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Anti-Inflammatory Screening of *Ulmus pumila L* ethanolic extract

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Abstract
Deciduous trees of Ulmus species were reported to exhibit antibiotic, antifungal, antioxidant, anti-inflammatory, hepatoprotective, neuroprotective, antiangiogenic, and antiviral effects that rich in flavonoids. In this study, phytochemical analysis and pharmacological activities of *Ulmus pumila L* were studied. The upper parts of *Ulmus pumila L* plants were grinded and extracted in 80% ethanol with a ratio of 1:10, infusing by maceration method. The total crude extract was fractioned into a group of solvents with increasing polarity: hexane, chloroform, ethyl acetate, and n-butanol. The treatment with ethanolic and ethyl-acetic fractions reduced inflammatory symptoms showing protective effects for carrageenan-induced paw edema. These preliminary findings may support its traditional medicinal use and could be promising candidate with application in the treatment of inflammatory symptoms.

Key words: Dwarf elm leaves /*Ulmus pumila L.*/, carrageenan, rat, anti-inflammatory, toxicity

Introduction
Ulmus species are deciduous trees found mostly in Asia, Europe, and North America in the northern temperate zone. [1]. Among them 3 species of elms are grown in Mongolia: dwarf elm (*Ulmus pumila L.*), large-seeded elm (*Ulmus macrocarpa Hance.*), and Japanese elm (*Ulmus davidianavar.japonica*) [2]. As reported, *Ulmus* species were to exhibit antibiotic, antifungal, antiangiogenic, anti-inflammatory, hepatoprotective, antioxidant, neuroprotective, and antiviral effects. Moreover, studies on *Ulmus* genus have shown the presence of various types of phytoconstituents like terpenoids, steroids, phenolics, and polysaccharides [1].
Ulmus pumila L., dwarf elm or Siberian Elm, is widely distributed across Mongolia [3], [4] which possess large amounts of phenols and flavonoids with potent antioxidant activities [1].
According to literature, *Ulmus pumila L* is used as effective components for the treatment of glycosuria, cancer, acquired immunodeficiency syndrome (AIDS), as well as pathogenic virus diseases and possess anti-inflammatory and immune reinforcing ability [5], [6]. Its leaf and stem bark extracts are employed as diuretic, demulcent, antipyretic, and laxative remedies [1].
Especially, the elm fruit is rich in vitamins (vitamin B1, vitamin B6, nicotinic acid, and ascorbic acid) and some minerals (calcium, potassium, magnesium, copper, iron)[7].
The present study was aimed to prepare extracts from the fresh leaves of *U. pumila* using different solvents, and subsequently used to determine the toxicity and anti-inflammatory activities.

Materials and Methods
Sample preparation
The aerial parts (leaves) of *Ulmus pumila L* have been collected from the prairies of Khatanbulag soum, Dornogovi province in during May and June, 2019. The plant samples were identified by Dr. Urgamal from the Institute of Botanic Garden, Mongolian Academy of Sciences. Subsequently, the plat materials were dried at room temperature in an air exchange condition and then powdered with an electric blender.

Extraction and fractionation

The leaves were reduced to a fine powder and extracted by static maceration method with 80% ethanol for three times at room temperature. After filtration, the crude ethanol extract was concentrated in a rotary evaporator. To yield different fractions, the dried ethanol extract (Up-E) was suspended in ethanol/water (1:4), then partitioned then in separating funnel with solvents of increasing polarity: hexane (Up-H), chloroform (Up-C), ethyl-acetate (Up-A), and *n*-butanol (Up-B).

Thin-layer chromatography

Thin-layer chromatography was carried out using TLC pre-coated plates (silica gel F365), according to Wagner and Bladt method [8].The plates were developed in a chromatographic tank using the polar and non-polar solvent systems, then were dried and visualized under normal day light. Polar solvent system was sprayed with 2% ethanol and 0.4% vanillyl alcohol as well as 5% polyethylene glycol followed by heating at 105^{o} C for 5-10 minutes in an oven, then visualized under normal day light and ultraviolet light at λ=365 nm [9], [10]. On chromatograms, the content, color of bands or spots were determined by virtue of comparison with rutin as the standard substance.

Animals

In vivo experiments were performed with male Swiss mice (18–25 g) and Wistar rats (180-220 g) obtained from by the Institute of Veterinary Medicine, in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals. All procedures were performed following the ethical standards, after approval from the Institutional Animal Care and Use Committee, Mongolian University of Life Sciences. Animals were housed under standard laboratory conditions (temperature 23 ± 2˚C) with 12 h dark and 12 h light cycle. The animals had free access to standard dry pellet diet and tap water ad libitum.

Carrageenan-induced paw edema rat model

Fifty rats were divided randomly into four different groups. The first group is carrageenan alone as Control group (*n*=12 per group), which received no treatment. The second group of rats used as Standard group (*n*=12 per group) that were treated with Indomethacin at dose of 25 mg/kg. The third (*Ulmus pumila L* Ethyl extract) and fourth (*Ulmus pumila L* Ethyl-acetate extract) groups (*n*=13 per groups) were treated with 20% ethanol extract and ethyl-acetate extract at dose of 45.4 mg/ kg, respectively. A 100 L subplantar injection of freshly produced 1% carrageenan solution in PBS into the right hind paws was used to induce paw edema. The drug treatment in the experimental group was continued for 5 days and hind paws of all rats each group of animals were measured apparently with a vernier caliper prior to administration of the dose and at 0 h, 0.5 h, 2 h, 24 h, and 72 h after treatment with drugs, as in the previously published [11], [12].

Toxicity LD_{50} assessment

For LD_{50} to measure the short-term toxicity, in decreasing doses of 20% ethanolic extract, prepared from a crude extract of *Ulmus pumila L*. and intravenously (IV) injected into the lateral tail veins of of 40 healthy male mice (*Mus musculus*), weighing between 19 and 21 g at amount of 0.1 ml (*n*= 4 per group), 0.2 ml (*n*= 6 per group), 0.25 ml (*n*= 6 per group), 0.3 ml (*n*= 6 per group), and 0.4 ml (*n*= 6 per group) as described by V.B. Prozorovsky method. The median lethal dose (LD_{50}) of 20% ethanolic extract *Ulmus pumila* L was calculated using a dose volume that killed 50 percent of the experimental animals.

Statistical analysis

The statistical analysis was done by applying Student's t-test to the mean. P values less than 0.05 were considered significant and less than 0.005 were considered highly significant. The variables analyzed were expressed as mean ± standard deviation.

Results

Extraction yield and phytochemical analysis

The maceration at room temperature of the plant powder gave a crude ethanol extract of 291 g. Each fraction after evaporation to dryness yielded hexane fraction (13 g), chloroform fraction (14 g), ethyl acetate fraction (6 g) and n-butanol fraction (10 g).

Preliminary phytochemical screening of *U.pumila L*. extraction was performed by TLC, which is suitable rapid quantification method for the analysis of induced active substance, such as flavonoids.

By a visual evaluation, yellowish spots of flavonoids were more evident in the total crude extract of plant leaves and the ethyl acetate extract than the other fractions as compared to the standard substance rutin.

Therefore, crude dry ethanolic extract was suspended in 20% (v/v) ethanol; the ethanol and ethyl acetate fractions with predominant flavonoids were chosen for further study.

Anti-inflammatory activity of Ulmus pumila L

To examine the anti-inflammatory efficacy of *Ulmus pumila L* in solvent fractions, an *in vivo* carrageenan-induced paw edema model was used. Inflammation in right hind paws of each animal was triggered by subplantar injection of 1% newly prepared solution of carrageenan in PBS after oral treatment with 25 mg/kg of Indomethacin and 45.4 mg/kg of ethanol and ethyl-acetate extracts once a day for a week.

As expected, carrageenan-induced paw edema showed prominent symptoms of inflammation, such as redness and swelling of the paws (not shown). Edema was assessed by measurement of paw thickness by caliper and expressed as the increase in paw thickness area (mm^2) after injection relative to the pre-injection value for each animal.

In control group of carrageenan alone, the paw thickness before carrageenan administration was 0.037±0.002 mm, while carrageenan-induced paw edema was measured as 0.069±0.003 mm at 0h (time=0 baseline), 0.0751 ± 0.003 mm^2 at 0.5 h, and 0.0960±0.005 mm^2 at 2 h, showing a subsequent increase in the paw, succeeding administration of phlogistic agent points toward the significance of edema.

In contrast, the swelling of the paw in the group treated with 20% Up-E, the average area of paw was measured 0.067±0.002 mm^2 at 0 hours, while it was decreased till 0.0639±0.0008 mm^2 at 72 hours of treatment, and the inflammatory swelling was reduced by 1.06 times. In the group treated with Up-A extract, the mean area of the paw of rat model was measured as 0.0661±0.001 mm^2 at 0 hours, and 0.0651±0.001 mm^2 at 72 hours of treatment, showing 1.01-fold reduction of inflammatory edema.

The injection generated intense inflammation which peaked after 2 hours in all cases. Treatment with 20% Up-E and Up-A showed significant reduction in paw thickness area (mm^2) in 72 h compared to control (ρ=0.0008 and ρ=0.001) by 1.1 and 1.05 times, respectively.

According to the results of the study, the parameters (area size) after creating pathological models with carrageenan were significantly (*P<0.005*) different compared to the healthy parameters (area size) of all groups of rats in the study.

Table 1.

Anti-inflammatory effects of *Ulmus pumila L*. extracts on carrageenan-induced paw edema in rats

Group	Dose, (мг/кг)	Healthy (Edema area mean, мм^2)	Changes in edema area mean, мм^2				
			Baseline, 0 h	0.5 h	2 h	24 h	72 h
Control	-	0.037±0.002 **	0.069±0.003 **	0.0751±0.002 **	0.0960±0.005 **	0.0766±0.001 **	0.0685±0.0009 ***
IND	25	0.0393±0.003 **	0.0663±0.002 **	0.0688±0.002 **	0.0753±0.001 **	0.0696±0.001 **	0.0645±0.001 **
Up-E	45.4	0.0405±0.001 **	0.067±0.002 ***	0.0778±0.002 **	0.0830±0.001 **	0.0708±0.002 **	0.0639±0.0008 ***
Up-A	45.4	0.0417±0.002 **	0.0661±0.001 **	0.0728±0.000 8***	0.0852±0.006 **	0.0764±0.003 **	0.0651±0.001 **

Values are presented mean ±SEM (n= 13 in treatment and n=12 in control groups)
**P<0.05, **P<0.005*

Toxicity (LD_{50}) study

The median lethal dose (LD50) of 20% ethanolic extract *Ulmus pumila L* prepared from the crude extract was evaluated according to V.B. Prozorovsky method. Briefly, different Up-E doses up to 0.45 ml were injected in to tail vein of white mice (six rats per group of 0.45-0.2 ml and 4 rats of 0.1 ml). The toxic symptoms in each group were recorded after 24 hours and continued for a week.

The results showed the death average dose (LD_{50}) of *U. pumila* extract was 0.454 g/kg which indicates the lower toxicity by Sidorov's classification [13], [14].

Table2.

The half lethal dose (LD_{50}) in mice

Dose, mL	Number of animals	Number of dead animals
0.45	6	6 dead
0.4	6	4 dead
0.35	6	4 dead
0.3	6	3 dead
0.25	6	3 dead
0.2	6	2 dead
0.1	4	-

Discussion

In recent years, the search for phytochemicals with pharmacological activity has increased due to their potential use in pharmaceutical production. Due to risk of side effects associated with the use of synthetic drugs, medicinal plants are the best alternative sources of active compounds such as polyphenols [15].

The fruit of *U. pumila* is used as traditional medicine and is regarded as a natural plant food full of nutrients like proteins, dietary fiber, vitamins, and minerals, as well as polyphenols [7].

The current study involves the investigation of the therapeutic potential of the ethanol extract of *U. pumila* leaves on carrageenan-induced paw edema in rats. The phytochemical analysis of *U. pumila* extract showed yellowish spots, indicating the abundance of phenolic compounds, as identified through thin layer chromatography analysis.

In this study, *Ulmus pumila L* was extracted by maceration method and its solvent extracts of ethyl-acetate fraction and 20% of ethyl extract fraction were determined for its toxicity (LD_{50}) and anti-inflammatory activity.

To screen the anti-inflammatory efficacy of *Ulmus pumila L.* solvent fractions, a carrageenan-induced paw edema rat model was employed. For this, 1% carrageenan was injected intraplantarly into the hind paw, which resulted in inflammatory signs. Carrageenan injection triggers an innate immune response characterized by edema, redness, and the continuing of neutrophil infiltration, and pro-inflammatory mediators and cytokines [16].

The results showed the death average dose (LD_{50}) of *U. pumila* extract was 0.454 g/kg which indicates the lower toxicity by Sidorov's classification [13], [14]. Throughout the experiment, the extracts had no effect on the general appearance or behavior of the mice.

Taken together the results, treatment with ethanolic and ethyl-acetic fractions decreased inflammatory symptoms and exhibited protective effects for carrageenan-induced paw edema. The presence of flavonoids and other biologically active compounds in the ethanol extract, are be responsible for anti-inflammatory action. The results supported those solvent fractions were able to alleviate inflammatory responses and could be clinically beneficial for treating inflammatory symptoms.

In summary, as the phytochemical analysis showed the presence of polyphenolic compounds in the plant extracts and the extracts alleviated inflammatory responses, *U. pumila L.* ethanolic extract may have beneficial anti-inflammatory effect. These preliminary findings may support its traditional medicinal use and could be promising candidate with application in the treatment of inflammatory symptoms. However, further studies are needed to identify the bioactive components of plant extracts.

Conflict of Interests

The authors declare no conflict of interests.

Authors' Contribution

B.S. carried out the experiment and performed the measurements; D.D. processed the experimental data, performed the analysis, drafted the manuscript; P.B conceived the original idea and supervised the project.

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