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Toxicity Analysis, Phytochemical and Pharmacological Study of the Plant Known as *Mora Herb*, Collected at the Environmental Education Center of Yautlica (CEA-Yautlica)

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Abstract: The "Mora herb" is an herbaceous plant that is frequently employed in the periphery of the "Santa Catarina" mountains, Mexico. In the present research, the "Mora herb" was typified as two different species that belong to the same gender, *Solanum nigrescens* Mart. and Gal. and *Solanum cervantesii* Lag. Extracts from the aerial part of the species were obtained by direct maceration using hexane, ethyl acetate and methanol. Phytochemical and pharmacologic studies were carried out afterwards. These studies found that the species have different pharmacological and chemical patterns in the studied experimental models and therefore, determined that they might possess different medical properties, thus explaining the wide variety of therapeutic qualities that had been attributed to the "Mora herb". This indicates the need to carry out a study that revises the pharmacological properties of this plant, placing an emphasis on identifying the specie.

Key words: Mora herb, *Solanum nigrescens* Mart. and Gal., *Solanum cervantesii* Lag, toxicology assay

INTRODUCTION

The traditional medicine represents a symbol of identity in the indigenous communities worldwide and Mexico is not an exception. When someone gets sick, firstly he chooses for some sort of traditional medicine and afterwards sees a doctor. In this context, the richness and widespread use of medicinal plants in Mexico have favored, for its great diversity of herbal treatments and healing practices, a culture that trusts the value of a medicinal plant and the witch-doctors or experts in the use of medicinal plants. Some of the reasons that promote the use of medicinal plants in the Mexican culture are: The accessibility, the availability and the viability of the costs, as well as the identity of the beliefs and customs.

However, risks have been reported that come with the popular use of medicinal plants, speciallly those related with: Confusion of the species, posology and interactions with other medicines (Almaguer and Mas-Oliva, 2009). This problem has increased in recent years, from the moment the herbal medicine reached the urban zones of the country and the traditional medicine lost its control, a control that was established through a delicate process that included from the time and the place of cutting the plant, to the preparation of the treatments, linking key points of the process to a ritual, while its effectiveness is based on trial and error (Loraine and Mendoza-Espinoza, 2010).

In this context, the studies of the wild plants in the surroundings of the Sierra de Santa Catarina are an important model because the populations close to the Valle de Mexico come from the interior of the republic, a fact that makes the the cultural mixture of how to handle the medicinal plants very rich and varied. An example of this can be seen in the study realized by the investigation group of the Universidad Autónoma de la Ciudad de México (UACM) in a journey through the foothills of Yautlica, accompanied by a group of experts in traditional medicine and use of wild plants in the area. In this survey, it came to our attention that the plant Mora herb was associated to pharmacological properties that had a wide range of medicinal properties. Therefore, the principal objective of this work was to carry out a taxonomic study of the Mora herb plant and determine the presence of some representative chemical compounds from its secondary metabolism related to the pharmacological properties of the plant, such as alkaloids, anthraquinones, volatile coumarins and saponins, as well as assess

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some of the pharmacological properties which correlate with the information with the ethnobotanical information in three different polarities (hexane, ethyl acetate and methanol).

MATERIALS AND METHODS

The research started with some fieldwork, to collect plants with in order to increase the stock of the herbarium of the UACM whose responsible is Loraine Schlaepfer PhD. From all the collected species, the Mora herb plant was the most interesting because of its pharmacological properties attributed by the villagers, as well as the way of how to identify the plant, only by the shape of the fruit. From there comes the interest of realizing a study of taxonomic identification that goes hand in hand with a pharmacological and phytochemical study.

Biological material: The medicinal plant "Mora herb" was collected at CEA-Yautlica. It was recognized by the collectors, residents of the surroundings of the Sierra de Santa Catarina Iztapalapa, Mexico City. Location dates were registered with the exact coordinates using a GPS.

Identification of the biological material and preparation of the extracts: The collected material with the common name of Mora herb was taken to the laboratory and identified according to (Calderon and Rzedowski, 2005), using a stereoscopic microscope. The reference samples were deposited and herborized by the personal of the herbarium of the UACM campus Casa Libertad. Once the collected samples were identified, the biological material was dried and macerated, in order to obtain the extracts, starting with hexane. After a week of maceration, the solvent was recovered by filtering and concentrated under reduced pressure. The filtered parts of the plant were macerated once again with ethyl acetate and with methanol afterwards. Finally, the samples were refrigerated till further analysis.

Determination of the presence of alkaloids: Silica gel $60F_{254}$ plates were used with a dimension of 3×5 cm. Aliquots of the different extracts were placed on the plates for both *Solanum* species. The plates were eluted with a solvent system containing chloroform-methanol 95:05 ($CHCl$-CH_3OH). The eluted plates were revealed with the Dragendorff reagent, indicating the presence of alkaloid with the formation of red-brown spots (Espejo *et al*., 2001; Galindo *et al*., 1989; Wagner *et al*., 1973).

Detection of tannins: For this assay 0.002 mg of the obtained extract were dissolved in 10 mL of distilled water. Afterwards, the dissolution was divided into 3 test tubes and treated with: A gelatin solution (1% w/v) in test tube number 1; a gelatin-salt reagent in test tube number 2 (1 g of gelatin and 10 g of NaCl dissolved in 100 mL of distilled water); saline in test tube number 3 (10% (w/v) of NaCl). The appearance of a white precipitate in test tubes number 1 and 2 and the absence of such precipitate in test tube number 3 indicated the presence of tannins (Espejo *et al*., 2001; Galindo *et al*., 1989; Wagner *et al*., 1973).

Saponin determination: The presence of saponins in the *Solanum* extracts was determined using the phytochemical test that evaluates the levels of formed foam. 0.002 mg of the extract was placed in three different test tubes, each of them containing one of the three solvents used (hexane, ethyl acetate and methanol). The different tubes were placed in 10 mL of bidistilled water, in a bath. Afterwards, tubes were allowed to cool, stirred vigorously and left to stand for 15 to 20 min (Espejo *et al*., 2001; Galindo *et al*., 1989; Wagner *et al*., 1973). The presence of saponins was assessed by measuring the height of the foam.

Determination of the presence of free anthracene derivatives: The free anthracene derivatives were assessed using Thin Layer Chromatography (TLC). First, silica gel plates $60F_{254}$ of 3×5 cm were cut and then an aliquot of the extracts was places on the plates. The plates were eluted with the same solvents used for the determination of alkaloids, afterwards they were observed under UV were the yellow or red fluorescent spots indicated the presence of anthraquinones (Espejo *et al*., 2001; Galindo *et al*., 1989; Wagner *et al*., 1973).

Analysis of toxicity using *Artemia salina* bioassay: Cysts of *A. salina* (200 mg) were disinfested with a 1% sodium hypochlorite solution. Afterwards they were placed in a 20×20×30 cm container with 250 mL of salty water (20 g L^{-1}) at 37°C. The optimum percentage of hatching was obtained after 72 h. Ten *A. salina* were then placed in 15 mL tubes and 5 different concentrations (100.0; 10.0; 1.0; 0.1 and 0.01 μg mL^{-1}) of the *Solanum* extracts were added. Once the number of dead nauplii after 24 h was identified, the death rate was calculated using the equation (number of dead nauplii/number of all nauplii)×100. For statistical significance, the study was carried out in triplicate (Jaramillo *et al*., 2007; Skehan *et al*., 1990; Meyer *et al*., 1982).

Antimicrobial effect against *Escherichia coli*: To determine the antimicrobial effect of the plant extracts, *E. coli* bacteria was grown in agar medium in 18 Petri

dishes. Five different concentrations of the extract were prepared (2000, 1000, 100, 10, 1 ppm) and stored in vials. Then circles were cut from previously sterilized blotting paper and they were added to the vials with the extracts. Afterwards, the circles impregnated with the extracts were placed in Petri dishes, previously prepared with the bacteria in all four of its quadrants and incubated at 37°C for 24 h, if there are no signs of bacterial growing around the circles (inhibition halos), the test is considered to be positive. The inhibition halos were measured with a vernier (Rangel *et al*., 2001, Borges-Argaez *et al*., 2007; Miranda-Cruz *et al*., 2012).

Statistical analysis: The software Statdisk v 11.0.1 and Microsoft Excel 2007 were used for the statistical analysis (Banuelos-Hernandez and Mendoza-Espinoza, 2012).

RESULTS AND DISCUSSION

Botanical characterization of the biological material and local uses: In their journey through the Valle de Mexico, the group of experts in traditional medicine identified the undergrowth "Mora herb'' for its ripe fruits that have a shiny black color and a size of about 2.0 cm. It's important to note that these were the only parameters considered for the identification. The therapeutic use of Mora herb by the locals is very widespread, highlighting its use as an antibiotic, to treat boils, digestive disorders.

The taxonomic analysis of the collected samples like Mora herb revealed the confusion that exists between the experts in traditional medicine because the collected Mora herb comprises two different species from the same genus: the first, *Solanum nigrescens* Mart. and Gal., collected at the coordinates 2412 altitude, 19′54′′ latitude, 98°59′ 105′′ longitude, all at meters above sea level. The second specie, *Solanum cervantesii* Lag., was collected at 2411 altitude, 19°19′ 55′′ latitude, 98°59′ 105′′ longitude. The collected samples were deposited at the herbarium of the UACM, campus Casa Libertad and are shown in Fig. 1 and 2.

It seems that the confusion lies in the way the experts identify the Mora herb, considering only the shape, the size and the color of its fruits. The differences between these two species lie in the anatomical features of their blades, inflorescence, corolla and anthers (Fig. 1, 2). These anatomical differences can only be seen under a microscope (Calderon and Rzedowski, 2005).

This result explains why the properties associated with Mora herb are so diverse because they have a combination of pharmacological actions of *Solanum nigrescens* Mart. and Gal and *Solanum cervantesii* Lag. This finding highlights the importance of phytochemical and pharmacological studies of both species determine the properties and possible uses of the plant Mora herb species. Also, it will help to inform the locals about the positive and negative consequences of using a medicinal plant that has been identified only for the color and shape of its fruits.

General description of the plants *Solanum nigrescens* Mart. and Gal. and *Solanum cervantesii* Lag., collected as "Mora herb"

***Solanum nigrescens* Mart. and Gal. (Fig. 1):** It is a herbaceous plant that grows up to 1.5 m tall with branched stems. It possesses flowers with white or purple corolla and globose fruits that are black at maturity. It is widely distributed in the Valle de Mexico, principally in the secondary vegetation and sometimes as undergrowth and weed (Calderon and Rzedowski, 2005). Its popular name is Mora herb and it is used as a medicinal plant since the sixteenth century in infusions against erysipelas, abscesses, acne, dermatitis, eczema, rashes, wounds, leucorrhoea, sores, warts, pimples, ringworm, ulcers and vaginitis and orally in diseases such as bronchial asthma, tonsillitis, anemia, cirrhosis, colic, diarrhea, toothache, scurvy, constipation, anasarca, meningitis, nervousness, malaria, hypertension, urinary retention, arthralgia, pertussis and food uses (Boy-Rivera, 2002).

***Solanum cervantesii* Lag. (Fig. 2):** It has various common and ethnic names among which that of Mora herb. It belongs to the Solanaceae family and is described as a shrub of 1-2 m tall with velvety leaves shaped spearhead protruding on each side. It has white or lilac flowers that are grouped on a large stalk. The fruits are shiny black and oval shaped and have numerous round seeds. It is native to Mexico, inhabiting dry and mild climate between 2240 and 3000 m above sea level. It is associated with disturbed vegetation or derived from desert scrub, oak woodlands, pine, mixed pine-oak and juniper forest. It grows on fences, on the slopes, stones and any place with there is shade[1] so it is a plant that could be easily accessible in certain communities.

In Mexico, the popular use of *Solanum cervantesii* Lag. (ScL) for medicinal purposes is very broad and varied. For example, a decoction of the branches, or juice obtained from grinding the leaves, can be taken for fever, the plant can be boiled in water which is then used in baths against burns. Another medicinal use is based upon de decoction of the branches or grinding them with alcohol or vinegar, which is applied to the skin to remove grains.

Fig. 1(a-b): (a) Sample of *Solanum nigrescens* Mart. and Gal. herborized and deposited in the herbarium of the UACM and (b) *Solanum nigrescens* Mart. and Gal. the image shows the form of the anthers, the leaves and the fruit seen through a microscope in a photograph that was taken with a conventional camera

Fig. 2(a-b): (a) Sample of *Solanum cervantesii* Lag. herborized and deposited in the herbarium of the UACM and (b) *Solanum cervantesii* Lag. The image shows the form of the anthers, the leaves and the fruit seen through a microscope in a photograph that was taken with a conventional camera

Phytochemical analysis of *Solanum nigrescens* Mart. and Gal. and *Solanum cervantesii* Lag. species, collected as "Mora herb". In the phytochemical study of both species *Solanum nigrescens* and *Solanum cervantesii* Lag. some of the chemical groups that are often associated with the therapeutic properties of medicinal plants such as: alkaloids, tannins, saponins and free anthracene compounds were measured. Such compounds are some of the secondary metabolic routes of plant defense, emphasizing alkaloid content which some literature studies indicate are located in a great proportion in the Solanaceae family. This study indicated for both Solanum species that the ethyl acetate fraction is the one richest in saponins, coumarins volatile

Table 1: Table shows the phytochemical and pharmacological differences between *Solanum cervantesii* Lag. and *Solanum nigrescens* Mart. and Gal., species known as Mora herb

	Solanum cervantesii Lag.			*Solanum nigrescens* Mart. and Gal.		
Chemical groups	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
Alkaloids	+	+++	-	-	-	-
Anthraquinones	+	++	+	+	+++	+
Coumarins	+	+	-	+++	+++	+++
Tannins	-	+	-	++	+++	+
Saponins	-	+	-	-	++	-
Pharmacology						
LD_{50} *(Artemia salina)*	>100 ppm	>20 ppm	>100 ppm	>100 ppm	100 ppm	100 ppm
Microbial assay	2 mm, 2000 ppm	2 mm, 1000 ppm	-	-	-	-

-: Not observed, (+): Low, (++): Moderate, (+++): Abundant, ppm: Parts per million, LD_{50}: Lethal dose 50, Microbial assay in inhibition halo mm, concentration ppm

anthraquinones and tannins contents as compared to the other two fractions (Table 1) and that the extract of *Solanum cervantesii* Lag. contains alkaloids detected by Drangendorff technique by thin layer chromatography that are not observed in *Solanum nigrescens* Mart. and Gal. These chemical differences may cause differences in the pharmacological behavior.

Pharmacological analysis of the *Solanum nigrescens* Mart. and Gal. and *Solanum cervantesii* Lag. species, collected as "Mora herb". The therapeutic use of Mora herb by locals is extensive as described in paragraphs 1 and 2, so the selection of pharmacological studies was based on the importance of knowing the antibacterial and cytotoxic properties, these properties correlate with the treatment of infections and boils reported by the locals, thus proposing the bacterial growth inhibition assay to assess the antibacterial potential and the lethality assay for *Artemia salina* to assess the toxic potential as an indirect measurement of cytotoxicity, proposed by (Meyer *et al*., 1982). This, because in the local use, it is valid to associate compounds that have anti-boils activities with cytotoxic properties.

The toxicity study of *Artemia salina* indicated a higher toxicity in *Solanum cervantesii* Lag. with a Lethal Dose 50 (LD50) of 20 ppm for the ethyl acetate extract and higher than 100 ppm for the ethyl acetate fraction of *Solanum nigrescens* Mart. and Gal. this result makes *Solanum cervantesii* Lag. an interesting candidate for cytotoxic studies (Table 1). As for the potential of the *Solanum cervantesii* and *Solanum nigrescens* extracts to inhibit the growth of *E. coli*, potential was found only in two of the *Solanum cervantesii* extracts presenting inhibition halos of 2 mm in the order of 2000 ppm for the hexane extract and 1000 ppm for the ethyl acetate extract (Table 1), with the possibility that the purification of the extracts sheds compounds with higher activity because the concentration of the active ingredients in the mixture is diluted. The observed difference in the growth inhibitory potential between the two species of *Solanum* versus *E. coli* reaffirms the differences in pharmacology and therefore in the therapeutic differences between the species.

This difference in toxicity and in the pattern of chemical compounds indicates the need to reassess the properties attributed to the Mora herb, emphasizing the problem of confusion of species and the consequences of inappropriate use.

CONCLUSION

The use of medicinal plants in our country is steadily rising, but there are still many problems that arise from the confusion of species. In our study we found that the plant known in the community as Mora herb are two different species of the same genus *Solanum nigrescens* Mart. and Gal. and *Solanum cervantesii* Lag. Both species show a different chemical pattern and different lethality in *Artemia salina* and growth inhibition of *E. coli*, which aims at reassessing the medicinal attributes of these species, with special attention to the taxonomic classification.

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REFERENCES

Almaguer, G.J.A. and J. Mas-Oliva, 2009. Interculturalidad en Salud, Experiencia Y Aportes Para el Fortalecimiento De Los Servicios de Salud. 2nd Edn., Imprenta Universitaria, Spain, pp: 158-159.

Banuelos-Hernandez, A.E. and J.A. Mendoza-Espinoza, 2012. Study of cyclization of diphenylacetals derived from L-rhamnose and L-fucose: A theoretical approach: Research article. South Afr. J. Chem., 65: 84-90.

Borges-Argaez, R., C.I. Canche-Chay, L.M. Pena-Rodriguez, S. Said-Fernandez and G.M. Molina-Salinas, 2007. Antimicrobial activity of Diospyros anisandra. Fitoterapia, 78: 370-372.

Boy-Rivera, R., 2002. Eficacia del tratamiento topico de la tinea Pedis con *Solanum nigrescens*. Tesis de Licenciatura, Unidad de Investigacion de la Facultad de Medicina, Universidad Francisco Marroquin, Guatemala.

Calderon, R.G. and J. Rzedowski, 2005. Flora Fanerogamica del Valle de Mexico. 2nd Edn., Instituto de Ecologia, Mexico, Pages: 672.

Espejo, O., J. Ebrard, P. Hersch, A. Aguilar, A. Navarrete and M.C. Becerril , 2001. *Farmacopea herbolaria* de los estados unidos Mexicanos. Secretaria de Salud, Ejemplar No. 1339, Mexico.

Galindo, W.F., M. Rosales, E. Murgueitio and J.E. Larrahondo, 1989. Sustancias antinutricionales en las hojas de guamo, nacedero y mata raton. Livestock Res. Rural Develop., Vol. 1.

Jaramillo, B.E., J.T. Olivero and K. Munoz, 2007. Composicion quimica y toxicidad aguda (CL50) frente a Artemia salina del aceite del Croton malambo Colectado en la costa norte colombiana. Sci. Tech., 33: 299-302.

Loraine, S. and J.A. Mendoza-Espinoza, 2010. Las plantas medicinales en la lucha contra el cancer, relevancia para Mexico. Rev. Mex. Cienc. Farmaceuticas, 41: 18-27.

Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med., 45: 31-34.

Miranda-Cruz, E., J. Espinoza-Moreno, D. Centurion-Hidalgo, J.R. Velazque-Martinez and M. Alor-Chavez, 2012. Actividad antimicrobiana de extractos de L., *Psidium friedrichsthalianum*, *Pterocarpus hayesii* L., *Tynanthus guatemalensis* L. y *Spondias purpurea* L. Bol. Latinoam Caibe Plant Med. Aromat., 11: 354-361.

Rangel, D., I. Garcia, J. Velasco, D. Buitrago and E. Velazco, 2001. Actividad antimicrobiana de los extractos etanolico, acetonico y acuoso de Baccharis n?tida (Ruiz et Pavon) Pers. Rev. Fac. Farm., 42: 43-46.

Skehan, P., R. Storeng, D. Scudiero, A. Monks and J. McMahon et al., 1990. New colorimetric cytotoxicity assay for anticancerdrug screening. J. Natl. Cancer Inst., 82: 1107-1112.

Wagner, H., S. Bladt and E.M. Zgainski, 1973. Plant Drug Analysis. Springer-Verlag, Berlin, Heidelberg, New York.