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Microscopic analysis and histochemical observations of the medicinal root of Ilostephane heterophylla
(Cav.) Benth. ex Hemsl. (Asteraceae)
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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). 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-Bettolo, 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 drugs.
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 ethnomedical, 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”.
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(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), “hui-chocachuarua” (Michoacán), “hierba del oso” and “liga” (Estado de México), “tecpatlhi”, “tlacopati” (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 20 g per 150 l of water is drunk daily to treat diabetes, lung affictions, liver ailments, and gastrointestinal complaints such as dysentery; it may be drunk alone or in a mixture with *Pneumus boldo*, *Dydimaea alsinoides*, and *Ternstroemia* sp. Other local uses include post-partum 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 Nahuahtl name “chipoaocíztic” 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 water-holes 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).

Figure 2. Distributional range in Mexico of *Iostephane heterophylla*. 
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 artemisifolia* 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 semiamplexicaule* 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).](image-url)
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 potassium 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 Axiostop Zeiss photomicroscope, and measurements were taken with a micrometric ocular inserted to this microscope. The macroscopic 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 includes 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 conduits 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 epidermis 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 phellogenodermis 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 conduits 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-
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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).
Abundant insoluble polysaccharides (Kunth) Cass. and roots have also thick, soft and succulent 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 apotracheal 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 conduct, polyphenols were detected from their positive reaction to potassium 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 (lugo), 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 rhyzodermis, 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.

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