

Uses and protein content of green guaje seeds (*Leucaena leucocephala* (Lam.) de Wit offered in the markets of Southern Puebla State, Mexico

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Abstract. *Leucaena leucocephala*, commonly known as guaje, is a woody plant endemic to Mexico with edible seed pods. Despite its historical significance as a resource for Mesoamerican peoples, the nutritional value of guaje remains poorly documented. This study aims to explore the uses and protein content of green guaje seeds offered in markets in Southern Puebla State, Mexico. The research involved interviewing vendors at various markets in Southern Puebla, including Tepeaca tianguis, Revolución market, La Rotonda market, and El Moralillo tianguis, where a total of 127 stalls were surveyed. Information was gathered regarding the source, uses, and preparation of guaje seeds. Additionally, the protein content of the seeds was analyzed to determine its nutritional value. Green guaje seeds were available at diverse sales points, with distinct vendor profiles: regatoneros (local resellers) predominantly at the Revolución and La Rotonda markets, while self-producing vendors were more common at Tepeaca tianguis. Eleven culinary preparations featuring guaje were identified, with guaxmole being the most prevalent (40.66%) and various chili sauces with guaje following (38.52%). Although most vendors were unaware of the medicinal uses of guaje seeds, a few recognized their traditional applications, including as a dewormer and for alleviating coughs, stomach pains, and cramps. The protein content in guaje seeds varied significantly, ranging from 6.991 µg/µl in seeds from San Pablo Zoyatitlanapan at the Revolución market to 22.3766 µg/µl in seeds from Acatzingo at the Tepeaca tianguis. This

study highlights the underutilized potential of guaje seeds and provides valuable information on their protein content and culinary uses. The significant variability in protein content based on the seed's origin underscores the need for further research into factors affecting nutritional quality. Additionally, increased awareness of guaje's medicinal properties could enhance its utilization and benefits in local communities.

Key words: Guaxmole, traditional markets, edible plants, medicinal plants, protein.

1. Introduction

Plants are invaluable resources for rural Mexican communities, serving diverse human needs (Caballero and Cortés, 2001). Due to their diverse applications, methods of preparation, and nutritional and medicinal properties, these plants have been significant in human history, influencing cultural development (Osés, 2010). According to the Ethnobotanical Database of Mexican Plants (BADEPLAM, 2006), around 3,500 plant species are recorded, and it is noted that approximately half of them have more than one use, while about 25% have up to five different uses. Among the botanical families with a higher number of species with medicinal and edible uses, the Leguminosae family stands out (Caballero and Cortés, 2001).

Legume seeds, such as guaje, beans, and fava beans, are essential to the diets of urban, rural, and indigenous communities because they are a significant source of carbohydrates and proteins compared to other plant-based foods (Abair *et al.*, 2019). These seeds are culinarily versatile, allowing for their preparation and consumption in various ways.

The green guaje (*Leucaena leucocephala* (Lam.) de Witt) is an outstanding example, valued for its protein and carbohydrates, and is considered the species with the best flavor within the *Leucaena* genus (Zarate, 1994). These seeds are sold in markets, tianguis and plazas, benefiting low-income people. In this context, Mexican markets are important for exchange that provide essential information on production practices and the degree of domestication of the species. They also provide an opportunity to explore the provenance and history of the region's plant products, offering insights into the interaction of diverse socioeconomic groups (Casas *et al.*, 1987). Thus, the term 'guaje' is used in different contexts to refer to a variety of plants with food, forage, medicinal, and timber uses (Peralta *et al.*, 2017). This diversity highlights the importance of these plant resources in the daily life and culture of the region.

The guaje has sparked significant economic interest after spreading to the Philippines post-conquest. However, its relevance lies in its native distribution, particularly its presence in Mexico, where guaje has played a series of traditional roles (Zarate, 1994; Casas and Caballero, 1996). In different regions of Mexico, guaje is consumed as tender foliage, used in animal feed, soil improvement, and as a windbreak (Solorio and Solorio, 2008; Dalzell, 2019). In addition to its practical use, it has also found applications in traditional medicine (Peralta *et al.*, 2017).

In the state of Puebla, communities in various municipalities of the southern region have incorporated guaje into their diet as an essential resource in the preparation of sauces and guaxmole. It is commonly combined with goat, beef, or pork as part of traditional stews in the area (SDR, 2006). The Emiliano Zapata and General Rodolfo Sánchez Taboada markets in Puebla and Acatlán de Osorio, respectively, offer green guaje seeds year-round. At the Emiliano Zapata market, the consumption of guaje intensifies during the summer and autumn seasons. On the other hand, the consumption of guaje in the region is part of the local tradition and culture (Reyes *et al.*, 2016).

Solorio and Solorio (2008) highlight the genus *Leucaena*'s nutritional benefits, particularly in Michoacán's animal production and tropical regions. They report that *L. leucocephala* has a chemical composition with 25.6% crude protein and 52.2% forage degradation. For their part, Martínez *et al.* (2016) report protein content (20.2%) and carbohydrates (33.2%), along with additional values of fiber, ash, and fat, at 0.04%, 0.21%, and 1.26%, respectively. Román *et al.* (2014) provide data on the crude protein content for *L. esculenta* (red guaje) and *L. leucocephala* (green guaje), with values of 33.12% and 31.7%, respectively. Reyes *et al.* (2016) also report that guaje seeds contain significant amounts of fats, carbohydrates, fiber, and proteins.

Based on the above context, the study aims to document the uses and protein content of green guaje seeds (*Leucaena leucocephala*) in the markets of Southern Puebla State, Mexico. The research seeks to explore several hypotheses: (1) the protein content of green guaje seeds varies significantly based on their origin within Southern Puebla; (2) the availability and use of green guaje seeds in culinary preparations are influenced by vendor type and market location; and (3) knowledge of the medicinal properties of green guaje seeds is limited among vendors in Southern Puebla, with only a minority being aware of their traditional uses.

2. Materials and methods

2.1. Study area

The study was carried out from June 2021 to July 2022, included locations such as Tepeaca tianguis, Revolución market in Izúcar de Matamoros, El Moralillo tianguis in Tepexi de Rodríguez, and La Rotonda market in Atlixco (Figure 1).

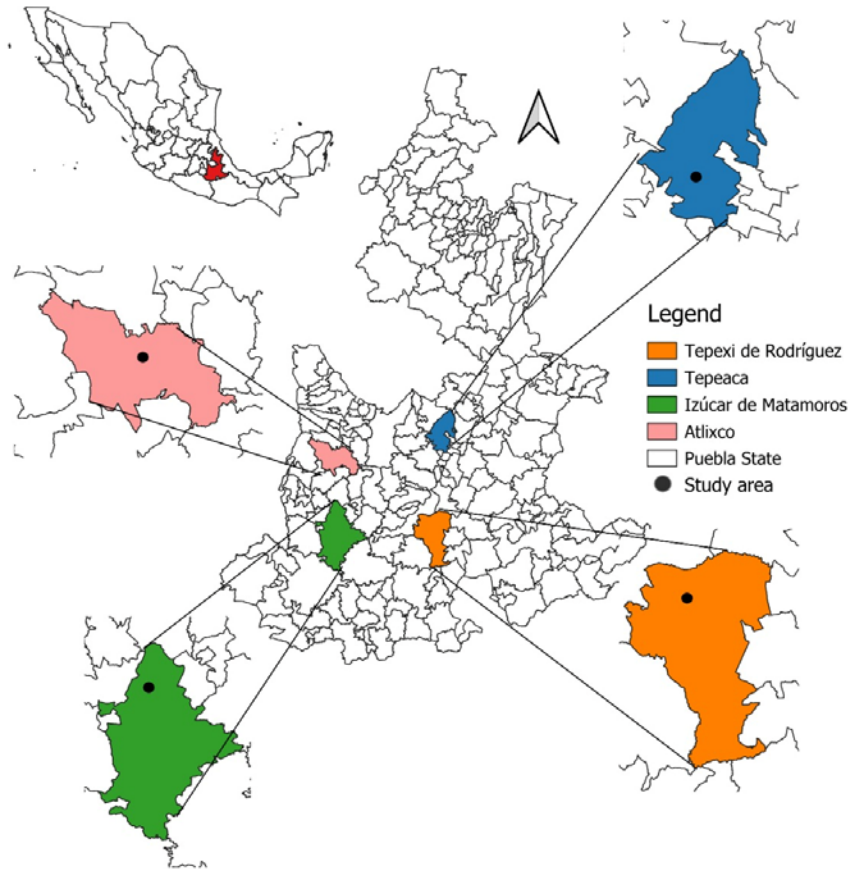


Figure 1. Location of the studied markets and tianguis in Southern Puebla, Mexico.

2.2. Data collection and analysis

A total of 107 open interviews were conducted with vendors of green guaje pods (*Leucaena leucocephala*) to gather information about the origin, sales methods, culinary uses, and medicinal applications of the pods. Each vendor was asked about the provenance of the pods, specifically whether they knew the origin and how they had been obtained-through

cultivation, garden, collection from edges, or wild gathering. The data collected from the surveys were analyzed by calculating percentages for each of the questions asked.

Additionally, a bundle of 30 pods from each vendor was purchased and labeled for further analysis of their protein content. From each bundle, 10 pods were randomly selected, ensuring representation from each origin and market. Repetitions of origin sites were also randomly sampled to ensure representativeness.

Then, 0.5 grams of seeds per pod were weighed, stored in microtubes, and frozen at -70°C. Maceration used a lysis buffer (200 mM Tris, 1.5M NaCl, PMSF, Triton X-100) for a uniform mixture. Once the homogenates were obtained, the quantification of total protein was determined by the Bradford method (1976) in accordance with the microtest protocol of Ernst & Zor (2010). This method is based on the differential color change of Coomassie Brilliant Blue G-250 in response to varying protein concentrations. Bovine Serum Albumin (BSA) was used as the standard. To determine the total protein content, measurements were performed in triplicate. The standard curve was constructed by sequentially adding solutions in the following order: Bovine Serum Albumin (BSA), Phosphate Buffer Solution (PBS), and finally Bradford reagent. A similar procedure was followed for the samples, starting with the guaje macerate, followed by PBS, and finally Bradford reagent. The samples were then homogenized, and absorbance readings were taken using a Hinotek spectrophotometer (Model 752) at a wavelength of 564 nm.

Data were analyzed using GraphPad Prism 6.0 software (GraphPad Software Inc.) to estimate protein amounts via linear regression, based on albumin standards. Due to non-normal distribution, data underwent Kruskal-Wallis analysis ($p \leq 0.05$), and protein medians across origins were compared using Wilcoxon tests ($p \leq 0.05$).

3. Results

Markets in southern Puebla have permanent facilities and operate daily, unlike tianguis, which are temporary street setups occurring once a week and allow for price negotiation. This study explores these diverse market environments by interviewing 107 vendors from 127 green guaje stalls across four markets, revealing diverse vendor types: 1) Resellers or professionals: these merchants acquire their products at the central supply market in Huixcolotla, Puebla, and offer them at the tianguis. 2) *Regatones* or *regatoneros*: these are

local residents who buy products from producers who arrive early at the plaza and resell them throughout the day. 3) Own producers: these merchants sell the products they themselves grow and occasionally exchange products from other regions for their own, and 4) Itinerant vendors: this group of merchants buys products for subsequent resale, but do not have a fixed sales location. Tepeaca tianguis had the most stalls at 41, while La Rotonda market had the fewest at 23 (Table 1).

Table 1. Merchant numbers, green guaje pod collections, and stalls across markets.

Market	Interviewed merchants	Regatoneros	Own producers	Pod collection	Stalls per market
Tepeaca tianguis	27	8	19	19	41
Revolución market	29	19	10	10	33
El Moralillo tianguis	29	18	11	9	30
La Rotonda market	22	19	3	3	23
Total	107	64	43	41	127

The majority of pods were sourced by *regatoneros*, notably at La Rotonda market in Izúcar de Matamoros, which had 19 such merchants (Table 1). In all markets, including Tepeaca tianguis, Revolución market, and El Moralillo tianguis, most vendors are producers themselves, selling pods typically by weight or bundle. It is important to point out that in the La Rotonda market, the price per bundle was set at \$20.00, which represented the highest price compared to the other three markets. On the other hand, in the Revolución market and El Moralillo tianguis, sales were mainly made by weight of the pods, with a price of \$40.00 per kilogram (Table 2).

Table 2. Prices and classification methods for green guaje pods at different markets.

Market	Price (\$)				Pod classification			
	Bundle (per unit)		Weight (kg)					
	Max.	Min.	Max.	Min.	Color*	Size (cm)	Quality*	They don't know
Tepeaca tianguis	10	3	35	20	2	6	13	6
Revolución market	10	5	40	25	0	11	10	8
El Moralillo tianguis	10	5	40	20	1	7	7	14

La Rotonda market	20	5	35	20	1	3	3	15
Total					4	27	33	43

*Number of interviewed merchants who appreciate this quality.

Merchants classify pods for sale primarily based on quality, color, and size. Quality is the most emphasized criterion, particularly among established sellers. In a study of 33 stalls across four markets, 13 in Tepeaca tianguis used this classification method extensively, while La Rotonda market had the fewest (Table 2). Guaxmole and diverse sauces are popular across all markets, as shown in Table 3. These products are popular due to their simplicity and the variety of chilies present in the regions where the markets are located.

Table 3. Distribution of merchants interviewed by type of green guaje-based dishes.

Preparation method	Interviewed merchants (%)	Markets per dish
Guaxmole	40.66	4 markets
Salsa	38.52	4 markets
Alone (snack)	6.42	4 markets
Stews with beef, pork, goat, and lamb	4.68	Tepeaca, Revolución and La Rotonda
Tostadas	1.74	Revolución and El Moralillo
Salsa with egg	1.6	Revolución, El Moralillo and La Rotonda
Fried with chili	1.6	Revolución and La Rotonda
Roasted	1.0	Tepeaca and El Moralillo
Guaje seeds in mincemeat	0.029	Revolución

Few vendors acknowledge the medicinal benefits of green guaje seeds, such as deworming and relief from coughs, stomach pains, and cramps (Table 4).

Table 4. Merchant insights on pod origin, acquisition, and medicinal uses of green guaje by market.

Market	Origin	Pod acquisition	Medicinal use*
Tepeaca tianguis	Central supply market	Purchase (8)	4
	Tehuacán	Orchard (11)	
	Izúcar de Matamoros	Cultivation (3)	
	Reyes de Juárez	Garden (5)	

	Huehuetlán El Grande		
	Zacapala		
	San Juan Ixcaquixtla		
Revolución market	Central supply market	Purchase (9)	4
	Edo. Morelos	Orchard (4)	
	Coatzingo	Cultivation (2)	
	Atlixco	Garden (3)	
	Tehuacán	Home (1)	
	Azochiapan	Cultivation (1)	
	San Pablo Zoquitlán	Irrigation (2)	
	Tezonteopan de Bonilla		
El Moralillo tianguis	Huixcolotla	Purchase (18)	4
	Coatzingo	Orchard (1)	
	Zacapala	Garden (6)	
	Mixteca Baja		
	Xayacatlán de Bonilla		
	San Jerónimo Xayacatlán		
	Acatlán de Osorio		
	Santiago Tenango		
	Tianguistengo		
	Atlixco		
	Tehuacán		
	Tecomovac		
La Rotonda market	Zotorgama	Purchase (19)	9
	Axocopa	Garden (2)	
	Coatzingo	Field (1)	
	Tehuacán		
	Izúcar de Matamoros		
	Edo. Morelos		
	Tepeaca		
	Tepexi de Rodríguez		
	Tezonteopan de Bonilla		
	San Juan Huiluco		
	Tlapanola		

*The column for medicinal use indicates the number of stalls aware of this use.

Protein content was analyzed in 41 green guaje pod collections from four markets. Due to non-normal distribution, a Kruskal-Wallis test was applied ($p \leq 0.05$), showing significant variability in protein content by origin ($r^2 = 50.723$, $df = 17$ y $p = 3259e^{-05}$) (Table 5). Pods

from Acatzingo had the highest protein levels at 22.376 $\mu\text{g}/\mu\text{l}$, while those from San Pablo Zoyatitlanapan had the lowest at 6.991 $\mu\text{g}/\mu\text{l}$.

Table 5. Comparative protein content of green guaje seeds by origin location.

Collection site	Place of origin	Average protein ($\mu\text{g}/\mu\text{l}$)	Sd	Median
Orchard	Acatzingo ¹	22.376618	11.74175	24.8115
Garden	Acatlán ³	16.7544	7.023499	15.331
Garden	Atlixco ²	14.8736	4.356087	14.7785
Orchard	Azochiapan ²	7.45975	3.067705	7.5125
Cultivation and orchard	Coatzingo ^{2,3}	12.41066	8.535778	10.6895
Garden	Huaquechula ⁴	18.3635	9.024086	17.153
Garden	Huehuetlán El Grande ¹	9.3861	6.851925	6.985
Cultivation and field	Izúcar de Matamoros ^{1,4}	15.44705	10.589439	10.5175
Cultivation	Reyes de Juárez ¹	16.7735	5.911062	16.436
Garden	San Jerónimo ³	14.6073	8.053415	12.612
Garden	San Juan Ixcaquistla ¹	14.2835	6.559036	13.143
Cultivation	San Pablo Zoyatitlanapan ²	6.99136	3.298885	6.0155
Cultivation	Santiago Tenango ³	8.3583	6.808536	7.0555
Orchard	Tehuacán ¹	12.02257	8.837743	9.3695
Garden	Tezonteopan de Bonilla ^{2,4}	11.23766	8.58354	8.531
Garden	Trinidad Tenango ²	10.7757	3.910722	10.9475
Garden	Xayacatlán del Bravo ³	12.35475	8.950369	7.8635
Orchard	Zacapala ¹	10.6638	2.770978	10.9035

Abbreviations: 1= Tepeaca tianguis; 2= Revolución market, Izúcar de Matamoros; 3= El Moralillo tianguis, Tepexi de Rodríguez; 4= La Rotonda market, Atlixco.

4. Discussion

The analysis of the results indicates that, unlike the four types of traders identified by Martínez *et al.* (2019), this study has identified only two categories of guaje vendors: regatoneros and producers (Table 1). This finding is important because it provides insights into the vendor chain in the various markets sampled, where many vendors primarily engage in reselling products within the market to earn profits with minimal effort.

Consistent with Zarate (1994), fresh guaje seeds are sold as food in local and regional markets across the country, such as the Emiliano Zapata market in Puebla and the Rafael Sánchez Taboada market in Acatlán de Osorio (Reyes *et al.*, 2016). The classification aspects mentioned earlier play a crucial role in the flavor of the seeds, as Zarate (1994) notes that green guaje seeds are consumed both raw and cooked in various regions of the country. In the studied markets, only *regatoneros* and producers sell guaje, aligning with Martínez *et al.* (2019), who note that these vendors also predominantly sell fruits and vegetables.

Eleven dishes using guaje seeds have been identified, with guaxmole being the most popular, preferred by 40.66% of respondents. The name "guaxmole" is derived from Nahuatl, combining "uaxin" and "molli." This dish is characteristic of the southern region of the state of Puebla, as mentioned by Reyes *et al.* (2016). Another widely consumed dish is salsa in various forms (green, red, and macha), made from chili, guaje seeds, garlic, and salt, which was preferred by 38.52% of respondents. Additionally, there are other dishes that are also consumed, albeit to a lesser extent. This is important as it demonstrates the value of these resources for increasing protein intake among people with low economic resources (Blancas *et al.*, 2013; García-Maceda *et al.*, 2023).

In the Tepeaca tianguis, seven dishes using green guaje seeds are prepared; in the Revolución market, nine dishes; in the El Moralillo tianguis, eight dishes; and in the La Rotonda market, six dishes (Table 3). These findings support hypothesis (2), which suggests that the availability and use of green guaje seeds in culinary preparations are influenced by vendor type and market location, as well as the variety and availability of guaje seed-based dishes. Protein content in green guaje seeds varied by origin. Seeds from Acatzingo sold at Tepeaca tianguis had the highest protein content at 22.3766 µg/µl. These orchard-collected seeds likely benefited from fertilization practices that increased nitrogen availability. On the other hand, the seeds collected in the Revolución market and coming from San Pablo Zoyatitlanapan presented the lowest protein content, with a value of 6.991 µg/µl (Table 5). It is important to note that these seeds were obtained from a crop; it is possible that in these sites, the plants have little access to fertilizer, since it is mainly used for fertilizing the crops. In relation to previous research, Román *et al.* (2014) and De Angelis *et al.* (2021) have reported a high content of crude protein in seeds of red guaje (31.7%) and green guaje (31.1%). Similarly, Reyes *et al.* (2016) obtained a value of 31.2% protein for green guaje

seeds in their studies. These results evidence significant variability in the protein concentration in green guaje seeds, supporting hypothesis (1) that protein content varies significantly based on the seeds' origin within Southern Puebla. This variation is possibly due to the diversity in the origin of the guaje pods, some of which come from orchards, while others are the product of crops or open-field collection (Durán-Puga *et al.*, 2020). This diversity of sources could influence the levels of protein quantification. Therefore, it is imperative to conduct studies that analyze the relationship between protein content, the origin of the seeds, and the impact of cultural practices to better understand the factors that contribute to the increase in proteins in green guaje seeds.

The use of non-traditional legume seeds, such as *leucaena*, in human nutrition is limited, primarily because their potential is not well known. While *leucaena* seeds have been extensively evaluated as an ingredient in livestock feed (Dalzell, 2019; Honda *et al.*, 2020; Zapata-Campos *et al.*, 2020), their use in human food has not been formally studied. However, they have been traditionally consumed in southern Mexico (De Almeida-Costa *et al.*, 2006).

The study also explored the knowledge of the medicinal properties of guaje seeds among vendors. The finding that vendors were generally unaware of the medicinal uses of guaje seeds, with only a few recognizing their use as dewormers and for relieving coughs, stomach pain, and cramps (Román *et al.*, 2014; Mohammed *et al.*, 2015; Mora-Villa *et al.*, 2021), supports hypothesis (3) that knowledge of the medicinal properties is limited among vendors in Southern Puebla. This lack of awareness is consistent with observations by Aquino *et al.* (2023), who highlighted the need for increased educational outreach regarding the potential health benefits and safe consumption practices associated with these traditional crops.

5. Conclusions

Green guaje (*Leucaena leucocephala*) is widely sold across various markets in the southern region of Puebla, with a total of 127 dedicated stalls. Vendors at these venues vary, with regatoneros being more common at the Revolución and La Rotonda markets and producers predominating at the Tepeaca tianguis.

Eleven different culinary preparations using guaje seeds were identified. The most popular dish was guaxmole, making up 40.66% of the preparations, followed closely by various

salsas (green, red, and macha) at 38.52%, made with chili, guaje seeds, garlic, and salt. Unique dishes included hip mole at the Tepeaca tianguis, battered guaje cakes at the El Moralillo tianguis, and fried seeds with chili at the Revolución market.

While many vendors were unaware of the medicinal properties of guaje seeds, some noted their use for deworming and alleviating coughs, stomach pain, and cramps.

There is significant variability in the protein concentration of green guaje seeds, influenced by their origin, whether from orchards, crops, or open-field collection. Seeds from Acatzingo sold at Tepeaca tianguis had the highest protein content, whereas those from San Pablo Zoyatitlanapan at the Revolución market had the lowest.

The observed differences in protein content suggest that seed origin plays a crucial role. Further research is necessary to explore the relationship between protein content, seed origin, and cultural practices to better understand the factors contributing to protein variability in green guaje seeds.

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