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HYPOGLYCEMIC ACTIVITY OF *JUNGIA RUGOSA* LESS ON INDUCED DIABETIC MICE (*MUS MUSCULUS*)

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Abstract

Jungia rugosa Less is a plant species that belongs to the Asteraceae family and it is distributed in the Andean region. This study has been focused on evaluate the hypoglycemic activity of *Jungia rugosa* leaves from Ecuador and determine its flavonoids content. The flavonic fraction (aglycones) was obtained from *J. rugosa* by reflux using ethanol 70%, methanol and ethyl acetate. The determination of flavonoids content was carry out through HPLC. The hypoglycemic activity of the flavonoid fraction was performed on induced severe and mild diabetic mice, using extracts at doses of 25, 100 and 400 mg/kg and standard controls (acarbose 20 mg/kg, insulin glargine 0.5 mmol/kg). Glucose levels were reduced in both severe diabetic and mild diabetic mice treated with *J. rugosa* extracts. In mild diabetes, the hypoglycemic activity of *J. rugosa* was statistically similar to acarbose. Flavonic fraction was not statistically similar to insulin treatment for severe diabetic mice. This activity is related to the presence of luteoline and apigenin, flavonoid aglycones found through HPLC analysis. Based on the results of this research, it can be concluded that *J. rugosa* is an interesting source of natural hypoglycemic compounds. Further detailed phytochemical, toxicity and clinical studies are needed.

Keywords: *Jungia rugosa, flavonoids, diabetes, hypoglycemic activity, luteolin, apigenin*

Introduction

Diabetes mellitus (DM) is a chronic disorder characterized by abnormal rise in blood glucose levels as well as alterations in both protein and lipids metabolism due to the pancreas inability to produce enough insulin and/or as a result of the body's dysfunction to effectively use the insulin it produces (1). In 2012, there were 1.5 million deaths worldwide directly caused by diabetes. Approximately, 420 million people were suffering from DM globally in 2014 and it is envisaged that this figure will increase up to 650 million by 2040 (1) (2). Currently, insulin and oral hypoglycemic agents are used for DM therapy, such as glibenclamide, metformin, sulfonylureas and α-glucosidase inhibitors. Nevertheless, these drugs are expensive and some of them have serious side effects (3). Due to the above, alternative therapies with little or no side effects are becoming the primary focus for the treatment and management of DM (1) and natural products, especially medicinal plants, have been considered as potential sources of safe and effective antidiabetic agents.

Plants were once the primary source of medicines in the world. Since they keep providing human being with new remedies, 50% of all drugs in clinical use in the world are derived from natural products, from which higher plants contribute 25% of the total (4). In developing countries, approximately 80% of people use traditional medicine because of its affordability and cultural acceptability (4), often representing the only therapeutic system to which certain people could refer (5).

Jungia rugosa Less (Asteraceae) is a vine or subshrub present in the Andean region of Ecuador, Peru and Bolivia (6). This species has four synonyms: *Cineraria stipulacea* Willd ex Less, *Jungia bullata* Turcz, *Jungia jelskii* Hieron and *Jungia malvifolia* Muschl (7). In Ecuador, it is commonly known as "*carne humana*", "*fompo*" or "*guayombo*" and it is traditionally used as medicine for the treatment of bruises, cuts and other external inflammatory processes (8). Similar ethno-medical applications have been described in Andean communities in the north of Perú (9).

There are only two pharmacological studies about *Jungia rugosa* (10) (11) focused on their anti-inflammatory and antioxidant activities. The results demonstrate an effective anti-inflammatory activity in acute and chronic inflammation on mice, supporting their traditional use. Similar pharmacological effects were found in other *Jungia* species: *Jungia paniculata* (12) and *Jungia sellowii* (13).

Jungia rugosa from Peru contains flavonoids in remarkable quantities (1.15 %) (10). Different studies show the antidiabetic effectiveness of phenolic and other compounds from plants both *in vitro* and *in vivo* models (14).

This study aimed to investigate the flavonoid content of leaves of *Jungia rugosa* grown in Ecuador and the *in vivo* hypoglycemic activity on mild and severe diabetic mice (*Mus musculus)* as animal models.

Methods

Collection of plant material

Leaves from *Jungia rugosa* were collected from Ecuador, in Pichincha province, in Mejía town (2945 meters above sea level). The plant material was taxonomically identified by the botanist Jorge Caranqui at the Herbarium of Escuela Superior Politécnica de Chimborazo (Riobamba, Ecuador). Collections were approved by the Ministerio del Ambiente del Ecuador under permission MAE-DNB-CM-2018-0086.

Flavonoid aglycones extraction

Leaves were selected, dried in a forced convection oven and ground in a knife mill until particle size of 2-3 mm, prior to the preparation of extracts. A 70% ethanolic extract was obtained by reflux for 4 hours, and then it was concentrated. The remaining aqueous suspension was dissolved with methanol (1:2). Finally, the flavonic fraction (aglycones) was extracted with ethyl acetate and concentrated until dryness (15).

Determination of flavonoids content by HPLC

The HPLC analysis was performed on Agilent Zorbax SB-C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of 0.1% formic acid, acetonitrile and methanol (60:16:24, v/v/v) at 30±1°C, in a constant flow rate of 1 ml/min. The wavelength was set at 350 nm for quantitative analysis. The

percentages of the standards components used were: 95% quercetin, apigenin 98% and 98% luteolin. Quercetin was selected as an internal standard (16).

Animals

Eight-week-old male mice *Mus musculus* BALB/C strain weighing 35–45 g were used in this study. They were kept under a 12 h light and 12 h darkness cycle. The animals were feed with a basic diet to rodents and water *ad libitum*.

Streptozotocin-nicotinamide-induced mild diabetes

Mice were fasted overnight before the experiments. Streptozotocin was dissolved in 50 mM citric acid buffer (pH = 4.5) before intravenous administration at 50 mg/kg. Nicotinamide (110 mg/kg) was dissolved in normal saline and intraperitoneally administered 20 minutes before streptozotocin. Normal control mice were administered normal saline (17) (18).

Streptozotocin-induced severe diabetes

After fasting 12 h, mice were rendered diabetic by intraperitoneal administration of multiple, low doses of streptozotocin (50 mg/kg) dissolved in citrate buffer (pH 4.5). The administration was done within 5 consecutive days (18) (19) (20).

Hypoglycemic activity

The hypoglycemic effect of *J. rugosa* was carried on overnight fasted diabetic mice, 8 days after the induction of diabetes. Experimental animals were divided randomly into groups of 5 mice. For each study of severe diabetes and mild diabetes, 6 groups of mice were used.

Group 1: Normal Control (non-diabetic mice + vehicle). Group 2: Diabetic control (diabetic + vehicle). Group 3: Standard Control (diabetic + conventional drug). From Group 4 to Group 6 served as test groups and received *Jungia rugosa* extract orally at the dose of 25, 100 and 400 mg/kg, respectively, using gastric gavage needle (21). The same Normal Control group was used in both types of diabetes in order to reduce the number of experimental animals.

In mild diabetic mice, acarbose (20 mg/kg) was the conventional drug administered; whereas in severe diabetic mice, insulin glargine was administered (0.5 mmol/kg). Vehicle was propylene-glycol 1% v/v.

Diabetic mice were treated orally daily up to 7 days. Blood samples were withdrawn at 0, 30, 60, 120, 240, and 360 min from the tail vein of mice and the blood glucose level was measured by using a glucometer (21). Blood samples were collected at the established time intervals from experimental animals in days 0, 1, 3, 5 and 7. On day 0, no conventional drugs or plant extracts were administered.

Data analysis

All values were expressed as mean ± SD (standard deviation) and data were analyzed by applying an analysis of variance (ANOVA) followed by Tukey test. The results were considered statistically significant if $p<0.05$

Results

Determination of flavonoids content by HPLC

HPLC chromatogram of the standard mixture of flavonoids is shown in Figure 1. The detected flavonoids in flavonic extract are presented in Figure 2 and Table 1. The HPLC analysis has shown that *J. rugosa* presented considerable amount of luteolin (195.70 µg/g extract) followed by apigenin (68.12 µg/g extract). Quercetin was absent in this extract. The remaining unknown peak was not characterized and it requires further study.

Streptozotocin-nicotinamide-induced mild diabetes

Table 2 shows the results of blood glucose levels during the week of treatment. Firstly, the effectiveness of the induction of diabetes has been identified since mice reached glucose levels > 190 mg/dl (22) after the administration of streptozotocin and nicotinamide. In healthy mice, the concentration of blood glucose is 80-110 mg/dl (22). This is seen in the significant difference between the normal control group and diabetic control.

On the other hand, both the standard treatment (acarbose) and flavonic fraction of *J. rugosa* in different concentrations reduced glucose levels significantly in diabetic mice. There was a statistical similarity between the treatment with acarbose and with the extracts of the studied species, which indicated an equivalent effectiveness of the

conventional drug and *J. rugosa* for the reduction of high glucose levels. Both acarbose and extracts allowed the reduction of glucose to basal levels (80-110 mg/dl), although there was no statistical similarity to the normal group.

There was no significant difference between the flavonic fractions of *J. rugosa* at 25 mg/kg and 100 mg/kg, nor between 100 mg/kg and 400 mg/kg. However, the treatments with 25 mg/kg and 400 mg/kg presented a slight difference, showing on day 7 glucose levels of 111.71 ± 7.6 mg/dl and 94.30 ± 8.5 mg/dl, respectively. Although the glycemia values are lower when the concentration is 400 mg/kg, the use of the flavonic fraction of *J. rugosa* at 25 mg/kg would be recommendable in order to avoid toxicity due to high concentrations.

Streptozotocin-induced severe diabetes

Table 3 shows the results of blood glucose levels during the treatment of severe diabetes. The administration of multiple doses of streptozotocin allowed the induction of severe diabetes; as a result, mice showed blood glucose levels around 290 mg/dl (23) on day 0.

A reduction in glucose levels was found in both the standard control group (insulin) and the groups treated with *J. rugosa* extracts. Nevertheless, fractions of *J. rugosa* do not achieve a decrease to basal glucose levels (80-110 mg/dl) and are not statistically similar to insulin treatment.

Moreover, there are no statistical differences between the three concentrations (25 mg/kg, 100 mg/kg, 400 mg/kg) of the flavonoid fraction of *J. rugosa.*

Discussion

Flavonoid aglycones found in *J. rugosa* from Ecuador, luteolin and apigenin, are also present in other species of Asteraceae family as *Helichrysum chasmolycicum*, *Tanacetum parthenium* or *Chrysanthemum sp.* (24) (25) (26).

J. rugosa from Peru presented 3', 5-dihydroxy-4', 7-di methoxy flavone, 4 ',5,6,8-tetramethoxy-7-O-sugar flavone and 3'- hydroxy-5,6,7-tri-methoxyflavone (10). The flavonoids luteolin and apigenin, determined in our study, belong to the group of flavones (27) as well as the compounds found by Enciso et al. The chemical variation of secondary metabolites in the same species could be related to several factors: habitat conditions, temperature, humidity, luminosity, altitude, pluviometry, ultraviolet radiation, soil and nutrient conditions, seasonality, circadian cycle, method of collection, drying and the part of the plant used (28) (29).

The flavonic fraction of *J. rugosa* allowed a remarkable decrease in blood glucose levels in both mice with moderate diabetes and severe diabetes. However, the use of *J. rugosa* was more convenient in moderate diabetes, since in this case baseline glucose levels were reached.

The hypoglycemic activity of *J. rugosa* can be attributed to the presence of the aglycone flavonoids detected: luteolin and apigenin. Since the early 1980s, the potential effects of flavonoids in diabetes mellitus have been studied extensively and these secondary metabolites have shown a potent antioxidant activity, suggesting to be beneficial in the treatment of this disease. The ability of antioxidants to protect against the deleterious effects of hyperglycemia, to enhance glucose metabolism and to uptake should be considered as a lead alternative in diabetes mellitus treatment. On top of their antioxidant effect, flavonoids may act on biological targets involved in type 2 diabetes mellitus such as α-glycosidase and DPP-4 and can effectively prevent and/or manage type 2 diabetes mellitus (30).

Luteolin (LU) is one of the natural flavonoids, common in many edible plants, such as pepper, celery, carrot, and spinach, that has been proved to possess strong antioxidant and anti-inflammatory activities (31) as well as the improvement of insulin resistance in diet-induced obese mice (32) (33). Luteolin and Luteolin-7-O-glucoside (LUG) had the abilities of anti-diabetes, but the effects of LU were stronger than those of LUG (34).

Cyclic AMP (cAMP) regulates insulin secretion from pancreas. An increase in cAMP level induced by an agent could be interpreted as inhibition of cAMP-phosphodiesterase enzyme activity which eventually reduces blood glucose concentration through stimulation of insulin secretion (35). Petkov et al. investigated inhibitory effect of some

flavonoids on cAMP-phosphodiesterase and reported that aglycone luteoline had potent inhibitor effect on this enzyme; whereas isoorientin and orientin, C-glycosides of luteoline, were inactive (36). These data reveal that the hypoglycaemic effect of luteoline depend on cAMP-phosphodiesterase enzyme inhibition (37).

Apigenin (4, 5, 7-trihydroxyflavone) is a naturally-occurring plant flavone, which exists in many fruits, vegetables, herbs and spices. Several studies have demonstrated that it possesses several biological functionssuch as anti-proliferative, anti-inflammatory, anti-obesity, antioxidant, and anti-cancer properties (38) (39) (40). Studies carry out on diabetic rats showed that apigenin significantly decreased the levels of blood glucose (41) (42), serum lipid, malonaldehyde, intercellular adhesion molecule-1 (ICAM-1) and insulin resistance index. This metabolite also increased superoxide dismutase activity and improved impaired glucose tolerance (41).

Apigenin was reported to enhance GLUT-4 translocation and downregulation of CD38 enzyme to improve diabetes, to possess a potent antihyperglycemic activity and to inhibit DPP-IV enzyme (43). Also, apigenin lowered pro-inflammatory cytokines and chemokines in plasma and controlled hyperglycemia, hyperinsulinemia and insulin resistance (44). The improved glucose metabolism by apigenin was described to be mediated through the inhibition of hepatic gluconeogenic enzyme activities (42) (44).

This research showed the significant hypoglycemic activity of the flavonic fraction of *Jungia rugosa* on diabetic mice during a period of 7 days, especially in moderate diabetes. This activity is related to the presence of luteoline and apigenin. Further studies are recommended to determinate the structure of the unknown compound and to investigate the toxicity of the extract of *Jungia rugosa.*

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Figure 1. HPLC chromatogram of a standard mixture of flavonoids. Peaks: 1, quercetin; 2, luteoline; 3, apigenin.

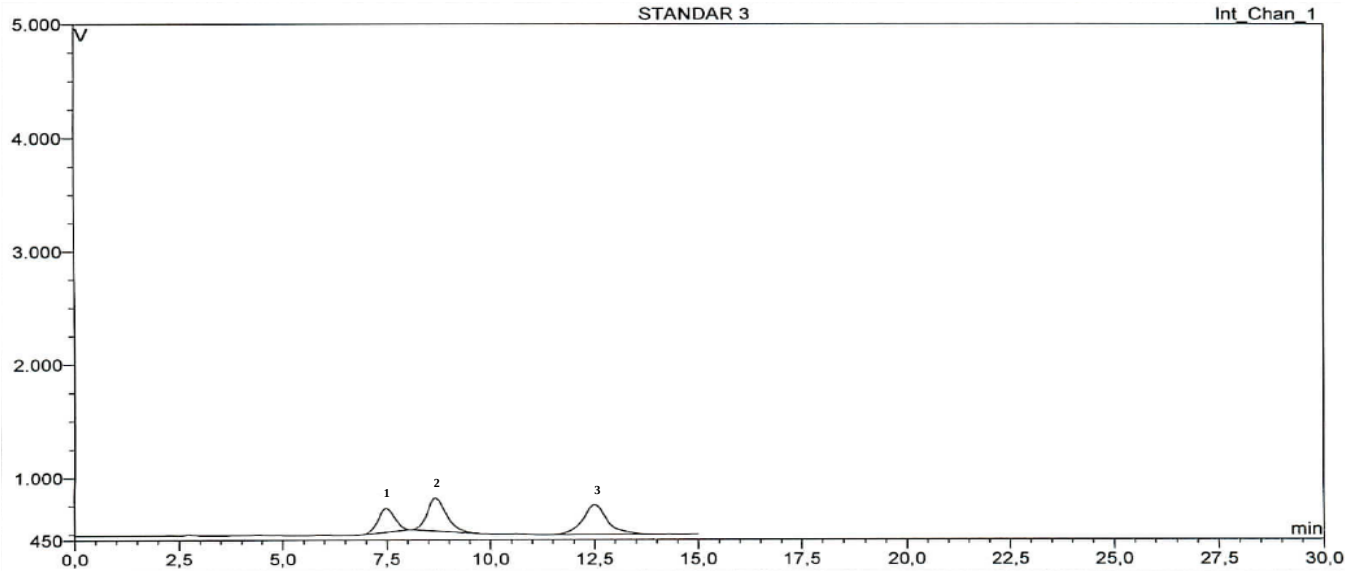

Figure 2. HPLC chromatogram of the flavonic extract of *J. rugosa*. Peaks: 1, luteolin; 2, apigenin; 3, not identified compound.

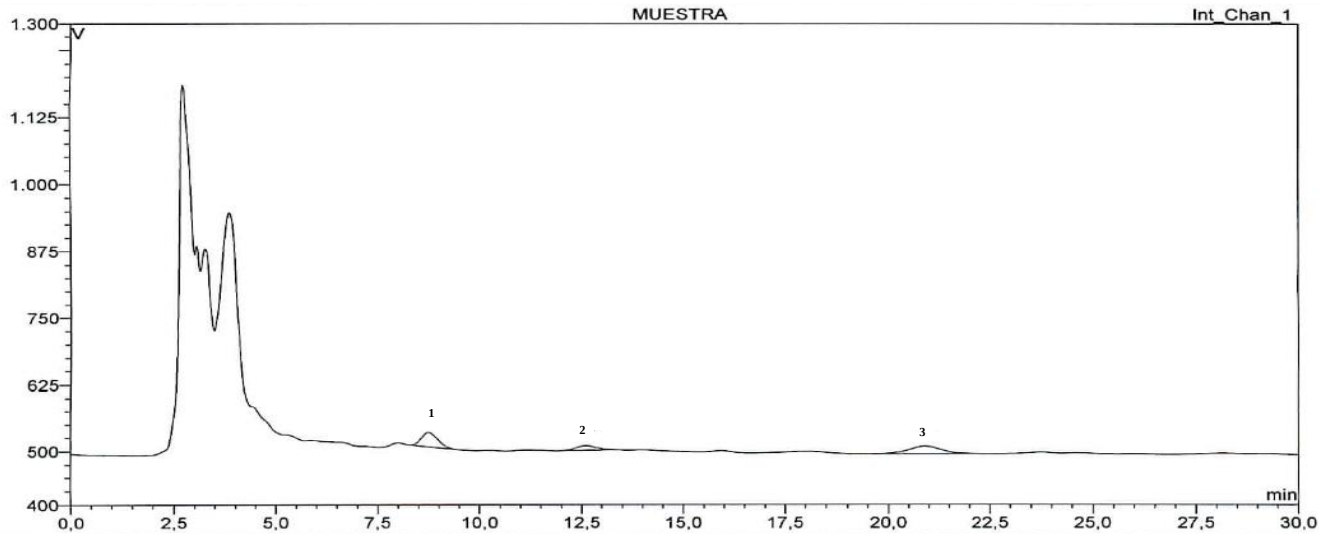

Table 1: Retention times and concentrations of compounds of the extract of *J. rugosa*

Flavonoids (aglycones)	RT (min) of standard compounds	Flavonic extract of *J. rugosa*	
		Rt (min)	**Concentration (µg/g extract)**
Quercetin	7.480	nd	nd
Luteolin	8.669	8.738	195.70
Apigenin	12.506	12.604	68.12
Unknown compound	---------	20.881	--------

Nd: not detected

Table 2: Effect of flavonic fraction of *Jungia rugosa* on blood glucose levels (mg/dl) in mild diabetes

Group	**Day 0***	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Normal control	80.52 ± 7.5	81.13 ± 8.3	81.05 ± 7.8	80.62 ± 7.4	82.01 ± 9^{d}
Diabetic control	262.47 ± 4.6	225.67 ± 9.0	230.34 ± 4,5	196.66 ± 7.5	214.33 ± 9.7^{c}
Standard Control (Acarbose 20 mg/kg)	265.62 ± 6.7	165.68 ± 2.1	149.32 ± 9.1	140.34 ± 9.9	113.00 ± 12.8$^{a, b}$
Diabetic + *J. rugosa* (25 mg /kg)	255.50 ± 5.2	168.32 ± 17.6	139.72 ± 6.8	114.02 ± 8.5	111.71 ± 7.6^{b}
Diabetic + *J. rugosa* (100 mg/kg)	259.53 ± 7.4	89.33 ± 15.00	104.62 ± 4.7	101.01 ± 3.6	100.28 ± 8.5$^{a, b}$
Diabetic + *J. rugosa* (400 mg/kg)	259.41 ± 6.2	105.70 ± 7.6	108.65 ± 2.9	102.31 ± 2.5	94.30 ± 8.5^{a}

Values are given as mean standard deviation. Different letters in the same column indicate significantly different ($p<0.05$) when analyzed by Tuckey test.

***** *before the administration of treatments*

Table 3: Effect of flavonic fraction of *Jungia rugosa* on blood glucose levels (mg/dl) in severe diabetes

Group	**Day 0***	**Day 1**	**Day 3**	**Day 5**	**Day 7**
Normal control	80.52 ± 7.5	81.13 ± 8.3	81.05 ± 7.8	80.62 ± 7.4	82.01 ± 9^{d}
Diabetic control	292.05 ± 8.4	239.00 ± 9.8	265.67 ± 10.7	230.33 ± 10.1	222.31 ± 2.1^{c}
Standard Control (Insulin 20 mg/kg)	294.12 ± 7.2	191.00 ± 19.3	123.00 ± 5.6	103.34 ± 7.6	90.00 ± 11.0^{a}
Diabetic + *J. rugosa* (25 mg /kg)	291.43 ± 6.3	249.00 ± 13.1	121.33 ± 3.5	121.00 ± 4.6	120.67 ± 7.8^{b}
Diabetic + *J. rugosa* (100 mg/kg)	296.07 ± 7.8	186.32 ± 20.6	126.00 ± 7.0	124.00 ± 3.0	123.33 ± 10.4^{b}
Diabetic + *J. rugosa* (400 mg/kg)	295.51 ± 5.6	181.00 ± 3.6	112.00 ± 11.4	111.67 ± 9.5	116.00 ± 4.4^{b}

Values are given as mean standard deviation. Different letters in the same column indicate significantly different ($p<0.05$) when analyzed by Tuckey test.

***** *before the administration of treatments*