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Mexican Plants Used in the Salmonellosis Treatment

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1. Introduction

Diarrhoeal diseases constitute a major public health problem, particularly in developing countries, where the rate of mortality and morbidity is very high (Bern et al., 1992). The World Health Organization (WHO) has estimated that 1.5 billion episodes of diarrhea occur every year in these countries, resulting in 3 million deaths (Alper 2003). In Mexico, intestinal infectious diseases are the second cause of morbidity, for which children younger than 5 years of age and adults over 65 years are the most affected (Secretaría de Salud, 2005). These diseases are the 18th leading cause of mortality in the general population and, more importantly, the primary cause of death in children (1 to 4 years) and the fourth for infants (1 to 12 months) (Secretaría de Salud, 2005). In addition, certain social groups are more prone to suffer intestinal infections. These diseases are the second cause of mortality among the Mexican rural population (Tapia-Conyer, 1994).

One study suggests that at least 37% of the Mexican population uses medicinal plants. From the 3034 plants used as medicine in Mexico, 1024 (34%) are used to treat diseases in the digestive tract (Argueta et al., 1994). The high percentage of species used to treat gastrointestinal diseases may reflect the high level of incidence of these illnesses among rural and native communities where the plants are their principal, if not only, available health resource (Tapia-Conyer, 1994; Waller, 1993).

The etiological agents of diarrhea described in epidemiological studies are transmitted as waterborne and foodborne. Some foodborne pathogens have been recently considered as emerging diseases (WHO Media Centre, 2007), despite the fact they have been known since a long time ago. For example, outbreaks of salmonellosis have been described for many decades, and yet their incidence has increased over the last 25 years. Diarrhoeal infections can be caused by many etiological agents, but mainly by enterobacteria such as *Escherichia coli*, *Salmonella spp*., *Shigella spp*., *Campylobacter jejuni* and *Vibrio cholerae*; as well as parasites such as *Entamoeba histolytica* and *Giardia intestinalis*, and some rotaviruses are also important agents (Guerrant et al., 1990). *Salmonella spp*. is a facultative, gram negative, flagellated member of the *Enterobacteriaceae* family. The most extensive accepted classification of

Salmonella strains is based on the diversity of two differentially expressed H flagellar antigens: flagellin phase I and phase II antigens (codified by fliC and fljB genes), and the O antigens of the bacterial lipopolysaccharide, both determined by serotyping. Until now, 2501 serotypes have been described; which turns Salmonella classification into a complex and laborious process in the clinical laboratory; therefore, several PCR based methods have recently been developed, and were reported to be a simple, highly sensitive, fast and reliable alternative when compared to traditional clinical laboratory methods.

As a part of our contribution in this area, we have studied an important plant used in this public health problem. *Piqueria trinervia* Cav. is a perennial herb of the family *Asteraceae* that grows commonly in open areas of the pine-oak forests of the mountains throughout Mexico and Central America. It is employed to treat diarrhea, dysentery, ''empachos'' (a cultural disease with manifestations of various gastrointestinal disorders), intestinal infections, stomach pain, and typhoid fever (Argueta et al., 1994). To cure diarrhea or intestinal infections, an infusion or decoction is prepared with 10 g of the aerial parts in 0.5L of water; the tea is consumed throughout the day until the sickness disappears (Linares, 1991). The biological and chemical study of this plant would be described in the present chapter.

2. Traditional medicine in the treatment of Salmonellosis

The use of medicinal plants has occurred in Mexico since pre-Hispanic times. Aztecs had traditionally viewed the disease and health as dynamic changes in the body. According with this, health is maintained when the body is balanced, and the disease comes when this balance is lost (Ortiz de Montellano 1987; Viesca Trevino 1986). The most common causes of illness were the “bad” spirits called “ehecatl”. This concept still remains until our days in the Nahua region. These spirits can get into the body of a person and bring bad luck, illness or even death. This concept can be easily associated etiologic with the popular cause of "mal aire" (bad air: offensive spirits in the air) found in different areas of Mesoamerica. Treatment for the disease among the Nahua is a series of techniques to get the offensive spirit of the patient's body, including complex rituals that use a combination of prayers, invocations, and medicinal plants. An important part of the ritual is the "limpia" or the ritual of the cleanliness, through which the body is cleaned of the spirits that cause disease. The specialist in healing restores the balance by removing the spirit from the body of the person, and then he use different formulations based on medicinal plants to complete the treatment of the patient. Aztec/Nahua healing specialists are very knowledgeable about plants that can be used medicinally and they have passed this knowledge from generation to generation.

The Yucatán peninsula is a region particularly recognized for its culture, endemic elements and ancient Mayan buildings. Furthermore, although less known, there is an important ancestral knowledge about the medicinal properties of plants, which is widely used by the general population (Ankli et al., 1999). The Mayan traditional healers keep the knowledge of the different properties of the plants, and how to make medicinal mixtures. These formulations (also known as potions) vary according to the disease to be treated and are used since ancestral times (Leonti et al., 2003) to cure some of the most common health problems in the population, such as diarrheal, cutaneous diseases and those from the respiratory tract as documented by Roys (1931). Among the infectious bowel diseases in the Mayan traditional medicine, there is a group known as dysenteries classified as white and red. The main symptom associated with the red dysentery is bloody diarrhea, and the main

symptom associated with the white dysentery is foamy diarrhea (Roys, 1931); other symptoms include abdominal cramps, fever or severe pain during defecation (Waldman et al., 1994). Since the traditional diseases are not easy to correlate with the causal agents of infectious bowel diseases, it has taken into account the most characteristic symptoms: bloody and/or mucous diarrhea, foamy diarrhea, and abdominal pain to select the microorganisms to be tested

With the presence of nearly 10,000,000 indigenous people speaking nearly 85 different languages, who still depend upon plants for primary therapy from the diverse flora (almost 5,000 medicinal plants), Mexico represent a good area to perform ethnopharmacological studies. Studying the biological diversity of plants related to their traditional use as medicines can lead us to understand how they act and to assure the rational exploitation of the resources and their further development as phytomedicines. Because medicinal plants continue to be culturally suitable as treatments for several illnesses, it is important to document their uses and perform studies about their pharmacological activities to assure their efficacy and safety. Despite the vast literature that exists in Mexico (in Spanish) about ethnobotanical studies, only a few efforts to publish these data in international journals have been done. However, Heinrich and his group published more than 18 works (i.e. Weimann and Heinrich, 1997; Heinrich et al., 1998) with a combination of adequate field work and appropriate interpretation of the data.

The people in Mexico still depend upon the use of medicinal plants to treat simple health problems, including those who live in regions where it is still possible to find people who speak the pre-Hispanic Nahua language. The cultural knowledge about the use of medicinal plants converges with the richness in the surrounding flora making possible to select different regions in Mexico to study selected traditionally used medicinal plants.

3. Mexican medicinal plants used in Intestinal diseases

Today in Mexico there is a strong attachment to the use of plants to cure various diseases. For large sectors of Mexican society, particularly for the indigenous, traditional medicine is the main or only source to address the health problems (Tapia-Conyer, 1994).

The presence of traditional medicine is clearly observed in the southern areas of Mexico such as Chiapas and Oaxaca, where native culture prevails (Lozoya, 1990). Although there are medical services in some rural communities, people prefer traditional medicine. In addition to the cultural, traditional medicine is more accessible to people living in rural communities, either because health facilities are far from the locality or because it is more expensive medical treatment with allopathic (Frei et al., 1998).

In a study from 1983 to 1985 in rural areas of Mexico, through surveys of the program IMSS-COPLAMAR clinics to traditional doctors, they enlisted the 1.950 most used medicinal species in the country. 140 plant species were highlighted for the frequency of use, of which 38.0% were used to cure digestive disorders (deworming, antispasmodics, laxatives, anti-diarrhea and cholera), 13.6% were used to cure diseases of the respiratory system and 13.5% for the treatment of skin lesions. (Lozoya et al., 1987).

Between 1994 and 1995, the most common diseases treated by traditional healers in rural areas of the country were digestive (44.0%), respiratory (11.0%) and injuries (9.0%) (Lozoya,

1990). Most of the medicinal plants used in three indigenous communities of Oaxaca (Maya, Nahua and Zapotec) were used to treat gastrointestinal, skin and respiratory diseases (Heinrich et al., 1992). Zapotec Healers from the Tehuantepec Isthmus used 205 plant species, of which 46.1% were used to treat skin problems and 39.6% to treat gastrointestinal diseases (Frei et al., 1998).

According to traditional Mexican medicine, there are diverse healing strategies, as well as different preferences for the plant parts used for various diseases. It is generally accepted that the beneficial effects of medicinal plants can be obtained from active constituents present in the whole plants, parts of plant (as flowers, fruits, roots or leaves), or plant materials or combinations thereof, whether in crude or processed state.

According with our etnobotanical research, the next plant are the most used in treatment of intestinal diseases: Buddleia scordioides, Byrsonima crassifolia, Geranium seemannii, Guazuma ulmifolia, Larrea tridentate, Persea americana, Piqueria trinervia, Psidium guajava.

3.1 Escobilla (*Buddleia scordioides* H. B. K)

Buddleja scordioides HBK (Buddlejaceae) is a shrub which grows in the Chihuahuan desert and in the state of Coahuila, Nuevo León, Tamaulipas, Durango, Zacatecas, Aguas Calientes, San Luis Potosí, Guanajuato, Queretaro, Hidalgo, Jalisco, México and Distrito Federal (Avila et al., 2005). *Buddleja scordioides* HBK (KUNTH), *Loganiaceae*, is commonly known as escobilla, butterfly-bush, mato, salvia real and salvilla (Martínez, 1979).

Its medicinal use includes the treatment of eating disorders, especially stomach aches and diarrhea. Also used as eupeptic, it is recommended to prepare branch or root infusions, these parts are boiled in milk and given to children when they have colic (Argueta, 1994).This plant is widely used for the treatment of diarrhea, stomacha che (colic) and gastrointestinal disorders.

It was reported that the amebicide activity of *B. cordata* is caused by linarin. This plant can be a source for this compound since it constitutes 24% of the methanol extract of aerial parts.

It was found that extracts of the aerial parts of *B. scordioides* and *B. perfoliata* showed antispasmodic activity and had a relaxing effect on rabbit jejunum and ileum of guinea pigs. Such activities may be the cause of its use in traditional medicine in the treatment of gastrointestinal pain, spasms and cramps (Cortés et al., 2006). Decoctions of this plant are commonly used orally or topically for treating several illnesses such as diarrhea, headache, and hurts (Avila et al., 2005).

In addition, a verbascoside with antibacterial activity, triterpenoid saponins and other glycosides, have been extracted from this species. The presence of some flavonoids such as rutine, quercetin and quercitrin has also been reported (Cortés 2006)

3.2 Nanche (*Byrsonima crassifolia* (L.) Kunth)

Byrsonima crassifolia (Malpighiaceae) is a tropical tree widely distributed in Mexico, Central and South America. The pharmacological activities of *B. crassifolia* extracts as a bactericide, fungicide, leishmanicide, and as a topical anti-inflammatory (Maldini et al. 2009) have been described. *B. crassifolia* is popularly known as "nanche" and it has been used medicinally

since prehispanic times, mainly to treat gastrointestinal afflictions and gynecological inflammation (Heinrich et al. 1998). The most often medicinal use of *B. Crassifolia* has been as an antidiarrheal, but has been also indicated to treat other disorders of the digestive system such as dysentery, stomach pain, indigestion and poor digestion. A decoction of the bark is usually used to treat these conditions (Martinez, 1959; Argueta, 1994). Some other reports indicate that *B. crassifolia* has been employed in the treatment of nervous excitement and to induce a pleasant dizziness (Maldonado 2008).

Different phytochemical studies have been carried out to isolate the main active compounds. The presence of terpenes, saponins, flavonoids and glycosides has been reported from the root of *B. crassifolia* and saponins, flavonoids, glycosides and tannins have been isolated from the stem as well.

3.3 Pata de León (*Geranium seemannii* Peyr)

Geranium seemanni Peyr is a perennial herbaceous plant, with flowers with different colours varying from purple to white (Rzedowski Rzedowski, 1995). It has been located in the states of Sinaloa, Chihuahua, Coahuila, Nuevo Leon, Tamaulipas, Durango, Zacatecas, San Luis Potosi, Aguascalientes, Guanajuato, Mexico City, Queretaro, Hidalgo, Guerrero, Jalisco, Michoacan, Morelos, Oaxaca , Puebla, Tlaxcala, Veracruz and Chiapas (Sanchez, 1979; Rzedowski and Rzedowski, 1995).

The Codigo Florentino, one of the most important books written in ethnobotany in the century XVI, mention that the ground plant applied in the face can remove stains on the skin face. Francisco Hernandez, in the same century says: "is astringent, cures dysentery and other flows, eye inflammation, hemorrhoids and indigestion, and cools down some fevers, among other diseases."

Currently the most common use of this plant is for digestive disorders such as vomiting and diarrhea. The decoction of the plant can be used as antigastralgic and the infusion of the leaves as a purgative. The decoction of the stem, leaves and flowers is usually used to relieve the itch (Argueta, 1994).

This specie has not been studied to analyze its bioactive compounds. However, different species of the *Geranium sp*, used with therapeutic purposes in Mexico, are reported in the literature (Calzada et al., 1999; Serkedjieva e Ivancheva, 1999; Akdemir et al., 2001). The geranin is the most abundant tannin founded in the *Geranium* genre. According with Okuda et al., in 1980, a 9.8 to 12% of this compound is present in dry leaves of the plant. (-)-Epicatechin, (+)-catechin, β-sitosterol-3-O-β-glucoside, tiramine y saccharose were isolated from the roots of the *G. mexicanum* (Calzada et al., 2005).

The aqueous and methanolic extracts prepared from the aerial part and root of *G. mexicanum* showed antimicrobial activity against strains of *E. coli*, *Shigella sonnei*, *S. flexneri* and *Salmonella sp*. (Alanis et al., 2005).

3.4 Guacimo (*Guazuma ulmifolia* Lam.)

Trees of *Guazuma ulmifolia* Lam. (*Sterculiaceae*), commonly known as guácimo, caulote, tapaculo, or aquiche, occupy dry lowlands from Peru, north and east to Venezuela and to

northern México. The species is common in pastures and fencerows and its foliage and fruits are valuable cattle and horse fodder (Seigler et al., 2005).

This plant is used in the Mexican traditional medicine to treat various diseases. In Guerrero, Puebla and Veracruz, is used to treat gastrointestinal disorders. The decoction of the bark, leaves or buds, are used to treat diarrhea. A tea prepared with guacimo shoots, shoots of guayaba (*Psidium guajava*), the stem of nanche (*Byrsonimia crassifolia*) and oak (Quercus sp.) is used in children suffering intestinal infection with diarhea (Argueta, 1994). *G. ulmifolia* is used in some Mixe communities of Oaxaca and Veracruz to treat diarrhea (Leonti et al., 2003). The species is widely used by the Zapotec of Oaxaca to treat gastrointestinal diseases. Indigenous healers recognize plants with astringent properties (high levels of tannins) as useful in the treatment of gastrointestinal disorders (Frei, 1998).

Tannins are the main components of *Guazuma sp*. The variation of these compounds depends on the part of the plant, thus the leaves has 0.145 mg/g, in the leaves with stems 0.115 mg/g, in the stems 0.087 mg/g, and were not detected in fruit (Ortega et al., 1998).

The following compounds were isolated from the ethanolic extract of the stem bark, tannin acid, (-)-epicatechin-[4β→8]-(-)-epicatechin-4β-benzilthioether, (-)-epicatechin-[4β→6]-(-)-epicatechin-4β-benzilthioether; (-)-epicatechin; the dimers procyanidin B2 and B5; the trimers procyanidin C1, (-)-epicatechin-[4β→6]-(-)-epicatechin-[4β→8]-(-)-epicatechin and (-)-epicatechin-[4β→8]-(-)-epicatechin-[4β→6]-(-)-epicatechin and the tetramer (-)-epicatechin-[4β→8]-(-)-epicatechin-[4β→8]-(-)-epicatechin-[4β→8]-(-)-epicatechin (Hörr et al., 1996).

3.5 Gobernadora (*Larrea tridentata* (DC) Cav.)

Larrea tridentata (Sesse and Moc. Ex DC, Zygophyllaceae) also known as gobernadora, coville, larrea, chaparral, or creosote bush, is a shrubby plant belonging to the family Zygophyllaceae. *L. tridentada* is a common shrub of North American warm deserts. Its dominance has increased within 19 million ha of lands previously considered desert grasslands in response to disturbances such as grazing (Arteaga et al., 2005). While often viewed as an indicator of desertified conditions and the focus of extensive control efforts it is also an important plant with a long history of medicinal use (Arteaga et al., 2005).

Tea brewed from the leaves of *L. tridentata* has been used in traditional medicine to treat digestive disorders, rheumatism, venereal disease, sores, bronchitis, chicken pox, and the common cold (Sinnott et al., 1998). This plant is often used to treat gynecological problems. In cases of infertility, the decoction of the leaves is used in vaginal washings or taken as a tea for nine days after the period (for three consecutive months)(Argueta, 1994). Among the proposed medicinal properties of creosote bush, the most prominent is its antioxidant effects (Sheikh et al., 1997).

Phytochemical studies carried out on *L. tridentate* showed that it contains a series of lignans, flavonoids, condensed tannins, triterpene saponins, and naphthoquinones (Abou-Gazar et al., 2004). The extracts or constituents of *L. tridentata* have been reported to possess antioxidant, anti-HIV, antimicrobial, enzyme inhibitory, anti-tumor, and anti-hyperglycemic (Abou-Gazar et al., 2004) activities. The plant contains the powerful antioxidant, nordihydroguaiaretic acid (NDGA) which is suspected to contribute to the toxic effects associated with the consumption of chaparral products (Sinnott et al., 1998).

3.6 Aguacate (*Persea americana* Miller)

Persea americana mill (lauraceae) is a tree plant also called avocado or alligator pear. It is chiefly grown in temperate regions and sparsely grown in tropical regions of the world. Since ancient times this plant has been valued for its nutritional and medicinal properties. The fruit is highly prized for its aroma and exquisite taste (Lozoya and Lozoya, 1982). Avocado is used in 25 states in Mexico to treat various digestive disorders (Lozoya et al., 1987) as dysentery, stomach pain, constipation, stomach gas, vomiting, among others (Argueta, 1994). Peel of the avocado is used in infusion for treating intestinal parasites (Martinez 1959; Lozoya and Lozoya, 1982; Argueta, 1994). It is recommended for anemia, exhaustion, hypercholesterolemia, hypertension, gastritis, and gastroduodenal ulcer. The infusion prepared from the leaves is used in the treatment of diarrhea and some cases of indigestion. The leaves have also been reported as an effective antitussive, antidiabetic, and relief for arthritis pain by traditional medicine practitioners. Analgesic and antiinflammatory properties of the leaves have been reported (Adeyemi et al., 2002).

The result of the phytochemical screening of the aqueous leaf extract of *Persea americana* revealed that the extract contained various pharmacologically active compounds such as saponins, tannins, phlobatannins, flavonoids, alkaloids, and polysaccharides.

From the aqueous extract of the avocado leaves, two new monoglycosyl flavonols were isolated, 3-O-α-D-of kaempferol and quercetin arabinopyranoside, along with the 3-O-α-L-ramnopiranosido-kaempferol (Afzeliana), 3-O-α-L-ramnopiranosido-quercetin (quercitrin), 3-O-β-gluco-pyranoside-quercetin and quercetin 3-O-β-galactopyranoside, quercetin (Almeida et al., 1998).

3.7 Guayaba (*Psidium guajava* L.)

Psidium guajava, a tropical fruit guava of the family *Myrtaceae*, is widely recognized as a plant of many herbal medicines. The leaf, root, and bark of *P. guajava* are used in indigenous herbal medicine for the treatment of various ailments including those that are bowel related. In 23 states of Mexico is used to treat gastrointestinal diseases (Lozoya et al., 1987), most notably diarrhea. Generally, a decoction or infusion prepared with the leaves of guava tree is taken against gastrointestinal diseases (Argueta, 1994). The Tzotzil prepare an infusion to relieve severe diarrhea, weakness, vomiting, stomach pain, when present watery or bloody stools that can last days (Argueta, 1994).

The decoction of the leaves has showed *in vitro* antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Shigella dysenteria*. Another paper showed the effectiveness of the leaf extract against *Staphylococcus aureus* (Gnan and Demello, 1999). It was shown to antibacterial in another study and in addition to *Staphylococcus aureus* was also useful against *Streptococcus spp*. The leaves are rich in tannin, and have antiseptic properties. Modern proof of the traditional use can be found in modern studies. The methanolic extract of *P. guajava* (leaves) showed significant inhibitory activities against the growths of 2 strains of *Salmonella*, *Shigella spp*. (*Shigella flexneri*, *Shigella virchow* and *Shigella dysenteriae*) and 2 strains of the enteropathogenic *Escherichia coli*. The results have confirmed the effectiveness of this medicinal plant as an antidiarrheal agent. Guava sprout extracts (P. guajava) by 50% diluted ethanol showed the most effective inhibition of *E. coli*, while those in 50% acetone were less effective. It is concluded that guava sprout extracts constitute a

treatment option for diarrhoea caused by *E. coli* or by *S. aureus* produced toxins, due to their quick therapeutic action, easy availability in tropical countries and low cost.

The leaves contains essential oil with the main components being α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene. The essential oil from the leaves has been shown to contain, nerolidiol, β-sitosterol, ursolic, crategolic, and guayavolic acids have also been identified. In addition, the leaves contain an essential oil rich in cineoland four triterpenic acids as well as three flavonoids; quercetin, its 3-L-4-4-arabinofuranoside (avicularin) and its 3-L-4-pyranoside with strong antibacterial action.

4. The *Asteraceae* (*Compositae*) family

The family *Asteraceae* (*Compositae*) is one of the largest families of flowering plants with about 1100 currently accepted genera and 25000 species (Heywood, 1977). It is of worldwide distribution particularly in semiarid region of the tropics and subtropics. The most members are evergreen shrubs or subshrubs or perennial rhizomatous herbs; biennial and annual herbs are also frequent. It is generally accepted that *Compositae* are a "natural" family with well established limits and a basic uniformity of floral structure imposed on all members by the common possession of charaters such as the aggregation of the flowers into capitula and the special features of the stamensand corolla.

Plants in *Asteraceae* are medically important in areas that don't have access to Western medicine. They are also commonly featured in medical and phytochemical journals because the sesquiterpene lactone compounds contained within them are an important cause of allergic contact dermatitis. *Asteraceae* (*Compositae*) are useful for therapeutic application due to their antihepatoxic, choleretic, spasmolytic, antihelminthic, antiphlogistic, antibiotic or antimicrobial activities. Some of them possess remarkable bacteriostatic and fungistatic properties and they probably participate in the pharmaceutical activity of some drugs and hence the elucidation of the structure of some members of the family. Some preliminary studies of *Piqueria trinervia* have demonstrated that active substances are present in these plants.

4.1 Piqueria trinervia Cav from the work "antibacterial activity of *Piqueria trinervia*, a Mexican medicinal plant used to treat diarrhea", Pharmaceutical Biology, 2007, vol. 45, No. 6, pp. 446–452

Piqueria trinervia Cav. is a perennial herb of the family *Asteraceae* that grows commonly in open areas of the pine-oak forests of the mountains throughout Mexico and Central America. It is usually called ''hierba de San Nicolás'' or ''hierba de tabardillo.'' During the 16th century, it was known by its Nahuatl name as ''cuapopolchi'' and was used as a febrifuge as well as in the treatment of various gastrointestinal ailments including diarrhea. Today, it is employed to treat diarrhea, dysentery, ''empachos'' (a cultural disease with manifestations of various gastrointestinal disorders), intestinal infections, stomach pain, and typhoid fever (Argueta et al., 1994). To cure diarrhea or intestinal infections, an infusion or decoction is prepared with 10 g of the aerial parts in 0.5L of water; the tea is consumed throughout the day until the sickness disappears. This preparation is used as an enema for 2 days, once in the morning and once at night (Torres, 1984). To cure ''empacho,'' people drink a half glass of the root decoction of the tea on an empty stomach for 2 days or until the sickness disappears.

4.2 Biological activity in salmonellosis

Because of its long history in traditional medicine in Mexico, the antimicrobial activity of *Piqueria trinervia* was investigated. Previous studies reported antibacterial activity only from the aerial parts. In our study, antimicrobial activity of extracts derived from both above-ground and root portions of ''hierba de tabardillo'' was evaluated using bacteria that are common to intestinal infections.

4.3 Plant material

The plant material used in this study was collected in the Ajusco zone, Distrito Federal, Mexico, during May and June. The voucher specimen is deposited in the National Herbarium (MEXU) located at the Instituto de Biología, UNAM. The plant was divided in three parts, thick (primary) roots, thin (secondary, 5mm or less) roots, and aerial parts (stem, leaf, flower, and fruit), and dried at room temperature. Each part was ground separately using a mill with rotary knives. The roots were extracted sequentially at room temperature with hexane, ethyl acetate, dichloromethane, and methanol.

4.4 Microbiological test

Test microorganisms. The microorganisms tested were strains of *Escherichia coli*, *E. coli* multidrug resistant (MDR), *Salmonella typhi*, *Shigella boydii*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Yersinia enterocolitica*, nontoxic *Vibrio cholerae*, *Bacillus subtilis*, *Enterobacter aerogenes*, and *Enterobacter agglomerans*. The strains used for this study were provided by Dr. José Guillermo Avila of the Laboratory of Phytochemistry, UBIPRO, Facultad de Estudios Superiores, Iztacala, UNAM.

Bioassay. *In vitro* antibacterial activity was evaluated using the agar disk diffusion method in which the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by microdilution assay in 96-well plates. Disks of 6-mm-diameter sterilized filter paper with concentrations of the extract 2 and 3 mg/disk were prepared as follows: (1) the extracts and fractions were dissolved in dimethyl sulfoxide (DMSO) and diluted with distilled water to obtain concentrations of 133 and 200 mg/mL; (2) then, 15 mL was applied to each disk. The methodology proposed by Bauer et al. (1966) and

Cáceres et al. (1990) was followed. A pure culture of each of the bacteria tested was incubated at 37 ^{o}C for 24 h in 5 mL of 1% peptone water (pH 7.2). Afterward, the culture was adjusted to a MacFarland value of 0.5 and diluted in a proportion of 1:20 with 0.15 M saline solution. An aliquote of 100 mL of the bacteria solution was spread over Müller-Hinton agar in the Petri dish, and the disks with the extracts were placed equidistant over the agar surface. After maintaining the Petri dish for 25 min at room temperature, incubation was followed at 37 ^{o}C for 24 h. The diameter of each inhibition zone is expressed in millimeters.

4.5 Results and discussion

Activity of organic extracts obtained by liquid-liquid separation. The partition of the aqueous extract of the aerial parts with dichloromethane and microbiological evaluation of the fractions showed activity only in the organic fraction. At a concentration of 3 mg/disk, this fraction was active against all the tested strains with an inhibition halo that ranged from

9 to 20 mm. *Y. enterocolitica* and *S. typhi* were the most sensitive bacteria with halos of 20 and 18 mm, respectively. Also, antibacterial activity was detected in the organic fraction of the aqueous root extract and inhibited the growth of *Y. enterocolitica*, *B. subtilis*, all the tested *Salmonella* strains, *Staphylococcus aureus*, and *Staphylococcus epidermidis* with halos ranging from 7 to 11mm. *Escherichia coli*, *Enterobecter aerogenes*, and *Enterobecter agglomerans* were resistant to the root extracts. These extracts produced bactericidal effects on *Salmonella dublin* (MIC= 3.0 mg/mL) and *Salmonella gallinarum* that produced diarrhea in chickens as well as on *Salmonella typhi* and *Staphylococcus aureus* with MBC ranging from 5 to 7.5 mg/mL.

Antibacterial activity of the organic extracts from the roots. The hexane extract from thick roots (TkR) at a concentration of 3 mg/disk produced antibacterial activity against all 11 strains assayed; meanwhile, the hexane extract obtained from the thin roots (TnR) was active only against *B. subtilis*, *E. agglomerans*, *S. aureus*, *S. epidermidis*, and *S. boydii* at the two tested concentrations (Tables 1 and 2). This TkR extract inhibited the growth of *V. cholerae* with a halo of 32.5mm as well as that of *Y. enterocolitica*, *S. boydii*, *E. aerogenes*, *S. epidermidis*, and *S. typhi* with inhibition halos between 24.0 and 11.5 mm. The activity of the extract at a concentration of 2mg/disk ranged from 14.0 to 11.5 mm, and the most sensitive strains were *S. epidermidis*, *S. aureus*, *B. subtilis*, *S. boydii*, *S. typhi*, and *E. Coli* (Table 1). In general, the TnR extracts had less antibacterial activity than the TkR extracts (Table 2). Nonetheless, *S. aureus* was highly sensitive to TkR and TnR hexane extracts with halos of 17.5 and 12 mm, respectively. Even at a higher dose of TnR hexane extract, the activity maintained the same order (Table 2). Dichloromethane extracts were less active than the hexane extracts (Table 2). In comparison with TnR, TkR dichloromethane showed the broadest antimicrobial action. At a concentration of 3mg/disk, the bacteria *V. cholerae*, *S. aureus*, *S. boydii*, *Y. enterocolitica*, *B. subtillis*, and *S. epidermidis* were sensitive to TkR, whereas TnR inhibited only *B. subtilis* and *E. agglomerans*.

	Inhibition zone (mm)						
	Organic Extract						
		2 mg/disk			3 mg/disk		
Strains	H	DM	EA	M	H	DM	EA
B. subtilis	13.0	14.5	12.5	9.5	12.0	10.0	12.0
E. coli	9.0	-	-	-	11.5	-	-
E. coli MDR	-	-	-	-	10.5	-	-
E. aerogenes	-	-	7.5	-	12.5	-	7.5
E. agglomerans	-	-	-	-	10.5	-	-
S. aureus	13.5	11.0	11.0	-	17.5	16.5	11.0
S. epidermis	14.0	12.0	13.0	-	11.5	10.5	8.0
S. boydii	11.5	12.0	11.0	-	17.0	16.5	11.5
S. typhi	11.0	-	-	-	11.5	-	8.5
Y. enterocolitica	NT	NT	NT	-	24.0	13.5	17.0
V. cholerae	NT	NT	NT	-	32.5	30.0	26.5

H, hexane; DM, dichloromethane; EA, ethyl acetate; M, methanol; –, no inhibition, NT, not tested.

Table 1. Antibacterial activity of the organic extracts of the thick roots of *Piqueria trinervia*.

The halo diameters for the effective dichloromethane extract of TkR at the concentration of 3mg/disk ranged from 16.5 to 10 mm, with the exception of the 30mm halo produced by V. cholerae. The ethyl acetate extract from TkR showed an inhibitory effect with halos between 17.0 and 8.0 mm. This extract was effective against most of the tested strains. TkR was inactive against *E. coli*, *E. coli* MDR, and *E. agglomerans*, and TnR was inactive against *E. Coli* MDR and *E. agglomerans*. Methanol extracts from TkR and TnR were generally inactive with the exception of the inhibition of *B. subtilis*, which produced a 9-mm halo (Tables 1 and 2).

In the majority of the inhibition zones, no bacterial growth was observed, but in some inhibitions zones we detected traces of bacterial growth. The inhibition zones without growth are indicated in the tables with bold numbers.

	Inhibition zone (mm)						
	Organic Extract						
		2 mg/disk			3 mg/disk		
Strains	H	DM	EA	M	H	DM	EA
B. subtilis	12.5	8.5	12.5	9.0	11.5	9.5	10.0
E. coli	-	-	-	-	-	-	7.0
E. coli MDR	-	-	-	-	-	-	-
E. aerogenes	-	-	-	-	-	-	8.5
E. agglomerans	8.7	-	-	-	9.0	8.0	-
S. aureus	12.0	-	11.0	-	12.0	-	9.5
S. epidermis	11.5	10.5	11.0	-	10.5	-	8.0
S. boydii	9.5	-	9.0	-	10.5	-	9.0
S. typhi	9.5	-	-	-	-	-	8.5
Y. enterocolitica	NT	NT	NT	-	NT	NT	12.5
V. cholerae	NT	NT	NT	-	NT	NT	27.0

H, hexane; DM, dichloromethane; EA, ethyl acetate; M, methanol; –, no inhibition; NT, not tested.

Table 2. Antibacterial activity of the organic extracts of the thin roots of *Piqueria trinervia*.

Activity of TkR hexane fractions. Nine fractions were obtained by TLC chromatography (hexane-ethyl acetate, 8:2) of the hexane TkR extract. *B. subtilis* and *V. cholerae* were highly sensitive to most of the isolated fractions, and the halos of the effect strains ranged from 8 to 18 mm. The greatest growth inhibition of *B. subtilis* was produced by F2, while that of *V. cholerae* (30 mm) came from the polar residue at the application zone. A moderate activity was found with fractions F1–F3 against *S. aureus*, *S. epidermidis*, and *S. boydii* (8 to 12 mm). *E. aerogenes*, *E. agglomerans*, and *S. typhi* were resistant to all the fractions. *E. coli* and *E. coli* MDR were inhibited by F1 and F2.

Chemical analyses. The organic fraction (dichloromethane) of the aerial part is a light oil, and the hexane extract of the roots is a dark yellow oil. Both oils were submitted for GC-MS study. According to the electronic database of the equipment, it was possible to identify 20 compounds besides Piquerol A (Fig. 1). The numbers are the proportion of each compound in the oil. The compounds present in both oils are the phenol, which was obtained also by Jiménez-Estrada et al. (1996) by chemical reaction of the piquerol; the other compound is the carquejol. The antibacterial activity is attributed to these compounds, as the antibacterial activity of the phenols have been reported. The carquejol and the piquerol A (Fig. 1)

monoterpenes have a biogenetic structural arrangement characteristic of this plant; the destitution of the group on the six member ring is in the vicinal positions 5, 6. Thus, we assigned aromatic compounds the same substitution to the aromatic compounds.

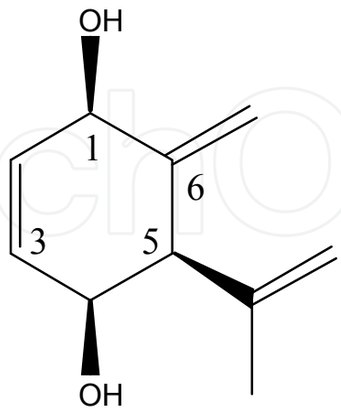


Fig. 1. Compound I: Piquerol A.

5. Conclusion

The organic portion of the aqueous extract prepared with the aerial part has highly activity and favors sustainable harvesting practices that would not damage the roots of plants in natural or cultivated populations. The extracts prepared with the thick root showed more activity with the hexane extract being the most active, followed by the ethyl acetate, dichloromethane, and methanol extracts, respectively. When the hexane extract of the thick root was subfractionated, the greatest antibacterial activity was retained in the residue at the application point and in the three most polar fractions. Based on these results, we conclude that P. trinervia exhibited antimicrobial activity.

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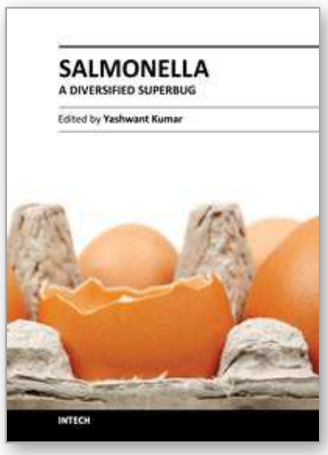
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