

Comparative pharmacobotanic study and ethnopharmacological uses of the “Botones de oro” from Argentinean folk medicine

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RESUMO: “Estudos farmacobotânicos comparativos e utilizações ethnopharmacológicas do “botões d’ouro” da medicina popular Argentina”. As partes aéreas de seis plantas argentinas - conhecidas geralmente como “botões d’ouro” - são usadas indistintamente na medicina popular ou para obter remédios ervais e fitoterápicos. No entanto, estas entidades pertencem a três gêneros da tribo Helenieae (Asteraceae): *Gaillardia*, *Helenium* e *Hymenoxys*, sendo as espécies mais utilizadas *Gaillardia megapotamica* var. *megapotamica* (Spreng.) Bak.; *G. megapotamica* var. *scabiosoides* (Arn.) Bak.; *G. megapotamica* var. *radiata* (Griseb.) Bak.; *Helenium donianum* (Hook. & Arn.) Seckt; *H. argentinum* Ariza e *Hymenoxys anthemoides* (Juss.) Cass. Neste trabalho, foram estudadas as relações entre os táxons mencionados a partir do ponto de vista morfológico, a sua coexistência em populações naturais e suas ações farmacológicas reputadas. As suas características histológicas são descritas e ilustradas, a fim de obter uma exata identificação botânica dos produtos encontrados no mercado, de forma a garantir a sua qualidade, especialmente porque as amostras são frequentemente já pulverizadas ou em pó.

Unitermos: Medicina popular, Asteraceae, Helenieae, botões d’ouro, estudos farmacobotânicos, utilizações etnofarmacológicas.

ABSTRACT: The aerial parts of six Argentinean plants - commonly known as “botones de oro”- are indistinctly used either in folk medicine or to obtain herbal remedies and phytotherapies. However, these entities belong to three different genera of the Tribe Helenieae (Asteraceae): *Gaillardia*, *Helenium* and *Hymenoxys*, being the most used species *Gaillardia megapotamica* var. *megapotamica* (Spreng.) Bak.; *G. megapotamica* var. *scabiosoides* (Arn.) Bak.; *G. megapotamica* var. *radiata* (Griseb.) Bak.; *Helenium donianum* (Hook. & Arn.) Seckt; *H. argentinum* Ariza and *Hymenoxys anthemoides* (Juss.) Cass. In this work, we studied the relationships between the mentioned taxa from the morphological point of view, their coexistence in natural populations and their reputed pharmacological actions. Their histological features are described and illustrated, in order to obtain an accurate botanical identification of the products found in the market so as to guarantee their quality, especially because the samples are often already powdered or grounded.

Keywords: Argentinean folk medicine, Asteraceae, Helenieae, “botones de oro”, pharmacobotanical studies, ethnopharmacological uses.

INTRODUCTION

The Asteraceae is the family of plants whose individual flowers are arranged in capitula. It includes the widest diversity amongst the plant families, comprising 1,620 genera distributed in 30 tribes and c. 22,750 species (Mabberley, 1997), many of which have had undeniable importance in ethnomedicine from ancient times (Del Vitto et al., 1997; Barboza et al., 2006; Agra et al., 2007 and 2008).

Thirteen tribes of the Asteraceae family (represented by 222 genera and 1,490 species) grow in Argentina. The tribe Helenieae is represented in Argentina by 12 genera with 41 specific and infraspecific

taxa (Petenatti, 1995; Petenatti & Ariza-Espinar, 1997) with high endemism degree. Six taxa belonging to the Tribe Helenieae are known under the popular name of “botones de oro” (“golden buttons”) and used as folk medicine in Argentinean and boundary countries like Brazil and Uruguay: *Gaillardia megapotamica* (Spreng.) Bak. var. *megapotamica*; *G. megapotamica* var. *scabiosoides* (Arn.) Bak.; *G. megapotamica* var. *radiata* (Griseb.) Bak.; *Helenium donianum* (Hook. & Arn.) Seckt; *Helenium argentinum* Ariza and *Hymenoxys anthemoides* (Juss.) Cass. (Del Vitto et al., 1997; Barboza et al., 2006).

Since these entities present a narrow morphological similarity, that their populations coexist

in the natural conditions and they are known with the same vulgar name “botones de oro”, this study was carried out to register its popular applications and to characterize commercial products of derived them, contributing to the determination of the botanical composition and to the certification of its quality, mainly when they are sold ground out or reduced to powder.

MATERIAL AND METHODS

Ethnopharmacological data

The ethnopharmacological data proceed of two sources: 1. The ethnomedical interviews carried out from some healers and regional merchants; and 2. ethnobotanical and ethnopharmacological bibliographical references.

The ethnobotanical interviews were carried out in several visits to field to “connoisseurs” made between the years 1990 and 1997. A total of 30 informants was interviewed using an interview semistructured according to Cotton (1996). The frequent questions were made on the use of the plant, leaves from the used plant, form of attributed preparation and use/s to the same ones in traditional medicine. Greater informants of 60 years selected themselves by virtue of which they were those that had the greater knowledge about the ethnopharmacological use.

The ethnomedical and ethnopharmacological bibliographical references were obtained from Barboza et al. (2006), Del Vitto et al. (1997), Núñez & Cantero (2000), Petenatti (1995), Steibel (1997), Bustos et al. (1996), Roig (2001), and Toursarkissian (1980).

Plant material

The reference material of the plants was gathered in company of qualified informers and their vernacular names were registered in each case. The collected material was identified by the authors and they are deposited in the Herbarium of the National University of San Luis (UNSL).

The aerial parts from these species were collected in the cities of Mendoza and San Luis, in the central-west region of Argentina or obtained from markets in the city of San Luis, Argentina. The following are the vouchers of such species:

Gaillardia megapotamica (Sprengel) Baker var. *megapotamica* - Del Vitto et al. 5825, San Luis Province: Pringles Department, near La Carolina, January 15, 1991.

Gaillardia megapotamica var. *scabiosoides* (Arn.) Baker - Del Vitto et al. 4592, San Luis Province: La Capital Department, San Luis city, December 28, 1989.

Gaillardia megapotamica var. *radiata* (Griseb.) Baker - Del Vitto et al. 7085, San Luis Province:

Ayacucho Department, 4 km east of L.N. Alem, 620 m above sea level, March 3, 1993.

Helenium argentinum Ariza - Del Vitto et al. 5499, San Luis Province: La Capital Department, crossroads of provincial road 20 and Aguada de Pueyrredón, December, 1990.

Helenium donianum (Hook. & Arn.) Seckt - Del Vitto et al. 9373, Mendoza Province: Uspallata december 2005.

Hymenoxys anthemoides (Juss.) Cass. - Del Vitto et al. 7454, San Luis Province: Belgrano Department, La Calera, km 784, gully of the creek, October, 1992.

Methodology

Plant material was fixed with formalin:acetic acid:ethyl alcohol (1:1:1) and analyzed exomorphologically and anatomically. Diaphanization (Dizeo de Strittmater, 1973) and quantitative micrographic techniques were applied. On the other hand, some materials were included in paraffin (Johansen, 1940), stained with safranin-fast green and mounted in DPX (D'Ambrogio, 1986). In the micrographic techniques, the following parameters were obtained: stomata number (SN) in the two epidermis (stomata.mm⁻²; Timmerman, 1927); stomata ratio among hypophyllum and epiphyllum (SR); stomata index (SI; Salisbury, 1927); palisade ratio (PR; Zornig & Weiss, 1925); vein-islet number (VIN, islets.mm⁻²; Levin, 1929) and veinlet termination number (VTN, terminations.mm⁻²; Hall & Melville, 1951), they were measured using a 20x objective (except for SN, with a 40x).

The microscopic observations as well as measurements and drawings were obtained by means of a Leitz DMRB optical microscope with drawing device, while the macromorphologic observations were made with a Leica M-10 stereomicroscope; in both cases, the photomicrographs were taken with a Leica interchangeable photographic device.

RESULTS AND DISCUSSION

Ethnopharmacological uses

The three varieties of *Gaillardia megapotamica* - especially their heads (capitula) - are used indistinctly as anticephalalgics, antineuralgics, antiseborrheics and antialopeics and against influenza and congestion and as infusions or decoctions for internal or external use respectively (Barboza et al., 2006; Hieronymus, 1882; Toursarkissian, 1980; Del Vitto et al. 1997; Steibel, 1997; Núñez & Cantero, 2000; Roig, 2001).

On the other hand, the aerial parts of *Helenium argentinum* is reputed as a digestive (Del Vitto et al.,

1997) and *Helenium donianum* as an anti-asthmatic, decongestant and stomachic (Roig, 2001), while the aerial parts of *Hymenoxys anthemoides* are used to treat catarrh and cephalalgia in popular medicine but there is some data about their toxicity in cattle (Ragonese & Milano, 1984; Núñez & Cantero, 2000). The scientific and vernacular name, synonymy, habit, plant part used and ethnopharmacological data on some *Gaillardia*, *Helenium* and *Hymenoxys* species from Argentina are summarized in Table 1.

Morphoanatomic features

Genus *Gaillardia*

Morphological features: Although these species are closely related from the morphological point of view, they can be distinguished especially by their receptacles. In fact, *Gaillardia* has rigid setaceous bristles, while the other species have smooth receptacles devoid of bristles.

G. megapotamica is a geophyte with gemmiferae roots to hemicyptophyte; aerial stems herbaceous, tending to be woody, growing up to 60 cm tall, densely leaved in the lower part, glandulose-pubescent, with alternate, lanceolate leaves, attenuating towards the base into a long petiole; the blade with an entire to lobate margin, bright green; the capitula are discoid, lonely in the apex of long aphyllous scapus, erect or flexuose, with hemispheric involucre with linear to lanceolate bracts, reflected in the fructification and the convex receptacle provided with conic and stiff bristles. The flowers are hermaphrodite and yellow. The turbinate achene has a red-brown and thick pubescence and a paleaceous pappus with 7-10 bidentate pieces.

G. megapotamica var. *scabiosoides*: The morphological differences with *G. megapotamica* var. *megapotamica* are the pinnatisect leaves and the foveolate receptacle surface because the bristles do not joint in the base.

G. megapotamica var. *radiata*: The typical variety differs by the radiate capitula and the toothed up to lobated leaves from the *scabiosoides* variety.

Anatomical features: The genus *Gaillardia* shows a unistrate epidermis with a strong cuticle. While the epidermal cell walls of *G. megapotamica* var. *scabiosoides* are irregular and sinuous, the other taxa of the genus have polyhedral ones with smooth walls varying in size and form. The stomata of all entities are *anomocytic* or ranunculaceous (Metcalf & Chalk, 1950), while the indument is formed both by eglandular (2-5 celled with a sharpened elongated apical cell) as glandular trichomes densely spread on leaves and inflorescence. The glandular trichomes are usually placed in a depression due to invaginations, with a 2-celled head, sessile or with 1-2 celled stalks. Both types of trichomes are present in the hypophyllus and

epiphyllous leaves, on the stems and inflorescences.

The leaves structure show their isolateral mesophyll with various layers palisade parenchyma towards both surfaces, while the center of the leaf structure is occupied by a loose spongy parenchyma. The vascular bundles are collaterally arranged; in the midrib they are protected by three or four layers of angular collenchyma towards both surfaces, while the lateral ones are immersed in a fundamental parenchyma.

The transverse section through ribbed stems shows a unistrate epidermis with a thick cuticle and simple eglandular and sessile glandular trichomes, while the cortex is formed by fundamental parenchyma that takes collenchyma in the angles. Usually the endodermis is darkly defined. The pericycle always including sclerenchyma, being the latter most frequent in the form of crescent-shaped strands at the outer boundary of the phloem groups of the vascular bundles. Some cortical vascular bundles are present, and the pith cells are thin-walled (Figure 1). The herbaceous stems show a single ring of collateral vascular bundles.

Genus *Helenium*

Morphological features: *H. argentinum* is an erect subshrub with pubescent striate stems. The leaves are linear, entire and glandulous. Radiate single head on long naked peduncles. The hemispherical involucre is covered with biserial bracts minutely pubescent. Flowers are dimorphous, yellow covered with gold-plated glands. Turbinate achenes, brown, with paleaceous pappus constituted by 8-11 acute, acuminate bristles, finished in an awn that exceeds the corolla.

H. donianum is a subshrub with many branches. The leaves are sessile, linear or lanceolated, usually entire. Radiate single head on long peduncles. Hemispherical involucre is covered with biserial bracts. Flowers are dimorphous and yellow. Turbinate achenes, brown, with paleaceous pappus constituted by 5-7 acuminate bristles, finished in an awn that exceeds the corolla.

Anatomical features: The *Helenium* shows similar anatomical characters from the *Gaillardia* in the epidermis, stomata, the indumenta and the structure of leaves and stems, but the endodermis shows a well defined starch sheath.

Genus *Hymenoxys*

Morphological features: *H. anthemoides* is an annual herb with ascendant or decumbent stems, 10-20 cm high, with pinnatisect or bipinnatisect leaves of narrow-linear segments. The heads are single, discoids, sparingly pedunculated, with globose involucre covered by biserial ovate, obtuse bracts. The receptacle is convex, glabrous. The flowers are isomorphic, tubulous and yellow. The hairy achenes show a pappus constituted by

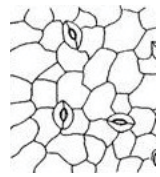
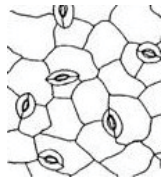
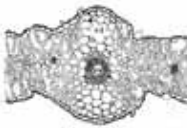



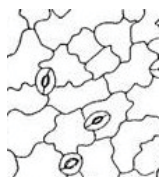

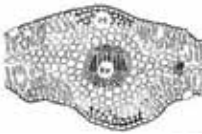




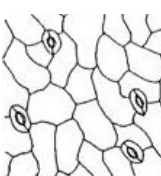
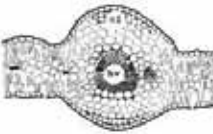





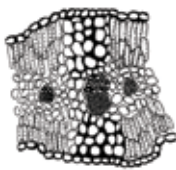



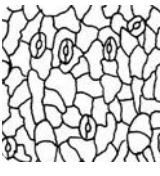
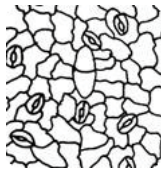




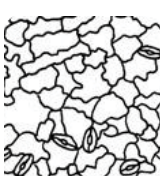
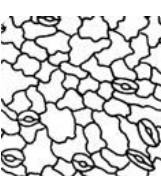




Taxa	Adaxial epidermis	Abaxial epidermis	Cross-section leaves	Cross-section stems	Leaves*	Stems*
<i>G. megapotamica</i> var. <i>megapotamica</i>						
<i>G. megapotamica</i> var. <i>scabiosoides</i>						
<i>G. megapotamica</i> var. <i>radiata</i>						
<i>Helenium argentinum</i>						
<i>Helenium dontianum</i>						
<i>Hymenoxys anhemoides</i>						

Figure 1. Comparative anatomical features on “botones de oro” of the medicinal folk in Argentina (* symbology after Metcalfe & Chalk (1950) modified).

Table 1. Summarized characteristics and ethnopharmacological data on some *Gaillardia*, *Helenium* and *Hymenoxys* species from Argentina.

Scientific name	Scientific synonymy	Vernacular names	Habit (Raunkiaer's life-form)	Plant part	Uses	Ethnomedical references
<i>Gaillardia megapotamica</i> var. <i>megapotamica</i>	<i>Gumtheria megapotamica</i> Spreng.	botón de oro, topasaire	Native perennial herb (geophyte with gemmiferous roots up to hemicyptophyte)	Aerial parts, capitula	Antialopecic, against dandruff and seborrhea	Barboza et al., 2006; Del Vitto et al., 1997; Núñez & Cantero, 2000; Petenatti, 1995; Steibel, 1997
<i>Gaillardia megapotamica</i> var. <i>radiata</i>	<i>Cercosylos scabiosoides</i> Arn. ex DC.; <i>Gaillardia scabiosoides</i> (Arn.) Benth & Hook. f.	botón de oro, topasaire	Endemic perennial herb (geophyte with gemmiferous roots up to hemicyptophyte)	Aerial parts, capitula	Antialopecic, against dandruff and seborrhea	Barboza et al., 2006; Del Vitto et al., 1997; Núñez & Cantero, 2000; Petenatti, 1995
<i>Gaillardia megapotamica</i> var. <i>scabiosoides</i>	<i>Gaillardia scabiosoides</i> (Arn.) Benth & Hook. f. var. <i>radiata</i> Griseb.	botón de oro, topasaire	Native perennial herb (geophyte with gemmiferous roots up to hemicyptophyte)	Aerial parts, capitula	Antialopecic, against dandruff and seborrhea	Barboza et al., 2006; Del Vitto et al., 1997; Núñez & Cantero, 2000; Petenatti, 1995
<i>Helenium argentinum</i>		botón de oro, topasaire	Endemic erect perennial herb to subshrub (hemicyptophyte up to suffrutescent chamaephyte)	Aerial parts	Digestive	Del Vitto et al., 1997
<i>Helenium donianum</i>	<i>Cephalophora doniana</i> Hook. & Arn.	botón de oro, topasaire	Endemic erect perennial herb to subshrub (hemicyptophyte up to suffrutescent chamaephyte)	Aerial parts	Anthasthmatic, digestive, pectoral and nasal decongestive	Bustos et al., 1996; Roig, 2001
<i>Hymenoxys anthemoides</i>	<i>Hymenopappus anthemoides</i> Juss., <i>Hymenoxys haenkeana</i> DC.	botón de oro, manzanilla cimarrona, mansanilla	Native annual herb (Therophyte)	Aerial parts	Against stomach-aches, flatulences and colics; toxic for cattle	Toursarkissian, 1980

Table 2. Quantitative micrographic parameters in *Gaillardia megapotamica*, *Helenium argentinum*, *Helenium donianum* and *Hymenoxys anthemoides*.

Taxon	SNue	Slue	SNle	Slle	PR	VIN	VTN
<i>G. megapotamica</i> var. <i>megapotamica</i>	2.6 ± 0.5	11.3 - 16.1	4.3 ± 0.5	16.3 - 21.9	2.3 - 2.5	2.2 ± 0.1	2.2 ± 0.3
<i>G. megapotamica</i> var. <i>scabiosoides</i>	4.9 ± 0.3	13.7 - 15.5	6.7 ± 0.5	19 - 21	2.0 - 2.8	2.7 ± 0.5	2.2 ± 0.5
<i>G. megapotamica</i> var. <i>radiata</i>	3.8 ± 0.4	12.0 - 13.6	6.3 ± 0.7	15.5 - 17.9	1.5 - 2.1	2.4 ± 0.4	1.7 ± 0.4
<i>Helenium argentinum</i>	3.75 ± 0.62	9.66 - 14.74	3.8 ± 0.93	13.02 - 17.02	2.37 - 3.02	16 ± 2.3	5.16 ± 0.75
<i>Helenium donianum</i>	2.5 ± 0.6	8.17 - 13.4	2.9 ± 0.6	9 - 12.3	2.37 - 2.8	7.4 ± 0.9	1.75 ± 1.4
<i>Hymenoxys anthemoides</i>	4.5 ± 0.75	6.58 - 9.18	6.5 ± 1	12 - 15.3	2.5 - 4.2	18.3 ± 2.3	4.3 ± 1.2

References: SN stomata number [stomata.mm⁻²]; ue upper epidermis; le lower epidermis; SI stomata index; PR palisade ratio; VIN vein-islet number [islets.mm⁻²] and VTN veinlet termination number [terminations.mm⁻²].

5-7 hyaline bristles, aristate in the apex, equal or longer than the corollas.

Anatomical features: The *Hymenoxys* shares the same anatomical characters of the *Gaillardia* and *Helenium* in the epidermis, stomata, the indumenta and the structure of leaves and stems, but the endodermis shows a well defined thick starch sheath. The herbaceous stems show a single ring of collateral vascular bundles. The cortical vascular bundles are present. The pith often consists of thin-walled cells (Figure 1).

Micrographic evaluation

These entities are differentiated by the values of micrographic parameters, which can be detected even when they are moltured, crushed or reduced to powder as they are usually found when integrating herbal or phytotherapeutic formulations. The quantitative micrographic parameters are summarized in Table 2.

CONCLUSIONS

The present study showed the variation of morphological features and especially the values of the micrographic parameters for three varieties of *Gaillardia megapotamica*, *Hymenoxys anthemoides*, *Helenium donianum* and *H. argentinum*. Thus, the infrageneric taxa of *Gaillardia* are easily distinguishable by their paleaceous receptacle, while the receptacles of *Helenium* and *Hymenoxys* do not have paleas. At a microscopic level, minor differences are observed.

The values of the micrographic parameters are interesting because the three varieties of *G. megapotamica* can be distinguished by the stomata number in both epidermis and the stomata index, while the vein-islet number and vein termination number present significant differences among the evaluated species of the three diverse genera.

We hope that these studies will lead to the correct use of each “*botones de oro*” in the traditional Argentinean medicine and at the same time, that it will facilitate the quality control of the commercial products which are out in the market under the vernacular name of “*botones de oro*”.

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