



Anti-urease activity of native species of genus *Piper* from Guatemala with potential application in infection control

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Abstract

Introduction: Urease is an enzyme that catalyzes the hydrolysis of urea in the gastrointestinal and urinary systems, related with chronic bacterial infection. *Piper* is a basal angiosperm genus with ethnobotanical application. The aim of this study was to determine urease inhibitory activity of extracts from ten Guatemalan *Piper* species.

Methods: Leaves from 7 species were collected in Suchitepequez (*P. amalago*, *P. auritum*, *P. hispidum*, *P. jacquemontianum*, *P. oradendron*, *P. retalhuleunse*, and *P. umbellatum*), and 3 in Alta Verapaz (*P. psilorhachis*, *P. sempervirens*, and *P. variable*), shade-dried and milled. Dichloromethane (DCM) and methanol (MeOH) extracts were prepared by percolation and concentrated by rotavapor. The inhibitory effect was determined by enzymatic assays, qualitative by thin layer chromatography developed by phenol red; and quantitative by spectrophotometric kinetic method evaluated by microcolorimetry at 630 nm with phenol red.

Results: Seventeen extracts out of 20 demonstrated anti-urease activity by TLC, showing one discoloration band with Rf 1.4-5.8; *P. psilorhachis* showed 2 discolorations bands at Rf 5.3 and 5.8, and the best activity (IC₅₀ MeOH 1.9 ± 0.08 µg/mL and DCM 2.1 ± 0.06 µg/mL), similar to positive control (hydrocyclohexanone IC₅₀ 1.4 ± 0.05 µg/mL). Two other species, *P. umbellatum* (IC₅₀ MeOH 2.5 ± 0.06 µg/mL) and *P. retalhuleunse* (IC₅₀ MeOH 4.1 ± 0.7 µg/mL, DCM 4.8 ± 0.06 µg/mL) showed a moderate activity.

Conclusion: Three species showed interesting anti-urease activity that deserves future studies by bio-guided fractionation. Literature review demonstrated that this is the first report about the urease activity of these *Piper* species.

Keywords: Urea, *Piper psilorhachis*, *Piper umbellatum*, *Piper retalhuleunse*, *Helicobacter pylori*



Introduction

Each organism breaks down nitrogen containing nucleic acids and proteins by specific enzymes, generating nitrogenous waste. Mammals, amphibians and some invertebrates excrete nitrogenous waste such as urea, which is produced in the liver and in small amount in the kidneys. Urea is an especially good compound for the removal of nitrogen because it is soluble in water and less toxic. It is a waste compound formed from ammonia, which is the main end product in protein metabolism and constitutes about one half of the total urinary solids. The proteins are simplified into amino acids; those that will not be used by the organisms go through a process of deamination that leads to the formation of ammonia, which form urea when combined with compounds present in the environment.^{1,2}

Urease is an enzyme that catalyzes the hydrolysis of urea for the production of ammonia; urea changes the local pH, in the gastrointestinal system it has been associated with *Helicobacter pylori* infection and related to the development of gastric ulcers and cancer³; and, in the urinary tract it has been demonstrated some association with chronic renal disease.⁴ For this mechanisms it is considered a virulence factor in the case of infection of the gastrointestinal system and the normal microbiota in the urinary tract, conducting to the chronic infection of both systems in a so call ureolytic bacterial infection.⁵

The use of this enzyme depends on the producing organism, for example *Helicobacter pylori* produces the accumulation of reactive oxygen species resulting from prolonged damage, and important levels of urease that



come from bacterial metabolism, changing the gastric pouch pH.⁶ An antioxidant and inhibitory effect of urease helps to diminish the effects of these infections and the severity of the clinical picture.

The compounds that inhibit the enzymes are of great importance in the prevention and treatment of a series of chronic diseases.⁷ Urease inhibitors can be broadly classified into 2 categories, active site directed (substrate-like), each inhibitor create a bridge between the nickel ions in the active site of the enzyme, and. mechanism-based directed, they affect the catalysis that leads to enzymatic inactivation; several plant-derived urease inhibitors have been described.^{8,9} Screening and confirmation studies have demonstrated urease inhibitory activity in gastrointestinal and urinary tract by traditional medicinal plants from China,¹⁰ Pakistan,¹¹ Iran,¹² Czech Republic,¹³ India,¹⁴ and other countries.

Important urease inhibitory activity associated with *H. pylori* inhibition has been demonstrated in several plant species, extracts or isolated compounds, particularly the phytochemical synergy of the water extracts, mainly the phenolics, of *Origanum vulgare* L, and *Vaccinium macrocarpum* Alton¹⁵; the oil extract of *Chamomilla recutita* (L.) Rausch¹⁶; the non-fermented and semi fermented *Camellia sinensis* L;¹⁷ and, the resveratrol and red wine.¹⁸

Piper is a basal angiosperm genus that belongs to the Piperaceae family with a thousand species and diverse ethnobotanical application; the genus is very diverse in its phytochemistry and biological activity, and 80% of the species of the genus are native of the Americas.¹⁹

The literature reviewed about *Piper* species with urease inhibitory activity demonstrated that there are no previous studies. Suggestive information found are studies carried out in Brazil, which demonstrated growth inhibition of *H. pylori* by the leaf extract of *Piper carpubya* Ruiz & Pav.²⁰ and in Panama, where a prenylated salicylic acid derivative was isolated from *Piper multiplinervium* C. DC.²¹ In both cases the mechanism by which the inhibition occurs was not evaluated, suggesting that it could be due to a possible

inhibition of the urease enzyme.

The present investigation is the first study conducted in Guatemala for studying urease inhibition activity in plant species from genus *Piper*.

Materials and Methods

Plants Collection and Extracts Preparation

Leaves from 10 species of genus *Piper* (*P. amalago*, *P. auritum*, *P. hispidum*, *P. jacquemontianum*, *P. oradendron*, *P. psilorhachis*, *P. sempervirens*, *P. umbellatum*, *P. retalhuleuense* and *P. variable*) were collected according to good collection practices in Samayac, Suchitepequez and Lachua, Alta Verapaz (Table 1). Flowering samples were identified by a biologist (Luis Alvarez) and confirmed by a botanist (Mario Veliz, BIGU Herbarium, USAC); voucher samples were deposited at Cemat-Farmaya Ethnobotanical Herbarium (CFEH). Plant material was dried for 48 hours at 40°C. Dichloromethane (DCM) and methanol (MeOH) extracts were prepared by sequential percolation technique and concentrated to dryness in rotavapor according to Sharapin.²²

Evaluation of Urease Inhibitory Activity

Qualitative determination of urease inhibitory activity was demonstrated by thin layer chromatography (TLC) bioautographic assay and developed with phenol red, with the following conditions.¹⁰ Extracts were applied to TLC and developed with mobile phase chloroform:methanol (200:1 v/v), after drying the plate was sprayed with jack bean urease and preincubated 2 hours, then sprayed with buffer solution (pH 6.8 100 mM) containing 500 mM of urea and phenol red (0.002%); omeprazol was used as standard. After incubation for 1 hour at 25°C, a red background was developed, the presence of white spots (decolorized) on the plate evidenced the enzymatic inhibition.

Urease inhibitory activity was quantitated by micro colorimetry with phenol red and urea with the following characteristics according to Tanaka et al²³: reacting elements were 100 mmol/L of phosphates buffer, 500 mmol/L of urea and 0.002% of phenol red; hydrocyclohexanone

Table 1. Provenance and Extraction Yield of 10 *Piper* Species from Guatemala

<i>Piper</i> Species	Place of Collection (Department)	CFEH Voucher	Extraction Yield (%)	
			Dichloromethane	Methanol
<i>P. amalago</i> L.	Samayac, Such.	1073	7.96	3.52
<i>P. auritum</i> L.	Samayac, Such.		6.26	7.54
<i>P. hispidum</i> Swartz	Samayac, Such.	1071	9.18	7.98
<i>P. jacquemontianum</i> Kunth	Samayac, Such.	1069	7.37	8.63
<i>P. ordendron</i> Trel. & Standl.	Samayac, Such.	1075	8.41	7.34
<i>P. psilorhachis</i> C. DC.	Chisec, AV	1143	10.71	5.42
<i>P. retalhuleuense</i> Trel. & Standl.	Sto. Domingo, Such.	1053	6.97	7.25
<i>P. sempervirens</i> (Trel.) Lundell	Chisec, AV	1113	11.49	6.33
<i>P. umbellatum</i> L.	Samayac, Such.	1070	8.89	7.43
<i>P. variable</i> C. DC. ex Donn.-Sm.	Chisec, AV	1140	15.53	3.29

Table 2. Urease Inhibitory Activity Determined by Micrometric Methodology with Phenol Red

Species	Solvent	Rf1	Rf2	Results	CI50 µg/mL	95% CI	µg EH/mg
<i>P. amalago</i>	MeOH	4.0	-	++	22.3±1.5	20.8-23.8	19.1±0.5
	CH ₂ Cl ₂	4.1	-	++	18.2±1.0	17.2-19.2	16.5±0.5
<i>P. auritum</i>	MeOH	3.0	-	++	30.3±1.3	29.0-31.6	25.6±1.2
	CH ₂ Cl ₂	2.4	-	++	12.2±0.9	11.3-13.1	13.7±0.8
<i>P. hispidum</i>	MeOH	1.7	-	++	17.9±1.1	16.8-19.0	18.1±1.0
	CH ₂ Cl ₂	1.4	-	++	29.4±1.5	27.9-30.9	23.0±1.6
<i>P. Jacquemontianum</i>	MeOH	2.6	-	+	15.7±1.0	14.7-16.7	18.6±0.4
	CH ₂ Cl ₂	4.0	-	++	26.3±1.8	24.5-28.1	24.7±0.8
<i>P. ordendron</i>	MeOH	1.3	-	++	31.6±1.2	30.8-32.8	22.1±0.7
	CH ₂ Cl ₂	1.6	-	++	27.5±1.0	26.5-28.5	30.8±1.6
<i>P. psilorhachis</i>	MeOH	5.3	5.8	+++	1.9±0.08	1.8-2.0	3.5±1.1
	CH ₂ Cl ₂	4.8	-	++	2.1±0.09	2.0-2.2	3.6±0.9
<i>P. retalhuleense</i>	MeOH	3.4	-	++	4.1±0.07	4.0-4.2	4.6±1.3
	CH ₂ Cl ₂	1.4	-	++	4.8±0.06	4.7-4.9	5.5±0.8
<i>P. sempervirens</i>	MeOH	2.5	-	++	13.7±0.9	12.8-14.6	12.2±0.6
	CH ₂ Cl ₂	3.6	-	++	12.9±0.9	12.0-13.8	11.3±1.1
<i>P. umbellatum</i>	MeOH	3.2	-	++	2.5±0.06	2.4-2.6	3.9±0.9
	CH ₂ Cl ₂	1.9	-	++	5.6±0.09	5.5-5.7	4.6±0.8
<i>P. variable</i>	MeOH	4.3	-	+	34.8±1.3	33.5-36.1	45.5±1.2
	CH ₂ Cl ₂	4.8	-	+	30.6±34.0	30.6-34.0	53.3±0.9
Omeprazol	Standard	5.0	-	+++	DA	DA	DA
Hydrocyclohexanone					1.4±0.05	1.3-1.5	DA

EO, Equivalent of hydrocyclohexanone per gram of extract; DA, Does not apply.

was used as standard; incubation at 37°C for 3 hours. Concentration of ammonium carbonate was measured spectrophotometrically (Awareness Technologies Stat-Fax-2109) at 630 nm.²⁴

Results and Discussion

To evaluate the urease inhibition activity, omeprazole was used as standard, which is an anti-ulcer drug used in ulcers associated with *H. pylori* since it performs a competitive inhibition at the active site of the enzyme.⁶ Omeprazole showed an IC₅₀ value of 1.4 µg/mL confirming its high efficacy.

In the qualitative analysis, all the dichloromethane DCM and MeOH extracts showed some degree of qualitative inhibition expressed by discoloration bands (Table 2), which indicates that the extracts contain some molecules capable of binding to the enzyme and avoiding urea hydrolysis. *P. psilorhachis*, showed that it has two types of active compounds since it presented two inhibition bands (Rf 5.3 and 5.8), which indicates that within the extract two molecules interact with the enzyme and prevent urea hydrolysis.

In the quantitative analysis when comparing the results of the extracts with the reference standard, it can be observed that the standard presents a greater inhibitory activity of urease than the analyzed extracts, the species that most closely resembles the IC₅₀ of the standards is the MeOH extract of *P. psilorhachis*, so the identification of

chemical compounds in this species is of great interest.

The best urease inhibitory activity was found in both extracts of *P. psilorhachis* (DCM IC₅₀ 1.9 µg/mL and MeOH 2.1 µg/mL), followed by the MeOH extract of *P. umbellatum* (IC₅₀ 2.5 µg/mL) and the MeOH extract of *P. retalhuleense* (IC₅₀ of 4.1 µg/mL) since they inhibited 50% of the urease with the least amount of extract.

There are only two previous reports of the anti-urease activity by *Piper* genus, specifically the activity by *Piper nigrum* L. seeds, in both cases showing a low inhibitory activity, in Iran by Bilgar et al²⁵ (IC₅₀ 603 µg/mL) and in India by Bai et al¹⁴ (inhibition 1.12%, no IC₅₀ was determined). The research by Quilez et al²⁰ suggest that flavonoids isolated from *P. carpunya* might influence the anti-inflammatory, antimicrobial and anti-ulcer activity.

According to the literature, the inhibition of urease is given by different compounds mainly by alkaloids, anthraquinones, saponins, cardiac glycosides, tannins, cyanogenetic glycosides and flavonoids. The main secondary metabolites isolated from the leaves of *P. psilorachis* and *P. umbellatum* are alkaloids and flavonoids,²⁶ *P. retalhuleense* has not been studied phytochemically. It is suggested by these author and others, that mechanism of action in the control of gastric ulcer by these species might be attributed to antibacterial,²⁷ antioxidant,²⁸ anti-inflammatory,²⁹ and immunomodulatory activity.³⁰

It must be remembered that this is the first screening of urease inhibitory activity in *Piper* species, but traditional

uses of *P. umbellatum* indicate activity to urinary and digestive tract infections, particularly peptic ulcer.³⁰ In a complementary research, anti-tyrosinase activity was studied in the same *Piper* species, demonstrating that two species (*P. psilorrhachis* and *P. umbellatum*) have anti-tyrosinase activity, as well as two other species (*P. jacquemontianum* and *P. variable*) that does not demonstrated urease inhibitory activity in this study.³¹ It can be concluded that there are two compounds in *P. psilorrhachis*, which act as inhibitors since they have different Rf values, although it is not necessarily an imidazole, but the Rf is close to omeprazole, the control compound (Rf 5.0). It is demonstrated that three species of *Piper* genus could have a potential application in the control of *H. pylori* and other chronic infections with urease-producing bacteria in the gastrointestinal and urinary tracts. This is the first report of urease inhibitory activity by any *Piper* species from the Americas.

Competing Interests

None.

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