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A COMPARATIVE STUDY OF THE ANTIBACTERIAL ACTIVITY OF *URTICA DIOICA* L. AND *URTICA DIOICAL*. VARIETY ANGUSTIFOLIA

R. Velasco [*1], S. B. Herrera [2], R. Tapia [1], J. Ramírez [3], R. Cerón [4] and J. Santana [4]

Departamento de Ciencias de la Salud [1], Posgrado de Biología Experimental [2], Licenciatura de Biología Experimental [3], Herbario Metropolitano"Ramón Riba y Nava Esparza" [4], División de Ciencias Biológicas y de la Salud-Iztapalapa, Universidad Autónoma Metropolitana, Mexico City, Mexico.

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Correspondence to Author:
Rodolfo Velasco-Lezama

Departamento de Ciencias de la Salud-Iztapalapa, Universidad Autónoma Metropolitana, México.

E-mail: rodolfo_velasco2003@yahoo.com.mx

ABSTRACT: *Urtica dioica* L. and *Urtica dioica L. angustifolia* are commercialized as the same plant under the name stinging nettles (*Ortiga*) but, to our knowledge, the antibacterial properties of the variety *angustifolia* are unknown. The antibacterial activity of *Urtica dioica* L., collected in the State of Mexico, was compared with that of *Urtica dioica L.* variety angustifolia, acquired in a market in Mexico City. The extracts were obtained by consecutive maceration of leaves in hexane, dichloromethane, methanol, and water for 48 h, dilutions of 5-0.03 mg/mL of each were added to culture plates with 4 x 10^6 Colony-forming units (CFU)/mL, resazurin, and Müeller-Hinton medium 3X. The cultures were incubated for 22 h/37°C. The bacteria employed were as follows: *Salmonella typhimurium*, *Shigella flexneri*, *Salmonella typhi*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. Each extract was evaluated on three occasions in triplicate. 1% Dimethyl sulfoxide (DMSO) and a solution of Penicillin-Streptomycin were used as negative and positive control, respectively. In terms of *Urtica dioica* L., the hexanic and dichloromethanic extracts inhibited *B. subtilis* and *S. aureus*. The methanolic extract inhibited *S. flexneri* and *S. typhi*. Antibacterial activity was observed in seven of the 28 seeded wells. *U. dioica* L. var. Angustifolia, with 10 wells exhibiting antibacterial activity. The hexanic, dichloromethanic, and methanolic extracts inhibited *B. subtilis* at 0.25, 1.25, 1.25 mg/mL, respectively. The dichloromethanic, methanolic, and aqueous extracts inhibited *S. typhi*. Also inhibited *S. flexneri*. The minimal inhibitory concentration (MIC) was 0.125 mg/Ml with *U. dioica* L.

INTRODUCTION: The diarrheic diseases are the second highest cause of death of children aged less than 5 years and they cause the death of 525,000 children each year worldwide [1]. In Mexico, infectious diseases have been a public health problem since the pre-Hispanic epoch.

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The ancient Mexicans utilized plants to cure such illnesses, as referred in the Florentine and De la Cruz-Badiano Codices, both written in the XVI century.

In the Florentine Codex, it is mentioned that the Mexica indigenous group utilized around 79 plants to treat gastrointestinal infections and 46 against cutaneous diseases, while in de la Cruz-Badiano Codex one acquires knowledge of the employment of 40 and 29 plants against these infections, respectively [2]. Infections are treated with antibiotics; however, many of these have lost their effectiveness due to that certain bacteria have

developed resistance to antibiotics, which has driven the search for new antimicrobial compounds, principally from medicinal plants and from marine invertebrate organisms [3]. Herbal remedies are still used to combat diverse infections; however, only 5% of plants, to our knowledge, have been studied chemically and biologically with the purpose of knowing their constituents and pharmacological actions [4]. One of the botanical genera employed in traditional Mexican medicine is the genus *Urtica*, which belongs to the Urticaceae Family. *Urtica dioica* L. and *Urtica urens* L. are the most prominent species of this genus due to their worldwide distribution [5]. Approximately 10 species of the genus *Urtica* are found in Mexico. Plants of with trichomes (stinging nettles). *U. dioica* L. is the most studied [6].

***Urtica dioica* L.:** *Urtica dioica* L. is of economic importance due to its medicinal, nutritional, and textile potential, it is cosmopolitan in distribution. In Mexico, it is known as *ortiga*, *chichicastle*, *mala mujer*, *etc.* It can achieve a height of up to 1 meter, the stem and leaves are covered with urticant (stinging) hairs, the leaves are round or elongated with a serrated edge, the flowers are green, small, with a herringbone-type pattern and are utilized to treat cystitis, and the flowers are employed to treat urethritis, urolithiasis, nephritis, benign adenoma of the prostate, gout, arterial hypertension, acne, diabetes, anemia, diarrhea, dysentery, and allergies. Among the trichomes, the following have been reported, including formic acid, acetylcholine, serotonin and histamine, in the flowers and leaves some glycosidic flavonoids and fatty acids, caffeic acid, and the carboxylic acids have been observed, as well as the acetic, butyric, citric, formic, fumaric, and ascorbic acids, in addition to tannins, mucilage, vitamins A,B_1, B_2, and C, folic acid, and salts, particularly iron. The aqueous extract of the aerial structures possesses antioxidant, antimicrobial, antiulcer, and analgesic activity [7, 8].

U. dioica L. variety angustifolia is known as *chichicastle* as well as *dominguilla*, reaches 2 meters in height, frequently with stinging nettles (hairs), leaves with narrowly triangular-to-subulated stipiules, oval elliptical segments from 6-12 cm in length and 1-4 cm in width, with leaves with serrated edges, and with influourescences 2-6 cm in length, in addition to pistillated flowers. This variety is distributed from central Mexico to Guatemala. In the literature, the angustifolia variety has been treated as a synonym of the ssp. *Gracilis* (Aiton). These plants are similar to each other and appear to be closely related; the angustifolia variety can be distinguished by its more southern distribution and by its hairless pistillated tepals [9]. *U. dioica* is cultivated with commercial purposes, is sold in tablets or capsules as a nutritional supplement and is employed alone or with *Serenoa repens* in the treatment of benign prostatic hyperplasia [10]. Considering that both plants grow in the same regions, they present a similar morphology, they are commercialized under the same name of *Ortiga* and they are utilized in Traditional Mexican Medicine to combat diverse types of infections. The objective of our work was to determine the antibacterial activity of *Urtica dioica* angustifolia variety, comparing it with *Urtica dioica* L.

MATERIALSAND METHODS:

Plant Material: *U. dioica* L. was collected in the Valley of Toluca and *U. dioica* variety angustifolia was acquired in August 2017 at the "Sonora" Medicinal Plants Market in Mexico City. Identification of the samples was carried out by Profs. Reyna Cerón and Jorge Santana of the "Ramón Riba y Nava Esparza" Herbolarium of the Metropolitam Autonomous University (UAM), where the samples were deposited with the following registry numbers: *U. dioica* L. (70446); and *U. dioica* L. var. angustifolia (70445), respectively.

Preparation of the Extracts: The plants were allowed to dry at room temperature protected from the sun and dust, the leaves were manually ground separately, and 500g of the pulverized material was macerated consecutively during 48 h in 3 liters of hexane, dichloromethane, methanol (J.T. Baker, USA), and water. The extracts were filtered, the solvents were eliminated under reduced pressure in a rotavapor (Buchi RII, Switzerland), and the water was eliminated by evaporation in a double boiler. The percentage of recovery of the extracts was assessed, the aqueous extract was subjected to a preliminary phytochemical study by means of colorimetric and precipitation reactions and their total protein content was determined by the Lowry method [11].

RESULTS: The percentage of recovery and the Total protein content of the extracts are presented in **Table 1.** The phytochemical analysis revealed the presence of flavonoids, phenolic compounds, and tannins.

TABLE 1: PERCENTAGE OF RECOVERY OF THE TOTAL PROTEIN EXTRACTS OF *URTICA DIOICA* L. AND *URTICA DIOICA* L. VAR. ANGUSTIFOLIA

***Urtica dioica* L.**				
Extract	**Hexane**	**Dichloromethane**	**Methanol**	**Water**
Recovery (g)	6.52	5.35	14.22	47.60
Percentage (%)	1.30	1.07	2.94	9.52
Proteins µg/mL	0.00	7.12	24.13	39.62
***Urtica dioica* L. variety angustifolia**				
Extract	**Hexane**	**Dichloromethane**	**Methanol**	**Water**
Recovery (g)	6.85	6.30	15.80	54.35
Percentage (%)	1.37	1.26	3.16	10.87
Proteins µg/mL	1.50	6.80	29.00	40.35

Antibacterial Activity:

***Urtica dioica* L.:** This presented the least antibacterial activity; in only seven of the 28 inoculated wells was there the presentation of antibacterial activity. The minimal inhibitory concentration (MIC) of 0.25 mg/mL was presented by the hexanic extract of *U. dioica*, inhibiting *B. subtilis* and *S. aureus*.

The hexanic and dichloromethanolic extracts inhibited the bacteria in the concentrations corresponding to the methanolic and aqueous extracts. Only 25% of the extracts tested presented inhibitory activity, preferentially on Gram-positive bacteria **Table 2.**

***Urtica dioica* L. variety Angustifolia:** *Urtica dioica* variety angustifolia presented greater activity than *U. dioica* in 10 reactive units against seven of *U. dioica*, representing 35% of bacterial activity. However, the antibacterial activity of the concentrations than those corresponding to the concentrations of the angustifolia variety. The average concentration of the extracts with antibacterial activity of *U. dioica* L. variety angustifolia was 0.625 mg/mL against 0.398 of mg/mL of *U. dioica.* Of the extracts tested, the methanolic extract of *U. dioica* presented the greatest activity on inhibiting the growth of four of the seven bacterial strains **Table 2.**

TABLE 2: ANTIBACTERIAL ACTIVITY OF *URTICA DIOICA* L. VAR. ANGUSTIFOLIA

***Urtica Dioica* L**	**Bacteria/ Extracts**	***Salmonella typhimurium***	***Shigella flexneri***	***Salmonella typhi***	***Proteus mirabilis***	***Escherichia coli***	***Bacillus subtilis***	***Staphylococcus aureus***
	Hexane	-	-	-	-	-	0.25	0.25
	Dichloromethane	-	-	-	-	-	0.125	0.5
	Methanol	-	1	1	-	-	-	-
	Water	-	-	1	-	-	-	-
***Urtica dioica* var. Angustifolia**	Hexane	-	5	-	-	-	5	-
	Dichloromethane	-	2.5	5	-	-	1.25	-
	Methanol	-	5	2.5	-	5	1.25	-
	Water	-	-	5	-	-	-	-

-No antibacterial activity.

DISCUSSION: *Urtica dioica* inhibits the growth of Gram-positive and Gram-negative bacteria, comparable to that of the inhibition of clavulinic acid/Amoxicillin and Gentamicin. The aqueous extract of the leaves, the root, or the seeds inhibit *B. subtilis*, *E. coli*, *Pseudomonas aeruginosa*, and *Lactobacillus plantarum* in concentrations of 36.21 mg/mL-76.43 mg/mL[13]. Gülcinand collaborators reported an inhibitory effect on *Proteus mirablis*, *Citrobacter koseri*, *Micrococcus luteuis*, and *Candida albicans* [14]. In counterpart, in our study, the aqueous extract of *U. dioica* solely inhibited *S. typhi* at the 1-mg/mL concentration, which is very low compared with that of the previously mentioned works. On the other hand, Modarresi-Chahardehand coworkers reported the antibacterial activity of the aqueous extracts of *U. dioica* obtained by means of two methods: a) consecutive

treatment with solvents of low and high chemical polarity, and b) partitioning with methanol, methanol chloroform, ethyl acetate, and water, reporting antibacterial activity within the range of 0.130- 66.66 mg/mL [15]. The extracts obtained by the former method presented less activity against Gram-negative bacteria, the resistance attributed to the complex structure of their cell wall, including the lipopolysaccharide (LPS) of the external membrane, in addition to other mechanisms of defense that permitted the neutralization of the action of antimicrobials, colorants, and diverse agents [16]. Different from that reported by these authors, in our previous studies, the aqueous extract of *U. dioica* presented low antibacterial activity, while *Urtica urens* and *Urtica mexicana* inhibited *Bacillus subtilis* only in the highest concentration employed of 5 mg/mL. This bacterium resulted more sensitive; it was inhibited by five extracts among those evaluated.

It is noteworthy that this bacterium was included in the study as an indicator of antimicrobial resistance due to its possessing endospores. The other Gram-positive bacterium utilized with *S. aureus* was inhibited in concentrations of 0.156-5.0 mg/mL in the study of Modarresi-Chahardehandours. In their work, these authors obtained extracts of 30-32°C during 72 h, while in our work, the average temperature was 25°C during 24 h. In terms of the evaluation method, this is a method that is less vigorous, that is, the obtention method of the MIC by diffusion, orthe resazurin oxido-reduction method, respectively.

In the work of Mahmoudiand collaborators [17], the aqueous extract of the *U. dioica* leaf inhibited the growth of *S. aureus* and *L. monocytogenes.* These authors reported that the alcoholic extract of the stem exhibited greater activity on Gram-positive bacteria than the leaf; notwithstanding this, it also inhibited Gram-negative bacteria and the yeast *Candida albicans,* its activity greater than in the extract of the root. According to those reported by Gulcin *et al.* [14], Joshi *et al.*[18], and Alqahtani *et al.*[19], the respective aqueous extracts of *U. dioica* exerted activity on *S. aureus*, *E. coli*, and *C. albicans*. Our results with the aqueous extract differ from those reported by other authors, principally in terms of the method of extraction. In the majority of the works, the method of extraction is by means of heating; in our case it was through maturation at room temperature, in addition to that in the present study, the plant was previously treated with hexane, dichloromethane, and methanol. Because the residual material comprised the aqueous extract, it is possible that during the maceration of the plant, methanolic compounds may have been extracted that are soluble in water, leaving the aqueous extract poor in components.

It is important to mention that in a comparative study of the antibacterial activity of *Urtica Mexicana* collected in the Valle de Toluca, *U. Mexicana* was gathered in Amecameca, both of these populations in the State of Mexico and having *Urtica dioica* L. as reference. The plant collected in Amecameca presented greater and better activity than that gathered in the Valle de Toluca, where *Urtica dioica* L. was also collected, which presented the least antibacterial activity in that study [20].

By virtue of the fact that the plants were collected or acquired with a difference of only a week in time, it can be affirmed that the results are attributable to the specific characteristics of each species and its habitat. In this study, our results are linked with the popular uses of the plant against gastrointestinal infections. This investigation forms part of a comparative study of four species of *Urtica* and of the variety angustifolia of *U. dioica* L. collected during the same period.

CONCLUSION: The angustifolia variety entertains a greater gamma of antibacterial activity than *U. dioica* L.

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