



Diuretic activity of methanolic extracts from *Jodina rhombifolia* aerial parts on Wistar rats

[Actividad diurética de extractos metanólicos de partes aéreas de *Jodina rhombifolia* en ratas Wistar]

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Abstract

Context: *Jodina rhombifolia* (Hook. & Arn.) Reissek (*Santalaceae*) it's used in folk medicine for treat a great diversity of health problems.

Aims: To evaluate the diuretic activity of aerial parts methanolic extract (leaves, bark, phloem, and branches of approximately three years) in Wistar rats.

Methods: The experimentation was organized with a negative control group (saline solution), a positive control (furosemide; 10 mg/kg) and for each methanolic extract were established three groups of animals that received doses of 125, 250 and 500 mg/kg of weight in normal saline solution.

Results: The maximum diuretic activity observed in-group administered with leaves methanolic extract followed of phloem extract. The dose 250 mg/kg of leaves methanolic extract was the more potent and equally important, result the dose 500 mg/kg, which supposes an interesting excretor effect of water for a phytodiuretic. These registers of diuresis in Wistar rats did not show significant statistically results with the positive control group. Furthermore, the onset of diuretic activity of leaves methanolic extract was extremely rapid, within the first hour of administration (for all doses).

Conclusions: This study contribute to scientific validation of the ethnomedical use of this botanic species in folk medicine of South America as diuretic agent, although further studies are necessary to evaluate the mechanisms involved in biological activity and safety following repeated use.

Keywords: bark; branches of approximately three years; diuretic activity; *Jodina rhombifolia*; leaves; phloem.

Resumen

Contexto: *Jodina rhombifolia* (Hook. & Arn.) Reissek (*Santalaceae*) es utilizada en la medicina tradicional para una gran diversidad de problemas de salud.

Objetivos: Evaluar la actividad diurética de extractos metanólicos de diferentes partes aéreas (hojas, corteza, floema y ramas de aproximadamente tres años) en ratas Wistar.

Métodos: La experimentación se organizó con un grupo control negativo (solución salina), un control positivo (furosemida; 10 mg/kg) y para cada extracto vegetal se establecieron tres grupos de animales que recibieron las dosis de 125, 250 y 500 mg/kg.

Resultados: La máxima actividad diurética se observó en el grupo de animales experimentado con el extracto metanólico de las hojas, seguido por el ensayado con el extracto de floema. La dosis de 250 mg/kg del extracto metanólico de las hojas fue el más potente, e igualmente importante resultó la dosis de 500 mg/kg, lo cual supone un interesante efecto excretor de agua para un fitodiurético. Estos registros de diuresis no mostraron diferencias estadísticamente significativas con el grupo control positivo. Además, el comienzo de la actividad diurética del extracto metanólico de las hojas fue extremadamente rápido, dentro de la primera hora de administración (para todas las dosis).

Conclusiones: Este estudio contribuye a la validación científica del uso etnomedicinal de esta especie botánica en la medicina tradicional de Sudamérica como agente diurético; además, futuros estudios son necesarios para evaluar los mecanismos responsables de la actividad biológica y la seguridad de sus uso repetido.

Palabras Clave: actividad diurética; corteza; floema; hojas; *Jodina rhombifolia*; ramas de aproximadamente tres años.

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INTRODUCTION

Jodina rhombifolia (Hook. & Arn.) Reissek (*Santalaceae*) is most commonly known in Argentina under the common names of "Peje", "Quebrachillo", "Quebracho flojo", "Sombra de toro" (Cabrera and Zardini, 1993; De la Peña and Pensiero, 2004; Dimitri, 2004). This specie is utilized in Argentine folk medicine for treat a great diversity of health problems that affect at metabolism and respiratory, digestive, musculoskeletal, cardiovascular and genitourinary systems. Also, it is used as antialcoholic, abortive, for carcinoma and in external applications (Hieronymus, 1882; Ratera and Ratera, 1980; Toursarkissian, 1980; Martínez Crovetto, 1981; Filipov, 1994; Lahitte and Hurrell, 1998; Núñez and Cantero, 2000; Roig, 2002; Vischi and Arana, 2002; Carrizo et al., 2005; Goleniowski et al., 2006; Martínez, 2007; Menseguez et al., 2007; Carosio et al., 2008; Arias Toledo et al., 2009; Barboza et al., 2009; Furlán et al., 2011; Hurrell et al., 2011; Martínez, 2011; Sharry et al., 2011; Madaleno, 2012; Campos, 2014; Alonso and Desmarchelier, 2015). Artisanal fishers in southern Brazil used the leaves for diseases of the genitourinary system (Baptista et al., 2013) and in traditional veterinary medicine of the Sierras de Cordoba (Argentina) was reported the use of aerial parts for urinary disorders (difficulty urinating) (Martínez and Luján, 2011). Furthermore, Romeo (2015) cites the use of leaves in Jujuy traditional medicine for urinary affections.

Despite the existence of multiples ethnopharmacological records, of the widespread use in different regions and of marketing of this plant, very few studies on pharmacological activities and referents to chemical composition of same were carried out.

Diuretic agents elicit increased in production of urine (diuresis) and in the strict sense, the term is applied to drugs with direct action at renal level. The most important indications for diuretics are mobilization of edemas, prophylaxis of renal failure, antihypertensive and congestive heart failure therapy (Lüllmann et al., 2005). However, the diuretic drugs produce several adverse reactions;

therefore, search of new active principles with diuretic activity are of interest to public health.

The aim of the present study was to evaluate the diuretic activity of aerial parts methanolic extract (leaves, bark, phloem and branches of approximately three years) of *J. rhombifolia* in Wistar rats to provide evidence about this ethnopharmacological utilization.

MATERIAL AND METHODS

Drug and chemical reagents

Furosemide (injectable) of registered mark Lasix® (Sanofi-Aventis Argentina S.A., La Tablada, Buenos Aires, Argentina) was used as reference diuretic drug whereas methanol was purchased from Biopack® (Buenos Aires, Argentina).

Plant material collection, identification and preservation

Aerial parts of *J. rhombifolia* were collected in San Luis Province, Coronel Pringles Department, Fraga locality, "Los Chañares" establishment. Bark by decorticating the main stem, leaves in previous stages to be flowering of plant, branches and phloem at time of maximum photosynthetic activity were obtained. Voucher specimens by triplicate were collected, prepared and conditioned for your conservation. Through the application of classical taxonomic methods was determined the botanical identification and certified by Dra. M.E. Petenatti (Herbarium UNSL). For future reference, the voucher specimen was deposited in the Herbarium of the Universidad Nacional de San Luis (Acronym: UNSL), San Luis, Argentine, under the registry No. 517. The collection of plant material was approved by the chief of "Biodiversity" program, Ministry Environment of the government of San Luis province (Resolution N° 588-PBD-2014).

Preparation of methanolic extracts

J. rhombifolia aerial parts (leaves, bark, phloem and branches of approximately three years) were collected and selected minutely. The vegetal mate-

rials were air dried, until to obtain a constant weight; dried material was cut and mechanically milled to powder in a mill with the help of a suitable grinder. The extract of each plant aerial parts was obtained separately from 100 g pulverized material at room temperature by maceration with 400 ml of methanol as solvent for 3 days. The mixture was stirred at intervals to ensure homogeneity during the extraction process. The whole mixture was filtered through filter paper, and the filtrate (methanol extract) obtained was concentrated by evaporating of methanol at 40°C under reduced pressure using a rotary evaporator until obtained a concentrated paste of extract. The residue of filtered was re-extracted twice (2; 72 h) and the methanolic extracts were combined.

Experimental animals

Eighty-four Wistar rats of both sex (180-200 g) were provided by the Central Bioterium of the Facultad de Química, Bioquímica y Farmacia of Universidad Nacional de San Luis (Argentina). All the animals were housed at a constant temperature of $22 \pm 3^\circ\text{C}$ (with periodic cycles of air changes) and a relative humidity of about 50-60%, with a 12-hour day-night cycle (lights on from 07:00 to 19:00 hours), with free access to tap water and standard laboratory rat food. The rats were acclimatized for three days before the commencement of the experiment.

Animal care and procedures were in compliance with the Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT, 1996) Disposition no. 6344/96 for animal care guide-lines and were also authorized by Institutional Committee for the Care and Use of Laboratory Animals (Acronym: C.I.C.U.A.) of our institution (protocol N° F-89/13 in Resolution 010-14).

Diuretic activity assay

Method of Lipschitz et al. (1943), with modification, was employed for the determination of diuretic activity. The rats were deprived of food but not water for 18 h before testing. The animals were divided in fourteen groups of six rats each: group

1, as negative control (received normal saline solution; oral administration); group 2, as positive control [furosemide (10 mg/kg), administered intraperitoneally]. Furthermore, three groups of animals for each *J. rhombifolia* methanolic extract (125, 250 and 500 mg/kg of weight in normal saline solution) were established. The samples were administered through a gastric catheter. All groups of experimental animals received an aqueous overload (10 mL/200 g) and was given in such a way so that fluid intake per kg of animal it was the same in all cases. Immediately after administration, animals were placed in metabolic cages (1 per cage), especially designed to separate urine and feces, and suitable for collection of urine in graduated measuring cylinders. During the three hours of experimentation period, no food and water was available to the animals. The animals were euthanized by inhalation of carbon dioxide.

The urinary excretion was monitored and quantified every 15 minutes for a period of 3 hours and was calculated the Urine Volumetric Excretion (U.V.E.) using the following formula:

$$\text{U.V.E.} = [\text{Collected vol. (mL)}/\text{Administered vol. (mL)}] \times 100 \quad (1)$$

Furthermore, the diuretic index (volume treated group/volume control group) was calculated for each group.

For the batches that received the *J. rhombifolia* leaves methanolic extract, and for negative and positive controls were determined in total urine collected, the ionic concentration of Na^+ and K^+ , pH, density and presence of urobilinogen, glucose, ketones, bilirubin, proteins, nitrite, blood, leukocytes and ascorbic acid. The concentrations of Na^+ and K^+ ions were determined by flame photometry using a Metrolab 315 (Metrolab, Buenos Aires, Argentina) photometer by method of patron aggregated. The pH, density and presence of urobilinogen, glucose, ketones, bilirubin, proteins, nitrite, blood, leukocytes and ascorbic acid were directly determined on fresh urine samples with the Urine strip 10 (Wiener Laboratorios S.A.I.C, Rosario, Argentina).

Statistical analysis

The statistical analysis was performed by using GraphPad Prism version 5.00 for Windows and GraphPad InStat version 3.00 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com). All data were expressed as the mean \pm SEM (Standard Error of Mean). A probability of $p < 0.05$ was considered significant. The figures were created with GraphPad Prism 5.00.

Data of UVE for each period of 15 minutes were evaluated separately by One-way Analysis of Variance (ANOVA) with Tukey-Kramer multiple comparisons test and by two-way [treatment (extract dose); time (period 15 min)] ANOVAs with repeated measures on the "time" factor, followed

by the Bonferroni post-test to compare replicate means by row.

Data of electrolyte excretion, pH and urinary density by were evaluated One-way Analysis of Variance (ANOVA) with Bonferroni multiple comparisons test.

RESULTS

In Table 1 are summarized all values of UVE for each dose of different *J. rhombifolia* extract, the diuretic index and significance of each *versus* negative and positive control groups.

In Tables 2 and 3 are exposed the different diuretic parameters tested for three doses of *J. rhombifolia* leaves methanolic extract, positive and negative control groups.

Table 1. Effect of *J. rhombifolia* aerial parts (leaves, bark, phloem and branches of approximately three years) methanolic extract on total urine excretion in rats and its statistic comparison *vs.* negative and positive control groups.

Group	Dose (mg/kg)	UVE	St. significance (vs. negative control) ^a	St. significance (vs. positive control) ^a	Diuretic index ^b
Control	-	46.06 \pm 4.64	-	-	1.00
Leaves extract	125	77.88 \pm 1.61	p<0.001	p<0.001	1.69
	250	92.72 \pm 1.74	p<0.001	ns	2.01
	500	91.08 \pm 3.02	p<0.001	ns	1.98
Phloem extract	125	77.29 \pm 3.62	p<0.001	p<0.001	1.68
	250	67.43 \pm 3.53	p<0.01	p<0.001	1.46
	500	75.74 \pm 3.76	p<0.001	p<0.001	1.64
Bark extract	125	58.93 \pm 4.33	ns	p<0.001	1.28
	250	66.70 \pm 2.06	p<0.05	p<0.001	1.45
	500	60.31 \pm 5.96	ns	p<0.001	1.31
Branches extract	125	81.61 \pm 4.14	p<0.001	p<0.001	1.77
	250	80.90 \pm 3.49	p<0.001	p<0.001	1.75
	500	61.99 \pm 3.03	ns	p<0.001	1.34
Furosemide	10	107.13 \pm 4.90	-	-	2.32

The values of UVE (Urine Volumetric Excretion) are expressed as mean \pm SEM ($n = 6$). ^aSt. significance: Statistical significance of p *versus* to negative control (0 mg/kg) and to positive control (furosemide; 10 mg/kg) groups for different groups of treatment (according Bonferroni post-test). ^bDiuretic index = volume treated group / volume control group.

Table 2. Effect of oral administration of *J. rhombifolia* leaves methanolic extract (125, 250 and 500 mg/kg), vehicle (negative control) and reference drug furosemide (positive control) on electrolyte excretion in rats.

Treatment	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Saluretic index		Na ⁺ /K ⁺ ratio
			Na ⁺	K ⁺	
Negative control	5.03 ± 1.33	10.19 ± 0.57	-	-	0.49
Positive control	51.44 ± 8.72***	18.40 ± 2.64*	10.22	1.80	0.35
Leaves extract					
125 mg/kg	15.14 ± 3.88	9.40 ± 1.32	3.00	0.92	1.61
250 mg/kg	11.20 ± 4.94	6.41 ± 1.62	2.22	0.62	1.74
500 mg/kg	10.21 ± 5.64	4.89 ± 0.55	2.02	0.47	2.08

The ions concentrations were determined by flame photometry using a Metrolab 315 photometer by method of pattern aggregated. Data are expressed as mean ± SEM of n = 6. Significance of *p* versus to negative control (0 mg/kg) for different groups of treatment (according Bonferroni post-test) (**p*<0.05; ****p*<0.001). Saluretic index = mmol/L treated group / mmol/L negative control group.

Table 3. Effect of single oral administration of *J. rhombifolia* leaves methanolic extract (125, 250 and 500 mg/kg), vehicle (negative control) and reference drug furosemide (positive control) on pH and density urinary in rats.

Treatment	pH	Density (g/mL)
Negative control	6.60 ± 0.10	1.006 ± 0.01
Positive control	7.50 ± 0.22	1.011 ± 0.01
Leaves extract		
125 mg/kg	7.00 ± 0.27	1.005 ± 0.01
250 mg/kg	6.60 ± 0.10	1.008 ± 0.01
500 mg/kg	7.10 ± 0.29	1.004 ± 0.01

The urinary pH and density were directly determined on fresh urine samples with the urine strip 10. Data are expressed as mean ± SEM of n = 6. Significance of *p* versus to negative control (0 mg/kg) for different groups of treatment (according Bonferroni post-test): all values with *p* no significative.

The reference diuretic (furosemide) significantly increased urine output (*p*<0.001) compared to the negative control group, with a diuretic index of 2.32. Administration of leaves extract at 125, 250 and 500 mg/kg also resulted in a significant increase in urine volume (*p*<0.001 for all doses *vs.* negative control) although less than that found with the positive control group. Furthermore, the increase in urine output induced by dose of 500 mg/kg was statistically significant from 45 minutes and doses of 125 and 250 mg/kg were significant since first hour (see Fig. 1), and lasted until the termination of the experiment.

Equally, administration of phloem extract at doses of 125, 250 and 500 mg/kg too resulted in a significant increase of urine volume for all doses,

with a diuretic index *vs.* negative group of 1.68 (*p*<0.001), 1.46 (*p*<0.01) and 1.64 (*p*<0.001), respectively. Furthermore, the increase in urine output induced by dose of 500 mg/kg was statistically significant from 75 minutes and doses of 125 and 250 mg/kg were significant from 90 min of experimentation (Fig. 2) and lasted until the termination.

Administration of branches extract was significant at two lower doses (125 and 250 mg/kg) with a diuretic index of 1.77 and 1.75 respectively (*p*<0.001 for two doses); the dose of 500 mg/kg was not significant *vs.* negative control group. The statistical significance of dose of 125 mg/kg was evident from 90 min of experimentation and since 75 min for dose of 250 mg/kg (Fig. 3).

Much less important result the effect on diuretic activity of bark extract, only dose of 250 mg/kg (diuretic index: 1.45) was significant vs. negative control group ($p < 0.05$). The statistical significance

of this dose was from the first hour of experimentation until 135 min and from 150 min to the end of experimentation (Fig. 4).

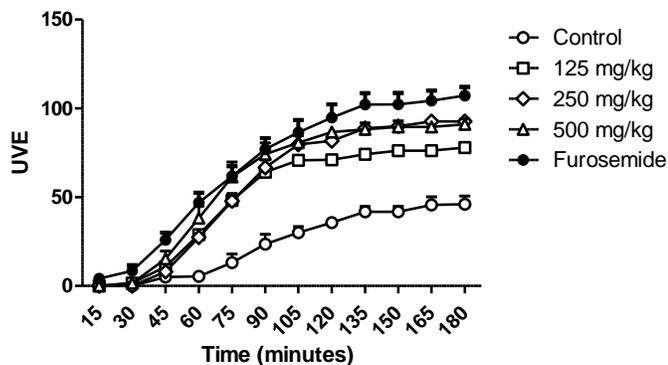


Figure 1. Time course of diuretic activity in Wistar rats treated with different doses of *J. rhombifolia* leaves methanolic extract (125, 250 and 500 mg/kg), vehicle (negative control) and reference drug furosemide (positive control).

Significance vs. Control: Dose 125 mg/kg: $p < 0.05$ (60, 75, 90 and 105 min) and $p < 0.001$ (120, 135, 150, 165 and 180 min); dose 250 mg/kg: $p < 0.05$ (60, 75, 90 and 105 min) and $p < 0.001$ (120, 135, 150, 165 and 180 min); dose 500 mg/kg: $p < 0.05$ (to 45 min) and $p < 0.001$ (60 to 180 min).

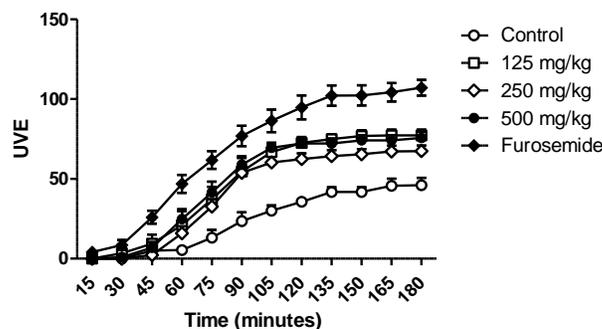


Figure 2. Time course of diuretic activity in Wistar rats treated with different doses of *J. rhombifolia* phloem methanolic extract (125, 250 and 500 mg/kg), vehicle (negative control) and reference drug furosemide (positive control).

Significance vs. Control: Dose 125 mg/kg: $p < 0.01$ (to 90 min) and $p < 0.001$ (105, 120, 135, 150, 165 and 180 min); dose 250 mg/kg: $p < 0.01$ (90 and 165 min) and $p < 0.001$ (105, 120, 135, 150 and 180 min); dose 500 mg/kg: $p < 0.05$ (to 75 min), $p < 0.01$ (90 min) and $p < 0.001$ (105, 120, 135, 150, 165 and 180 min).

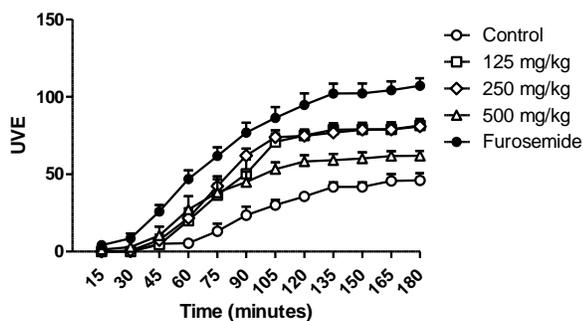


Figure 3. Time course of diuretic activity in Wistar rats treated with different doses of *J. rhombifolia* branches methanolic extract (125, 250 and 500 mg/kg), vehicle (negative control) and reference drug furosemide (positive control).

Significance vs. Control: Dose 125 mg/kg: $p < 0.05$ (90 min) and $p < 0.001$ (105, 120, 135, 150, 165 and 180 min); dose 250 mg/kg: $p < 0.05$ (75 min), $p < 0.01$ (90 min) and $p < 0.001$ (105, 120, 135, 150, 165 and 180 min); dose 500 mg/kg: $p < 0.01$ (105 and 120 min) and $p < 0.05$ (135, 150 and 180 min).

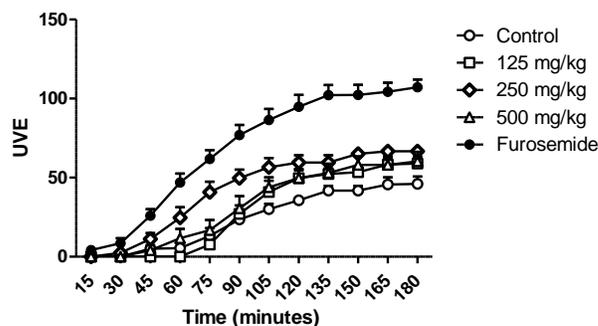


Figure 4. Time course of diuretic activity in Wistar rats treated with different doses of *J. rhombifolia* bark methanolic extract (125, 250 and 500 mg/kg), vehicle (negative control) and reference drug furosemide (positive control).

Significance vs. Control: Dose 125 mg/kg: $p =$ no significant (all values); dose 250 mg/kg: $p < 0.05$ (60, 90, 105, 165 and 180 min) and $p < 0.01$ (75, 120 and 150 min); dose 500 mg/kg: $p =$ no significant (all values).

In all cases each point represents the mean of six rats and vertical bars indicate SEM. The urinary excretion was monitored and quantified every 15 minutes for a period of 3 hours. UVE: Urine Volumetric Excretion [Collected volume (mL)/Administered volume (mL)] x 100.

With respect to the results for *J. rhombifolia* leaves methanolic extract on electrolytic excretion (Na^+ and K^+), was observed that these results compared with those of negative control group were no significant, and furthermore, the values of Na^+ and K^+ excretion decrease with the increase of dose administered of extract. On the other hand, there was absence of urobilinogen, glucose, ketones, bilirubin, proteins, nitrite, blood, leukocytes and ascorbic acid in animal's urine. The values of pH and urinary density too were no significant versus negative control group.

The statistics analysis of different methanolic extracts by two-way [treatment (extract dose); time (period 15 min)] ANOVAs with repeated measures on the "time" factor in comparison with negative control group, were:

***J. rhombifolia* leaves methanolic extract vs. control group (vehicle)**

ANOVA indicated a significant effect of treatment [$F(3,220) = 52.92, p < 0.0001$], and time [$F(11,220) = 707.74, p < 0.0001$], and a significant interaction [$F(33,220) = 17.96, p < 0.0001$] on diuretic activity of *J. rhombifolia* leaves methanolic extract in comparison with control group. The matching is considered extremely significant [$F(20,220) = 11.16, p < 0.0001$].

***J. rhombifolia* phloem methanolic extract vs. control group (vehicle)**

ANOVA indicated a significant effect of treatment [$F(3,220) = 14.00, p < 0.0001$], and time [$F(11,220) = 363.10, p < 0.0001$], and a significant interaction [$F(33,220) = 5.91, p < 0.0001$] on diuretic activity of *J. rhombifolia* phloem methanolic extract in comparison with control group. The matching is considered extremely significant [$F(20,220) = 12.95, p < 0.0001$].

***J. rhombifolia* bark methanolic extract vs. control group (vehicle)**

ANOVA indicated a significant effect of treatment [$F(3,220) = 6.94, p = 0.0022$], and time [$F(11,220) = 191.96, p < 0.0001$], and a significant in-

teraction [$F(33,220) = 2.55, p < 0.0001$] on diuretic activity of *J. rhombifolia* bark methanolic extract in comparison with control group. The matching is considered extremely significant [$F(20,220) = 8.70, p < 0.0001$].

***J. rhombifolia* branches methanolic extract vs. control group (vehicle)**

ANOVA indicated a significant effect of treatment [$F(3,220) = 11.44, p = 0.0001$], and time [$F(11,220) = 327.39, p < 0.0001$], and a significant interaction [$F(33,220) = 7.83, p < 0.0001$] on diuretic activity of *J. rhombifolia* branches methanolic extract in comparison with control group. The matching is considered extremely significant [$F(20,220) = 16.08, p < 0.0001$].

DISCUSSION

The experimental results demonstrated that *J. rhombifolia* aerial parts (leaves, bark, phloem and branches of approximately three years) methanolic extract acts as a diuretic agent in rats, with an increased excretion of urine total volume. The maximum diuretic activity was observed in the animals group administered with *J. rhombifolia* leaves methanolic extract followed of phloem extract.

The dose 250 mg/kg of *J. rhombifolia* leaves methanolic extract was the more potent, with a diuretic index of 2.01. Equally important, result the dose 500 mg/kg of leaves methanolic extract with a diuretic index of 1.98, which supposes an interesting excretor effect of water for a phytodiuretic. The registers of diuresis in Wistar rats with doses 250 and 500 mg/kg of *J. rhombifolia* leaves methanolic extract not showed significant statistically results with positive control group that received the reference diuretic, furosemide (10 mg/kg). Furthermore, the onset of diuretic activity of the *J. rhombifolia* leaves methanolic extract was extremely rapid, within first hour of administration (for all doses), as observed with synthetic loop diuretics used clinically (Rang et al., 2012).

The minimal diuretic activity was observed with *J. rhombifolia* bark methanolic extract (diuretic index of 1.28, 1.45 and 1.31 respectively for doses of 125, 250 and 500 mg/kg), therefore assumed

that active metabolites responsible for diuretic activity are not found in the bark of this plant species, or well, these are in low concentrations.

The administration of *J. rhombifolia* leaves methanolic extract to Wistar rats failed to show a significant increase in electrolytic excretion of Na⁺ and K⁺ ions, despite observing a significant increase in urine total volume collected, that is to say, was not found a parallelism into increase of urine volume when they were administered increasing doses of leaves extract and the ionic excretion, since the same decreased. These results obtained could indicate a probable aquaretic effect of extract of this vegetal organ and not a natriuretic action.

The mechanism of action by which *J. rhombifolia* exerts its effect on diuretic activity cannot be explained with the present data. Phytochemically, leaves contain tannins, phenolic compounds, organic acids, flavonoids, steroids, gums and mucilages (Barboza et al., 2009); additionally, was revealed the presence of C-glycosyl flavonoids identified as vicenin-2, vitexin, orientin, and swertisin (Montanha et al., 2009) and isovitexin (Carballo de la Peña, 2015). However, it is difficult to attribute the diuretic activity to C-glycosyl flavonoids because they are poorly absorbed in digestive tract, suggesting the necessity of a time prolonged in contact with the mucosa so that they can exert their pharmacological action (Talhi and Silva, 2012; Zeng et al., 2013). Leaves extract contains various groups of components that may contribute to its effect, even may result from the combined mechanism of several constituents. Further pharmacological studies with *J. rhombifolia* are necessary to a firm conclusion about the mechanism of action.

CONCLUSIONS

These results contribute to scientific validation of the ethnomedical use of this botanic species in folk medicine of South America as diuretic agent, although further studies are necessary to evaluate the mechanisms involved in biological activity and safety following repeated use. Finally, these results stimulate further investigations to identify the ac-

tive chemically compounds responsible of effects observed in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION:

Contribution	Teves MR	Pacheco PH	Bazán C	Wendel GH
Concepts or ideas	x	x	x	
Design	x	x	x	
Definition of intellectual content	x	x		
Literature search	x			
Experimental studies	x	x		
Data acquisition	x	x		x
Data analysis	x	x	x	
Statistical analysis	x	x	x	
Manuscript preparation	x	x		
Manuscript editing	x	x	x	
Manuscript review	x	x	x	

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