), 160.95 (C-5), 97.53 (C-6), 163.62 (C-7), 92.51 (C-8), 156.71 (C-9), 104.71 (C-10), 122.84 (C-1'), 126.97 (C-2', C-6'), 115.38 (C-3', C-5'), 159.37 (C-4').By comparing the spectroscopic records with that stated in the literature [40]the isolated compound was established as apigenin.

Compound 2was isolated as yellow crystals, from mixture of chloroform: methanol (85:15) with R_f of 0.86, in chloroform: methanol (9:1), m.p.181 ºC. UV-λmax nm; MeOH (260, 335); MeOH+NaOMe (268,300sh, 395); MeOH+$AlCl_3$ (271,306, 355,390); MeOH+$AlCl_3$/HCl (270,307, 350); MeOH+NaOAc (261, 371); MeOH+NaOAc/ H_3BO_3 (260,368).EI-MS showed molecular weight at *m/z*432 for molecular formula $C_{21}H_{20}O_{10}$, in addition to m/z 270 for apigenin aglycone, and other mass fragments at *m/z*213 & 153. ^{1}H NMR (400 MHz, DMSO, δ ppm): 6.76 (1H, s, H-3),6.48 (1H, d, J= 2.6 Hz, H-6), 6.63 (1H, d, J= 2.6 Hz, H-8), 8.03(2H, d, J= 7.6 Hz, H-2′,6′), 7.47 (2H, d, J= 7.6 Hz, H-3′, 5′), 5.04 (1H, d, J= 6.9 Hz, H-1″), 3.16-3.74 (m, glucose protons). The glycosidic hydrolysis of the isolated compound gave glucose in the aqueous phase. The obtained spectral data harmonized to Sezen Karaoğlan et al. [43]**.** Based on spectroscopic data, the isolated compound was concluded as apigenin 7-*O*-β-D-glucopyranoside.

Compound 3was isolated as light yellow crystals from mixture of chloroform: methanol (75:25) with R_f of 0.79, m.p. 188 ºC. UV-λmax nm; MeOH (249, 261, 352); MeOH + NaOMe (253, 264, 386), MeOH + $AlCl_3$ (271, 366, 378); MeOH + $AlCl_3$ + HCl (273, 368 sh, 381); MeOH + NaOAc (254, 268 sh, 413); MeOH + NaOAc + Boric acid (257, 269, 411), EI-MS showed molecular weight at m/z 578 which assumed to the molecular formula $C_{27}H_{30}O_{14}$. ^{1}H NMR (400 MHz, DMSO, δ ppm):6.51 (1H, d, J=2.3 Hz, H-6), 6.79 (1H, d, J=2.3 Hz, H-8), 7.89 (2H, d, J=7.9 Hz, H-2′,6′), 7.26 (2H, d, J=7.9 Hz, H-3′,5′), 5.03 (1H, d, J=1.5 Hz, anomeric proton of 3-Rhamnose), 5.12 (1H, d, J=1.5 Hz, anomeric proton of 7-Rhamnose), 1.08 (3H, d, CH3 of 3-Rhamnose), 1.17 (3H, d, CH3 of 7-Rhamnose). ^{13}C-NMR spectrum (250 MHz, CD3OD,ppm): 160.1 (C-2), 129.4 (C-3), 180.3 (C-4), 162.7 (C-5), 99.2 (C-6), 164.5 (C-7), 93.5 (C-8), 155.2 (C-9), 103.8 (C-10), 120.4 (C-1′), 126.8 (C-2′, 6′), 113.6 (C-3′, 5′), 158.2 (C-4′); 100.4, 68.3, 71.2, 73.5, 72.9, 15.7 (for 3-Rhamnose); 98.7, 69.7, 70.3, 72.1, 68.8, 15.9 (for 7-Rhamnose). The glycosidic hydrolysis of the compound resulted in presence of rhamnose in the aqueous phase. By

comparison with the previous literatures [40, 44]**,** the isolated compound was confirmed as kaempferol 3, 7-*O*-dirhamnopyranoside.

Compound 4 was recovered as yellow amorphous powder from mixture of chloroform: methanol (70:30) with R_f of 0.81, m.p. 177 ºC. UV-λmax nm; MeOH (253, 268, 346); MeOH + NaOMe (248, 267, 399), MeOH + $AlCl_3$ (276, 360, 390); MeOH + $AlCl_3$ + HCl (277, 358 sh, 390); MeOH + NaOAc (258, 270 sh, 352, 410); MeOH + NaOAc + Boric acid (255, 269, 351. EI-MS showed molecular weight at *m/z*462 which assumed to the molecular formula $C_{22}H_{22}O_{11}$.In addition to m/z 300 after glucose loss, 285, 257, 151, 148, 137, 133, 123, 116 , 95. ^{1}H NMR (400 MHz, DMSO, δ ppm):7.01 (1H, s, H-3), 6.68 (1H, s, H-6), 6.53 (1H, s, H-8), 7.46 (2H, d, dd, J=2.5, 8.6, H-2`, H-6`), 6.87 (1H, d, J=8.6, H-5`), 5.11 (1H,d, J=8.3, H-1``), 3.21-3.64 (4H, m, H-2``/H-5``), 3.72 (1H, m, H-3``), 3.78 (1H, m, H-4``), 3.65 (1H, m, H-6``), 3.76 (3H, s, O-CH3). ^{13}C-NMR spectrum (250 MHz, CD_3OD,ppm): 165.26 (C-2), 105.04 (C-3), 183.47 (C-4), 160.15 (C-5), 98.11 (C-6), 163.52 (C-7), 94.52 (C-8), 154.82 (C-9), 102.86(C-10), 120.61 (C-1`), 111.45 (C-2`), 149.74 (C-3`), 153.17 (C-4`), 116.23 (C-5`), 123.86 (C-6`), 100.42 (C-1"), 76.92 (C-2"), 75.38 (C-3"), 65.79 (C-4"), 74.17 (C-5"), 62.63 (C-6"), 54.73 (O-CH_3). After reviewing the previous reports [43], the isolated compound was characterized as chrysoeriol 7-*O*-glucopyranoside.

Compound 5 was isolated as yellow needles from fraction of chloroform: methanol (70:30), with R_f of 0.74, m.p. 241 °C. UV λmax, nm; MeOH (254,263sh, 351); MeOH+NaOMe (267, 299, 389); MeOH+$AlCl_3$ (274, 305sh, 335, 436); MeOH+$AlCl_3$/HCl (273,304sh, 336,433); MeOH+NaOAc (275,326sh, 375); MeOH+NaOAc/H_3BO_3 (261,305sh, 385). ESI-MS revealed molecular weight at m/z 610 for molecular formula $C_{27}H_{30}O_{16}$, beside to significant fragment at m/z 302for quercetin aglycone and m/z284 for $C_{15}H_8O_6$ after water loss, beside to m/z 274 and 246 for $C_{14}H_{10}O_6$ and $C_{13}H_{10}O_5$ as a result of sequential loss of CO from quercetin (m/z 302). ^{1}H-NMR (500 MHz, CD_3OD, δ / ppm) displayed 6.36 (1H, *d*, *J* = 2.4 Hz, H-6), 6.21(1H, *d*, *J* = 2.4 Hz, H-8), 7.18 (1H, *d*, *J* =2.2 Hz, H-2'), 6.03 (1H, *d*, *J* =8.7Hz, H-5'), 7.54 (1H, *dd*, *J*=2.2, 8.7Hz, H-6'). The anomeric proton at δ 5.13 (1H, *d*, H-1") andrutinoside protons at δ3.28-3.69, and δ 1.34 (3H, *d*, CH_3-Rhamnose). ^{13}C-NMR spectrum (250 MHz, CD_3OD,ppm):8.01 (C-2), 135.3 (C-3), 179.5 (C-4),163.7 (C-5), 103.7 (C-6), 166.3 (C-7), 95.8(C-8), 158.7 (C-9), 104.8 (C-10),122.7 (C-1'), 119.1 (C-2'), 147.3 (C-3'), 150.4 (C-4'), 115.8 (C-5'), 123.7 (C-6'), 102.6 (C-1"),77.2 (C-2"), 76.4 (C-3"), 72.6 (C-4"), 79.3 (C-5"), 70.4 (C-6"), 101.4 (C-1"'),74.8 (C-2"'), 75.2 (C-3"'), 73.8 (C-4"'), 68.4 (C-5"'), 55.0 (C-6"').In the water phase, glucose and rhamnose were produced by the glycosidic hydrolysis of the purified compound. Therefore, the isolated compound was recognized as rutin (quercetin 3-*O*-rutinoside) based on the spectrum studies, and comparison with the previously reported information [46]**.**

As well, the methanolic extract of *A. tinctoria* aerial parts experimentally proved to have compounds 2, 4&5 after successively application on TLC slica gel alongside the isolated flavonoids from the flowers using chloroform: methanol (9:1) as developing system.

3.4. *In Vitro* Biological Activities

3.4.1. Antioxidant activity

Free radicals or reactive oxygen speciesare the main risk factor for various diseases such as cancer, diabetes, neurological disorders, atherosclerosis, and chronic inflammatory diseases [47]**.** Results in table(5) illustrated that the methanol extract of *A. tinctoria*flowers remarkably inhibited DPPH by 69.01% & 70.00% at concentration of 0.01 & 0.05 µg/ml, respectively. Followed by the petroleum ether extract of the aerial parts with values of 61.35% and 67.10% at concentration of 0.01 & 0.05 µg/ml, respectively, in comparison to ascorbic acid as a reference standard drug. Antioxidant compounds found in plant-based medicines can potentially protect human beings from many widespread and metabolic illnesses [48]**.** Recently, it has been reported thatconsumption of plant-based antioxidant is directly correlated with decreasing the probability of illness and death from infectious diseases and other chronic diseases [49]**.** Antioxidant capability is determined by reaction mechanisms and is directly related to the number and structural diversity of the phytoconstituents [50]**.**

Table 5: Antioxidant activity of both petroleum ether and methanolic extracts of *A. tinctoria* flowers and aerial parts.

Concentration (µg/ml)	DPPH inhibition (%)				
	Pet. ether extract		Methanol extract		Ascorbic acid
	Flowers	Aerial parts	Flowers	Aerial parts	
0.01	52.11±0.21 ^{d}	**61.35±0.21** ^{c}	**69.01±0.21** ^{c}	26.06±0.82 ^{f}	81.00±0.82 ^{a}%
0.05	55.01±0.21 ^{d}	**67.10±0.21** ^{b}	**70.00±0.63** ^{b}	39.44±0.91 ^{e}	89.00±0.82 ^{a}%

Values were calculated from three replicates and expressed as mean ± SE.

3.4.2. Anti-diabetic activity

Phytoconstituents that exist in therapeutic plants have a wide range of promising biological effects. Investigation for herbal medicines and naturally occurring substances produced from plants is now very important for medication development [51]. Dietary starch is digested by alpha-amylase into maltase, which is then broken down into glucose by intestinal alpha-glucosidase. Through the inhibition of these two enzymes, carbohydrate metabolism could be controlled, leading to reducing the postprandial blood glucose levels [52]. In this context, the*in vitro* anti-diabetic evaluation of both petroleum ether and methanolic extracts of *A. tinctoria* flowers and aerial parts was assessed in comparison with acarbose as a standard drug.

Results in table (6) demonstrated that the petroleum ether extract of *A. tinctoria* aerial parts and methanol extract of theflowers significantly inhibited α-amylase and α-glucosidase enzymes by 62.72% & 58.72 %, and 52.22% & 49.22 % respectively.

Table 6: The *in vitro* anti-diabetic activity of both petroleum ether and methanolic extracts of *A. tinctoria* flowers and aerial parts.

Tested enzymes	**Inhibition (%)**				
	Pet. ether extract		**Methanol extract**		**Acarbose**
	Flowers	**Aerial parts**	**Flowers**	**Aerial parts**	
α-amylase (µg/mL)	30.96 ± 0.03^{d}	**62.72 ± 0.02^{a}**	**58.72 ± 0.02^{b}**	44.72 ± 0.07^{c}	**69.17 ± 0.01^{a}**
α-Glucosidase (µg/mL)	20.46 ± 0.03^{d}	**52.22 ± 0.02^{a}**	**49.22 ± 0.02^{b}**	34.22 ± 0.07^{c}	**58.67 ± 0.01^{a}**

Values were calculated from three replicates and expressed as mean ± SE.

3.4.3. Anti-Alzheimer's activity

Key enzymes play a chief role in the development and progression of various diseases. Inhibiting the activity of these enzymes is a medical strategy for management or prevention of these kinds of disorders. For instance, cholinesterase inhibition is a treatment plan for the management of Alzheimer's disease. Therefore, extensive and continued research on bioactive natural materials and medicinal plants is necessary to develop treatments with greater effectiveness with fewer side effects[53]**.** The *in vitro* anti-Alzheimer assessment of both petroleum ether and methanolic extracts of *A. tinctoria* flowers and aerial parts was carried out in comparison with donepezil as a reference drug. The results in table (7) illustrated that the petroleum ether extract of *A. tinctoria* aerial parts and methanol extract of theflowers considerably inhibited acetyl cholinesterase by 63.85%&47.99%, respectively. The various phenolic and flavonoid components, which operate in a synergistic manner, may be responsible for this action.

Table 7: Anti-Alzheimer effect of both petroleum ether and methanolic extracts of *A. tinctoria* flowers and aerial parts.

Tested enzyme	**Inhibition (%)**				
	Pet. ether extract		**Methanol extract**		**Donepezil**
	Flowers	**Aerial parts**	**Flowers**	**Aerial parts**	
AChE (mg/L)	32.89 ± 0.01^{d}	**63.85 ± 0.01^{b}**	**47.99 ± 0.02^{c}**	31.89 ± 0.02^{d}	**72.14 ± 0.02^{a}**

Values were calculated from three replicates and expressed as mean ± SE.Statistical analysis is conducted utilizing the SPSS software, specifically employing One Way Analysis of Variance (ANOVA) with Least Significant Difference (LSD) post hoc, alongside the co-state software. Significance is determined at $p \leq 0.05$, with different letters indicating significant differences.

3.4.4. Anti-inflammatory activity

Inflammation is a prevalent condition that affects around one-fifth of the world's population specially people above 50 [54]**.** Different kinds of medications including non-steroidal anti-inflammatory medicines, and corticosteroids are used to treat osteoarthritis and other inflammatory conditions, which may cause many gastrointestinal complications such as stomach ulcers. Besides, continued NSAID use can adversely affect liver and kidney function, putting the patient at risk for cardiovascular problems. As a result, researchers are continuously looking for natural medications made from plants to be used as a safe and effective source of anti-inflammatory and antioxidant compounds[55] such as phenolics, flavonoids, sterols and terpenoids [56]**.**

The anti-inflammatory activity of both petroleum ether and methanolic extracts of *A. tinctoria* flowers and aerial parts was observed in association with diclofenac sodium as a classic anti-inflammatory drug. The examination ascertained that the methanolic extract of *A. tinctoria* flowers have proteinase denaturation value with 45.12%, and inhibition of proteinase enzyme by 42.62% as shown in table (8)

Recent studies affirmed that both petroleum ether and methanolic extracts of aerial partsand flowers of*A. tinctoria* possessed antioxidant, antidiabetic , *Anti-Alzheimer's* and anti-inflammatory activity, The fore mentioned activities possibly will be attributed to presence of numerous phytoconstituents, as hydrocarbons, fatty alcohols, triterpenes as α-amyrin (pentacyclic triterpene) which ameliorates hyperglycemia and dyslipidemia by improving glucose tolerance in mice and reduces atherogenic risk factor by its anti-inflammatory and antioxidant effects[57]and lupeol (The Pentacyclic triterpenes) can be used as chemopreventive to avoid several diseases as it exhibits broad spectrum pharmacological activities against inflammation, cancer, arthritis, diabetes, heart diseases, renal toxicity and hepatic toxicity [58]beside sterols, *β*-sitosterol acted as a 5-α-reductase inhibitor and it takes part as anti-inflammatory effect due to blocking of 5-lipoxygenase pathways of arachidonic acid [59]and lanosterol which presented in the petroleum ether extract of the aerial parts as well as many

phenolics and flavonoidal compounds as apigenin, which is found in *Anthemis tinctoria* flowers, exhibits strong antioxidant potential because it interacts with particular target sites in a way that prevents lipid peroxidation in cell membranes to prevent oxidative damage to proteins andDNA so it high antioxidant capacity as a result of conjugation, control of particular pathways and gene expression, and apigenin's structural characteristic for metal chelation and free radical scavenging[60] Due to presence of high amount of polyphenols as apigenin 7-*O*-β-D-glucopyranoside, kaempferol 3,7-*O*-dirhamnopyranoside, chrysoeriol 7-*O*-glucopyranoside, and rutin in the methanolic extracts of both *Anthemis tinctoria* flowers and aerial parts, they had potential application in anti-Alzheimer's therapy**.** Polyphenols considered as possible active neuro-protectants can reach the brain and proved that, phenolic compounds play an important role in neuroprotection associated with the number and position of hydroxyl groups in molecules which played a minor role, aglycons showed more effective cholinesterase inhibitors than their corresponding glycosylated forms [61].Regarding previous biological activity studies performed on *A. tinctoria*, a former study indicated that polar extracts including ethylacetate, methanol and aqueous extracts of *A. tinctoria*aerial partsdemonstrated strong inhibitory effects against important enzymes implicated in type II diabetes, Alzheimer's disease, and hyperpigmentation conditions, as well as notable antioxidant activity. In particular, methanol and eyhylacetate extracts exhibited greater enzyme inhibitory activity than water extracts [62]**.**

Table 8: Anti-inflammatory effect of both petroleum ether and methanolic extracts of *A. tinctoria* flowers and aerial parts.

Tested enzymes (µg/ml)	Inhibition (%)				
	Pet. ether extract		Methanol extract		Diclofenac Sodium
	Flowers	Aerial parts	Flowers	Aerial parts	
Proteinase Denaturation	29.30 ± 0.03^{b}	29.15 ± 0.02^{b}	**45.12 ± 0.02^{a}**	29.64 ± 0.02^{b}	**49.45 ± 0.02^{a}**
Inhibition of Proteinase	26.80 ± 0.03^{c}	26.65 ± 0.02^{c}	**42.62 ± 0.02^{a}**	27.14 ± 0.02^{b}	**46.95 ± 0.02^{a}**

Values were calculated from three replicates and expressed as mean ± SE.Statistical analysis is conducted utilizing the SPSS software, specifically employing One Way Analysis of Variance (ANOVA), alongside the co-state software. Significance is determined at $p \leq 0.05$, with different letters indicating significant differences.

3.5. *In-vivo* biological activity

3.5.1. Acute toxicity study

According to the previous *in-vitro* the petroleum ether extract of aerial part and methanolic extract of flower are chosen for *in-vivo* studies. Acute toxicity was determined using serial concentrations of two extracts up to 5000 mg/kg.b.wt., there were no signs of mortality or toxicity, for 48 h, in addition to the absence of toxicity or behavioural problems. The dosage used for this study was 500 mg/kg.b.wt [33]**.**

3.5.2. Effects of *A. tinctoria* petroleum ether extract of aerial part and methanolic extract of flower on Ankle Swelling in MSU Crystal-Gouty Arthritis Rats

Rats with MUS-induced arthritis (positive group) had significantly higher uric acid levels than the control group, with a percentage increase that reached271.8%.The proportion of the protective group thatchange in uric acid level reached to 90.63% and 103.13% for Protective group with P.E extract and Protective group with methanolic extract respectively as compared to control group. However, arthritic rats treated with P.E. extract and methanolic extract showed a noticeable change in uric acid levels, reaching 143.75% and 147% respectively.Indomethacin changed the uric acid level by 93.75% as compared to control group (see Table 9). Additionally, the baseline level of ankle swelling did not vary. After seven days of MSU injection, MSU crystal raised ankle swelling levels in MSU-induced arthritis by 1272.73% when compared to the control group.

The ankle swelling in the protective group with P.E extract and methanolic extract showed change by 254.55% and 261.82%, respectively as compared to control group after 7 days. However, arthritic rats treated with P.E extract and methanolic extract showed change in ankle swelling by 627.27% and 663.64%, respectively after 7 days of treatment as compared to control group. Indomethacin changed ankle swelling by 260% as compared by control group after 7 days (Table 10).

Rats with MUS-induced arthritis showed significant increases in all inflammatory markers when compared to controls, with percentage increases of692.08% and 107.55% for TNF – α and IL-1β, respectively. Inflammatory marker levels were noticeably lower in the MUS-protected groupwith methanolic extract with percentages of reduction reached to 384.60% and 391.20%, respectively for TNF –α and with percentage change reached to 233.96% and 234.18%, respectively for IL-1β compared to the MUS- group. However, rats treated with P.E and methanolic extract recorded significant reduction in TNF –α reached to 469.64% and 480.75%, respectively and by 298.92% and 300%, respectively for IL-1β (Table 10).

Table 9: Effects of *A. tinctoria* petroleum ether extract *of aerial part and methanolic extract of flower* on uric acid levels, and ankle swelling reduction in serum of MSU Crystal-Gouty Arthritis Rats.

Parameters / **Groups**	**control**	**Control rats with P.E**	**Control rats with M.E**	**Msu Group**	**Protective rats with P.E**	**Protective rats with M.E**	**Treated rats with P.E**	**Treated rats with M.E**	**Treated rats with R.D**
Uric acid (mg/dl)	3.20 ± 0.13 ^{d}	3.50 ± 0.12 ^{d}	3.30 ± 0.08 ^{d}	11.90 ± 0.10^{a}	6.10 ± 2.10 ^{c}	6.50 ± 1.20 ^{c}	7.80 ± 0.90 b	7.90 ± 0.90 ^{b}	6.20 ± 0.90 ^{c}
% change		9.38	3.12	271.88	90.63	103.13	143.75	147.88	93.75
%of improvement					181.25	168.75	128.13	125	178.13
Ankle Swelling (mm) Post 7 Says	0.55 ± 0.02 ^{d}	0.57 ± 0.10 ^{d}	0.56 ± 0.09 ^{d}	7.55 ± 0.16 ^{a}	1.95 ± 0.18 ^{c}	1.99 ± 0.19 ^{c}	4.00 ± 0.90 ^{b}	4.20 ± 0.90 ^{b}	1.98 ± 0.90 ^{c}
% change		3.64	1.82	1272.73	254.55	261.82	627.27	663.64	260
%of improvement					1018.18	1011	645.45	609.09	1012.73

Data are mean ±SD of eight rats in each group. Statistical analysis is brought out utilizing SPSS computer program (One-way Analysis-of-Variance, ANOVA; IBM SPSS (version 8) (SPSS Inc., Chicago, IL, USA) connected with co-state computer program, where different letters at the same column are significant at $p \leq 0.05$. % Change is calculated compared to control group as: (normal control mean – treated mean /normal control mean× 100. % improvement: (mean of positive control − mean of treated group/mean of control) × 100. P.E: petroleum ether extract, M.E: methanolic extract,R.D: reference drug.

Table 10: Effects of *A. tinctoria* petroleum ether extract of aerial part and methanolic extract of flower on Inflammatory Markers TNF-α and IL-1β in Synovial Tissues Homogenate.

Parameters / **Groups**	**Control**	**Control rats with P.E**	**Control rats with M.E**	**Msu Group**	**Protective rats with P.E**	**Protective rats with M.E**	**Treated rats with P.E**	**Treated rats with M.E**	**Treated rats with R.D**
TNF-α (pg/mL)	90.90 ±9.00 d	93.50 ± 0.12 ^{d}	93.30 ± 0.08 ^{d}	720.00± 10.00 ^{a}	440.00± 6.90 ^{c}	446.50 ± 1.30 ^{c}	517.80 ± 0.70 ^{b}	527.90 ± 0.50 ^{b}	416.20 ± 0.30 ^{c}
% change		2.86	2.64	692.08	384.60	391.20	469.64	480.75	357.87
%of improvement					308.03	300.88	275.42	211.33	334.21
IL-1β (pg/mL)	18.55 ±1.40 d	18.57 ± 0.10 ^{d}	18.56 ± 0.09 ^{d}	107.55 ± 0.26 ^{a}	61.95 ± 0.18 ^{c}	61.99 ± 0.19 ^{c}	74.00 ± 0.90 ^{b}	74.20 ± 0.90 ^{b}	61.98 ± 0.90 ^{c}
% change		0.11	0.05	479.51	233.96	234.18	298.92	300	234.12
%of improvement					245.55	245.34	180.59	179.51	245.39

Data are mean ± SD of eight rats in each group. Statistical analysis is brought out employing SPSS computer program (One-way Analysis-of-Variance, ANOVA; IBM SPSS (version 8) (SPSS Inc., Chicago, IL, USA) connected with co-state computer program, where different letters at the same columnare significant at $p \leq 0.05$. TNF-α: Tumor-Necrosis-Factor Alpha and IL-1β: Interleukin-1-beta. % Change is calculated compared to control group as: (normal control mean – treated mean /normal control mean× 100. % improvement: (mean of positive control − mean of treated group/mean of control) × 100. P.E: petroleum ether extract, M.E: methanolic extract,R.D: reference drug.

3.5.3. Effects of *A. tinctoria* petroleum ether extract of aerial part and methanolic extract of flower on Oxidative Stress in Synovial Tissues Homogenate.

GSH and SOD levels were significantly reduced in arthritic rats (61.11% and 43.90%, respectively) in Table 11. While a significant increase in MDA level (206.10%) was found. GSH, MDA, and SOD levels in the arthritic-protected group with both extracts changed by 32.22 and 35%, respectively for GSH, while by 69.51 and70.49% for MDA and by24.39% and 22.44 %, respectively for SOD. Furthermore GSH, MDA, and SOD levels in arthritic- treated rats treated with both extracts improved by18.69% and 19.89%, respectively, for GSH, by135.61% and 130.49% for MDA and by 8.78% and 9.76% for SOD when compared to standard drug (34.67%, 135.85%, and 17.07%, respectively).

Table 11: Effects of *A. tinctoria* petroleum ether extract of aerial part and methanolic extract of flower on Oxidative Stress and Antioxidant Markers in Synovial Tissues Homogenate.

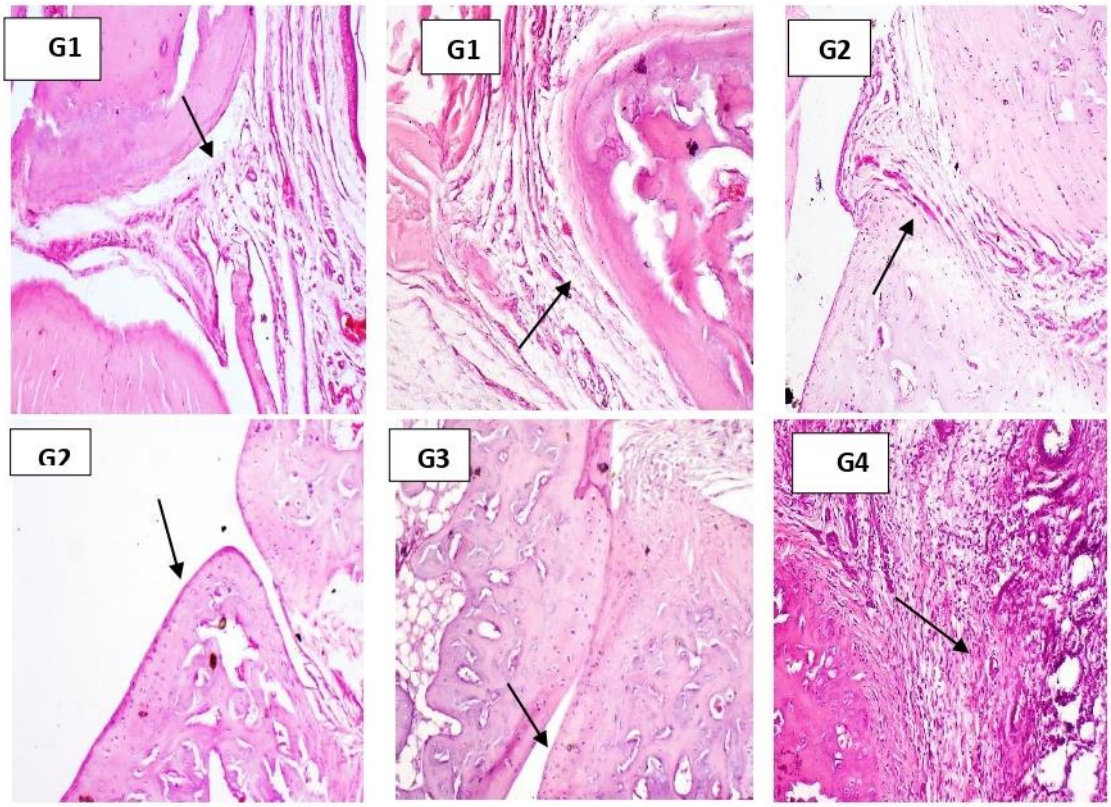
Parameters / **Groups**	**Control**	**Control rats with P.E**	**Control rats with M.E**	**Msu Group**	**Protective rats with P.E**	**Protective rats with M.E**	**Treated rats with P.E**	**Treated rats with M.E**	**Treated rats with R.D**
GSH (mg/g tissue)	90.00 ±9.00 ^{a}	89.50 ± 0.12 ^{a}	87.30 ± 0.08 ^{a}	35.00± 10.00 ^{d}	64.00± 6.90 ^{b}	66.50 ± 1.30 ^{b}	51.80 ± 0.70 ^{c}	52.90 ± 0.50 ^{c}	66.20 ± 0.30 ^{b}
% change		0.56	3.00	61.11	28.89	26.11	42.44	41.22	26.44

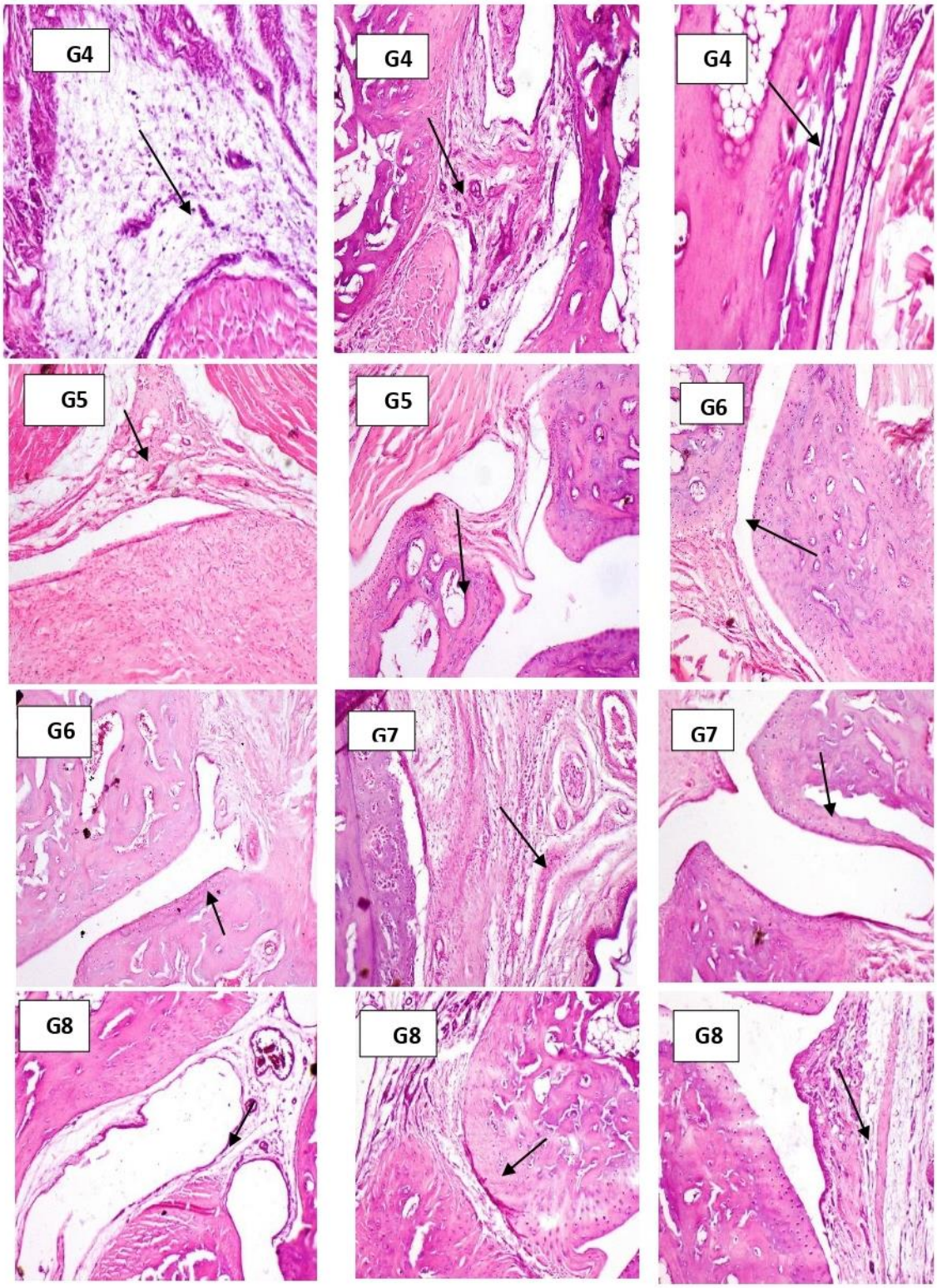
%of improvement					32.22	35	18.67	19.89	34.67
MDA(µmol/g tissue)	4.10 + 0.18 d	4.57 ± 0.10 [d]	4.56 ± 0.09 [d]	12.55 ± 0.26 [a]	6.95 ± 0.18 [c]	6.99 ± 0.19 [c]	7.00 ± 0.90 [b]	7.20 ± 0.90 [b]	6.98 ± 0.90 c
% change				206.10	69.51	70.49	70.73	75.61	70.24
%of improvement					136.58	135.61	135.37	130.49	135.85
SOD(µmol/g tissue)	205.00 + 12.0 [a]	203.00 + 12.0 [a]	201.00 + 10.0 [a]	115.00 + 22.0 [d]	155.00 + 12.0 [b]	159.00 + 32.0 [b]	133.00 + 0.08 [c]	135.00 + 0.08 [c]	150.00 + 22.0 b
% chage		0.98	1.95	43.90	24.39	22.44	35.12	34.15	26.83
% of improvement					19.51	21.46	8.78	9.76	17.07

Data are mean ± SD of eight rats in each group. Statistical analysis is brought out utilizing SPSS computer program (One-way Analysis-of-Variance, ANOVA; IBM SPSS (version 8) (SPSS Inc., Chicago, IL, USA) connected with co-state computer program, where different letters at the same column are significant at $p \leq 0.05$. GSH: Glutathione, MDA: malondialdehyde and SOD: Superoxide dismutase. % Change is calculated compared to control group as: (normal control mean – treated mean /normal control mean× 100. % improvement: (mean of positive control − mean of treated group/mean of control) × 100. P.E: petroleum ether extract, M.E: methanolic extract: R.D: reference drug.

3.5.4. Histopathological Findings

Rat paw showing few inflammatory cells infiltration in synovial membraneand showed normal synovial membrane with normal articular surface.Photomicrograph (6) of protective group with petroleum ether extract showed nearly normal synovial membrane and smooth articular surface. Photomicrograph (7) of Arthritic rats treated with petroleum ether extract showed moderate infiltration of synovial membrane with inflammatory cells and relatively regular articular surface. Photomicrograph (8) of Arthritic rats treated with ethanol extract rat paw showed hemorrhage of synovial membrane, moderate edema of synovial membrane and irregular articular surface with infiltration of synovial membrane with few inflammatory cells. Photomicrograph (9) of protective group with ethanol extract rat paw showed normal articular surface, few inflammatory cells infiltration in synovial membrane and mild edema of synovial membrane.

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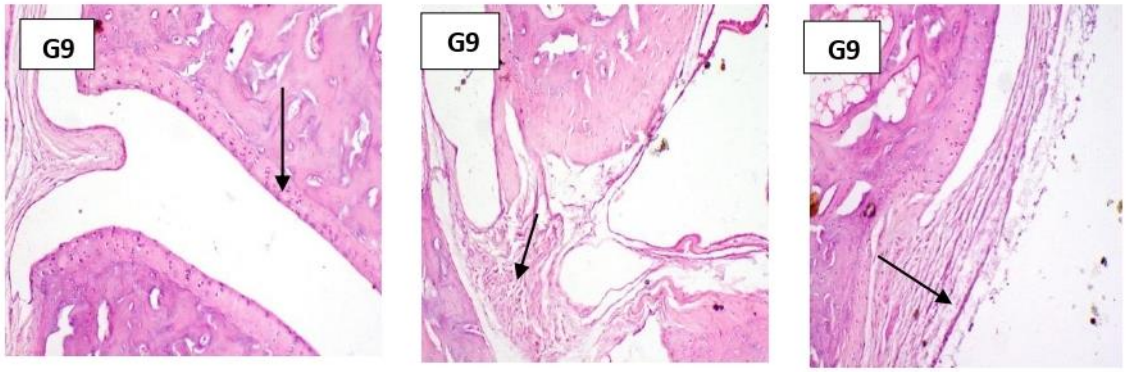

Figure 3. Histopathological alterations of Arthritic and treated rats. Photomicrograph (G1), of control rats showed synovial membrane showing normal histological structure. Photomicrograph (G2), of control rats treated with petroleum ether extract showed synovial membrane showing normal histological structure. Photomicrograph (G3), of control rats treated with ethanol extract showed synovial membrane showing normal histological structure. Photomicrograph (G4), of positive arthritic rats, rat paw showed heavy infiltration of inflammatory cells in synovial membrane, edema of synovial membrane, irregularity of articular surface with widening of inter-trabecular space and multiple bony defects. Photomicrograph (G5), arthritic rats treated with reference drug.

3.5.5. Histopathological lesion scoring

The severity of each lesion discovered in the bone tissue was assigned a number. When compared to the Arthritic group, Table (12) demonstrates a significant decrease in lesion score in both the treated and protective groups.

4. Discussion

In the current work, *A. tinctoria* was subjected to *in vitro* antioxidant and enzyme inhibition evaluation. furthermore, its chemical composition was investigated and the results illustrated that the flowers and aerial parts have large amounts of triterpenes, sterols, phenolic acids and flavonoids, in addition to the great free radical scavenging effects, anti-diabetic, anti-Alzheimer, and anti-inflammatory effects which are attributed to variety of triterpenes (α-amyrin and lupeol), sterols (β-sitosterol and lanosterol) and flavonoids such asapigenin, apigenin 7-*O*-β-D-glucopyranoside, kaempferol 3,7-*O*-dirhamnopyranoside, chrysoeriol 7-*O*-glucopyranoside, and rutin. Triterpenes and sterols make excellent candidates for the development of anti-inflammatory medications and their application in cancer chemotherapy or chemoprevention via the interaction with therapeutic targets related to inflammation [63]**.** Triterpenes and sterols continue to show significant ameliorative effects against a number of metabolic disorders, including diabetes mellitus [64]**.** Flavonoids are naturally occurring polyphenolic secondary metabolites which have a wide range of pharmacological properties, most importantly, antidiabetic and anti-inflammatory effects [65]**.** The structures of flavonoid molecules have a direct impact on their anti-oxidative activity. The number and location of the hydroxyl, the ring-C yoke system, the glycoside or methylation of the hydroxyl, the lipid solubility and the charge distribution are the main factors affecting the anti-oxidative activity of flavonoids [66]**.**

According to a number of studies,flavonoids compounds significantly improve cognitive functions and inhibit or delay the amyloid beta aggregation or neurofibrillary tangles (NFT) formed in Alzheimer's disease AD [67]**.**

It could be established that *A. tinctoria* can be considered for a promising adjuvant therapy in treatment of many common health problems due to valuable constituents and promising bioactivities, further pharmacological and phytochemical researches on *A. tinctoria* are required. Because MSU crystals accumulate in and around joints, gout is an auto-inflammatory disease linked to increased blood urate levels [68] which in turn causes a rise in neutrophils. Increased levels of IL-1β and TNF-α, which are important mediators in the pathophysiology of gout and essential cytokines in inflammation, are correlated with neutrophils [8]**.** Multiple cytokines are regulated by a network that promotes NF-κB interpretation. TNF-α and IL-1β are just a few of the many cytokines that can have their expression increased by NF-κB, a crucial regulator of proinflammatory gene expression [69]. We measured TNF-α and IL-1β levels in arthritic rats caused by MUS in a rat model in the current study which showed significant increase in arthritic rats, while both extracts petroleum ether and methanolic management or protection showed significant decrease. Moreover, oxidative stress is another inflammatory process linked to gout [70].The results of the present study showed a marked decrease in GSH and SOD, along with a significant increase in MDA levels. The oxidative state is brought about by the generation of reactive oxygen species (ROS) and pro-inflammatory cytokines [71]. This leads to the depolymerization of hyaluronic acid, the breakdown of collagen and proteoglycans, the breakdown of

proteins, and the inhibition of proliferation. It is well known that these cells contain low concentrations of antioxidants like SOD and GSH[72].The release of cytokines like GM-CSF and the activation of NF-κB, which encourages osteoclast differentiation and cellular invasion of the cartilage-affected surface, speed up the destruction of bone [73] **.** TNF-α and IL1β can facilitate the regulation of adhesion molecules, additional inflammatory cytokines, and the activation of chondrocytes and osteoclasts, all of which are involved in the degeneration of joints [74] **.**In this study, the GC/MS composition, amino acid content, and phytochemical composition of FE were examined. Its anti-inflammatory and antioxidant potential were assessed in relation to treating or protecting the joints of Wistar albino male rats with arthritis. Furthermore, the present results indicated that all parameters listed above demonstrated a significant improvement in either treatment- or protective-arthritic inflamed rats with both extracts, Furthermore, the synovial membrane and articular surface displayed almost normal structure with low lesion scores in terms of irregularities and articular surface deformities. Chondrocyte necrosis, inflammatory cells infiltrating the synovial membrane, and trabecular space widening (table 1-3, figure 1).Additionally, rats treated with both extracts showed improvements in their inflammatory, clinical, and histological settings when cytokine modulation was present, suggesting that *A. tinctoria*is a viable treatment for arthritis.These results considered noteworthy because, although anti-arthritic drugs lessen inflammation, they do not consistently stop or reverse bone loss and cartilage degradation.The modulatory activity of these inflammatory mediators, such as the downregulation of TNF-α and IL-1 may be linked to the decrease of inflammatory infiltrates in the cartilage of animals treated with both extracts. This suggests that *Anthemis tinctoria*decreased inflammatory infiltrate, synovial hyperplasia, and bone erosion [75]**.**

5. Conclusion

Nutrition plays a crucial role in an individual's overall health. The research has revealed that the petroleum ether extract of aerial part and methanolic extract of flower of *A. tinctoria*, are rich in phenolics, and methanolic extracts of *A. tinctoria*, which are rich in phenolics, flavonoids, and sterols, can effectively mitigate inflammation. The absence of adverse side effects, combined with the beneficial health effects of *A. tinctoria*, underscores its remarkable therapeutic potential demonstrated inour study involving arthritis-induced rats. The mechanisms behind the effects of both extracts appear to involve the inhibition and modulation of nitric oxide and cytokine production and release, both of which are integral to the development of various diseases. Consequently, we anticipate that *A. tinctoria* will serve as a valuable therapeutic alternative for arthritis, contributing to the alleviation and management of this chronic inflammatory disorder.

Conflict of interest: The authors declare that no personal or other potential conflicts of interest.

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