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EVALUATION OF HEMATOPOIETIC AND ANTIBACTERIAL ACTIVITIES OF *Urtica urens* L.

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Abstract

The plant was collected in Amecameca, Mexico. The dry leaves were crushed and consecutively soaked in hexane, dichloromethane, methanol, and water. For the hematopoietic activity assays, the femur and spleen of female CD_1 mice 8-12 weeks of age were isolated to prepare cell suspensions of 4.5 x 10^5 cells/mL, which were cultured in RPMI-1640 medium-10% calf serum. The extracts were added to final concentrations of 1, 10 and 100 µg/mL. The cultures were incubated for 72 and 48 h, respectively, at 37°C, with 5% CO_2. Extract-free cultures with dimethylsulfoxide (DMSO) and colchicine were included as negative and positive witness, respectively. Cell proliferation was evaluated by the sulfrhodamine method. For antibacterial activity and to determine the Minimum Inhibitory Concentration (MIC), double dilutions of extracts were performed from 5-0.039 mg/mL of extracts, which were later added to cultures of *Salmonella typhimurium*, *Shigella flexneri*, *Salmonella typhi*, *Escherichia coli*, *Proteus mirabilis*, *Bacillus subtilis*, and *Staphylococcus aureus* (5 x 10^6 CFU/mL) cultured with RPMI-1640 medium and resazurin sodium salt. The cultures were incubated 24 h at 37°C.

The hexanic extract inhibited the proliferation of spleen cells. Dichloromethanic and methanolic extracts stimulated cell proliferation as the extract-free in bone-marrow cultures, and 1- and 10 µg/mL of dichloromethanic and aqueous extracts and 1 µg/mL of hexanic extract inhibited cell proliferation. In contrast, 1 µg/mL of the methanolic extract and 10 and 100 µg/mL of the hexanic extract stimulated cell proliferation of bone marrow (p <0.001). The remaining extracts and concentrations acted cytostatically. The lowest MIC (0.039 mg/mL) was obtained with the hexane, dichloromethane, and methanol extracts against *B. subtilis* and hexane and dichloromethane extracts against *S. aureus* (0.156 mg/mL).*U. dioica* showed least activity. *U. urens* and *U. dioica* produced 14 and 7 inhibitory extracts, respectively.

Keywords: *Urtica urens, hematopoietic activity, antibacterial activity.*

Introduction

The Urticaceae family is made up of 52 genera and nearly 1,900 species It is widely distributed in tropical regions. The genera with the largest number of species are as follows: *Pilea* (500-715 species): *Elatostema* (300 species); *Urtica* (80 species), and *Cecropia* (75 species). Plants of the genus *Urtica* are annual and perennial herbs, characterized by stinging trichomes, opposing leaves, and the flowers are green with yellow stamens. Male and female flowers are on separate floors. The most representative species worldwide are the following: *Urtica dioica* L.; *Urtica urens* L.; *Urtica pilulifera* L.: *Urtica cannabina* L.; *Urtica membranacea* Poiret.; *Urtica haussknechtii* Boiss.; *Urtica atrovirens* Req.*Urtica rupestris* Guss.; *Urtica chamaedryoides* Pursh., and *Urtica ferox* Forst. Among these, *U. dioica* and *U. urens* have been known for a long time as medicinal plants (1).

Urtica dioica L. is of economic importance because of its potential medicinal and nutritional qualities and potential in the textile industry. . It is widely distributed in Mexico and is known as stinging nettle, Chichicastle, and wicked woman. It is located in the states of Hidalgo, Jalisco, Morelos, Puebla, and Oaxaca, and grows up to one meter in height. The stem and leaves are covered by stinging hairs. The leaves are round or elongated with serrated edged. The flowers are small, green, with grouped spikes arising from the axils of the leaves. The plant is traditionally used to treat genitourinary conditions (cystitis, urethritis, urolithiasis, nephritis), benign adenoma of the prostate, gout, hypertension, edema, disorders of the skin (acne, burns, skin ulcers, alopecia), diabetes, anemia (vitamin or mineral deficiency), internal bleeding, diarrhea, dysentery, and gastric hyperacidity, musculoskeletal pains, and osteoarthritis (2).

In the stinging trichomes, the presence of formic acid, acetylcholine, serotonin, and histamine has been reported. In the flowers and leaves, some glycosidic flavonoids are found, including rutin, isoquercetin quercetin, and kampferol. The presence of fatty acids, caffeic. butyric, acetic, citric, formic, and fumaric and ascorbic acids, proteins, essential oils, tannins, mucilage, vitamins A, B1, B2, and C, folic acid, Pantothenic acid, iron, sulfur, magnesium, manganese, copper, zinc, cobalt, potassium and calcium salts, nitrates, etc. has been determined The aqueous extract of the plant's aerial structures possesses antioxidant, antimicrobial, analgesic, and antiulcerative activities (3).

Its seeds are used to reduce the levels of serum creatinine and, in tests with mice, it prevents carbon tetrachloride-induced hepatotoxicity (4). As mentioned previously, *U. dioica* is the most studied species in the genus, is grown for commercial purposes, and is sold in tablets or capsules as a nutritional supplement and used alone or along with *Serenoa repens* in the treatment of benign prostatic hyperplasia (5).

This is a monoecious, annual herb, 10-60 cm in height, pale green in color, 1-4, 1-6 cm, ovate, and deeply serrated leaves. Male

and female flowers are numerous, centrally glabrous, and the fruits are achenes. It has smaller leaves and shorter flowers shorter *U. dioica*. It is often used instead of *U. dioica* and it is known by the names girl nettle, Chinese nettle, and black nettle, preferably. *U. urens* has a distribution pattern similar to that of *U. dioica*. Medicinally, it is used as an expectorant, laxative, diuretic, hemostatic, hypoglycemic, and as an antibacterial, and in rheumatism, hemorrhoids, bronchitis, hyperthyroidism, and cancer.

U. urens is native to Europe, but has worldwide distribution, grows in more than 50 countries or locations and at high altitudes. The plant is adapted to many environments. infesting a wide range of horticultural areas, especially where there is summer rain or irrigation. It may become prevalent in grasslands rich in organic matter, such as landscapes, playgrounds, or water points (6). It is an annual or a perennial herb with a few stinging hairs. Size: Up to 80 cm in height. Stem: Simple or branched leaves: A pair of tiny blades called stipules usually occurs at the base of each leaf. Opposing leaves of a variable, but usually ovate-to-triangular, shape, up to 6 cm in length although usually shorter, long and pointed, and with teeth on the edges, sometimes a heart-shaped base, with tiny blisters on top of an irregular shape but mostly cylindrical and whitish, around long, slender petioles. Inflorescences: many flowers arranged in small groups, subglobose or elongated, flowers usually shorter than the petioles of the leaves. Two or more of these inflorescences located in the axils of the leaves. Distribution altitude in the Valley of Mexico up to an altitude of 3,200 m (7). There are scientific reports that of *U. urens* has a similar pattern of distribution as *U. dioica*, and there are also scientific reports on the ability of plants of the genus *Urtica* to treat hematological alterations, particularly anemia (8). However, it is not known whether *Urtica urens* possesses hematopoietic properties.

Methods

The plant was collected at Bosque Esmeralda in Amecameca, Mexico, in November 2017, and was authenticated by Jorge Santana- Carrillo and Reyna Cerón-Ramírez from the Herbario Metropolitano "Ramón Riba y Nava Esparza" of the Universidad Autónoma Metropolitana (UAM) Iztapalapa, where a voucher specimen of the plant (79750) is stored.

Male $CD_{1,}$ mice, 8-12-weeks in age from the UAM-Iztapalapa Animal Facilities were used. Four mice per box were housed at a constant temperature of 24°C with a 12-h light/12-h dark photoperiod and were allowed free access to food and sterilized water by filtration through 0.22 µm Millipore membranes (USA). The handling of laboratory animals and experimental procedures was performed according to the national and international rules (U.S. National Institutes of Health [NIH] Guidelines for the Handling and Care of Animals), including the Official Mexican Regulation 2001 (9). In addition, the study was approved by Institution's Ethical Committee.

The aerial parts were dried at room temperature, protected from dust and sunlight. The leaves were ground; 100 g of such this material was macerated with 600 ml of hexane for 48 h at room temperature. After that, the extract was filtered, with the recovered plant material, and the process was repeated using dichloromethane and methanol (J.T. Baker, USA) and water. The organic solvents were evaporated to dryness under reduced pressure at 35°C in a rotaevaporator (Buchi RII, Switzerland). The four extracts were diluted to a concentration of 20 mg/mL with Dimethylsulfoxide (100% DMSO); each of these solutions were diluted 1:10 initially with bidistilled water, and another two decimal dilutions were performed with DMSO 10%, thus containing the test concentrations of 2,000, 200, and 20 has been determined µg/mL in 10% DMSO. Prior to each assay, a dilution was performed of each decimal using RPMI-1640 medium supplemented with 10% newborn calf serum (NCS), thus obtaining summary test concentrations of 200, 20, and 2 µg/mL of the extract/DMSO 1%.

A preliminary phytochemical study of methanol and the watery extracts of both plants was performed by coloring and precipitation assays as reported. Proteins were determined by the Lowry method (10). In addition, the total polyphenols were quantified of the aqueous extract using a colorimetric oxide-reduction reaction. The oxidizing agent utilized was the Folin-Ciocalteau reagent. A standard solution of gallic acid (0.1 mg/mL) was employed. Absorbance was measured at 760 nm (11). Results are expressed in mg as the equivalent of gallic acid per g of the extract (mg/GA7g extract).

In order to know the effect of extracts on the proliferation of normal cells, cultures of hematopoietic cells from spleen and bone marrow were performed. Mice were sacrificed in a CO_2 chamber, the femur was isolated under sterile conditions, the epiphysis and diaphysis were cut, 1 mL of physiological saline solution was injected through the bone-marrow channel, and cells were collected in a 4.8-mL cryotube (Nunc, USA). A cell suspension aliquot was diluted with the Turk solution (1:20) in white-cell pipettes in order to count the total nucleated cells with the aid of hemocytometer under a clear field microscope. Cell viability was determined using 0.2% of Trypan Blue. The cell concentration was adjusted to 4.5×10^5 in RPMI-1640 medium-FCS-1%.

One hundred µl was added to the 96-plate wells containing 100, 10, and 1 µg/mL of each extract in DMSO 0.5%. RPMI-1640 medium-10% fetal calf serum (FCS), and incubated at 37°C during 72 h into an atmosphere of CO_2 5% and 90% humidity.

The needle of a 3-ml syringe with 3 ml of RPMI 1640 medium with 10% FCS was introduced into spleen: The cells were collected in plastic tubes and centrifuged at 1,500 rpm at room temperature for 5 min. the supernatant was removed by decanting, and the cell button was resuspended with one from the same medium. Each cell suspension obtained was quantified with total nucleated cells, and cell viability was determined with Turk and

Trypan Blue solutions 0.2%, respectively. Finally, the concentration was adjusted to 4.5 x 10^5/mL in RPMI 1640 medium with 10% of FCS. Additionally, DMSO 10% was employed as the control solution (Note: Wells framing the plate were filled with 200 µl of sterile distilled water). Ready-made plates were maintained in in incubation in anticipation of cell suspension for the crop (bone-marrow cells) or spleen cells. Each experiment included extract-free cultures. Each extract was tested five times by triplicate in independent experiments. To evaluate cell proliferation, the Sulphorhodamine B (SRB) method was used (ED_{50} <50 µg/mL) (12). Results are expressed as mean ± standard of error. The extract-treated cultures were compared with control cultures utilizing ANOVA analysis.

The bacteria used were *Salmonella typhimurium* ATCC 13311, *Shigella flexnerii* ATCC 29003, *Salmonella typhi* ATCC 6539, *Escherichia coli* SOS, *Proteus mirabilis*, *Bacillus subtilis*, and *Staphylococcus aureus* ATCC 6538. To determine the antibacterial activity and the minimum inhibitory concentration (MIC), we followed the protocol of Drummond and Waigh, modified by Satyajit, which employed 96-multiwell plates and resazurin as an indicator of viability. This method is based on the reduction of the resazurin-to-resorufin capability by oxide-reductase enzymes of the surviving bacteria: When the extract inhibits bacterial growth, there is rust-reductase bacterial activity; thus, blue, while when they survive, reduction-to-resorufin gives a pink color to the culture medium by the shift in the indicator (13).

The bacteria were grown in Mueller-Hinton broth for 24 h and the concentration was adjusted to 4 x 10^6 CFU/mL, with a turbidity of 0.5 (McFarland Nephelometer), and this incubated for 24 h at 37°C. *U. urens* and *U. dioica* were prepared separately with solid extracts, an initial dissolution of 5 mg/mL in DiMethylSulfOxide (DMSO, J. T. Baker, USA) to 0.8% and comprised two-fold dilutions. We deposited, in 50 µl/well plates, the 10 µl bacterial suspension; 10 µl of sodium resazurin were added (0.675% w/v to distilled water, as well as 30 µL of Müller-Hinton medium (320 mosm). As negative controls, we used DMSO 8% and sterile distilled water sterile, and as positive control, 1 x 10^4 Penicillin-Streptomycin solution IU/mL-1 x 10^4 mg/mL (Sigma Chemical Co., USA). The culture plates were incubated at 37°C for 22 h.

Results

The recovered material and the total content of proteins are shown in table 1. For the phytochemical analysis were included methanol and the watery extracts, tannins, sugars, and saponins, and flavonoids were presents. No triterpene or alkaloid were detected. Total polyphenol content was 2.5%.

The hexanic extract of *Urtica urens* L. at concentrations of 10 and 100 µg/mL and the aqueous extract at 1 µg/mL presented hematopoietic activity in bone-marrow cell cultures similar to the control-free extract, which was considered as 100% of proliferation (p <0.1). Meanwhile, the 10-

and 1-µg/mL concentrations of the dichloromethanic and aqueous extracts exhibited great inhibitory activity and similiar to that of the colchicine (positive control) (Figure 1). Due to the dispersion of the mean, the concentration of 1 ug/mL of the hexanic extract also inhibited the proliferation of bone-marrow cells (p <0.25), exhibiting a cytotoxic effect.

Regarding spleen-cell cultures, the dichloromethanic and methanolic extracts exhibited hematopoietic activity similar to negative control (DMSO 0.05%) (p <0.1), but the hexanic extract at concentrations of 100, 10, and 1µg/mL inhibited cell proliferation, causing a reduction of 38, 30, and 27%, respectively, activity that was similar to that of colchicine, which reduced cell proliferation by 34% compared to the negative witness. p <0.0025. (Figure 2).

Urtica urens L. The minimum inhibitory concentration was 0.039 mg/mL, which was presented by the hexanic, dichloromethanic, and methanolic extracts of *U. urens* acting on *B. subtilis*. These extracts also inhibited *S. aureus* at concentrations ranging from 0.156-5 mg/mL, the maximal dose employed. At the same concentration, the previously mentioned extracts also inhibited *S. flexneri* and *S. typhi*. The aqueous extract inhibited *Escherichia coli* and *B. subtilis* (Table 2).

Urtica dioica L. This plant that is the reference that exhibited the lowest antibacterial activity of seven inhibitory extracts compared to the 14 extracts of *U. urens*. None of the extracts inhibited the development of *Salmonella typhimurium* and *Proteus mirabilis* (Table 2).

Discussion and Conclusions

Urtica urens L. is used instead of *U. dioica* L. In the Americas, both frequently used in South America (Bolivia, Chile, Uruguay, Argentina, and Colombia) in Central and North America, particularly in Mexico (14). Experimentally, its activity has been shown as antinoceptive and anti-inflammatory, and more abundantly by its antibacterial, antiviral, and antifungal properties. Among the first articles linking the use of *Urtica dioica* against anemia was published in 1938, reporting that after, inducing anemia in rabbits by bleeding administered *Urtica* juice; after 20 days the animals regained levels of erythrocytes measured by the increase of porphyrins in urine (15). In 2003, other investigators reported that the administration of *Urtica* seed oil in rats previously treated with CCl_4 caused in them increased levels of erythrocytes, hemoglobin and volume, mean cell volume, and leukocytes, concluding that the oil of the seeds was able to restore erythropoiesis (16).

Due to its high iron content, *Urtica dioica* is recommended in traditional medicine to avoid anemia during pregnancy. In 2013, the authors showed that *U. chamaedroydes* Pursch was able to restore hematopoiesis in pregnant female mice by restoring, at 15 and 34 days, normal levels of erythrocytes and platelets. Reinforcing this, it also has been reported that *U. dioica* var. Angustifolia was able to restore hematopoiesis in pregnant anemic female mice, thereby preventing the development

of malformations in the products of gestation (8).

It is well known that anemia is a malnutrition-linked syndrome that causes a state of immunodeficiency, exposing those afflicted to fatal outcomes due to the aggravation of infections, development of cancers, internal and external bleeding, etc. Herrera and collaborators reported that L. (0.2 g/ml) reverses the effects of malnutrition in newborn rats malnourished by food competition, noting the increase in lymphocyte total count and in CD4+ CD8+ monocytes, and increments in red blood-cell counts, hemoglobin, and white blood-cell counts, due to its acting as a stimulant of the proliferation and maturation of lymphocytes (17). However, in our studies, only concentrations of 10 and 100 µg/mL and the 1µg/mL concentration of the aqueous extract had similar activity in bone-marrow cultures compared with the extract-free witness culture, which indicated that there was no hematopoietic activity. Even more so, the dichloromethanic extract and the concentrations of 10 and 1 µg/mL of the aqueous extract inhibited the cell proliferation equivalent to colchicine, as depicted in Figure 1.

Regarding spleen culture, Figure 2 demostrates that the hexanic extracts of 100 and 10 ug/mL exerted inhibitory activity similar to that of colchicine. The dichloromethanic and methanolic extracts possessed hematopoietic activity similar to that of the extract-free witness culture. This means that the presence of plant extracts for the proliferation of spleen cells in the culture is not necessary. *U. urens* has no hematopoietic activity: some extracts inhibit the proliferation of bone-marrow and spleen cells, while other extracts are cytostatic but not stimulating.

Inhibiting cell proliferation of some extracts of *U. urens* in spleen and bone-marrow cultures reveal cytotoxicity, which has been reported for *U. dioica.* The plant's aqueous extract inhibited the growth of MCF-7 and MDA-231 cells (derived from breast cancer); in the MDA, the Cytotoxicity Index of 50% (IC50) with a concentration of 34 g/mL (18). *U. dioica* is cytotoxic for transformed cells, while *U. urens* is cytotoxic for normal cells of the hematopoietic and immune systems.

Concerning the antibacterial activity of the genus *Urtica*, *U. dioica* demonstrates notable activity against Gram-positive and Gram-negative bacteria, comparable to that exhibited by compounds such as Amoxicillin-clavulanic acid and Methylenomycin. The aqueous extracts of leaves, roots, and seeds inhibited *B. subtilis*, *E. coli*, *Pseudomonas aeruginosa*, and *Lactobacillus plantarum* within a range of concentration of 36.21 mg/mL-76.43 mg/mL (19). An inhibitory effect on *Proteus mirabilis*, *Critrobacter koseri*, *Micrococcus luteus*, and *Candida albicans* have been reported (20). In contrast, in our results, with the aqueous extract of *U. dioica* only inhibited *S. typhi* at a concentration of 1 mg/mL, very low compared with the work mentioned previously. The antibacterial activity of aqueous *U. dioica* extracts was obtained by consecutive treatment with low-to-high chemical polarity solvents, such as hexane, ethyl acetate, chloroform,

methanol and water, and methanol-chloroform, ethyl acetate, and water, reporting antibacterial activity in ranges of 0.130-66.66 mg/mL.

In general, the raw extracts obtained demonstrated better activity against Gram-positive than against Gram-negative bacteria (21). The resistance of the latter has been associated with the more complex structure of their cell wall, with lipopolysaccharides in the outer membrane and other defense mechanisms that allow them to neutralize the action of antimicrobials, dyes, and various agents that affect the viability of these bacteria (22).

Unlike in the study of these authors, in our study the aqueous extract of *U. dioica* presented low antibacterial activity, while those corresponding to *U. urens* inhibited *Bacillus subtilis* at the highest concentration used: 5 mg/mL. This bacterium was the most sensitive, as it was inhibited in its development by 10 of the 12 extracts evaluated at concentrations of 0.039-5.0 mg/mL. The other Gram-positive bacterium employed, *S. aureus*, was inhibited only by six extracts at concentrations of 0.156-5.0 m g/mL The work of Modarresi-Chahardeh work and our work differ in terms of their extraction method at a temperature of 30-32°C for 72 h and that of our work at an average temperature of 25°C for 24 h, and in the method of evaluation and obtaining the MIC (Minimal Inhibitory Concentration) by diffusion, while in ours, these were conducted with the oxide-reduction method of resazurin.

Mahmoudi and collaborators reported that the aqueous extract of the *U. dioica* leaf acted as a powerful antimicrobial on *S. aureus* and *L. monocytogenes*, these the most sensitive bacteria. These authors reported that the alcoholic extract from the stem possesses better activity than that of the leaf on Gram-positive bacteria. This extract also inhibited Gram-negative bacteria and *Candida albicans* yeast, its activity higher than that of the root extract (25). According to the results reported by Gulcin et al. (23), Joshi et al., (26), and Koszegi et al. (27), the respective aqueous extracts of *U. dioica* exhibited activity on *S. aureus, E. coli*, and *C. albicans*. Based on MIC data and data for other species of the genus *Urtica*, antibacterial activity has also been reported in the ethanolic extract of *U. urens* (6); *U. pilifera* had been highlighted in reports that its aqueous extract of the seeds and the methanolic extract of the leaves, roots, and seeds have antibacterial activity on several species of Gram-and Gram-negative bacteria (28). In our study, the methanolic extract from the leaves of *U. urens* inhibited *Shigella flexneri*, *Salmonella typhi*, and *Bacillus subtilis.*

Our results with the aqueous extract differ from what other authors reported, mainly in terms of the extraction method since, in the majority of works, this is by heating, while in our case, it is at room temperature, together with that, in the present study, the plant was previously treated with hexane, dichloromethane, and methanol. The aqueous extract comprised the residual material, as it is known that methanol and water are chemically polar compounds that

extract the same compounds. It is possible that, in the maceration of the plant with methanol, compounds were extracted, thus leaving the aqueous extract poor in components. Among the outstanding components of *Urtica dioica* and other species of the genus *Urtica* we find alkaloids, flavonoids, phenols, tannins, and saponins mainly, compounds to which antibacterial activity is attributed. Alkaloids are considered to have antibacterial activity because of their ability to react with the amino, carboxyl, sulfhydryl groups of bacterial proteins, as well as with nucleic acids (29).

Tannins precipitate proteins, including structural or bacterial enzymatic slams, preventing the microorganism from nourishing itself. The antibacterial action of flavanoids is attributed to their ability to form complexes with extracellular and soluble proteins and to form complexes with the bacterial cell wall; moreover, flavonoids can disorganize the lipids of bacterial membranes (30, 31). It is noteworthy that *U. urens* was collected in Amecameca, Mexico, at an altitude of 2,420 meters above sea level (masl) and at a latitude of 98°45 x 46 West, while *U. dioica* was collected in the Valley of Toluca Valley at an altitude of 2,667 masl and at an altitude of 99°39 '38 West. Both localities have cold weather. The results obtained are attributable to the specific characteristics of each species in these habitats. These results showed that the environment of Amecameca was more favorable to the antibacterial activity of plants than that of Toluca, which with more in agreement rope with the preliminary results of a comparative study of the antibacterial activity of *U. mexicana* collected in Amecameca and Toluca, obtaining greater activity with the former, which presented 15 active extracts vs. that of Toluca with seven extracts with antibacterial activity (results not included).

This study demonstrates a relationship between the ethnomedical use of plants of the genus *Urtica* in the treatment of gastrointestinal infections and the experimentation findings. It is important to note that in our study, this species had the lowest antibacterial activity. According of the findings we conclude. Thar *U. urens* L. did not modify substancially the hematopoietic myeloid or lymphoid activity. Even more dichorometane and watery extracts are cytotoxic and could be faced against cells from carcinomas. *U.urens* L. had best antibacterial activity than *U. doica L.*

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Table 1. Recovery and total protein content of extracts

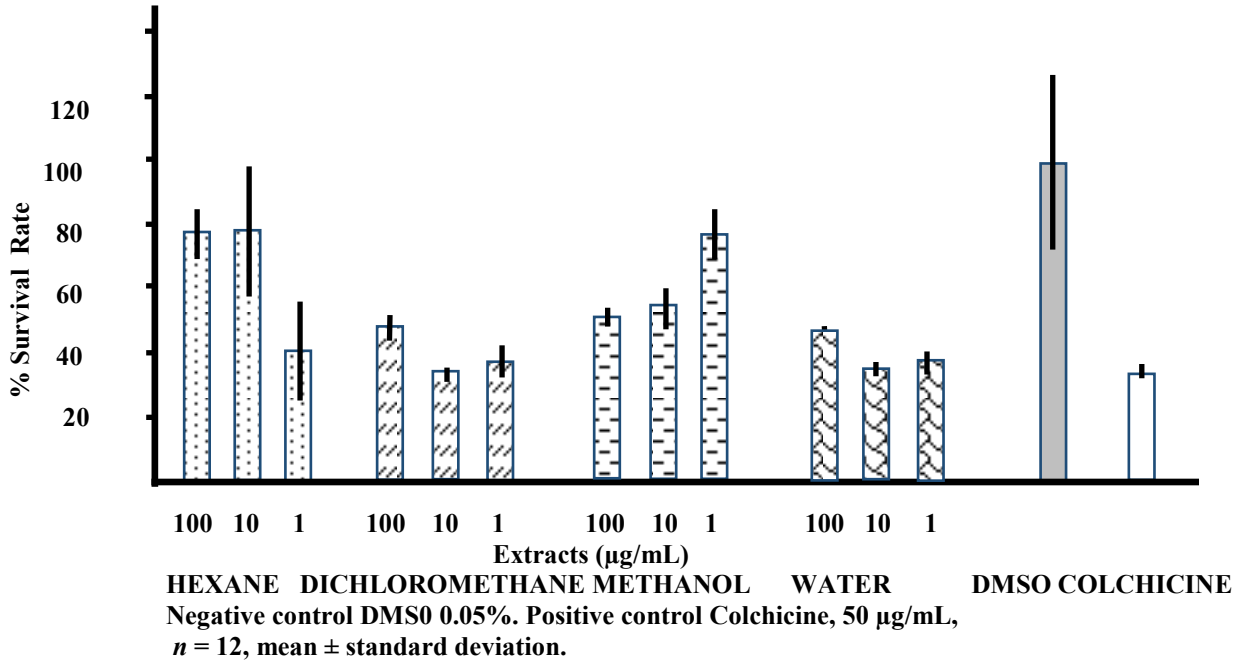
Extract	Recovery % *U. dioica* L.	Proteins µg/mL	Recovery % *U. urens* L.	Proteins µg/mL
Hexane	1.30	0.00	2.80	0.00
Dichloromethanic	1.07	7.12	0.90	5.37
Methanol	2.94	24.13	2.70	32.22
Water	9.52	39.62	4.6	42.27

Table 2. Antibacterial activity of *Urtica urens* L. and *Urtica dioica* L.

Bacterial strains	*A*	*B*	*C*	*D*	*E*	*F*	*G*
***Urtica urens* L.**							
Hexane	-	5	5	-	-	0.039	0. 312
Dichloromethane	-	5	5	-	-	0.039	0.156
Methanol	-	5	5	-	-	0.039	2.5
Water	-	-	-	5	-	5	-
***Urtica dioica* L.**							
Hexane	-	-	-	-	-	0.50	0.25
Dichloromethane	-	-	-	-	-	0.25	0.5
Methanol	-	1	1	-	-	-	-
Water	-	-	1	-	-	-	-

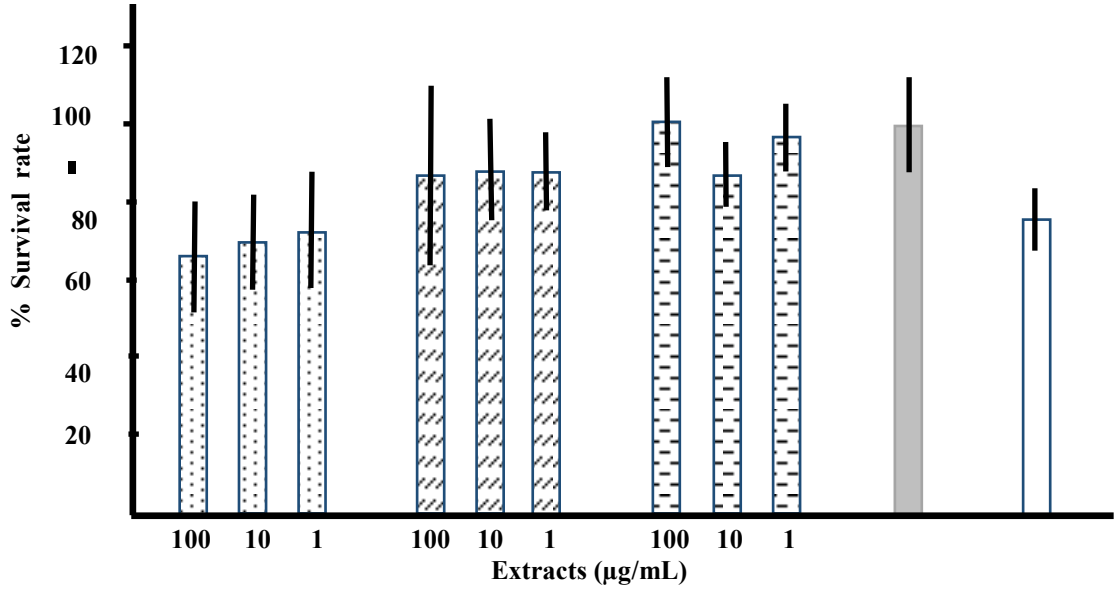
A = *Salmonella typhimurium*, B = *Shigella flexneri*, *C* = *Salmonella typhi*, *D* = *Escherichia coli*, *Proteus mirabilis*, *F* = *Bacillus subtilis*, *G* = *Staphylococus aureus.* No inhibition was observed.

Figure 1. Hematopoietic activity of *Urtica urens* L. extracts on mouse bone-marrow cells



Negative control DMS0 0.05%. Positive control Colchicine, 50 µg/mL, *n* = 12, mean ± standard deviation.

Figure 2. Hematopoietic activity of the extracts of *Urtica urens* L. on mouse spleen cells



Negative control DMS0 0.05%. Positive control Colchicine, 50 µg/mL, *n* = 12, mean ± standard deviation.