Bol.Soc.Bot.Méx. **77:** 65-73 (2005)

MICROSCOPIC ANALYSIS AND HISTOCHEMICAL OBSERVATIONS OF THE MEDICINAL ROOT OF *IOSTEPHANE HETEROPHYLLA* (CAV.) BENTH. EX HEMSL. (ASTERACEAE)

ESTELA SANDOVAL[1]*, ROBERT A. BYE[1], GRISELDA RÍOS[2] AND MARÍA ISABEL AGUILAR[2]

[1] Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad Universitaria, México 04510, D.F., México. Tel 5622-8987, fax 5622-9046.
[2] Departamento de Farmacia, Facultad de Química, Conjunto "E", Universidad Nacional Autónoma de México, Ciudad Universitaria, México 04510, D.F., México.
* Author to whom reprints requests should be addressed; e-mail: esz@ibiologia.unam.mx

Abstract: The roots of *Iostephane heterophylla* are popular in Mexican traditional medicine and as such are a good candidate to develop herbal drug preparations to be used as phytomedicine. International criteria for validation and standardization of a herbal product as phytomedicine include, among others, the integration of microscopic and histochemical characteristics of the raw material, as in this case the herbal drug, to guarantee its authenticity. As an original contribution to the knowledge of the root structure of this species, fresh roots fixed in FAA, were processed with conventional histological techniques (paraffin embedment and subsequent transversal and longitudinal sections that were stained with safranin-fast green) and stained with histochemical markers for identification of cellular contents. The root description includes dermic, fundamental and vascular tissues as well as cellular contents (proteins, polysaccharides, polyphenols, condensed and hydrolyzed tannins, starches and lipids, some of which have been isolated in previous phytochemical studies). These characteristics are compared to those of other species of Asteraceae as an initial comparative study to contribute to identify medicinal plants based upon their underground parts.
Key words: Asteraceae, *Iostephane heterophylla*, medicinal plants, microscopical studies, roots.

Resumen: Las raíces de *Iostephane heterophylla* son empleadas en la medicina tradicional mexicana y constituyen buenos candidatos para la posterior utilización de sus preparados como fitomedicamentos. Los criterios internacionales para la validación y estandarización de un producto vegetal como fitomedicamento involucran, entre otros, la integración de características microscópicas e histoquímicas del material vegetal para garantizar su autenticidad. Como una contribución original al conocimiento de la estructura de la raíz de esta especie, las raíces frescas fueron fijadas en FAA, procesadas de acuerdo con las técnicas histológicas convencionales y de manera adicional teñidas con marcadores histoquímicos para la detección de sus contenidos celulares. La descripción de la raíz incluye a los tejidos dérmico, fundamental y vascular, así como a los contenidos celulares (proteínas, polisacáridos, polifenoles, taninos condensados e hidrolizados, almidones y lípidos, algunos de los cuales han sido aislados en estudios fitoquímicos previos). Estas características son comparadas con las de otras especies de Asteraceae como un estudio inicial comparativo para contribuir a la identificación de plantas medicinales basadas en sus estructuras internas.
Palabras clave: Asteraceae, estudios de microscopia, *Iostephane heterophylla*, plantas medicinales, raíces.

The systematic ethnobotanical study of the flora traditionally used has led to the identification of plant species with bioactive compounds that could eventually be developed as new drugs (Spjut and Perdue, 1976; Malone, 1977; Pereda-Miranda, 2003). Studies of plants from an ethnobotanical point of view have shown that more species are used for medicines than for any other purpose (Bennett and Prance, 2000). In fact, during the last 20 years, research on the secondary metabolites in plants has led to the discovery of new pharmaceuticals (Galeffi and Marini-Bettólo, 1988; Hamburger and Hostettman, 1991). Because they are widely appreciated and used by both rural and indigenous people (Duke and Ayensu, 1985), medicinal plants are very important material to be developed as new

commercial drugs and to introduce them into the traditional pharmacopoeias (Lozoya *et al*., 1999). Therefore, to guarantee the validation and standardization in the quality of the preparation of phytopharmaceuticals it is necessary to document its healing properties, as well as other attributes of the plant including ethnobotanical, pharmacological, chemical, anatomical, toxicological and clinical aspects.

Among the quality control methods for medicinal plant materials recommended by the World Health Organization (1998), the Mexican Herbs Pharmacopoeia (Secretaría de Salud, 2001) and by Evans (2002), is the visual macroscopic and microscopic verification of the botanical identity of the vegetal drug. Since the macroscopic characteristics are mostly subjective and substitutes or adulterants exist which closely resemble the genuine material, it is often necessary to substantiate the findings of the macroscopic examination by microscopy and/or physicochemical analysis (WHO, 1998). The microscopy analysis compares diagnostic features of fragments of the vegetal part used as "medicine" with taxonomically verified material using a stereoscopic microscope. The anatomical comparison of a plant used as a drug includes histochemical and hystological studies of disassociated and sectioned tissue. However, an examination by microscopy alone cannot always provide complete identification, though when used in association with data from other analytical methods it can frequently provide invaluable supporting evidence (WHO, 1998). In our case, a spectrophotometric method to quantify total phenols and a gas chromatography coupled to mass spectrometry method were used to analyze the metabolic content of the root (Aguilar *et al*., submitted).

In the course of our chemical (Aguilar *et al*., 1993), pharmacological (Ponce-Monter *et al.*, 1999; Campos *et al*., 2000; Aguilar *et al.*, 2001; Mata *et al*., 2001), and anatomical (Luna *et al*., 1986; Manzanero, 1995) studies of several plants used in Mexican traditional medicine, we have examined the roots of *Iostephane heterophylla* (Cav.) Benth. ex Hemsl. (figure 1), a plant belonging to the Heliantheae tribe of the Asteraceae family and well known in the Mexican traditional medicine (Bye, 1985; Martínez, 1989; Argueta *et al*., 1994). *Iostephane heterophylla* is a perennial herb of the pine-oak and oak forests of the mountains (1,500 to 3,000 m) in central and northern Mexico, ranging from the north in Chihuahua to the south in Oaxaca. Of the four species of the genus restricted to Mexico, this taxon has the widest biogeographic distribution, while the other taxa are limited to smaller regions in the Sierra Madre Occidental or the mountains of Oaxaca and Chiapas (figure 2). The subscapiform perennial herb has a fleshy spindle-shaped taproot that grows to more than 45 cm in length and up to 8 cm in width, and is covered by longitudinal corrugated ridges. The root gives rise to the upright, woody caudex, 2 to 7 cm long, from whence grows the basal rosette of leaves. The alternate leaves measure up to 40 cm long and 15 cm wide, are linear-lanceolate to ovate in shape, some broadly lobed, covered with strigose pubescence, and have slightly winged petioles. The flowering scape grows from 0.5 m to 1.5 m tall, and usually has a solitary head (although up to five additional heads may be found on some individuals). The inflorescence has 2 to 3 seriate phyllaries, 15 to many yellow disk flowers (sometimes with purplish lobes), and 8 to 21 uniseriate ray flowers with white to pink colored ligules that are up to 5 cm long and 15 mm wide.

The plant is commonly known throughout its geographic range by the common names "escorcionera" or "manso"

Figure 1. *Iostephane heterophylla*. **A.** Plant. **B.** Roots.

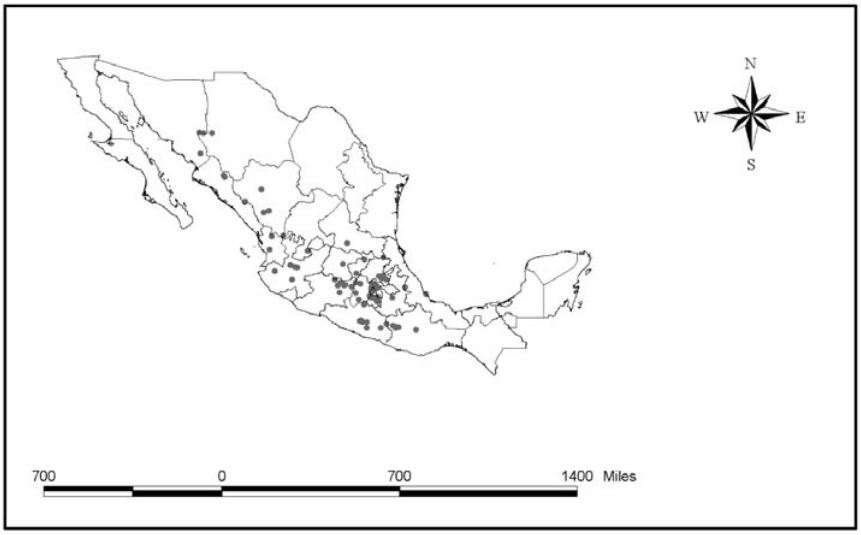


Figure 2. Distributional range in Mexico of *Iostephane heterophylla.*

(including "hierba de manso", "raíz de manso"); the former name apparently was applied during the early Colonial Period although it was recognized as morphologically different from the European "escorzonera" used in treating poisonous animal bites (*Scorzonera hispanica* L.) (Hernández, 1959; Nuez and Hernández, 1994). Other common names used on a more local basis include: "bauji" (Nayarit), "corsonera" (Sonora), "coyorí" (Chihuahua), "cuauhtolotlanenci" (Hidalgo), "cuatolotlanenzi" (Hidalgo), "gentiana de país" (Distrito Federal), "huichocachuarua" (Michoacán), "hierba del oso" and "liga" (Estado de México), "tecpahtli", "tlacopatli" (Jalisco), "tlalpopolote", and "zacapal" (Morelos) (Martínez, 1989; Villavicencio, 1995).

The root, fresh or dried, is usually used medicinally throughout Mexico for alleviating back and kidney pain; the cataplasm is applied topically to the site of the pain. Another widespread application is to promote cicatrisation for flesh wounds and sores; the cataplasm of the fresh root is used as a topical while the dried powdered root or the ash of the burnt root is sprinkled over the affected area and wrapped. Poisonous animal bites are treated with root cataplasms or decoctions. Another general use is the rubbing of a tincture of the root on the body joints to relieve pains associated with arthritis, rheumatism, and dislocated bones or on other painful areas of the body. In central Mexico, a decoction of *ca*. 20 g per 150 l of water is drunk daily to treat diabetes, lung afflictions, liver ailments, and gastrointestinal complaints such as dysentery; it may be drunk alone or in a mixture with *Pnemus boldo*, *Dydimaea alsinoides*, and *Ternstroemia* sp. Other local uses include postpartum bath treatment in "temascal" (indigenous Mexican sweat baths) in Morelos. In colonial Morelos, gangrene was arrested using powder from the toasted root (Martínez, 1989). This species (reported by the Nahuatl name "chipaoacíztic" or "hierba contraria a los venenos") has been identified in the early Mexican Colonial period as an effective treatment for wounds, bites and pain (Valdés and Flores, 1985). Normally the root is collected from the wild populations during the senescent period during the dry season after the leaves and scapes have withered and before the resprouting.

Only a few non-medicinal uses are reported. In central Mexico, the fresh roots exude a red-brown, resin-like substance that bird hunters apply to shrub branches near waterholes so as to trap small songbirds that are later sold in the cities. The Tarahumara Indians of Chihuahua use the root as a source of vegetal dye for wool and tanned skins.

Chemical studies of the roots of *I. heterophylla* have identified sesquiterpenes, diterpenes, coumarins, glycosides and chromenes as the main constituents (Aguilar *et al*., 1993). Some of these metabolites displayed diverse biological actions. Diterpene trachylobanoic acid showed marginal antimicrobial activity against dermatophytes such as *Trichophyton mentagrophytes* and *Microsporum gypseum* and the yeast *Candida albicans* (Aguilar *et al*., 2001). This same compound showed cytotoxic activity against UISO-SQC-1 cells. In addition, the sesquiterpene xanthorrhizol exhibited marginal cytotoxic effect against KB cells (Aguilar *et al*., 2001), and this latter compound as well as trachylobanoic acid and dihydroxy xanthorrhizol glycoside inhibited the tonic-induced contraction of rat uterus (Ponce-Monter *et al.*, 1999). Xanthorrhizol itself induces endothelium-independent relaxation of rat thoracic aorta (Campos *et al*., 2000).

Many Asteraceae have been used in traditional medicine, but only a few anatomical descriptions are available to support their commercialization as a phytomedicine (Heinrich, 2000).

Detailed microscopical observations of the flower, stem and leaf on numerous species of *Artemisia* were described by Obermeyer (Metcalfe and Chalk, 1972), who found to be of specific diagnostic value. Other microscopical details concerning members of the Asteraceae which are of minor medicinal importance or have been used as herbal remedies or as adulterants for more important drugs have been recorded for *Eupatorium perfoliatum* L., *Grindelia squarrosa* (Pursh.) Dunal, *Solidago odora* Ait, *Ambrosia artemisaefolia* L. (Metcalfe and Chalk, 1972), for North American species (Metcalfe and Chalk, 1972); for species of *Antennaria*, *Gnaphalium* and *Helichrysum*; and for the anatomy of the foliage leaves of officinal and pharmaceutically important species of Asteraceae (Metcalfe and Chalk, 1972). The anatomical descriptions for the Mexican medicinal roots of *Psacalium peltatum* (Kunth) Cass. and the tuberous rhizome of *Roldana sessilifolia* (Hook. et Arn.) H.Robins et Brett, have been made (Manzanero, 1995). On the other hand, the Mexican Herbs Pharmacopoeia reports the monography of some Asteraceae: flowers of *Arnica montana* L., leaves of *Artemisia absinthium* L., leaves and stem of *Conyza filaginoides* (DC.) Hieron., leaves and stem of *Echinacea purpurea* (L.) Moench, flowers, leaves and stem of *Heterotheca inuloides* Cass., flowers of *Gnaphalium semiamplexiacaule* DC., flowers of *Matricaria recutita* L., leaves and stem of *Tanacetum parthenium* (L.) Sch.Bip, for which there are partial microscopical descriptions of the used parts (Secretaría de Salud, 2001).

Although traditional knowledge of the Mexican plants in general and of medicinal plants in particular is highly appreciated, there is little information about the anatomical and morphological characteristics regarding the medicinal qualities of most plants (Luna *et al*., 1986). Such is the case of *Iostephane heterophylla*. For this reason, we describe the anatomical and some histochemical characteristics of this medicinal root as part of our program to provide botanical and phytochemical basis for quality control of Mexican medicinal plants under the national and international standards.

Materials and methods

Fresh roots of *Iostephane heterophylla* were collected in the state of Puebla, México, and deposited in the National Herbarium (MEXU, Bye and Linares 26,535). The material was processed as follows: the fresh roots were fragmented and small parts from the central and peripheral regions of the root's middle part (figure 3) were fixed in FAA, and then softened in a glycerin-absolute alcohol-water solution (1:1:1) for 30 days. They were then dehydrated in gradual alcohol-terbutanol-water solutions for 48 h each, and kept under continuous movement, at 25ºC. They were afterwards submerged in 100% terbutanol (TBA) three changes at intervals of 48 h. Once dehydrated, the material was imbibed and placed in blocks of histological paraffin (58 - 60ºC) (Sandoval *et al*., 2005). Transverse and longitudinal sections approximately 20 µm thick were obtained with a

Figure 3. Roots of *Iostephane heterophylla*. Transversal longitudinal and radial sections of the middle part (arrow).

rotary microtome. The sections were stained with safranine-fast green and mounted in Permount to leave them as permanent preparations and kept at the collection of anatomical preparations at the Research Support Laboratory of the Botanical Garden of the Instituto de Biología, Universidad Nacional Autónoma de México. Cellular contents were searched by means of histochemical tests on both, hand cut longitudinal and transverse sections from the fresh root and from sections obtained with a rotary microtome of imbibed material. Proteins were tested by means of bromophenol blue, insoluble polysaccharides with peryodic acid and Schiff reactive, pectin with ruthenium red, lignin with floroglucine and hydrochloric acid, polyphenols with potasium permanganate, condensed tannins with ferric sulfate, hydrolizable tannins with vanillin, starch with lugol, and lipids with "O" red (Sandoval *et al*., 2005). Observations and micrographics were done with the Axioskop Zeiss photomicroscope, and measurements were taken with a micrometric ocular inserted to this microscope. The macroscopical description was based on the criteria established in WHO (1998) and the microscopical on Metcalfe and Chalk (1972). The images shown in this paper were digitalized (scanned) and then edited using the Paint Shop Pro version 7.

Results

Morphology. Fresh Root. - The root is a fleshy spindle-shaped taproot that grows to more than 45 cm in length and up to 8 cm in width, and is covered by longitudinal corrugated ridges. This gives rise to an upright, woody caudex, 2 to 7 cm long, from whence grows the basal rosette of leaves (figure 3).

Bark. - Without persistent root hairs; the bark is 2 mm thick; it has a sweet, slightly astringent flavor; it differentiates in outer and inner bark. The outer bark is dark reddish brown, pleated, fissured, scaly and slightly more than 1 mm thick; the inner bark is light reddish brown and approximately 1 mm thick. With a magnifying lens of 10× it is possible to see dispersed canals of circular outline, more abundant in the inner bark, which makes it shinier; externally, there are longitudinal ridges, 0.5 to 1.0 cm wide and 0.2 to 0.6 cm high.

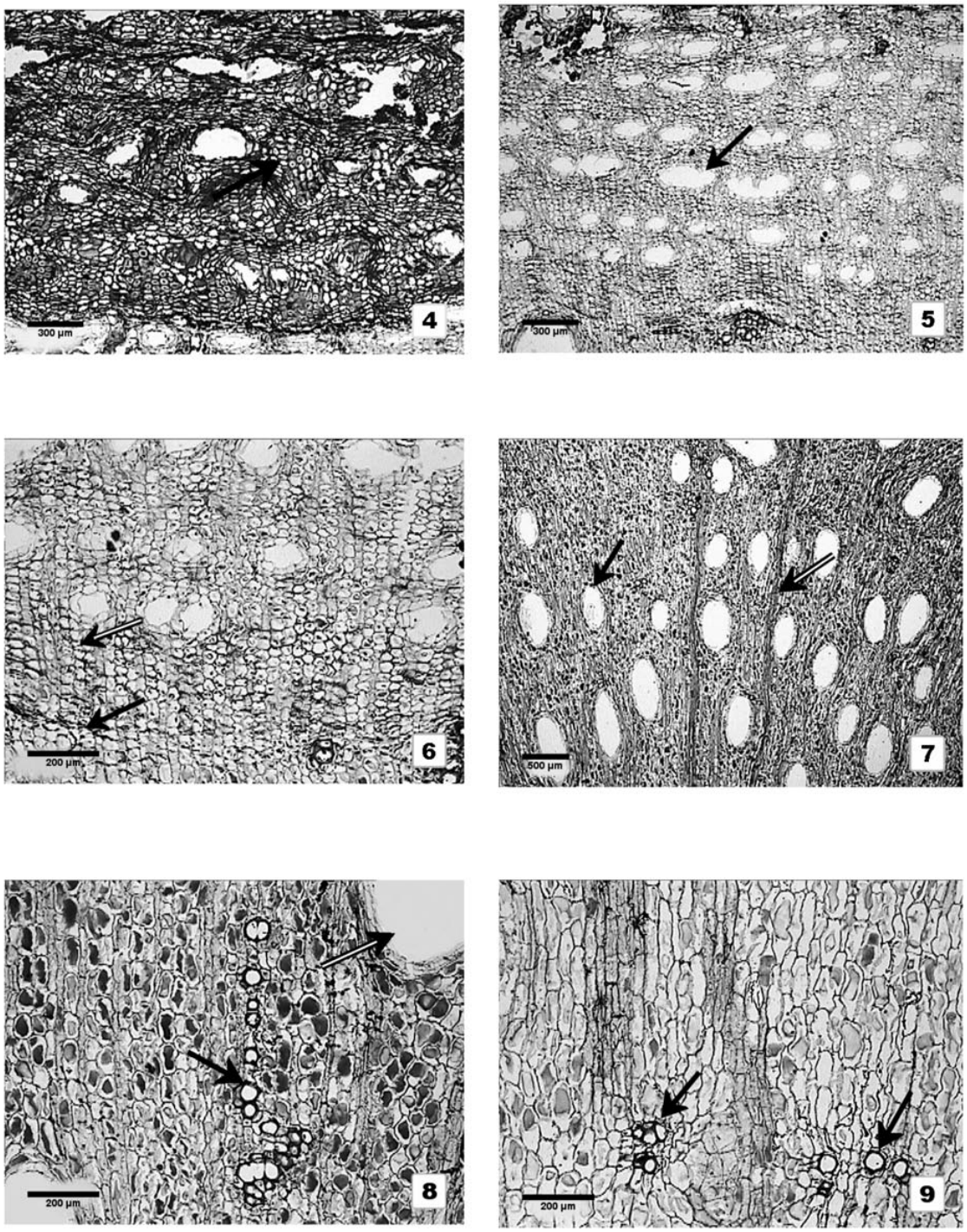
Internal tissue. It has a bitter, resinous and astringent flavor, a pleasant sweet smell and it is cream colored. It is slightly soft, of rough and opaque texture. Structure included conspicuous canals, single, abundant, organized in ring-like bands. At first sight, no growth rings or rays can be seen. The root oxidizes fast when exposed to air or light.

Anatomy. Dermic Tissue. Rhyzodermis.- Transverse section (figure 4). Well developed, arises in the outer part of the cortex. Phellem or complex suber has two alternating cellular types in the form of bands: a continuous one, with rectangular cells, tangentially elongated, organized in well defined radial rows, with thin suberized walls, some obliterated; immersed in this band are little schizogenic laticiferous conducts of circular or oval outline, their epithelium formed by only one layer of small thin-walled cells, with abundant primary pits and cell content; the walls of the epithelium cell closest to the phellogen are not suberized, while the epithelia of the most external laticiferous have suberized cell walls, and some cells even look obliterated. The other band is discontinuous and constituted of sclerenchyma cells like macrosclereids organized in packs of 1 to 25 cells, of two types: some are smaller, round, with thickened walls and reduced lumen, located near the phellogen zone; the other type of cells are larger and located in the peripheral zone of the rhyzodermis, walls which are not too thick, wide cellular lumen and tangentially elongated. Phellogen and phellodermis are inconspicuous.

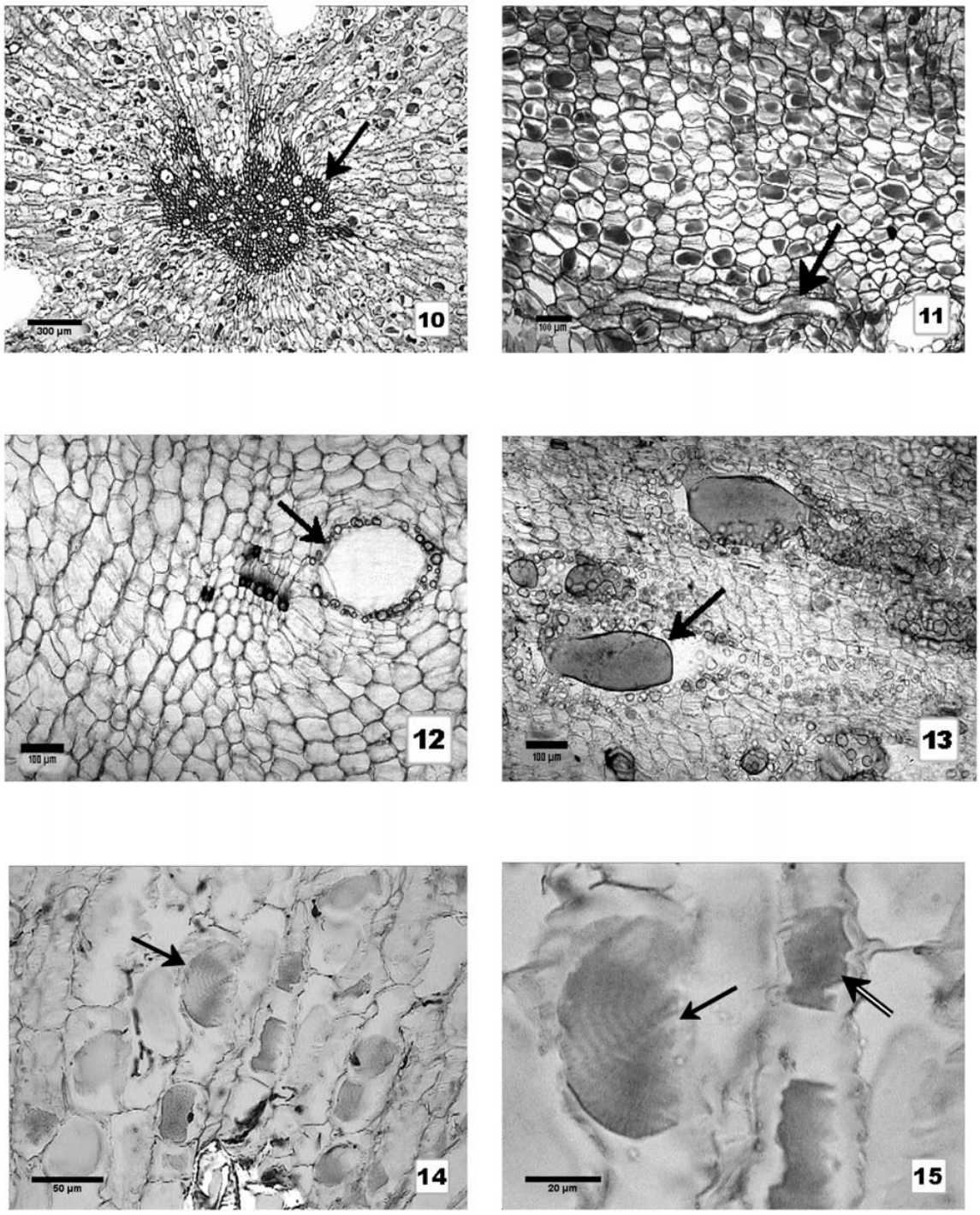
Fundamental tissue. Cortex.- Transverse section (figures 5 and 6). The cortex is more developed than the secondary phloem. It has a width of 575 µm; most of its cells are isodiametric, another with sinuous borders, some close to the primary phloem are tangentially elongated. Small and numerous schizogen laticiferous conducts similar to those observed in the rhyzodermis, but concentric and the cells of the epithelium with thin walls and abundant cell content. Few packs of macrosclereids similar to those seen in the rhyzodermis; the largest packs with cells arranged in radial rows and located near to the rhyzodermis.

Internal section.- Transverse section (figure 7). Occupies the most part of the root. With parenchyma cells radially enlarged, thin walls and some with abundant and dense cellular content. Abundant schizogen laticiferous similar to those observed in other parts of the root, but larger and concentrically oriented, can be seen immersed in this tissue. Rays inconspicuous, but the medullar rays conspicuous, tall, wide and composed of large, pitted cells.

Vascular tissue.- Transverse section. Vascular cambium conspicuous (figure 6), with two cellular layers, rectangular cells and conspicuous nucleus. Phloem is slightly lignified, of the accumulative and stratified type, 1 mm thick (figure 6). Primary phloem with sieve elements and parenchyma cells of similar tangential diameters. Secondary phloem well developed, with sieve tubes members, companion cells, large parenchyma cells and like the cortex with small, concentric and abundant schizogen laticiferous conducts, all of them distributed in radial rows. In longitudinal section some sieve tubes members are nucleated with an inconspicuous sieve plate; the companion cells are scarcely evident as well. Phloematic radios are evident, with two to four rows of scarcely thick-walled cells. Secondary xylem (figures 8 and 9) less developed, organized in small disperse packs, each pack with one to ten ves-



Figures 4-9. Root of *Iostephane heterophylla*. Transverse section. **4.** Rhyzodermis, group of macrosclereids (arrow). **5.** Cortex, schizogen conducts (arrow). **6.** Vascular cambium (arrow), phloem (double arrow). **7.** Fundamental tissue, schizogen conducts (arrow), rays (double arrow). **8.** Secondary xylem, vessels radially arranged (arrow), schizogen conducts (double arrow). **9.** Secondary xylem, vessels organized in small packs (arrow).



Figures 10-15. Root of *Iostephane heterophylla*. **10.** Transverse section of the central zone, fundamental tissue and primary xylem forming a diarch (arrow). **11.** Longitudinal section of fundamental tissue, cells with abundant insoluble polysaccharides and polyphenols, vessels (arrow). **12.** Transverse section of fundamental tissue, schizogen monostratified conduct, epithelial tissue with abundant content of polyphenols (arrow). **13.** Longitudinal section, schizogenic conducts with abundant polyphenols (arrow). **14.** Transverse section of fundamental tissue, cells with amiloplasts (arrow). **15.** Transverse section of fundamental tissue, amiloplasts (arrow) and polysaccharides (double arrow).

sel elements, the peripheral ones with more and larger vessel elements, these with 65 to 80 µm of tangential diameter and distributed in radial chains; the central part of the mature root occupied by a core of xylem, including numerous smaller vessels. In longitudinal section, the vessel elements of 80 to 130 µm long, secondary wall with alternate circular bordered pits on the lateral walls and simple perforation plates on its terminal walls, these perforation plates have an inclination angle of less than 45º. Axial apotraqueal parenchyma is scarce and the cells with conspicuous nuclei. Primary xylem located in the root's central zone and substituting one pith. Organized in two opposing packs or diarch (figure 10); with vessel elements and fibers, the first ones measuring 45 to 65 µm in tangential diameter and with a 5 µm thick wall, the largest vessel elements corresponding to the metaxylem located towards the periphery of the diarch. Tissue surrounding the xylem mostly consisting of parenchyma, with concentrically arranged strands of articulated schizogen laticiferous embedded in it.

Cellular content.- Abundant insoluble polysaccharides detected from their positive reaction to Schiff reactive were seen in the fundamental tissue (figure 11). In the same tissue, and in the epithelium of the schizogen conducts, polyphenols were detected from their positive reaction to potasium permanganate (figures 12 and 13); abundant storage starch like amiloplasts of large and fan shapes were detected by means of polarized light (figures 14 and 15), and their positive reaction to potassium iodide (lugol). Lignin was detected with fluoroglucine in the macrosclereids of the rhyzodermis (figure 4) and in the wall vessel elements (figures 8 and 9).

The following contents were not detected: proteins, condensed and hydrolizable tannins and lipids. Even though pectin is a constitutive element of the wall cells, it was not evident with ruthenium red.

Discussion

By means of this study it was possible to provide the anatomical characterization of the root of *Iostephane heterophylla*, in addition to specific histochemical tests for the corroboration of the presence of some of its secondary metabolites.

Like other Asteraceae studied by Metcalfe and Chalk (1972), *I. heterophylla* roots have also thick, soft and succulent roots, without a central pith surrounded by a radiate xylem. However, in other cases such as *Taraxacum officinale* Weber, the central part of the mature root is occupied by a core of primary xylem, including numerous vessels, and the tissue surrounding the xylem mostly consists of secondary phloem, fundamental tissue with articulated laticiferous vessels embedded in it. Although *I. heterophylla* has the same characteristics mentioned, it lacks secondary phloem at this level. Another characteristic of some members (usually herbaceous rather than woody) of the Asteraceae is the persistence of root hairs for up to three years (Metcalfe and Chalk, 1972). In the case of the perennial herbaceous species of this study, persistent root hairs were not observed.

From a comparative study of *I. heterophylla* with other species of Asteraceae (Manzanero, 1995), specifically in *Psacalium peltatum* (Kunth) Cass. and *Roldana sessilifolia* (Hook. et Arn.) H.Rob. et Brettell, it was found that *I. heterophylla* has anatomical characteristics of specific diagnostic value that contribute to its identification. Such is the case of its glaucous roots, with abundant macrosclereids in rhizodermis, cortex less developed, absence of calcium oxalate, inconspicuous endodermis, scarce developed xylem without fibers, with isolated rays and inconspicuous axial parenchyma, large tangential diameter vessels and absence of pith. Another characteristic found in this species is the presence of big sized, fan shaped amiloplasts, not commonly found in other Asteraceae roots. The presence of polyphenols previously isolated (Aguilar, 1993), and not to-date detected in other *Iostephane* species, was histochemically corroborated by their positive reaction to potassium permanganate as a diagnostic character (Delgado *et al*., 1994); polysaccharides, lignin and starch were also detected. However pectin, proteins, condensed and hydrolizable tannins and lipids were not perceived.

Even when the microscopic characterization of the medicinal species is necessary as an additional method of quality control, to this date there is no information on the detailed anatomical description of most of the recorded medicinal species in the Mexican Herbolaria. In fact, in 15 of the 40 species of the medicinal plants reported in the Mexican Herbs Pharmacopoeia (Secretaría de Salud, 2001), the roots constitute the medicinal part and only few of them have partially been microscopically described. The well-known use of plants as an alternative within the traditional medicine requires the rigorous identification of the plant resource to eliminate the risk of mistakes between species and the use of adulterants. Under this scope and according to Bye and Linares (1987), *I. heterophylla* is included in the Cachana complex of medicinal plants that groups different species with similar morphological characteristics to solve the same health problem; this type of research together with other evidences contributes to the unequivocal identification of the species. The present study represents the beginning of a series of descriptive anatomical studies towards a comparative analysis in order to identify some diagnostic characteristics of some medicinal species of the Mexican Herbolaria.

Acknowledgements

The authors thank M. Alejandro Vallejo Z. for his technical support in obtaining the morphometric data, and to the

Dirección General de Asuntos del Personal Académico of UNAM (IN-206900) for financial support.

Literature cited

Aguilar M.I., Delgado G., Bye R. and Linares E. 1993. Bisabolene, polycyclic diterpenoids and other constituents from the roots of *Iostephane heterophylla. Phytochemistry* **33**:1161-1163.

Aguilar M.I., Delgado G., Hernández M.L. and Villarreal M.L. 2001. Bioactive compounds from *Iostephane heterophylla* (Asteraceae). *Natural Product Letters* **15**:134-136.

Aguilar M.I., Rios G., Castro R., Navarrete A., Bye R., Guzmán M. and Duarte-Lisci G. Submitted. Determination of xanthorrhizol from *Iostephane heterophylla* (Cav.) Benth. ex Hemsl. and its quantification by two analytical methods. *Phytochemical Analysis.*

Argueta V.A., Cano A.L. and Rodarte M.E. 1994. *Atlas de las Plantas de la Medicina Tradicional Mexicana, Vol. II*. Instituto Nacional Indigenista, México, D.F.

Bennett B.C. and Prance G.T. 2000. Introduced plants in the indigenous pharmacopoeia of Northern South America. *Economic Botany* **54**:90-102.

Bye R. 1985. Medicinal Plants of the Tarahumara Indians of Chihuahua, México. In: Tyson R.A. and Elerick D.V. Eds. *Two Mummies from Chihuahua: a Multidisciplinary Study*, pp. 77-104, San Diego Museum, San Diego.

Bye R. and Linares E. 1987. A study of four medicinal plant complexes of México and adjacent United States. *Journal of Ethnopharmacology* **19**:153-183.

Campos M.G., Oropeza M.V., Villanueva T., Aguilar M.I., Delgado G. and Ponce-Monter H. 2000. Xanthorrizol induces endothelium-independent relaxation of rat thoracic aorta. *Life Sciences* **67**:327-333.

Delgado G., Cano A.E., Ruiz-Cancino A., Bye R. and Linares E. 1994. Polycyclic diterpenes from the roots of *Iostephane heterophylla. Planta Medica* **60**:493.

Duke J.A. and Ayensu E.S. 1985. *Medicinal Plants of China. Vol. 1.* Reference Publications, Alginac, MI.

Evans W.C. 2002. *Trease and Evans Pharmacognosy*. W.B. Saunders, New York.

Galeffi C. and Marini-Bettólo G.B. 1988. New approaches to the utilization of plants in the preparation of pharmaceuticals and insecticides. *Fitoterapia* **59**:179.

Hamburger M. and Hostettman K. 1991. Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry* **30**:3864.

Heinrich M. 2000. Ethnobotany and its role in drug development. *Phytotherapy Research* **14**:479-488.

Hernández F. 1959. *Historia Natural de Nueva España. Vol. 1.* Obras Completas, Tomo II. Universidad Nacional Autónoma de México. México, D.F.

Lozoya X., Rivera-Arce E. and Domínguez F. 1999. *Phytopharmaceuticals and the Medicine of the New Century.* Symposium 3, IMSS-Farmasa Schwabe. The Phytopharmaceuticals of the New Century. México, D.F.

Luna S., Sandoval E. and Lozoya X. 1986. Estudio anatómico de la hoja del guayabo (*Psidium guajava*) para propósitos de control de la calidad del medicamento herbolario. *Fitoterapia* **LVII**:223-229.

Malone M.H. 1977. *New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutic Activity*. Wagner H. Wolff, Springer Verlag, Berlin.

Manzanero G.I. 1995. Estudio de siete raíces medicinales frescas del mercado de Sonora. D.F., México. Thesis (M.Sc.), Facultad de Ciencias, Universidad Nacional Autónoma de México, México, D.F. 173 pp.

Martínez M. 1989. *Las Plantas Medicinales de México*. Botas. México, D.F.

Mata R., Martínez E., Bye R., Singh M.P., Janso J.E., Maiese W.M. and Timmermann B. 2001. Biological and mechanistic activities of xanthorrizol and 4-(1', 5'-dimethylhex-4'-enyl)-2-methylphenol isolated from *Iostephane heterophylla. Journal of Natural Products* **64**:911-914.

Metcalfe C.R. and Chalk L. 1972. *Anatomy of the Dicotyledons Leaves, Stem and Wood in Relation to Taxonomy with Notes on Economic Uses. Vol. II*. Claredon Press, Oxford.

Morman D.E. 1994. North American food and drug plants. In: Etkin N.L. Ed. *Eating on the Wild Side: the Pharmacologic, Ecologic and Social Implications of Using Noncultigens*, pp. 166-181, University of Arizona Press, Tucson.

Nuez F. and Hernández J.E. 1994. Neglected crops: 1492 from a different perspective. In: Hernando J.E. and León J. Eds. *Plant Production and Protection Series*, no. 26, pp. 303-332, FAO, Rome.

Pereda-Miranda R. 2003. Biodynamic constituents in the Mexican morning glories: purgative remedies transcending boundaries. *Current Topics in Medicinal Chemistry* **3**:111-131.

Ponce-Monter H., Campos M.G., Aguilar I. and Delgado G. 1999. Effect of xanthorrhizol, xanthorrhizol glycoside and trachylobanoic acid isolated from the cachani complex plants upon the contractile activity of uterine smooth muscle. *Phytotherapy Research* **134**:1-4.

Sandoval E., Rojas A., Guzmán C., Carmona L., Ponce R., León C., Loyola C., Vallejo M. and Medina A. 2005. *Técnicas Aplicadas al Estudio de la Anatomía Vegetal.* Instituto de Biología, Universidad Nacional Autónoma de México, México, D.F.

Secretaría de Salud. 2001. *Farmacopea Herbolaria de los Estados Unidos Mexicanos*. 2001. Secretaría de Salud. México, D.F.

Spjut R.W. and Perdue R.E. 1976. Plant folklore: a tool for predicting source of antitumor activity. *Cancer Treatment Reports* **60**:979-981.

Valdés J. and Flores H. 1985. Historia de las plantas de Nueva España. In: *Comentarios a la Obra de Francisco Hernández. Obras Completas, Tomo VII*, pp. 7-222, Universidad Nacional Autónoma de México, México, D.F.

Villavicencio M.A. 1995. *Plantas Útiles del Estado de Hidalgo.* Universidad Autónoma de Hidalgo, Pachuca.

WHO [World Health Organization]. 1998. *Quality Control Methods for Medicinal Plant Materials*. World Health Organization, Geneva.

Received: April 12, 2005
Corrected version: November 7, 2005
Accepted: November 7, 2005