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# CHEMICAL COMPOSITION AND CURRENT DISTRIBUTION OF "AZAFRÁN DE BOLITA" (*DITAXIS HETERANTHA* ZUCC; EUPHORBIACEAE): A FOOD PIGMENT PRODUCING PLANT<sup>1</sup>

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> Méndez-Robles, Ma. Dolores, Claudia Flores-Chavira, Ignacio Orozco-Ávila, Eugenia Lugo-Cervantes (Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, Av. Normalistas 800, Colinas de la Normal, 44270 Guadalajara Jalisco, México; e-mail elugo@ciatej.net.mx), and Ma. Eugenia Jaramillo-Flores (Departamento de Alimentos, Escuela Nacional de Ciencias Biológicas del IPN, Carpio y Plan de Ayala, 06470 México D.F.). CHEMICAL COMPOSITION AND CURRENT DISTRIBUTION OF "AZAFRÁN DE BOLITA" (DITAXIS HETERANTHA ZUCC; EUPHORBIACEAE): A FOOD PIGMENT PRODUCING PLANT. Economic Botany 58(4):530-535, 2004. Ditaxis heterantha Zucc. (azafrán de bolita), a plant of the Euphorbiaceae family, grows wild in the semiarid regions of Mexico. Its seeds are used by the inhabitants of the regions where it grows to give color and to enhance the flavor of food. It has been recently cultivated locally, however, the plant has not been cultivated extensively, and it is considered that this crop could have an economic potential. The purpose of this work is to validate the reported geographical distribution of the plant and its chemical composition. The endosperm of the seeds has an intense yellow color, indicating the presence of pigments of the carotenoid family. The pigment was extracted using hexane, and seven fractions were obtained by HPLC. The spectra of the fractions produce three maxima,  $\lambda_{max}$  characteristic of carotenoids. Chemical composition of the seed showed average oil content of 39.4% and 18.8% of protein. The oil and protein content are similar to that of cottonseed and sunflower seed, respectively. Its composition allows the seed to be used as a source of natural pigment, vegetable oil, and protein. The seed has a competitive price and a promising profit margin in local markets, and its cultivation requires little care, growing especially well under limited water conditions.

> COMPOSICIÓN QUIMICA Y DISTRIBUCIÓN ACTUAL DEL AZAFRÁN DE BOLITA (DITAXIS HETERANTHA: EUPHORBIACEAE): UNA PLANTA PRODUCTORA DE COLORANTES USADOS EN ALIMENTOS. Ditaxis heterantha Zucc. (azafrán de bolita) es una planta que pertenece a la familia de las Euphorbiáceas, se encuentra en las regiones semiáridas de México en forma silvestre. Sus semillas son utilizadas por los habitantes de las regiones donde crece, para dar color y sabor en la preparación de sus alimentos. Recientemente se ha iniciado su cultivo en forma local, sin embargo, la planta no ha sido cultivada extensivamente pero puede ser un cultivo económicamente potencial. El objetivo de este trabajo es validar la distribución geográfica de la planta y determinar su composición química. El endospermo de las semillas es de color amarillo intenso indicando la presencia de pigmentos de la familia de los carotenoides. La extracción del pigmento se llevó a cabo con hexano, y se lograron identificar 7 fracciones por HPLC. El espectro de estas fracciones presentó un espectro con 3 absorbancias máximas, lo cual es característico de los carotenoides. Los análisis de la composición química de la semilla, mostraron que el contenido de grasa en promedio es del 39.4%, mientras que de proteína es del 18.8%. El contenido de grasa y proteína es semejante a las semillas de algodón y girasol respectivamente. Su composición puede permitir la utilización de la semilla como fuente pigmentos naturales, aceite vegetal y proteína. La semilla alcanza precios competitivos en los mercados locales y tienen una promisoria rentabilidad económica debido a que su cultivo requiere pocos cuidados específicamente baja demanda de agua.

> **Key Words:** Euphorbiaceae; azafrán de bolita; carotenoids; *Ditaxis heterantha*; *Argithamnia heterantha*.

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A great diversity of plants, mainly of the Cactaceae and some Euphorbiaceae (Rzedowsky 1983), are found in the semiarid regions of Mexico. The Euphorbiaceae is a large family that includes 310-320 genera and 7500 species (Walters and Keil 1996). Two centers of distribution for the family are tropical America and tropical Africa. Members of the family have extremely variable life spans and habits, being found as annual and perennial herbs, vines, succulents, shrubs, and tall trees. Members of the Euphorbiaceae are found in many habitats, from deserts to rain forests. The family is absent from regions with severe cold winters (Walters and Keil 1996). Ditaxinae, a sub-tribe including the genus Ditaxis, is native to the New World; it grows from sea level to an altitude of about 2450 meters, and essentially between latitudes 40° N and 40° S. The genus Ditaxis is closely related to the genus Argythamnia. Some authors have therefore divided the genus Argythamnia into three sub-genera: Argythamnia, Ditaxis, and Chriropetalum (Ingram 1980). However according to Webster (1994), evidence, including pollen morphology, indicates that they are three different genera. In fact, Radcliffe-Smith (2001) separates the genus Ditaxis from Argythamnia even though he mentions that these genera are closely related; they can be differentiated by an androcium with 8-10 stamens placed in two whorls and their pollen is bilaterally symmetrical

Ditaxis heterantha Zucc. was first gathered at Tolimán, Querétaro, by Karwinski von Karwin in 1827 (McVaugh 1980), and classified by Joseph Zuccarini, a botanist, who published its name, Ditaxis heterantha, and its description in the Abhandlungen der Matematisch—Physikalischen Classe der Königlichen Bayerishen Akademie der Wissenschaften (1:290) in Munich. Nevertheless Müller described and classified the plant in 1866, calling it Argythamnia heterantha Müll. Arg (Müller 1866). Currently, it is found under both names in herbaria.

*Ditaxis heterantha* is endemic to Mexico. It is found in semiarid regions in coarse sandy soils. Its distribution is limited, hence it is not widely used. It is a bush with heights ranging from 2.5 to 3 meters (Vázquez 2000). Its stems are ligneous, leaves are alternate, petiolate, laceolate, acuminated, from 4 to 8 cm long. The abaxial surface is puberulent and whitish, mainly on the young leaves. The inflorescences are extensively pedicelated, with small pale yellow unisexual flowers. The fruit is a green glabrous trilocular capsule and the seeds are black or dark brown. Locally, it is known as "azafrán," "azafrancillo," or "azafrán de bolita" (Martínez 1937; Martínez 1959; Nowicke et al. 1999; Standley 1924).

Azafrán is distributed in central and western Mexico in the states of Sinaloa, Querétaro, Hidalgo, San Luis Potosí, Guanajuato, Jalisco, and Guerrero (Martínez 1959; Standley 1924; Herbario Nacional de México (MEXU), Herbarium of the Instituto Ecológico del Bajío (IEB), Herbarium of the Escuela Nacional de Ciencias Biológicas of the IPN (ENCB), Herbarium of the Instituto de Botánica of the Universidad de Guadalajara (IBUG)). In Mexico it grows at an altitude ranging from 1600 to 2200 m above sea level and between 16° and 23° North latitude and 99° and 136° West longitude, usually in semiarid regions.

Seed of *Ditaxis* is consumed locally in the regions where it grows. Inhabitants gather it yearly and keep the seed, using it as yellow coloring for their food, such as rice, chicken, or fish. Each plant produces from two to three kg of seeds per year. Its price reaches up to US \$35/ kg. Currently, it is used in the villages of the semiarid regions of the states of Jalisco, Hidalgo, and Querétaro, and it is only traded at local markets. However in other states, such as Michoacán, its use has been forgotten by all but the elderly population.

The purpose of this research is to determine the chemical composition of the "azafrán de bolita" seed, identify the type of pigment it produces, and verify its current distribution.

# MATERIAL AND METHODS

#### PLANT MATERIAL

The samples analyzed of *Ditaxis heterantha* were collected in the municipalities of Huichapan (Hidalgo State), Totatiche, and Cuquío, and at a market in Guadalajara City (Jalisco State).

## Chemical Composition

The seeds were ground in a Thomas Wiley 3383-L10 mill with a number 20 mesh. The protein, fat, fiber, and ash content were determined using the techniques reported by the Association of Official Analytical Chemists (AOAC 1997). The analyses were performed in triplicate.



Fig. 1. Distribution of Ditaxis heterantha in México.

#### Pigment Extraction and Identification

Two samples, 50 g each, of endosperm seed were ground. Hexane (200 mL) was added and maintained in agitation for 30 minutes during a first extraction. The suspension was filtered, recovering the solvent, while the solids were further extracted under the same conditions. The same operation was repeated three times mixing the recovered solvent and discharging the exhausted solids. The recovered solvent was evaporated, resulting in orange-colored oil. This sample was analyzed using a Beckman-126 HPLC, with a System Gold diode detector. A Supelco 13229-03, Phenomedex Prodigy 5  $\mu$  ODS (2)  $250 \times 4.60$  mm column was used. The mobile phase was methanol:water (9:1) at a flow speed of 1 mL/min.

# Herbaria Consulted

The following herbaria were initially consulted: Herbario Nacional de México (MEXU); the Instituto de Ecología del Bajío (IEB); the Escuela Nacional de Ciencias Biológicas of the IPN (ENCB); and the Instituto de Botánica of the University of Guadalajara (IBUG) to determine where the plant was located. At the same time, local markets were asked the origin of their merchandise. These results confirmed the need to visit some municipalities of the states of Jalisco, Michoacán, and Hidalgo to verify that the plant was still grown and used there.

#### **RESULTS AND DISCUSSION**

#### DISTRIBUTION OF THE PLANT

The most important herbaria of Mexico were consulted to determine the current distribution of the plant (Fig. 1). In addition, the origin of the "azafrán" was also questioned at regional markets where it is sold. It was found that only Jalisco had started to cultivate it. In the northern part of the state, the plant is normally found in back yards, where it grows without being groomed. However, wherever the plant grows, the nearby villagers gathered it for their own consumption. Municipalities in the states of Hidalgo, Querétaro, Michoacán, and Zacatecas were visited. The presence of plants was verified in the states of Hidalgo and Querétaro, however, fruits could not be collected since it was not cropping season. It is consumed by the Otomi

	D. heterantha (Huichapan Hidalgo State)	D. heterantha (Totatiche, Jalisco State)	D. heterantha (Cuquio, Jalisco State)	D. heterantha (Moyahua, Zacatecas State)	D. heterantha (Market)
Oil, %	38.4	42.3	36.9	35.1	39.0
Protein, %	23.7	15.3	21.4	20.22	19.7
Crude fiber, %	26.7	21.0	22.7	26.80	21.2
Moisture, %	6	3.6	4.0	4.35	3.5
Ash, %	2.6	2.6	2.5	2.32	2.4

TABLE 1. CHEMICAL COMPOSITION OF D. HETERANTHA FROM DIFFERENT PLACES.

Indians in the states of Hidalgo and Querétaro. In several villages of the states of Michoacán and Zacatecas, this plant is only known by the elderly, significantly decreasing its consumption. No fruit samples could be gathered in these states.

#### COMPOSITION OF THE SEED

The "azafrán de bolita" seed has a hard dark husk covering the yellow endosperm. Chemical composition of the seed indicated that the protein content varied from 15.3% to 23.7%, and the oil content from 36.9% to 42.3%, depending on the source of the samples (Table 1). The variations are probably due to environmental factors, such as soil type, climatic conditions, and cultivation conditions (e.g., irrigation, if any). The average oil content is similar to that of cottonseed (35%). Nevertheless, it surpasses the oil percentages reported for species commercially cultivated for producing edible vegetable oils, such as corn and soybean (Table 2). The protein content of Ditaxis seed is low compared to soybean, but similar to that of sunflower (23%) (Abraham and Hron 1992).

The high content of protein in the "azafrán" seed also offers another possibility: once the pigment and oil have been extracted, the pulp residue may be used as animal feed, such as with soybean. The "azafrán de bolita" offers a stor-

age advantage since its low moisture content prevents microbiological attacks. In comparison to other edible seeds of the same Euphorbiaceae family, the oil content of *Ditaxis heterantha* is higher than *Colliguaya integerrima* (35%), but similar to *Pluketia volubilis* (35%–60%) and *Caryodendron orinocense* (34%–41%) (Jiménez and Bernal 1992). These plants do not compete with *Ditaxis heterantha* since *Caryodendron orinocense* and *Colliguaya integerrima* are native to South America, and *Pluketia volubilis* grows in South America and Africa and neither one grows in Mexico, or at least it has never been reported.

A final consideration is the possibility of toxic effects of *Ditaxis heterantha*. The Euphorbiaceae is a family that includes some species with toxic characteristics. We assume that *Ditaxis* does not present any toxicity problems since it has been used for years. This is an aspect that requires further studies.

### IDENTIFICATION OF THE PIGMENT

In order to determine the chemical group to which the pigment of *Ditaxis heterantha* seed belongs, as well as the number of its compounds, the extract obtained by hexane was fractioned by HPLC identifying seven compounds. Two predominant compounds were observed at

Corna Cottonseed<sup>a,b</sup> Soybeana Sunflower D. heterantha Oil, % 20.0  $38.9 \pm 2.72$ 3.6 36.0 47.0Protein, % 8.0 33.0 36.0 23.0  $21.2 \pm 3.15$  $24.1 \pm 0.93$ Crude fiber, % 2.5 2.05.0 4.2 Moisture, % 16.0 47 8.5 5.4  $5.7 \pm 0.26$ Ash. % 1.2 4.6 4.9  $2.6 \pm 0.1$ 

TABLE 2. CHEMICAL COMPOSITION OF D. HETERANTHA COMPARED TO VARIOUS OILSEEDS.<sup>A</sup>

<sup>a</sup> Encyclopedia of Food Science and Technology (1992).

<sup>b</sup> Delinted seed.

TABLE 3.	ABSORPTION	SPECTRUM	OF	THE	SEVEN
FRACTIONS	FOUND BY HP	LC.			

Peak	$\lambda_{max \ 1} \ (nm)$	$\lambda_{max \ 2} \ (nm)$	$\lambda_{max 3} (nm)$
1	370	395	409
2	370	396	406
3	369	393	409
4	428	443	466
5	412	433	457
6	425	445	464
7	412	431	450

9- and 15-minute retention times. These compounds constitute 80% of the pigment.

The UV-Visible spectrum of each fraction was determined. The presence of carotenoids was considered positive since each fraction produced three maxima in their spectra. Britton (1991) has reported that is characteristic of carotenoids is to have three maxima spectra.

The maximum absorption wavelengths of each fraction can be observed in Table 3. The spectra can be grouped according to the wavelength of their maxima in: 1, 2, 3; 4, 6; and 5, 7. It may be thought that the fractions within the groups would be the same compound, however it has been observed that some carotenes, with a different number of carbon atoms or with a different functional group, show the same absorption spectrum. An example of this is  $\beta$ -carotene and zeaxanthin, which have the same chromophore, but have different hydroxy groups and show an equal absorption spectrum. Another example is tunaxanthin C40 and decaprenoxanthin C<sub>50</sub>, which have virtually identical spectra, as does neurosporene  $C_{40}$ . The characteristic of these carotenoids is that they have the same chromophore (Britton 1991, 1995; Gross 1991). The authors suggest that even if the fractions of each group have a common chromophore that defines the maxima of their spectra, the chromatographic behavior may be determined by differences in the carbon number or the presence of different functional groups between them.

#### **CONCLUSIONS**

The seeds of *D. heterantha* have been consumed for generations in the semiarid regions of Mexico. *Ditaxis* is a valuable plant, not only for its high protein and oil content, but also for its potential as an additive (colorant) for the food industry. The presence of carotenes in the seed may be an alternative source of natural colorants for food use.

It is a plant with great potential, as it does not require special cultivation attention. This type of plant may be introduced in the barren semiarid regions as an alternative crop where the yield of traditional crops, such as corn and beans, is low. Its low production costs offer the possibility of expanding and promoting its cultivation.

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