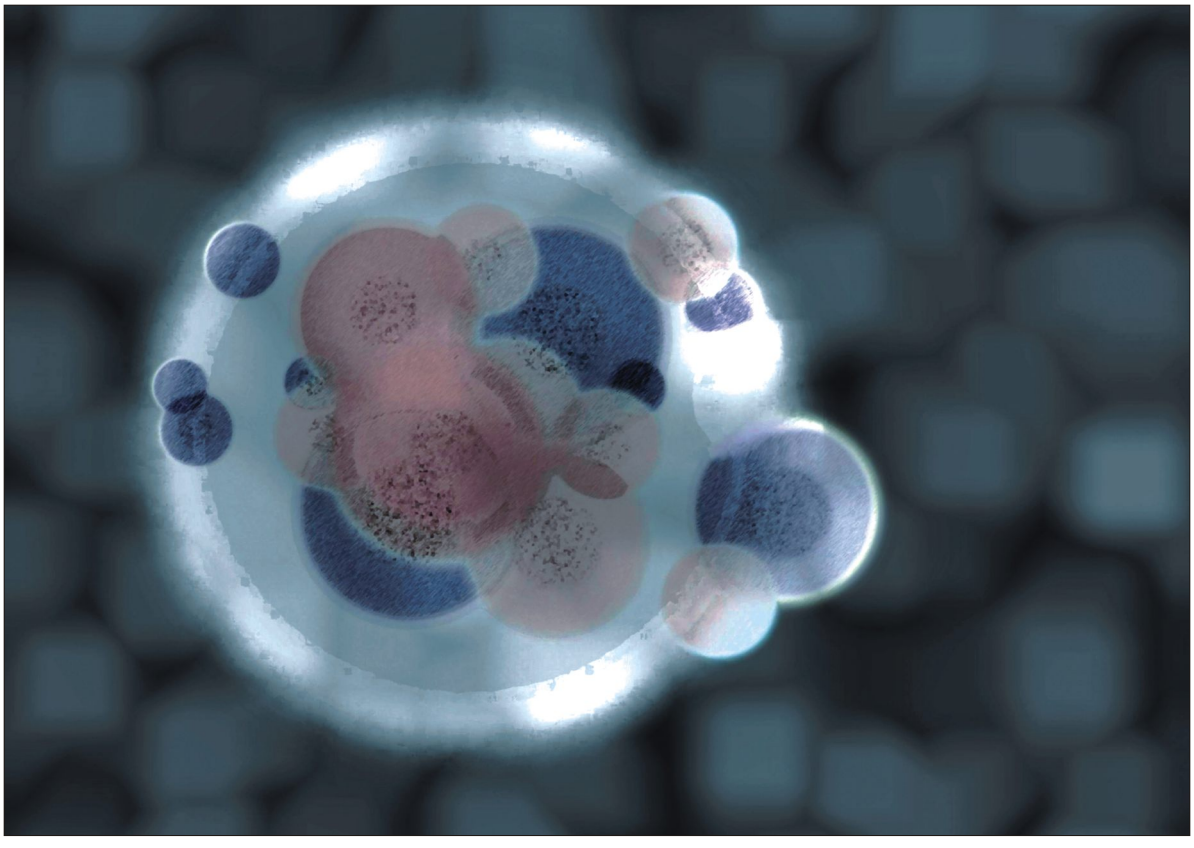

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Research Article
Evaluation of Antioxidant, Anti-inflammatory and Anti-arthritic Activities of Yarrow (*Achillea millefolium*)

[1]Doha A. Mohamed, [2]Eman A. Hanfy and [1]Karem Fouda

[1]Department of Nutrition and Food Sciences, National Research Centre, Dokki, Cairo, Egypt
[2]Department of Biochemistry, Faculty of Agriculture, Cairo University, Cairo, Egypt

Abstract

Background and objective: Rheumatoid arthritis is an autoimmune disease associated with elevation of oxidative stress and inflammatory markers. The aim of the present research was evaluation of the antioxidant, anti-inflammatory and anti-arthritic activities of yarrow aerial parts (YAP). **Materials and Methods:** Proximate composition of YAP was assessed. Total phenolic compounds, total flavonoids, tannins and alkaloids contents of YAP were determined. Fatty acids methyl esters of petroleum ether extract of YAP were analyzed. The antioxidant activity of YAP was assessed. Acute anti-inflammatory activity of petroleum ether and ethanol extracts of YAP was evaluated. Anti-arthritic activity of ethanol extract of YAP was evaluated using adjuvant-induced arthritis (AIA) in rats. **Results:** Chemical compositions of YAP clarified presence of carbohydrate (58.29%), protein (10.33%), fat (3.85%), crude fibers (17.32%) and ash (3.37%). Total phenolic, total flavonoids, tannins and alkaloids were presence in YAP by 0.43±0.003 mg GAE g^{-1}, 0.31±0.008 mg QE g^{-1}, 0.96±0.006 mg and 1.52%, respectively. Ethanol extract of YAP showed free radical scavenging activity ranged from 47.8-87.8%. Fatty acids methyl esters analysis revealed that saturated and unsaturated fatty acids were presented by 50.55 and 16.16%, respectively. Linoleic acid and linolenic acid was present by 8.44 and 7.72%, respectively. Ethanol extract of YAP was promising as anti-inflammatory agent in carrageenan induced rat paw edema and xylene-induced mice ear edema. Ethanol extract of YAP possess anti-arthritic effect in AIA in rats through inhibition of swelling in arthritic rats, reduction of oxidative stress and inflammation. **Conclusion:** Ethanol extract of YAP showed promising antioxidant, anti-inflammatory and anti-arthritic activities. These activities may be attributed due to the presence of total phenolic, total flavonoids and tannins. Ethanol extract of YAP may be used as anti-arthritic agents in rheumatoid arthritis patients.

Key words: Yarrow aerial parts, *Achallea millefolium*, antioxidant, anti-inflammatory, anti-arthritic, adjuvant-induced arthritis

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Corresponding Author: Doha Abdou Mohamed, Department of Nutrition and Food Sciences, National Research Centre, Cairo, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by inflammation and proliferating synovitis. RA is distinguished by synovial inflammation followed by destruction of joint. It leads to damage of cartilage and bone destruction, resulting in joint deformities and functional disability[1]. Arthritis affects approximately 0.5-1% of the worldwide population and it commonly results in detriment of the quality of life of the patients. The pathogenesis of arthritis involves immune imbalance of the endogenous system (autoimmune disease). Causative factors include immune imbalance, oxidative stress, genetics and environment[2]. Oxidative stress plays an important role in RA. In RA as one of the autoimmune diseases cellular immune system and body's endogenous and/or exogenous antigens interaction produce reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS include highly toxic superoxide (O_2^-) and peroxynitrite ($ONOO^-$) radicals, which activate the signaling cascades of inflammatory cells to synthesize pro-inflammatory cytokines and chemokines[3]. ROS can activate different signaling pathways having a vital importance in the pathophysiology of RA[4]. Non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs and corticosteroids are the effective drugs used in the treatment of rheumatoid arthritis. Using synthetic anti-inflammatory drugs produce side effects such as gastrointestinal disorders, liver damage, water retention and renal failure[5,6]. So the development of alternative anti-inflammatory agents and antioxidants from natural sources has attracted considerable attention. Plants are major source of phytochemicals, which play an important role in drug discovery[7]. *Achillea millefolium* L. (also known as yarrow) family Asteraceae has been used in many applications such as medicine, veterinary science and cosmetics[8]. The flowering herbs were reported as possessing tonic, antispasmodic, vulnerary and diaphoretic activities, among others and therefore are recommended for colds, flatulence, hysteria, spasmolytic, haemostatic, antidiabetic, cholagogue, antitumor, antifungal, antiseptic and liver protective effects[9,10]. *Achillea millefolium* can be consumed as essential oil, infusion or alcohol extract, decoction, hydroalcoholic, or aqueous extract[11]. The aim of the present research was evaluation of the antioxidant, anti-inflammatory and anti-arthritic activities of yarrow aerial parts.

MATERIALS AND METHODS

Materials

Plant sample: Aerial parts of Yarrow (*Achallea millefolium*) were obtained from Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, Giza.

Chemicals: λ-Carrageenan, type IV (Sigma-Aldrich, USA): One-percent carrageenan suspension was prepared freshly for induction of acute inflammation. Xylene and all chemicals and reagents were HPLC grade (purity 99%) obtained from Sigma chemical Co. (Sigma-Aldrich, USA). Freund's complete adjuvant (Sigma-Aldrich, USA) was used for induction of adjuvant arthritis in rats.

Animals: Male Sprague Dawley rats 120-150 g was used in carrageenan-induced rat hind paw edema and in adjuvant induced arthritis rat model. Animals were kept individually in stainless steel cages at room temperature of 25±2°C and a relative humidity of about 55%, water and food were given *ad-libtium*. Adult normal male albino mice of 25-35 g body weight were used in xylene-induced mice ear edema.

Diets: A balanced diet composed of 10% protein supplemented from casein, 10% corn oil, 23.5% sucrose, 47% maize starch, 5% fiber, 3.5% salt mixture[12] and 1% vitamin mixture[13] was prepared for feeding animals all over the experimental period.

Methods: All phytochemical screening were done in Department of Biochemistry, Faculty of Agriculture, Cairo university, while all the biological experiments were done in Nutrition and Food Sciences Department, National Research Centre, Cairo, Egypt at the period from April, 2017 till January, 2018.

Preparation of plant material: The aerial parts of Yarrow were washed with tap water then dried in an air-circulated oven at 40°C to dryness.

Preparation of plant extracts: The dried powder of aerial parts of yarrow were placed in a continuous extraction apparatus (Soxhelt) and subjected to successive extraction using petroleum ether (40-60°C) then ethanol. The solvent was completely removed by evaporation under reduced pressure at a temperature not exceeding 40°C. All extracts were kept in deep-freeze till used.

Proximate composition of yarrow aerial parts: Proximate compositions including crude protein content, crude fiber, ash and crude fat were analyzed according to AOAC[14].

Determination of total phenolics, total flavonoids, total tannins and total alkaloids in yarrow aerial parts: Total phenolics, total flavonoids and total tannins were determined according to the method described by Meda *et al.*[15], Zhuang *et al.*[16] and Saxena *et al.*[17], respectively. Gallic acid and quercetin were used as standard in the case of total phenolic and total flavonoids, while tannic acid was used as standard in the case of total tannins. The total phenolic content expressed as mg gallic acid equivalents (GAE)/g dry weights and total flavonoids were expressed as mg quercetin equivalents (QE)/g dry weights, while total tannins was expressed as mg/g dry weight. Total alkaloids were determined according to Krishnaiah *et al.*[18]. Total tannins were expressed as mg/g dry weight. Three replicates were measured and the results were expressed as Mean±SE.

Evaluation of free radical scavenging activity: Free radical scavenging activity was determined using 2,2 diphenyl-1-picryl hydrazyl (DPPH) method as described by Gyamfi *et al.*[19]. The reaction mixture contained sample ethanol extract 40, 60, 80 and 100 μg mL^{-1} or 80% MeOH as a blank and 1 mL of 0.002% (w/v) solution of DPPH in methanol. Discoloration was measured at 517 nm after incubation for 30 min. This test was carried out in triplicate and the DPPH radical's concentration was calculated using the following Eq.:

$$\text{DPPH scavenging effect (activity) (\%)} = \frac{A_o - A_1}{A_o} \times 100$$

Where:
A_o = Absorbance of the control
A_1 = Absorbance of the samples[20]

Assessment of fatty acids of yarrow aerial parts petroleum ether extract: Fatty acid methyl esters of the studied extracts were prepared according to AOAC[21] to be subjected to GLC analysis of fatty acids. Assessment of the methyl ester was carried out by injecting 2 uL into a Hewlett Packard HP-system 6890 gas chromatograph equipped with FID. HP-5 capillary column (30 m×0.32 mm i.d., 0.25 um film thickness) was used to separate the different methyl esters. The chromatographic analysis conditions were: Initial temperature 70 C with a hold for 1 min, then rose to 120°C at a rate of 40°C min^{-1} with 2 min hold then the temperature was finally raised to 220°C at a rate of 4°C min^{-1} with another 20 min hold. The injector and detector temperatures were 250 and 280°C, respectively. Identification of the fatty acid methyl esters were carried out by direct comparison of retention times of each of the separated compounds with standards of the fatty acid methyl esters analyzed under the same conditions. Quantization was based on peak area integration.

Preparation of dosage form: Ethanol extract of the aerial parts of yarrow was dissolved in water, while petroleum ether extract was emulsified separately in water using gum acacia as emulsifying agent.

Studying the anti-inflammatory effects of the petroleum ether and ethanol extract of yarrow aerial parts: The anti-inflammatory activity of the studied extracts was evaluated using carrageenan-induced rat hind paw edema model and xylene-induced mice ear edema.

Carrageenan-induced rat hind paw edema: Eighteen rats were divided into three groups each group contains 6 rats. Before any treatment, the volume of the paw of each animal was determined using vernier calipers. Two groups were given one oral dose of ethanol or petroleum ether extracts of aerial parts of yarrow (300 mg kg^{-1} rat body weight). The third group was served as control group[22]. One hour after these administrations, each rat received in its left hind paw a subplantar injection of 1% carrageenan suspension (0.1 mL per animal). Then the thickness of the paw of each rat was measured at 1, 2, 3 and 4 h after the injection of the inflammatory agent. The thickness of inflammation was calculated by subtracting the thickness of paw before inflammation from that of the inflamed paw at the different times intervals. The percentage of inhibition compared with the control group was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Thickness of edema (control)} - \text{Thickness of edema (test)}}{\text{Thickness of edema (control)}} \times 100$$

After the last measurement of the paw thickness, 4 h from carrageenan injection.

Xylene-induced mice ear edema: The acute inflammatory activity of petroleum ether and ethanol extracts of yarrow aerial parts was evaluated using xylene-induced mice ear edema as previously described[23,24]. The mice were randomly divided into three groups of eight animals each. Group one

vehicle control, group two and three mice were given oral dose of petroleum ether or ethanol extracts of aerial parts of yarrow (300 mg kg^{-1} body weight). These treatments were administered orally for six consecutive days. On day 7 at 1 h after oral administration, 30 µL of xylene was applied on both surfaces of right ear to induce edema. The left ear served as a control. After 30 min from xylene treatment the mice ears were removed. Each ear was weighed and the edema weight difference between the two ears was measured. The percentage of inhibition of inflammation at the end of the study compared with the control group was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Weight of edema (control)} - \text{Weight of edema (test)}}{\text{Weight of edema (control)}} \times 100$$

Evaluation of the anti-arthritic activity of yarrow ethanol extract in rats using adjuvant induced arthritis: Eighteen rats were divided into 3 groups each comprised six rats. The first group was given daily oral dose 300 mg of ethanol extract of the aerial parts of yarrow kg^{-1} rat body weight. The other two groups served as control (one group as control normal and the second served as arthritic control rats). A day after starting oral medication, arthritis was induced in all rats (except one of the control groups which is normal group) by subcutaneous injection of Freund's complete adjuvant into the subplantar region of the right hind-foot paw[25]. The oral medication continued for 14 days. Rats were maintained on the balanced diet all over the experiment. The inflamed paws of all arthritic rats were measured before induction of arthritis and three times per week using vernier calipers. Inflammation volume was calculated for all arthritic rats by subtracting the foot volume in the final of the experiment from its volume in the starting of the study. Food intake and body weight of all rats were recorded all over the experiment. Blood samples were collected at the end of the study from overnight fasting rats. Blood hemoglobin was determined in blood samples using the method of Van Kampen and Zijlstra[26]. Each blood sample was divided into two parts. The first part mixed with trisodium citrate (109 mmol L^{-1}) for determination of erythrocyte sedimentation rate (ESR)[27] as an inflammatory biomarker. The second part was mixed with heparin for separation of plasma and determination of plasma tumor necrosis factor-α (TNF-α)[28] (an inflammatory biomarker), malondialdehyde (MDA)[29] as indicator of lipid peroxidation and total antioxidant capacity (TAC) Koracevic *et al.*[30] as an antioxidant biomarker. Plasma level of creatinine[31] and urea[32] were determined as indicator of kidney function, while the activity of aspartate transaminase (AST)[33] and alanine transaminase (ALT)[33] were determined as indicator of liver function.

All animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Cairo, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Statistical analysis: The results of animal experiments were expressed as Mean±SE and they were analyzed statistically using one-way analysis of variance ANOVA followed by Duncan's test. Student's t-test (2 tailed) was applied to compare the results of swelling volume in control arthritic rats and arthritic rats given ethanol extract of yarrow.

RESULTS

Chemical compositions of yarrow aerial parts are shown in Fig. 1a. The results revealed that yarrow aerial parts contain high percentage of carbohydrate (58.29%) and crude fibers (17.32%). Percentage of protein was 10.33%, fat was presence by 3.85% and ash content was 3.37%.

Alkaloids, tannins, total phenolic and total flavonoids contents of yarrow aerial parts are presented in Fig. 1b. Alkaloids were presented in yarrow aerial parts by 1.52%. Tannins, total phenolics and total flavonoids were presented in yarrow aerial parts by 0.96±0.006 mg g^{-1}, 0.43±0.003 mg GAE g^{-1} and 0.31±0.008 mg QE g^{-1}, respectively.

Fatty acids present in petroleum ether extract of yarrow aerial parts are shown in Table 1. Saturated fatty acids were presented by 50.55%, while unsaturated fatty acids were presented by 16.16%. Arachidic acid was the highest content of saturated fatty acids (20.11%), while palamitic acid (3.14%) was the lowest one. Linoleic acid and linolenic acid was present by 8.44 and 7.72%, respectively in petroleum ether extract of yarrow aerial parts.

Table 1: Fatty acids contents of petroleum ether extract of YAP (as percentage of total fatty acids)

Fatty acids	Fatty acids (%)
Myristic acid (C14:0)	11.10
Palmitic acid (C16:0)	3.14
Linoleic acid (C18:2)	8.44
Linolenic acid(C18:3α)	7.72
Arachidic acid (C20:0)	20.11
Behenic acid (C22:0)	7.98
Lignoceric acid (C24:0)	8.22
Total saturated fatty acids	50.55
Total unsaturated fatty acids	16.16

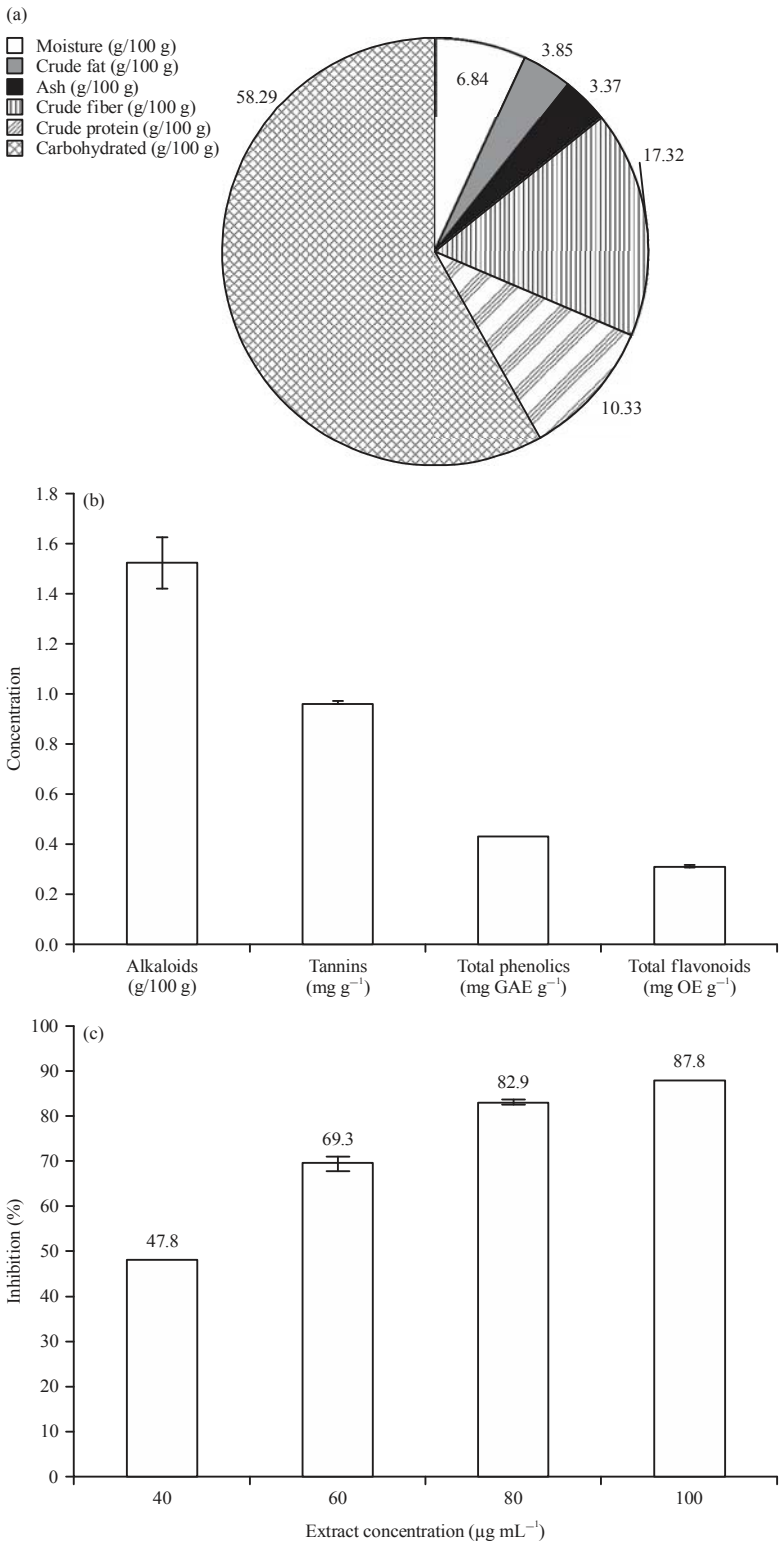

Fig. 1(a-c): (a) Proximate composition of yarrow aerial parts, (b) Alkaloids, tannins, total phenolic and total flavonoids contents of yarrow aerial parts and (c) Free radical scavenging activity of ethanol extract of yarrow aerial parts

Four concentrations ranging from 40-100 µg mL^{-1} of the ethanolic extract of yarrow aerial parts were tested for their antioxidant potential using free radical scavenging activity (DPPH) method. It was observed that free radicals were

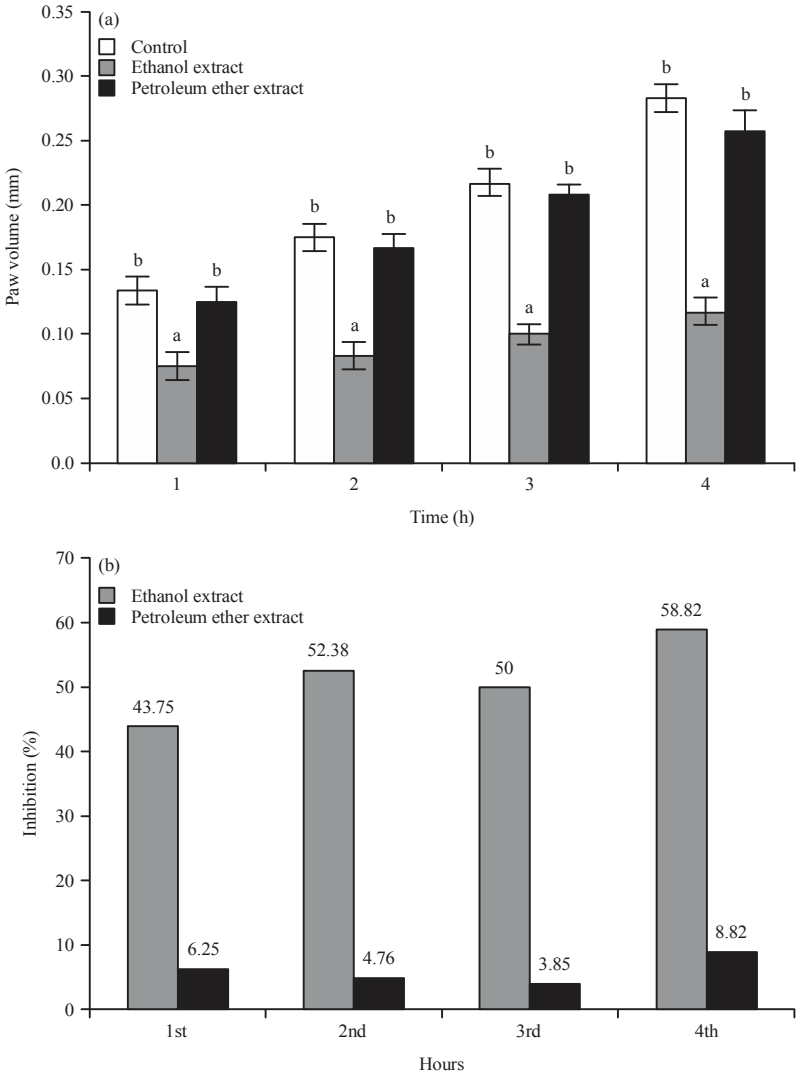

Fig. 2(a-b): (a) Swelling of different experimental groups after carrageenan injection and (b): Inhibition percent of inflammation after carrageenan injection

scavenged by ethanol extract of yarrow aerial parts at different concentrations. Ethanol extract of yarrow aerial parts showed free radical scavenging activity ranged from 47.8-87.8% (Fig. 1c).

Studying the anti-inflammatory activity of the petroleum ether and ethanol extract of Yarrow aerial parts: Two models of acute anti-inflammatory were used in the present study, the first one is carrageenan induced edema in rats paw and the second is xylene-induced mice ear edema.

Carrageenan induced edema in rats paw: Swelling of different experimental groups after carrageenan injection are shown in Fig. 2a. In control group, injection of carrageenan in rat paw induced inflammation from the 1st h till the 4th h. Paw swelling was increased gradually to reach its maximal intensity 4 h after carrageenan injection. Pretreatment by ethanol extract of yarrow aerial parts significantly reduced the carrageenan-induced edema. Oral administration of petroleum ether extract of yarrow aerial parts reduced the carrageenan-induced edema non-significantly compared with control. The highest inhibition percent (Fig. 2b) observed by ethanol extract after 4 h from carrageenan injection (58.8%). Also the petroleum ether extract showed the maximum inhibition percent after 4 h from carrageenan injection (8.82%). Ethanol extract of yarrow aerial parts inhibit inflammation in the 1st h from carrageenan injection and continued till the end of the experimental period (4 h from carrageenan injection). The results indicated that ethanol extract of yarrow aerial parts is effective as anti-inflammatory

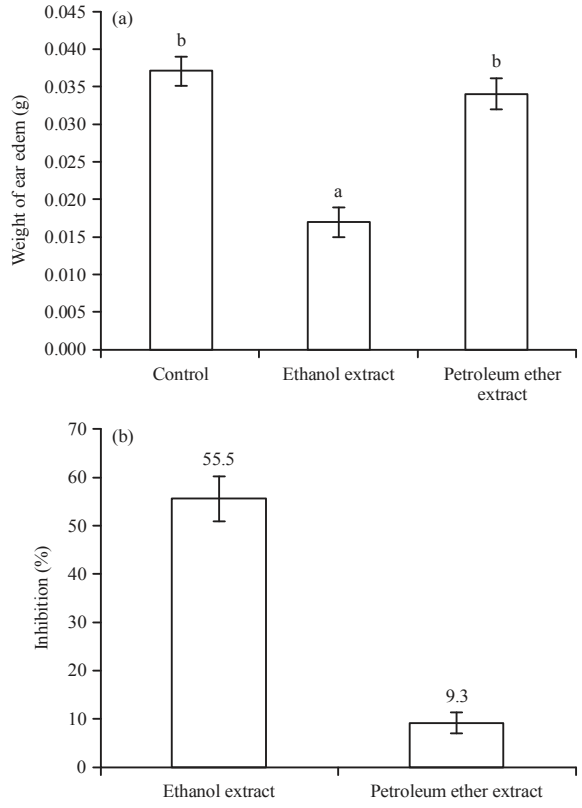

Fig. 3(a-b): (a) Edema weight of different experimental groups in xylene-induced mice ear edema and (b) Inhibition percent of ethanol and petroleum ether extract of yarrow aerial parts against xylene induced mice ear edema

In each column same letter means non-significant difference while different letter means significant difference at 0.05 probability

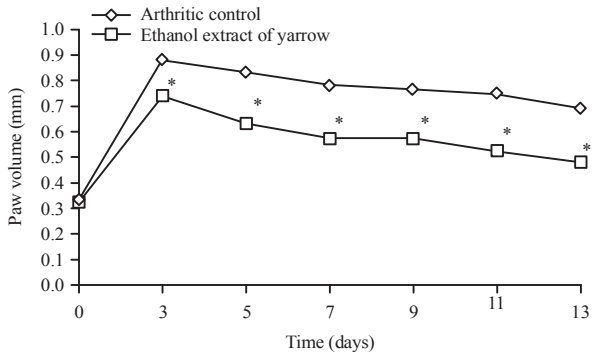

Fig. 4: Paw swelling (mm) of different arthritic rats groups

Values significantly differ from arthritic control: *p<0.001

agent against carrageenan induced inflammation, while petroleum ether extract was non-effective towards this model of inflammation in rats.

Xylene-induced mice ear edema: Topical application of xylene in mice ear produced increase in mice ear weight. Oral administration of ethanol and petroleum ether extracts of yarrow aerial parts before xylene application attenuate the increase in the weight of mice ears by 55.5 and 9.3% (Fig. 3b). The reduction of weight of mice ears was significantly only in the group of mice given ethanol extract of yarrow aerial parts (Fig. 3a).

Studying the anti-arthritic effect of ethanol extract of yarrow aerial parts: From the results of acute inflammation, ethanol extract \of yarrow aerial parts was the promising in both anti-inflammatory models used in the present research. So we decided to study ethanol extract of yarrow aerial parts only as anti-arthritic agent.

Effects of ethanol extract of yarrow aerial part on rat hind paw volume: Rats paw swelling of arthritic rats are shown in Fig. 4. The swelling of the hind paw increased over time up to the thirteen day. Oral administration of ethanol extract of the aerial parts of yarrow significantly reduced paw edema compared with arthritic control ($p<0.001$). Ethanol extract (0.167 ± 0.017) of aerial parts produced 53.4% inhibition of swelling in arthritic rats compared to arthritic control (0.358 ± 0.008).

Evaluation of biochemical and nutritional changes in arthritic rats: Blood erythrocyte sedimentation rate, hemoglobin and plasma MDA, TAC and TNF-α of normal and arthritic rats are presented in Table 2. ESR elevates significantly in arthritic rats compared with normal control. Ethanol extract of yarrow aerial parts attenuate the elevation in ESR in arthritic rats significantly compared with arthritic control. Blood hemoglobin reduced significantly in arthritic rats compared with normal rats. Administration of ethanol extract of aerial parts of yarrow elevates hemoglobin concentration in blood but still significantly lower than normal control. This reduction in blood hemoglobin in arthritic rats is indicator to anemia. Plasma level of MDA as indicator of lipid peroxidation showed significant elevation in arthritic rats when compared with normal rats. TNF-α plasma levels as inflammatory marker increased significantly in arthritic control rats in comparison to normal rats. Arthritic rats given oral administration of ethanol extract of yarrow aerial parts reduced the elevation in MDA and TNF-α compared to arthritic control but still significantly higher than normal rats. Plasma total antioxidant capacity as indicator of antioxidant status showed significant reduction in arthritic rats compared to normal rats. This reduction in antioxidant status was significantly attenuated by oral administration of ethanol extract of yarrow aerial parts.

Table 2: Blood ESR, Hb and plasma MDA, TNF-α and TAC of normal and arthritic rats

Groups	ESR (mm h^{-1})	Hb (g dL^{-1})	MDA (nmol mL^{-1})	TAC (mg dL^{-1})	TNF-α (pg mL^{-1})
Normal	1.33±0.211[a]	14.5±0.278[c]	7.5±0.428[b]	2.11±0.077[b]	13.8±0.469[a]
Arthritic control	5.00±0.258[c]	7.4±2680[a]	21.2±0.945[a]	1.16±0.064[a]	30.8±1.195[c]
Ethanol extract of YAP	3.20±0.307[b]	8.8±0.422[b]	15.2±0.477[b]	1.99±0.111[b]	23.0±0.816[b]

In each column same letter means non-significant difference while different letter means significant difference at 0.05 probability. The data are expressed as Mean±Standard error

Table 3: Effect of different methanol extracts on liver and kidney functions of rats

Groups	ALT (U m L^{-1})	AST (U m L^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})
Normal control	12.8±0.946[a]	43.3±0.882[a]	27.3±0.882[a]	0.627±0.024[a]
Arthritic control	13.9±0.764[a]	45.0±0.894[a]	28.3±0.667[a]	0.645±0.027[a]
Ethanol extract of YAP	13.2±0.601[a]	43.8±1.014[a]	27.7±0.715[a]	0.62±0.035[a]

In each column same letter means non-significant difference while different letter means significant difference at 0.05 probability. The data are expressed as Mean±Standard error

Table 4: Nutritional parameters of different experimental groups (Mean±SE)

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total food intake (g)	Feed efficiency ratio
Normal control	129.7±5.313[a]	176.8±2.993[b]	47.2±4.384[b]	164.8±3.389[b]	0.288±0.030[b]
Arthritic control	129.7±5.167[a]	147.0±4.073[a]	17.3±2.859[a]	128.3±4.957[a]	0.133±0.020[a]
Ethanol extract of YAP	129.5±4.136[a]	151.2±5.002[a]	21.7±2.603[a]	137.2±3.069[a]	0.158±0.018[a]

In each column same letter means non-significant difference while different letter means significant difference at 0.05 probability. The data are expressed as Mean±Standard error

In the present study creatinine and urea plasma levels as kidney functions indicator showed non-significant changes in all the studied groups (Table 3). Transaminases activity (AST and ALT) as liver function indicator in the present study showed non-significant changes in all the studied groups (Table 3).

Nutritional parameters results are shown in Table 4. Adjuvant induced arthritis in rats reduced final body weight, body weight gain and feed efficiency ratio significantly compared with normal rats. Also total food intake showed significant reduction in arthritic rats when compared with normal rats. Arthritic rats orally administrated by ethanol extract of yarrow aerial parts showed improvement in all the studied nutritional parameters but still lower than normal rats.

DISCUSSION

In the present research aerial parts of yarrow (*Achillea millefoilum* L.) family Asteraceae were successively extracted using petroleum ether and ethanol. Both yarrow extracts were evaluated in acute inflammation, while in chronic inflammation only ethanol extract was evaluated. Acute inflammation was assessed through carrageenan induced paw edema and xylene-induced mice ear edema. Chronic inflammation was evaluated in adjuvant-induced arthritis as rat model mimic to rheumatoid arthritis in human.

Chemical composition of the dried aerial parts of yarrow revealed presence of phenolic compounds, flavonoids and alkaloids. The aerial parts of yarrow possess free radical scavenging activity using DPPH method. This activity may be attributed to the presence of phytochemical compounds such as total phenolic compounds and flavonoids as observed from the chemical composition in the present study. Various flavonoids were reported from yarrow such as resveratrol, morin, myricetin, naringin, quercetin and kaempferol[34-36]. Methanol extract of yarrow aerial parts showed significant antioxidant activity against DPPH[37]. Also five phenolic compounds isolated from yarrow (caffeic acid, neochlorogenic acid, ferulic acid, luteolin and apigenin) observe high antioxidant activity using DPPH[34].

Acute inflammation beings very quickly and lasts a few h and associated with exudation of fluid and plasma proteins and leukocyte emigration, while chronic inflammation is associated with presence of lymphocyte and macrophages, vascular proliferation, fibrosis and tissue destruction[38]. Macrophages promote the production of inflammatory mediators such as IL-1b , TNF-α, prostaglandins (PGE_2 especially), reactive oxygen spices, nitric oxide in chronic inflammatory diseases such as rheumatoid arthritis[39]. So, one of the mechanisms of action of anti-inflammatory agents is slowing down the production of inflammatory markers. The development of alternative anti-inflammatory agents and antioxidants from natural sources has attracted considerable attention. In the present research yarrow aerial parts extracts were used as an antioxidant and anti-inflammatory agents.

In the present study petroleum ether and ethanol extract of yarrow aerial parts were evaluated as anti-inflammatory agents using carrageenan induced rat paw edema an *in vivo* test for the evaluation of acute inflammatory activity and xylene-induced mice ear edema. In the

carrageenan-induced paw edema model, the subcutaneous injection of carrageenan into the rat paw produced plasma exudation associated with the migration of neutrophils into the inflamed site and increased arachidonic acid product release[40]. Carrageenan induced edema in rats is a biphasic, the first one has been attributed to histamine and serotonin release. The second phase is attributed to prostaglandin release. Histamine and/or serotonin play an important role in the 1st h of carrageenan injection[41].

Anti-arthritic activity of yarrow aerial parts ethanol extract was evaluated in the present study using adjuvant-induced arthritis (AIA) in rats, which used previously as model for evaluation of compounds that may be used as drugs for RA therapy. Freund's complete adjuvant is used for induction of AIA. Complete adjuvant injection produce an immune reaction associated with inflammatory destruction of cartilage, bone of the distal joints and swelling of surrounding tissues. Complete adjuvant produced a series of cellular events leading to T-cell activation and polyarthritis[42]. Inflammatory cytokines such as IL-6 and TNF-α have been implicated in AIA[43]. AIA is characterized by inflammation, oxidative stress and reduction of body weight[44].

Yarrow aerial parts ethanol extract possess anti-arthritic effect through inhibition of paw inflammation and reduction of oxidative stress and inflammatory markers (reduced TNF-α plasma levels).

Plasma levels of MDA as lipid peroxidation marker elevates in arthritic rats, ethanol extract of yarrow aerial parts attenuate this elevation. This reduction of MDA levels in rats given oral administration of yarrow ethanol extract was companied by elevation of TCA as antioxidant status indicator. Oxidative stress is chemical imbalance between antioxidants and pro-oxidants leading to damaging effects. Oxidative stress is closely related to RA[45,46]. TNF-α showed significant elevation in arthritic rats, which reduced significantly by administration of ethanol extract of yarrow aerial parts. Arthritis is associated with production of inflammatory cytokines such as IL-6 and TNF-α[47]. TNF-α plays an important role in RA, in addition, therapies targeting TNF-α is also recognized as effective treatments for patients with RA[48].

ESR elevates significantly in arthritic rats. Ethanol extract of yarrow aerial parts attenuate the elevation in ESR in arthritic rats. ESR as inflammatory marker in RA is correlated positively with TNF-α[49]. ESR is an excellent index of assessing the therapeutic effect of anti-inflammatory agents[50]. In the present study ESR reduced significantly in arthritic rats given oral administration of ethanol extract of yarrow aerial parts and this reduction is accompanied by reduction in plasma levels of TNF-α.

In the present study hemoglobin reduced significantly in arthritic rats as indicator of anemia. Hemoglobin concentration can be dissociated from other disease activity components due to a slower fluctuation than acute phase reactants in response to inflammation. From this perspective, hemoglobin may be of utility for monitoring chronic conditions such as RA[51]. Anemia is present in 75% of rheumatoid arthritis patients and positively is related with articular inflammation[52]. Anemia is one of the most infirm extra-articular complications in RA patients[53]. Anemia of chronic disease and iron-deficiency anemia are the mainly cause of anemia in RA. Inflammatory cytokines such as IL-6 and TNF-α play an important role in anemia in RA through hepcidin an iron regulatory hormone and are associated with the impairment of erythropoiesis as well as blunted erythropoietin response[54-56]. In inflammation, cytokines such as TNF-α or interleukin-6 may both lower blood hemoglobin concentrations[57]. Ethanol extract of yarrow aerial parts elevate hemoglobin concentration in arthritic rats. The elevation in blood hemoglobin concentration in rats given oral administration of ethanol extract of yarrow aerial parts may be attributed to its anti-inflammatory activity through reduction of the inflammatory cytokine TNF-α as found in the present research.

The anti-inflammatory and anti-arthritic activities of ethanol extract of yarrow aerial parts may be attributed to the presence of total phenolic, flavonoids and tannins in the extract. High intake of phenolic-rich food is responsible to reduction rate of chronic diseases. Phenolic compounds are responsible for this beneficial effect[45]. Some phenolic compounds have exhibited anti-inflammatory properties[58]. The mechanism of action of phenolic compounds as anti-inflammatory agent is similar to non-steroidal anti-inflammatory drugs through inhibition of cyclooxygenase (COX) also inhibiting gene expression of pro-inflammatory mediators. Phenolic compounds can up/down regulate transcriptional factors, like nuclear factor-kB (NFkB) or Nrf-2, in inflammatory and antioxidant pathways[59,60]. Dietary flavonoids have shown to modulate inflammatory mediators such as IL-6 and TNF-α Stote *et al.*[61]. Phytochemical possess strong antioxidant ability as well as anti-inflammatory action. Antioxidants can reduce inflammation through inhibition of the production of inflammatory mediators[62,63].

Arthritic rats showed significant reduction in all nutritional parameters this reduction observed in nutritional parameters was expected since rheumatoid arthritis is associated with loss of lean tissues that contain most of the body's protein[64]. Reduction of body weight gain in arthritic rats observed in the present study may be due to tissue destruction in

adjuvant arthritic rats. AIA is usually associated reduction of body weight through wasting muscle due to enhanced protein breakdown by the ubiquitin-proteasome proteolytic pathway[65,66]. Reduction of total food intake may be also responsible of the reduction in body weight gain observed in the present study. RA is associated with anorexia[64]. Ethanol extract of yarrow aerial parts produced improvement in all the studied nutritional parameters but still lower than normal control. Improvement in body weight gain in arthritic rats is an indicator for improvement of the adjuvant arthritis[67].

CONCLUSION

Ethanol extract of YAP showed promising antioxidant, anti-inflammatory and anti-arthritic activities. These activities may be attributed due to the presence of total phenolic, total flavonoids and tannins. Ethanol extract of YAP may be a potential therapeutic candidate for the treatment of rheumatoid arthritis.

SIGNIFICANT STATEMENT

This study confirmed that ethanol extract of yarrow aerial parts as an alternative treatment for rheumatoid arthritis. The extract contains high content of phenolic compounds and flavonoids which possess antioxidant in-vitro in DPPH method. It's so important to use this rich source of phenolic compounds and flavonoids in preparation of alternative treatment to enhance human health and reduce the risks of rheumatoid arthritis a major public health problem.

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