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Traditional use and phytochemical analysis in extracts of sauce (*Salix humboldtiana* Willd) in Mexico

Uso Tradicional y Análisis Fitoquímico en Extractos de Sauce (*Salix humboldtiana* Willd) en México

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Abstract:

The objective of this research was to determine the traditional use and chemical compounds of leaves and branches of *Salix humboldtiana* collected in Chumatlan, Veracruz, Mexico by measuring the cultural significance index and the main phytochemicals obtained from crude extracts and analyzed by Thin Layer Chromatography, respectively. Crude extracts biosynthesize phytochemicals in function of the vegetable organ (leaf and branches). On the other hand, traditional knowledge of species *Salix* is scarce in the municipality of Chumatlan. In the interviews carried out, several traditional uses of the willow tree were found.

Keywords:

Index of cultural significance, interculturality, thin layer chromatography, traditional medicine.

Resumen:

El objetivo de esta investigación fue determinar el uso tradicional y compuestos químicos de hojas y ramas de *Salix humboldtiana* colectada en Chumatlán, Veracruz, México por medio del índice de significancia cultural y los principales compuestos fitoquímicos obtenidos a partir de los extractos crudos se analizaron por Cromatografía en Capa Fina, respectivamente. Los extractos crudos biosintetizaron a los compuestos fitoquímicos en función del órgano vegetal (hojas o ramas). Por el otro lado, el conocimiento tradicional de la especie de *Salix* es escaso en el municipio de Chumatlán. En las entrevistas realizadas se encontraron varios usos tradicionales del sauce.

Palabras Clave:

Cromatografía en capa fina, índice de significancia cultural, interculturalidad, medicina tradicional.

Introduction

The genus *Salix* (willow) comprises about 500 species, which are distributed worldwide in temperate regions at high altitudes of tropics [1]. These species are rich in phenolic constituents such as salicylates, flavonoids, and tannins, which have important pharmacological activities and

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medicinal uses [2]. Willows from Mexico represent important forest resources that have not been sufficiently valued. The information recorded about them is scarce and very dispersed. Willows are trees with multiple benefits, such as: shade and ornamental plants, the use of their wood for firewood and charcoal, in agroforestry systems, in the medicinal and artisanal field, as well as their recent use in plantations for the generation of fuels for electric power in the United States. In research work in France, shows that these trees also absorb nitrates and phosphates, which would help in the future to purify waste water from large cities. In addition, the active ingredient acetylsalicylic acid is extracted from the willows for the manufacture of aspirins [3]. The services provided by ecosystems are valued differently by different actors according to socio-ecological contexts and cultural and economic interests [4]. The measure of this value can be ecological, economic and sociocultural [5]. This last category has become an important tool to know the importance and benefits that ecosystem provide to human communities, resulting in a combination of social perception and the capacity of an ecosystem to meet the needs of human groups. Currently, studies on the use and management of medicinal plants serve as a source of information to treat diseases, generating great discussions about the use of these in allopathic medicine, due to the isolation of active ingredients they contain to generate new patents with greater activity biological and/or lower toxicity. Therefore, the importance of traditional medicine is born, since the World Health Organization (WHO) 80% of the world population uses this health system [6]. However, in the Municipality of Chumatlan, Mexico research has not yet been carried out on the use of plants and on the use that is given to the willow tree *S. humboldtiana* (Willd) in traditional medicine, this due to multiple factors including lack of interest and misinformation of people on the management of medicinal plants. The aim of this research was to determine the traditional use of the willow tree specifically by the traditional medical of the municipality and peasants of the Tutunaku ethnic group, which have a wealth of traditional knowledge about the use and management of plants and medicinal trees. Will also be investigated the main phytochemical compounds of the willow tree to be able to relate to the healing properties of this species.

Materials and Methods

Area of study. The municipality of Chumatlan is located in the northern area of the state of Veracruz, Mexico. Between parallels 20° 11' and 20° 15' north latitude; meridians 97° 33' and 97° 37' west longitude; altitude between 100 and 500 m adjacent (Figure 1).

Figure 1. *Geographical location of the study site.*

It borders to the north with the municipalities of Coyutla and Coxquihui; to the east with the municipality of Coxquihui; to the south with the municipalities of Coxquihui and Mecatlan; to the west with the municipalities of Mecatlan and Coyutla [7].

Collection of biological material. A tour was made in the areas of gallery forest with willow species to select the species, with the best characteristics to collect the vegetative material that was used in the phytochemical characterization for the identification of secondary metabolites. In the same way, the georeferencing was carried out, for this a Garmin-650 GPS was used, with which

coordinates of the collection area were determined (Figure 2).

Figure 2. *Sample collection of S. humboldtiana (Willd.).*

The botanical identification of the species was carried out by Dr. Heike Vibrans Lindemann (Germany) of the Postgraduate School, Campus Montecillo, Texcoco, State of Mexico.

Interviews with traditional medical. The interviews were applied to traditional medical of the municipality of Chumatlan and three locations: Lazaro Cárdenas, La Vega and Zapote, belonging to the same municipality. 25 semi-structured interviews were applied using the snowball sampling technique by using the methodology purposed by Basir, in order to know the therapeutic uses of the willow species, in addition to assess and to determine the index of cultural significance this as a tool to calculate the value given to this tree [8]. To measure the Index of Cultural Importance of plants, the formula takes into account seven variables or indices that expressed the frequency of the contribution index (QI), the availability index (ALI), the frequency of utilization index (FUT), Index of plant parts used (PUI), multifunctional food use index (MFFI), index of appreciation of the taste score (TSAI) and the index of food/medicinal function (FMRI). However, adjustments were made towards the options and scores of some sub-index variables. The cultural significance index was calculated following the formula: $CSI = AI \times FUI \times PUI \times TSAI \times FMRI \times 10^{-2}$. Where CSI= Cultural Significance Index, AI= Appreciation Index, FUI= frequency of use index, PUI= Index of used part, TSAI= Flavour Appreciation Index and FMRI= Medicinal Role Index [8].

Analysis of data. A database was created with the help of a Microsoft Excel® sheet (2018), later the data obtained in Statistical Analysis Systems (SAS 2011) [9]. The result was adjusted to a linear regression model to compare the data between the age of the interviewees and the Index of Cultural Significance in order to assess whether the value of the index given by the doctors depends on or correlates with the age of the interviewees. Based on the above, a dendrogram was carried out where the data were grouped in the program to observe how many types of groups are formed with a similar level of knowledge.

Preparation of crude extracts and phytochemical analysis. 100 g of dry plant material was weighed (leaves and branches). The dry material was crushed in a manual mill to be able to fractionate into small particles and improve the absorbance of the particles with the solvent. It was placed in a test tube, and 90 ml of 80% methanol was added. The extract was placed in an ultrasonic bath for 10 min. It was left to stand for 5 min. Again, it was placed in the ultrasonic bath for a further 5 min. The ultrasonicated extract was filtered and collected in 25 mL flasks. The above was done in triplicate. In order to identify the main secondary metabolites, present in leaves and branches of *S. humboldtiana* (Willd.) in order to provide knowledge to the community about the composition of this species. For the identification of phenols, flavonoids, terpenes, alkaloids, saponins and tannins, an analysis of secondary metabolites in dehydrated leaves and branches was carried out [10, 11]. In this study, Thin Layer Chromatography was also performed with the extracts of said species to identified the secondary metabolites present. Table 1 shows the solvent system used and the detection reagents for TLC (Thin Layer Chromatography) of *S. humboldtiana* extracts.

***Table 1**. Solvent systems and detection reagents for Thin Layer Chromatography in extracts analyzed of S. humboldtiana. NP-PEG: Natural products (Polyethylene glycol reagent).*

Secondary Metabolites	Basic Solvent Systems	Proportion (v/v)	Detection Reagent	Remark
Tannis	Ethyl acetate and methanol	1:1	Ferric Chloride	
Phenols	Ethyl acetate and methanol (v/v)	9:1	Folin-Ciocalteu Reagent	
Flavonoids	Ethyl acetate and methanol	1:1	NP-PEG	Before the development is exposed for 4 minutes at 110 °C in a stove
Terpenoids	Hexane and ethyl acetate	8:2	Vainillin in sulfuric acid/ethanol	After revealing, it is exposed to 110 °C in an oven.

Results and discussion

Traditional knowledge. In the municipality of Chumatlan there are very few connoisseurs of the medicinal use of the *S. humboldtiana* species, so this work is a precedent of current research to rescue the intercultural knowledge that exists in the area. The interviewees (traditional medical, midwives and healers) mention that they use the leaves in greater proportion (72%) as a medicinal plant than as a combination with other tree tissues (Figure 3a), leaves and bark (16%), and root (12%). Regarding the medicinal use conferred on S. humboldtiana, they use it to treat hot and cold diseases (72%), followed by fever and headache (16%), and prevention of abortion (12%) (Figure 3b). Regarding the common use of wood, in the municipality they use it as firewood (60%), medicinal (32%) and combination of wood and medicinal (8%) (Figure 3c).

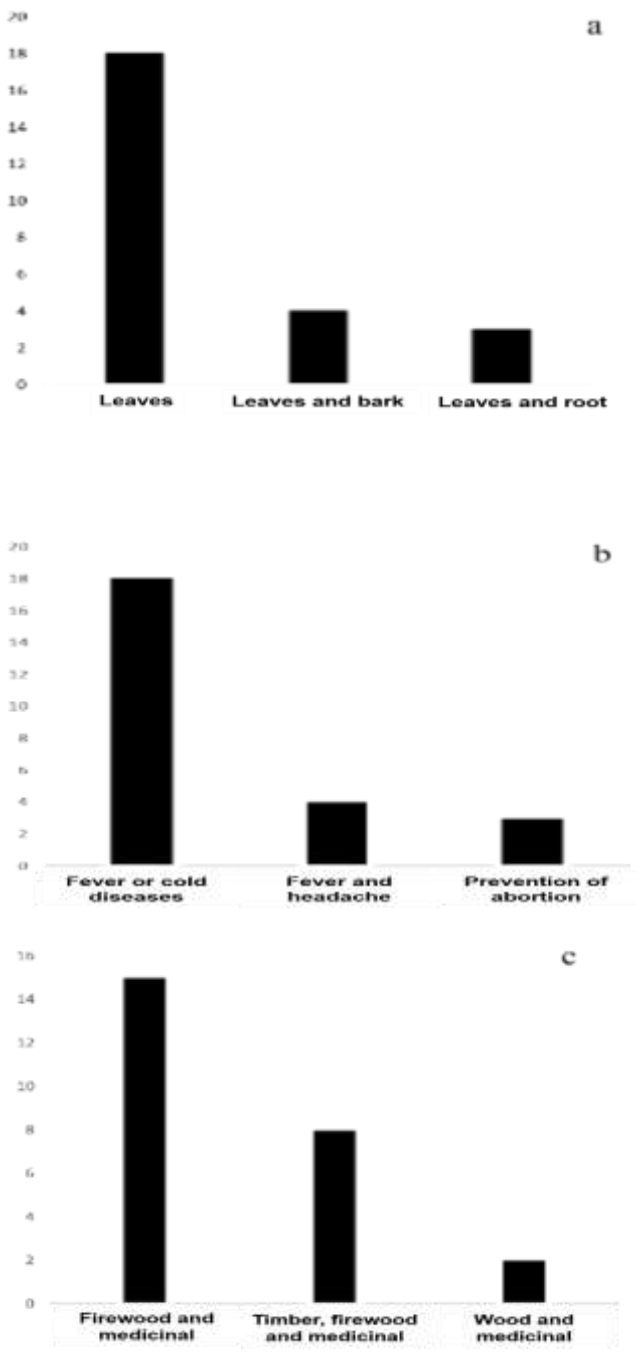

***Figure 3.** Traditional knowledge of S. humboldtiana tree. a) Part of the S. humboldtiana tree used; b) Use of S. humboldtiana in traditional medicine and c) Different types of use of S. humboldtiana.*

A short time ago, the existence of traditional knowledge was still disqualified. Exist the idea that it is only about the survival of ancient practices and is disqualified by Western scientific knowledge because it is linked to religious practices; current social changes have led to the disintegration and degradation of local knowledge systems, increasing the risk of loss due to the absence of

records of this type of knowledge. The disqualification of traditional knowledge about Western science has led to authors such as Pérez and Argueta (2011) [12], first exposed the need to legitimize, systematize, write, formalize or validate traditional knowledge, assuming that Western scientific instruments are required for this, to pass from a diffused knowledge to an objective knowledge, that is, to pass from local validity to universal validity. The contribution of ancestral knowledge has been of great importance, as it has been mentioned in the world conference on science. That traditional and local systems of knowledge, as dynamic expressions of the perception and understanding of the world, can contribute, and have done in the course of history, a valuable contribution to science and technology, and that it is necessary to preserve, protect, investigate and promote this cultural heritage and that empirical knowledge. Based on the previous argument, in this research the integration of merging western science and traditional knowledge is proposed, in this way having a way to disseminate traditional knowledge about the management and use of medicinal trees within Totonac families. At the same time, it is important to mention the importance of knowing the active ingredients, this in order to inform traditional medical about the present metabolites contained in the species, which acts to cure certain diseases, since traditional medical you are unaware of the phytochemistry of plants and their action against diseases. Much remains to be done in the research process of herbal remedies used primitively and by the current rural and indigenous populations. There is a consensus opinion that in this field, research is strong today and some attribute this level of interest to several factors that, among others, include: the need for new therapies or to normalize some existing ones and therefore, the search for new clinical agents for various conditions and the possibility of finding solutions to solve the demands of nutritional supplements or herbal remedies, duly validated.

Phytochemistry analysis and Thin Layer Chromatography (TLC). Figure 4a shows a collection of *S. humboldtiana* trees, of which only the leaves and branches (renewable source of the trees) were analyzed. Figure 4b shows four TLC chromatoplates that allow analyzing and identifying the content of secondary metabolites present in the foliar extracts of *S. humboldtiana*. The chromatoplate of Figure 4a shows the identification of flavonoids; terpenes (Figure 4b); phenols (Figure 4c) and tannins (Figure 4d).

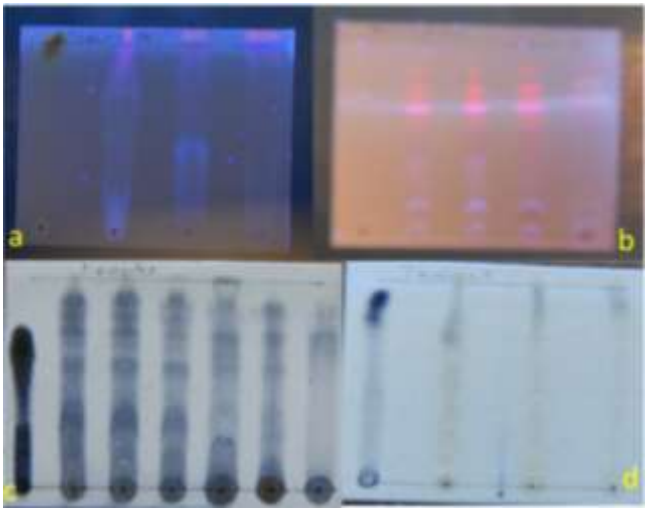

Figure 4. *Thin Layer Chromatography from extracts of S. humboldtiana (Willd.).*

Table 2 shows the results obtained from the extracts of leaves and branches of *S. humboldtiana.*

Table 2. *Phytochemical analysis of secondary metabolites of S. humboldtiana (Willd.) extracts.*

Secondary metabolites	Treatments					
	Sample 1		Sample 2		Sample 3	
	Leaves	Branches	Leaves	Branches	Leaves	Branches
Alkaloids	+	+	+	+	+	+
Tannines	+++	-	+++	-	+++	-
Saponins	-	-	-	-	-	-
Phenols	+++	+++	+++	+++	+++	+++
Flavonoids	-	+	-	++	-	+
Terpenoids	+++	-	++	-	+++	-

Concentration scale: (+) weak, (++) medium, (+++) high and (-) absence.

The presence of alkaloids was found in very low concentration, this reflects that traditional medical can continue to recommend the therapeutic use of *S. humboldtiana*, because the alkaloids in high doses, most of them are toxic. However, in low doses they have a high therapeutic value as a muscle relaxant, tranquilizer, antitussive or analgesic [13]. The presence of tannins in the leaves of *S. humboldtiana* in the study revealed a high degree of concentration, this means that the tannins in general according to Travieso (2011) [14], have demonstrated with preclinical and clinical results their activity and effectiveness as a healing, antiseptic and antioxidant mainly due to the presence of tannins. In the preliminary study of saponins, a null concentration of this secondary metabolite was found, according to the foam test that was performed on the leaf and branch simples. With respect to the analysis of the phenols, a great concentration was found in the extracts of leaves and stems, being the type of secondary metabolites present in very high concentration. In the case of flavonoids, leaf extracts showed no concentration in any of the samples. In samples 1 and 3 of branches a low concentration was found, in sample 2 a medium or moderate concentration is observed. In the group of terpenoids in leaf extracts samples 1 and 3 a high concentration was observed and in sample 2 a low concentration. In the extracts of the branches there was no presence of this metabolite. On the other hand, studies conducted by Mohd and Fehmeeda (2014), present a result *S. alba*, which showed that alkaloids, phenols, tannins, glycosides and steroids were present, while saponins were absent in the extract of the proof [15]. As in this study conducted in the *S. humboldtiana* species, no presence was found with regard to saponins in leaf and stem extracts. The results of the *S. mucronata* analysis in leaf extracts showed that the concentrations have high amounts of flavonoids, tannins, phenols, cardiac glycosides, moderate amounts of sterols and saponins, as well as small amounts of alkaloids [16]. Comparing with the study conducted in *S. humboldtiana*, in the flavonoid group in the leaf extract there was no presence of this metabolite, but if a high concentration in tannins and phenols was found, in this case no saponins were found, but there was a slight concentration of alkaloids. Another study conducted by Niemeyer, (2014), these authors found that there is a high concentration of alkaloids in *S. humboldtiana* in the native flora of Chile where the aerial part was occupied to perform the analysis [17]. El-Shazly et al. (2012) analyzed the methanolic extract of the *S. tetrasperma* (Roxb.), bark by column chromatography isolating various metabolites [18]. From the dichloromethane fraction of the leaves, catechol and tremulacin were isolated. Salicin and its derivatives were isolated from the ethyl acetate fraction of the leaves. The genus *Salix* have been used in ethnomedicine for the treatment of fever, pain, and inflammation [19]. Several species in this genus are known to contain salicin, which is a precursor of a potent nonsteroidal anti-inflammatory drug (NSAID), acetylsalicylic acid (aspirin). Previous phytochemical investigations on the genus *Salix* have led to reports on phenolic compounds, flavonoids, terpenoids, and lignans, which have been associated with biological activities including cytotoxic, neuroprotective, and antiplasmodial activities [20]. *Salix glandulosa*, also called “Korean King Willow”, is distributed in Korea, Japan, and mainland China, although several salicin derivatives and flavonoids were isolated from this plant [21]. Chung et al. (2018) as a part of an ongoing search for bioactive constituents from Korean medicinal plants, the phytochemical investigations of the twigs of *S. glandulosa* afforded 12 new phenolic glycosides [22]. On the other hand, Figure 5 shows the grouping of data with respect to the knowledge of traditional medical. The first group G1 represented by midwives-healers (PA-CUA) with two occupations at the same time, made up of 6 members, this group shares the same knowledge regarding the value of the Cultural Significance Index (CSI).

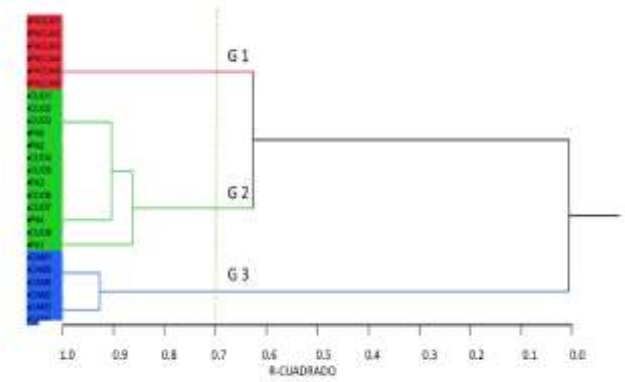

Figure 5. *Dendrogram of data clusters.*

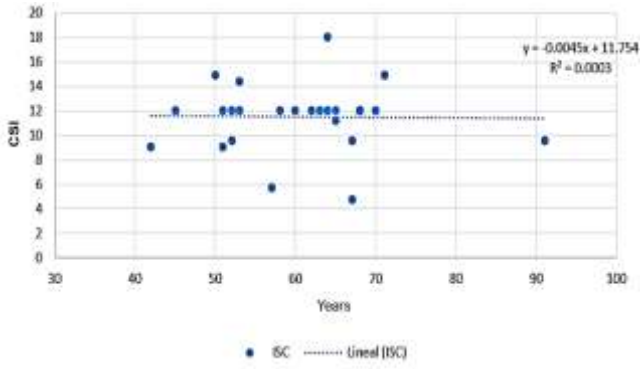

Figure 6. *Correlation graph between the CSI and the age of traditional doctors with respect to the traditional knowledge of S. humboldtiana***.**

The second group G2, of green color, grouped by healers and midwives made up of 13 traditional doctors, who share similar knowledge regarding the use, management and application of the willow tree in alternative traditional medicine. Group 3 G3, of blue color, made up of 6 farmers is the only group that does not share knowledge with respect to the two groups G1 and G2, there is no similarity, it is the group with the low value of knowledge with respect to the two groups, reflecting that they are not experts in that area of knowledge, simply dedicating themselves to the work of the field, in contrast to this, the peasants give more value to what is the use of the tree within the environment and the use of wood in its various areas, also the use of firewood as fuel.

Figure 6 shows that the people interviewed are in the age range of 42-91 years, the youngest person being a lady of 42 years and a man of 91 years, the only one with the highest age in the range of interviewed, the latter, retaining a wealth of ancestral knowledge in the use and management of plants for use in traditional medicine, taking advantage of plants and trees rationally.

The relevant thing about the result of the graph is that it is observed that there is no positive correlation, the points are not adjusted to the trend line, this means that age is not a factor that depends on the value of (CSI), in contrast to this result, the most logical thing should be that at a higher age, more knowledge, but in this study, it was not so, knowledge does not depend on age. The opinion expressed by traditional medicals is that everyone gives the significant value they think they deserve to give to plants and trees used in traditional medicine or ethnomedicine.

Conclusions

Because there are no works on the traditional knowledge of *S. humboldtiana* among the population of the municipality of Chumatlan, Veracruz, interviews with traditional doctors allowed to have extensive knowledge about the use and management of *Salix*, such as firewood, medicinal plant and timber, appreciated for its rapid growth and for the low density of wood. The value of the Index of Cultural Significance of the species evaluated in this research reflects that the midwives and the healers are those who give it a high medicinal value, however, the farmers have low knowledge regarding the use and use of Salix. S. humboldtiana, studied in this research, presents important active principles where they stand out; tannins, phenols, flavonoids and terpenoids, which can be used in the field of allopathic medicine to treat certain diseases, so the null hypothesis is

rejected. Therefore, this research is the first reference regarding the intercultural and phytochemical approach of *S. humboldtiana*. Therefore, the management and preservation of this species is important, because it is in danger of extinction.

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