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COMPOSICIÓN FITOQUÍMICA DE EXTRACTOS DE *Salvia hispánica* L. Y SU EFECTO DE SACIEDAD

PHYTOCHEMICAL COMPOSITION OF *Salvia hispánica* L. EXTRACTS AND THEIR SATIETY EFFECT

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Resumen

Se realizó un estudio fitoquímico de los extractos de hexano, acetato de etilo y metanólico de semillas de chia (*Salvia hispanica* L). Se determinó la capacidad antioxidante de los extractos metanólicos obtenidos de las semillas y de la parte aérea de la planta de chía. También se analizó el efecto del extracto acuoso de las semillas de chía en la ganancia de peso ponderado de ratas Wistar. El extracto metanólico de la parte aérea de la planta mostró 268 veces más flavonoides totales (268 ± 6 mEQ) comparado con el extracto de semillas de chía (1 ± 0.2 mEQ). Sin embargo, la capacidad antioxidante del extracto metanólico de la parte aérea de la planta fue bajo (2.0 ± 0.1 TEAC) comparado con los reportados para otros extractos vegetales. También no se observó toxicidad en larvas de *A. salina* en todos los extractos analizados. Por otra parte, no se observaron diferencias significativas ($p > 0.05$) en el peso entre el grupo que consumió el extracto acuoso de las semillas de chía y el grupo testigo que solo consumió agua. Tampoco se observaron cambios en los consumos de alimento y de soluciones, por lo que estos resultados indican que los extractos acuosos de las semillas de chía no produjeron el efecto de saciedad reportado en otros trabajos y atribuido a las semillas.

Palabras clave: chía, composición química, planta medicinal.

Abstract

A phytochemical study of the hexane, ethyl acetate and methanolic extracts of chia (*Salvia hispanica* L.) seeds was conducted. The effect of chia seeds aqueous extract on the weight gain of Wistar rats was also analyzed. The methanolic extract of the aerial part of the plant showed 268 fold more total flavonoids (268 ± 6 mEQ) compared to the chia seeds extract (1 *pm* 0.2 mEQ). However, the antioxidant capacity of the methanolic extract of the aerial part of the plant was low (2.0 ± 0.1 TEAC) compared to those reported for other plant extracts. Also, no toxicity was observed in *A. Salina* larvae for all the analyzed extracts. On the other hand, there were no significant differences ($p > 0.05$) of weight between rats that consumed the aqueous extract of chia seeds and the control group that consumed water. There were also no changes in the consumption of food and liquids indicating that chia seeds aqueous extract did not produce the satiety effect that has been attributed to the seeds in other studies.

Keywords: chia, chemical composition, medicinal plant.

1 Introduction

Through mankind history, man has used countless plants for his benefit and at some point he discovered that some of them could be useful for treating medical affections. In Mesoamerican cultures, this practice of using plants therapeutically was very well developed; they put together this practice with religious rites obtaining great benefits in treating and curing several illnesses. In Mexico, the use of medicinal plants in pre-Hispanic cultures have been extended to our days; Mexican population preserves a rich tradition on the use of plants for healing purposes (Linares *et al.*, 1994; Lozoya, 1994). Currently, Mexican medicinal flora contains between 3,000 and 5,000 species of plants with therapeutic potential (Lozoya, 1994). A total

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of 3,103 species have been compiled in an atlas of Mexican medicinal plants used by diverse ethnical groups. According to the Latin American Society of Natural and Traditional Medicine, the Mexican herbal industry commercializes about 1,200 tons monthly, among which the Sonora Market in Mexico City participates with 160 tons. Gutiérrez and Betancourt (2005) reported that fresh and dehydrated plants from around 250 species, mainly from the center and the South of the country, are commercialized daily. Furthermore, Mexico is considered the second country in the world with the largest number of inventoried medicinal plants with almost 4,500 species, followed by China, which has 5,000 species, according to the Latin American Society of Natural and Traditional Medicine. Erick Estrada, the president of this society, points out that 80% of the Mexican population frequently uses herbs for treating their illnesses (Schlaepfer and Mendoza-Espinoza, 2010). According to his own words "Mexican people have always been herbalists, since the pre-hispanic era and even earlier, we have always consumed medicinal plants and these are used in combination with other active substances" (Morón *et al*., 1991; Meckes *et al*., 2004; Cáceres *et al.*, 2005; Muñetón-Pérez, 2009). It is in this context, that the importance for studying the seed of *Salvia hispanica* L., commonly known as chia in the South-Center of Mexico, arises. Chia belongs to the family Labiatae, genus Salvia and the species is *Salvia hispánica* L. Figure 1 shows a botanized specimen of this plant collected in Sierra de Santa Catarina that belongs to the Herbarium of the Universidad Autónoma de la Ciudad de México (UACM). The plant measures from 90 to 100 cm of height and has leaves of 4-8 cm long and 3-5 cm wide; it has hermaphrodite purple or white flowers occurring as terminal clusters. The plant blooms in summer in the months of July and August on the North Hemisphere producing fruits and seeds in this season (Cahill, 2003; Hernández-Gómez *et al.*, 2008). Several pharmacological properties are attributed to chia seeds. In recent years there has been increased the interest in evaluate the antioxidant potential of fruits, vegetables, plants and seed extracts and their possible application as functional foods and nutraceuticals (Morales-Delgado, 2014; Martínez-Palma *et al.*, 2015).

Figure 1. Chia (*Salvia hispanica* L.) botanized specimen belonging to the Herbarium of the UACM, Campus Casa Libertad.

2 Materials and methods

2.1 Biological material and experimental set up

Chia seeds were bought in Puebla State a local producers and transported to the Laboratory of Natural Products of the Universidad Autónoma de la Ciudad México (UACM), where flour was obtained by using a hammer mill. *Salvia hispánica* plant was collected in Sierra de Santa Catalina del Monte and transported to the herbarium of UACM, campus Casa Libertad, where it was botanized and taxonomically characterized by the taxonomist Susana Peralta.

2.2 Chemical analysis

2.2.1 Qualitative phytochemical evaluation

Qualitative phytochemical evaluation was performed to determine the chemical constituents present in chia seeds and aerial parts of the plant. Presence of alkaloids, anthraquinones, coumarins, saponins and tannins was determined. Analysis of the constituents was carried out on a) three extracts of the flour of chia seeds obtained in Puebla State using solvents of different polarity, hexane, ethyl acetate and methanol and, b) a methanolic extract of the aerial part of the plant collected in Sierra de Santa Catarina. Extract preparation and the evaluation of the main chemical constituents was carried out using the methodology reported by our research group (Peralta-Gómez *et al*., 2013; Mendoza-Espinoza *et al*., 2016).

2.2.2 Quantitative phytochemical study

2.2.2.1 Determination of total flavonoids

Total flavonoids were determined using the method reported by Chang *et al*. (2002) with some modifications and reported as equivalents of quercetin (EQ) per mg of dry extract.

2.2.2.2 Determination of total phenols

Total phenols were determined using the method reported by Singleton and Rossi (1965) based on the Folin-Ciocalteu reagent and reported as equivalents of gallic acid per mg of dry extract (EGA).

2.2.2.3 Determination of caffeic acid and chlorogenic acid

Analysis of these two compounds was conducted by High Performance Liquid Chromatography (HPLC). Chia seed flour (20 g) were defatted with hexane and then, 125 mL methanol were added for maceration during 2 h at room temperature. Afterwards, the solvent was recovered and concentrated at reduced pressure for obtaining an extract enriched of high polarity compounds; This extract was injected (20 μL) in the High Performance Liquid Chromatograph (Agilent 1260 coupled to a Multiple Wavelength Detector). The mobile phase was acetic acid in water (0.1 % v/v) and acetonitrile, the elusion run started with the initial proportion of 85% and 15% , the polarity gradient of the mobile phase was gradually adjusted until reaching 10 and 90% as shown in Table 1 (Pellati *et al*., 2011).

2.2.2.4 Antioxidant capacity determination with radical 2,2-diphenyl-2-picryl-hidracyl (DPPH) method

The DPPH method was carried out as described by Brand-Williams *et al*. (1995), and Romero-López *et al*., 2015). A methanolic solution of DPPH 0.1 mM was prepared, the vegetal extract was diluted 1:10 in methanol (80% v/v). The reaction mixture contained 50 μL of the diluted extract and 950 μL of DPPH 0.1 mM. The antioxidant capacity was determined in the decrement of the visible light absorption at 515 nm. The results were expressed as Trolox equivalent antioxidant capacity (TEAC).

2.3 Acute test method based on Artemia Salina (A. salina)

For the toxicity assay, 200 mg *A. salina* cysts were disinfected with 1% (v/v) hypochlorite solution and then placed in a 20 × 20 × 30 cm container with 250 mL saltwater (20 gL^{-1} table salt) and incubated at 37°C for 24 h. After the hatching, ten Artemia salina nauplii were transferred to 15 mL tubes and the extracts were added at five different concentrations 100, 10, 1, 0.1 and 0.01 mgmL^{-1}. Nauplii death after 24 h were counted. Mortality rate was calculated by the formula: (dead nauplii/total number of nauplii) × 100, the assay was performed in triplicate (Rivero-Pérez *et al*., 2007).

Table 1. Elution gradient utilized for the separation of the extracts by HPLC.

Time in minutes for reaching the gradient	0.1% acetic acid solution (%)	Acetonitrile (%)
0	85	15
10	70	30
18	35	65
25	20	80
30	10	90
35	10	90

2.4 Effect of Salvia hispánica L. extracts on satiety in wistar male rat

Growth assessment in Wistar male rat

A chia aqueous extract was prepared with 0.5 g of chia flour in 700 mL of water. This solution was administered to the rats for 23 days. Two groups of male Wistar rats (n = 9) with an approximately weight of 40 g weaned at 20 days were used for this assay. The first group was treated for one night with *ad libitum* chia extract solution prepared with 20 mg of chia flour in one liter of potable water while the second group only drank water. Both groups have free access to food. The amount of food consumed by each rat was measured as well as its state of health using as indicators the color of the eyes and the fur conditions (Lenzi de Almeida *et al*., 2008).

3 Results and discussion

3.1 Qualitative phytochemical analysiso

The results of the phytochemical characterization of chia seed are shown in Table 2, where the presence of the analyzed chemical groups obtained with solvents of different polarity are presented. A higher amount of alkaloid-like compounds were detected in the hexane extract compared to the other extracts, which indicates that this type of compounds present in chia seed belong to a group of low polarity. Such compounds are insoluble in water, soluble in ether and contain at least one nitrogen atom in its molecule; the majority of these alkaloids are heterocyclic, however, some aliphatic alkaloids were detected. Several alkaloids are found in vascular plants. Most of the pharmacological properties in humans that have been attributed to these compounds are related to the central nervous system (Ávalos and Pérez-Urria, 2009). Regarding the anthraquinones, two types were detected in chia seed, of low and high polarity (found in the hexane and methanolic extract, respectively). The commonly reported anthraquinones in the literature are derived from phenolic compounds and have a wide variety of pharmacological properties such as antifungal, antimicrobial, anticarcinogenic, antioxidant and recently they have been associated to analgesic properties (Singh *et al*., 2016, Mayer *et al*., 2016). Anthraquinones, coumarins and tannins, are products of the shikimic acid pathway. Tannins were found in the high polarity fraction, however, coumarines were not detected in any of the fractions. Finally, it is interesting to remark the high content of saponins found in the polar fraction. Steroid glycosides and triterpenes are among the most common saponins reported in the literature. Triterpenes are precursor of steroids, and these latter may contain one or more sugar molecules in their structure and some of them are used in the food industry as additives. One of the biological properties frequently associated to saponins are their capacity to break cellular membranes once they have been incorporated in the bloodstream. Regarding to the analysis of the aerial part extract of the plant, the profile obtained for alkaloids, anthraquinones and saponins was similar to the seed extracts, but different for coumarins and tannins. The difference in composition between leaves and seed will be discussed with detail in the results of the quantitative analysis of some secondary metabolites in the methanolic extract.

Table 2. Qualitative phytochemical analysis of chia ({*Salvia hispánica*} *L.) seed and the aerial part of the plant.*

Extract	Hexane (C_6H_{14})	Ethyl acetate ($C_4H_8O_2$)	Methanol (CH_3OH)
		Seed	
Alkaloids	+++	++	+
Anthraquinones	+++	++	+++
Coumarins	-	-	-
Tannins	-	-	+++
Saponins	-	-	++
		Aerial	
Alkaloids			+
Anthraquionines			+++
Coumarins			++
Tannins			-
Saponins			++

- = absent, + = present, ++ = moderately, +++ = concentrated.

Table 3. Caffeic acid, chlorogenic acid, total phenols and total flavonoids in chia ({*Salvia hispánica*} *L.)* methanolic extracts from the seeds and the aerial part of the plant.

Chemical analysis	**Seed**	**Aerial part**
Cafferic acid (ppm)	1 ± 0.01	2 ± 0.01
Chlorogenic acid (ppm)	Not detected	Not detected
Total phenols (EGA)	612 ± 24	640 ± 25
Total flavonoids (EQ)	1 ± 0.2	268 ± 6
Antioxidant capacity	Not detected	2 ± 0.1
Toxicity assay in {*A. salina*}	Not observed	Not observed

3.2 Quantitative phytochemical analysis

Total flavonoids content in chia seeds methanolic extract was 1.026±0.23 mEGA, lower than the value obtained for the aerial part methanolic extract, 268±6 mEGA. This difference might be due to the fact that these compounds are generally stored in the leaves. In contrast, total phenols content was in the same concentration range for both extracts from seeds and from the aerial part (Table 3). Regarding to caffeic acid and chlorogenic acid, these metabolites are frequently related to the antioxidant capacity, HPLC analysis revealed the presence of a similar amount of caffeic acid in both seeds and aerial parts of the plant. Caffeic acid concentration found in chia was lower than the values reported for coffee (an approximate average of 800 ppm) (Gutiérrez, 2002). Chlorogenic acid was not detected in any of the extracts.

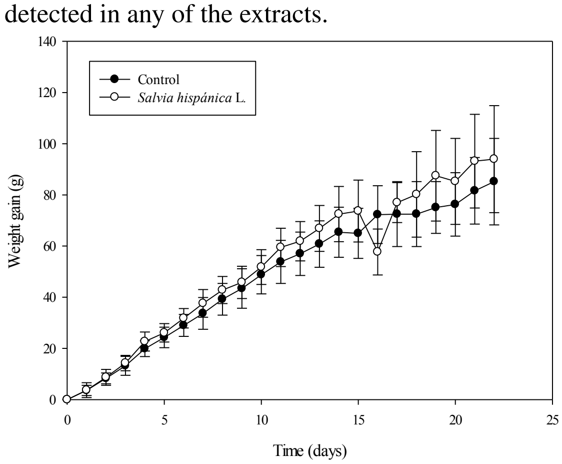

Figure 2. Effect of *Salvia hispánica* L. extracts on weight gain in Wistar rats. Means of weigth ± SD

3.3 Toxicity on A. salina, antioxidant capacity and the effect of Salvia hispánica L. extracts on satiety in wistar male rat

Toxicity of the extracts obtained from chia seeds and the aerial part of the plant was evaluated using the *A. Salina* model. No toxicity was observed at the evaluated concentration (10 and 500 ppm) showing the innocuousness of *Salvia hispanica* L. extracts. It is important to mention that this assay only indicate the toxicity of polar compounds. The antioxidant capacity determined by the DPPH method of the extract showed a low activity as a radical scavenger. These results correlate well with the low levels of caffeic acid and total polyphenols detected in the extracts. The evaluation of the effect of chia seed aqueous extracts in the Wistar rat growth model showed a similar growth and weight gain compared to the control (Figure 2), which only consumed water. In addition, there were no changes in the consumption of food and liquids in the animal model. These observations did not support the expected weight decrease as a result of the satiety effect in treated animals and agree with those reported by Nieman *et al*. (2009). Thus, no effect of chia seeds on satiety was found.

Conclusions

A difference in the main chemical constituents were observed in chia (*Salvia hispánica* L.) seeds and the aerial part of the plant. Hexane extract had the highest amount of alkaloid-like compounds compared to the extracts obtained with ethyl acetate and methanol from chia seeds, as well as the methanolic extract prepared from the aerial part of the plant. On the other hand, leaves are richer in flavonoids than seeds by two orders of magnitude. The presence of caffeic acid was low and chlorogenic acid was not detected in any extracts. These results correlate with the low antioxidant capacity observed in the extracts. None of the analyzed extracts showed a toxic effect on the *A. salina* model. Regarding the capacity to produce satiety and growth weight gain, in this work were not differences observed. Therefore, the results obtained under the conditions used in the present study do not support a satiety effect in the animal model that was used.

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Notation

EQ	Equivalents of quercetin
TEAC	Trolox equivalent antioxidant capacity
A. salina	*Artemia salina*
EGA	Equivalents of gallic acid
HPLC	High Performance Liquid Chromatography
DPPH	Radical *2,2-diphenyl-2-picryl-hidracyl*
ppm	Parts per million

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