Ethnobotanical study and antimicrobial activity of *Galphimia glauca* Cav.

Estudo etnobotânico e atividade antimicrobiana de *Galphimia glauca* Cav.

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**ABSTRACT**

*Galphimia glauca* (Malpighiaceae) has been widely used in traditional Mexican medicine. This study is the first to report on the root’s antimicrobial activity based on traditional knowledge from Xochipala, Guerrero. Two preliminary interviews were conducted with key informants. Final surveys were then prepared and distributed in three schools in the community. The information was organized in a hierarchical list according to importance and frequency of use to apply the Friedman index. The antibacterial and antifungal activity of the extracts were evaluated using the double serial dilution method in agar. Four main plants were reported for the treatment of skin pimples. *G. glauca* or "Tespancolole" was the most frequently used (54.9 %), followed by *Hintonia latifolia* or "Copalchi" (27.4 %), *Dorstenia drakena* or "Gallito" (0.87 %), and *Ziziphus amole* or
"Corongoro" (7.83 %). The antimicrobial effect of the methanolic and dichloromethane extracts on *Trichophyton. rubrum* and *T. menthagrophytes* was observed at a concentration of 4 µg/mL. Growth inhibition was higher when compared to miconazole, the reference drug used, which was evaluated at 8 µg/mL. This study supports the traditional knowledge of the community of Xochipala, Guerrero that uses the root of *G. glauca*, showing antimicrobial activity for the treatment of dermal lesions that may be due to infectious agents such as *Staphylococcus. aureus*, *T. rubrum*, and *T. menthagrophytes*.

**Keywords**: antifungal, traditional medicine, medicinal plants, *Trichophyton menthagrophytes*, *Trichophyton rubrum*.

**RESUMO**

*Galphimia glauca* (Malpighiaceae) tem sido amplamente utilizada na medicina tradicional mexicana. Este estudo é o primeiro a relatar a atividade antimicrobiana da raiz com base no conhecimento tradicional de Xochipala, Guerrero. Foram realizadas duas entrevistas preliminares com informantes-chave. Os inquéritos finais foram então distribuídos a três escolas da comunidade. As informações foram organizadas em uma lista hierárquica de acordo com a importância e frequência de utilização para aplicação do índice de Friedman. A atividade antibacteriana e antifúngica dos extratos foi avaliada pelo método de dupla diluição seriada em ágar. Quatro plantas principais foram relatadas para o tratamento de espinhas na pele. *G. glauca* ou “Tespancolole” foi a mais utilizada (54.9%), seguida de *Hintonia latifolia* ou “Copalchi” (27.4%), *Dorstenia drakena* ou “Gallito” (0.87%) e *Ziziphus amol* ou “Corongoro” (7.83%). O efeito antimicrobiano dos extratos metanólico e diclorometano em *Trichophyton. rubrum* e *T. menthagrophytes* foi observado na concentração de 4 µg/mL. A inibição do crescimento foi maior em comparação ao miconazol, medicamento de referência utilizado, que foi avaliado em 8 µg/mL. Este estudo corrobora o conhecimento tradicional da comunidade de Xochipala, Guerrero que utiliza a raiz de *G. glauca*, mostrando atividade antimicrobiana para o tratamento de lesões dérmicas que podem ser causadas por agentes infecciosos como *Staphylococcus aureus*, *T. rubrum* e *T. Menthagrophytes*.

**Palavras-chave**: antifúngico, medicina tradicional, plantas medicinais, *Trichophyton menthagrophytes*, *Trichophyton rubrum*.

**RESUMEN**

*Galphimia glauca* (Malpighiaceae) ha sido ampliamente utilizada en la medicina tradicional mexicana. Este estudio es el primero en informar sobre la actividad antibacteriana y antifúngica de la raíz basado en el conocimiento tradicional de Xochipala, Guerrero. Se realizaron dos entrevistas preliminares con informantes clave. Posteriormente se distribuyeron encuestas finales en tres escuelas de la comunidad. La información se organizó en una lista jerárquica según importancia y frecuencia de uso para aplicar el índice de Friedman. La actividad antibacteriana y antifúngica de los extractos se evaluó mediante el método de doble dilución seriada en ágar. Se informaron cuatro plantas principales para el tratamiento de las espinillas de la piel. *G. glauca* o “Tespancolole” fue la más utilizada (54.9 %), seguida de *Hintonia latifolia* o “Copalchi” (27.4 %), *Dorstenia drakena* o “Gallito” (0.87 %) y *Ziziphus amol* o “Corongoro” (7.83 %). El efecto antimicrobiano de los extractos metanolítico y diclorometano sobre *Trichophyton. rubrum* y *T. menthagrophytes* se observó a una concentración de 4 µg/mL. La inhibición del crecimiento fue mayor en comparación con el miconazol, fármaco de referencia utilizado,
que fue evaluado en 8 µg/mL. Este estudio respalda el conocimiento tradicional de la comunidad de Xochipala, Guerrero que utiliza la raíz de *G. glauca*, mostrando actividad antimicrobiana para el tratamiento de lesiones dérmicas que pueden deberse a agentes infecciosos como *Staphylococcus aureus*, *T. rubrum* y *T. menthagrophytes*.

**Palabras clave:** antifúngico, medicina tradicional, plantas medicinales, *Trichophyton menthagrophytes*, *Trichophyton rubrum*.

### 1 INTRODUCTION

Medicinal plants have always been an important resource for humanity. Around 70-95% of people worldwide use medicinal plants to meet their health needs (Robinson and Zhang, 2011). In Latin America, especially among indigenous populations, the use of medicinal plants is deeply rooted as it is component of health care and an important element in rituals within indigenous cultures (Montenegro and Stephens, 2006). In Mexico, a country recognized for its high biodiversity, it is estimated that there are around 3,000 to 4,500 plants with medicinal uses (CONABIO, 2020). The utilization of plant extracts in developing countries is based not only on traditional herbal knowledge but also on a growing body of preclinical and clinical research. The elderly population of Xochipala has a deep herbal knowledge. Xochipala community is located in the state of Guerrero, Mexico, and belongs to the municipality of Eduardo Neri. It has a territorial extent of 1,289 km² and is characterized by an average annual temperature of 25 °C with 684 mm of rainfall.

There are two main types of vegetation in the region surrounding Xochipala, including tropical deciduous forest and *Quercus* forest, with trees reaching heights of 10 to 15 m (Rzedowski, 1978; Jiménez *et al.* 2003). The total number of inhabitants is 3,989, mostly children from 0 to 14 years old. Economic activities include agriculture, mainly maize production and livestock (INEGI, 2020). The community has an outpatient unit and private medical offices (INEGI, 2020). However, patients also go to nearby cities such as Iguala or Chilpancingo for medical attention (Barrera and Chino, 2001). The most common medical conditions reported include dermatological, gastrointestinal, respiratory infections, malnutrition, and dengue.

The deep herbal knowledge is no longer shared by younger generations. González (2012) reported 77 species with medicinal uses grouped in 35 botanical families, including *Galphimia glauca*.
**Galphimia glauca** (Malpighiaceae), known locally as “calderona amarilla” or “arnica roja”, is a 1 to 3 m high shrub (Figure 1). The leaves are ovate or elongated, green above and bluish green below. The yellow flowers appear in clusters (Biblioteca de la Medicina Tradicional Mexicana, 2009).

![Figure 1: Galphimia glauca](image)

Figure 1: *Galphimia glauca*

Photography Margarita Aviles

Previous studies showed the effectiveness of *G. glauca* for the treatment of anxiety and depression (Guzmán-Gutiérrez *et al.* 2005; Herrera-Arellano, 2007; Sharma *et al.* 2012). *G. glauca* main component with sedative and relaxant properties is β-galphimine. Dorsch *et al.* (1992) reported the use of the methanolic extract for the treatment of bronchial reactions to allergens and as an anti-asthmatic agent. *G. glauca* has also exhibited anti-inflammatory effects (Sharma *et al.* 2012).

In Xochipala, Gómez (1981) reported four plants with hypoglycemic properties, including *G. glauca*. The root, bark and flowers are used to treat pimples on the penis and vagina. The treatment is carried out by applying vaginal washes with the boiled root. The washes with boiled root are also used to treat wounds caused by childbirth (Flores, 1990).

Ethnobotanical research was carried out in the community of Xochipala, Guerrero to gather information on the medicinal use of *G. glauca* as well as to determine the plants used in the community for the treatment of infectious diseases of the reproductive system (skin pimples). The antimicrobial activity of crude extracts of the root was also evaluated.
2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The root of *G. glauca* was collected in April in the archaeological zone "La Organera" (17° 47' 478" N; 99° 37' 877" W). The material was dried in a place at room temperature and kept in a box to avoid degradation of the photosensitive compounds. Specimens were prepared and deposited at this institution under the code (registration number 0001782), Faculty of Sciences (FCME), UNAM.

Plant roots were shade-dried at room temperature, powdered material was extracted sequentially using Soxhlet equipment with polarity gradient solvents such as n-hexane, dichloromethane, and methanol (40 g per 1000 mL) each for 8 hours at room temperature were performed. The extracts were evaporated under reduced pressure using a rotary evaporator (BUCHI B490). Finally, the resulting crude extracts were stored at -20 °C until they were tested.

For the aqueous extract, 40 g of dried and grounded root was added to 1 L of water for 10 hours, protected from light at room temperature. The resulting liquid was filtered using a funnel, a Kitasato flask, and cotton. A total volume of 800 ml filtrate was obtained, which was distributed into six ball flasks and frozen with dry ice. The frozen solution was lyophilized and the aqueous extract was stored at 4 °C.

2.2 ETHNOBOTANIC METHOD.

For the collection of ethnomedicinal information, we followed the approach proposed by Gispert *et al.* (1971). Two preliminary interviews were conducted with key informants. Final surveys were then prepared and distributed in three schools in the community. Home visits were conducted to clarify any ambiguous information in the answers. A database was prepared for data analysis and the preparation of graphs.

The informants were asked to describe the uses and names of plants used for the healing of pimples and skin wounds. The information was organized in a hierarchical list according to importance and frequency of use to apply the Friedman index (Friedman *et al.* 1986). This index indicates the potentially most effective plant species. The Friedman index is expressed from 0 to 100, with a high value indicating the high effectiveness of the plant. The Friedman index is computed as follows:
\[ FL = (I_p / I_t) \times 100 \]

Where:

- \( I_p \) is the number of informants mentioning a species with medicinal use (frequency of mention),
- and \( I_t \) is the total number of informants (Friedman et al. 1986).

2.3 ANTIMICROBIAL ACTIVITY

The microorganisms used for the antimicrobial evaluation from the American Type Culture Collection (ATCC, Rockville, MD) were obtained from the microbiology laboratory of the Centro de Investigación Biomédica del Sur CIBIS, IMSS (Xochitepec, Morelos). The bacterial strains used were *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 43071), and *Salmonella Typhi* (ATCC 06539). The fungal strains were *Candida albicans* (ATCC 10231), *Trichophyton mentagrophytes* (ATCC 28185), and *Trichophyton rubrum* (ATCC 28188). The antibacterial and antifungal activity were evaluated using the double serial dilution method in agar suggested by Rojas and collaborators (2001).

The hexanic, dichloromethane, methanolic and water extracts were individually dissolved in 2% dimethylsulfoxide (DMSO Merck). Further two-fold dilutions of each extract were performed using sterile distilled water and were added to melted agar culture medium in 100 mm x 15 mm Petri dishes (Falcon) at the final concentration of 4 and 8µg/mL. Agar culture medium and DMSO 2% were used as negative controls.

2.4 ANTIBACTERIAL ACTIVITY

Bacterial suspensions for each bacterium were prepared by transferring five or six colonies, chosen after overnight growth on Tryptic Soy agar, to 5 mL of Mueller-Hinton broth (MHB Merck). Cultures were incubated at 36 °C until they were visibly turbid, and the suspensions were then diluted until the turbidity matched the 0.5 McFarland standard turbidity equivalents (108 colony-forming units (CFU)/mL). Microbial suspensions were further diluted 1:10 to obtain a concentration of 10⁷ CFU/mL. The diluted inoculum of each bacterium was applied with a loop calibrated to deliver 0.002 mL, resulting in a
spot covering a circle of 5 to 8 mm diameter and containing $10^4$ CFU/mL. The plates were incubated for 24 h at 36 °C. Gentamicyn (Sigma) was used as reference standard. Observations were performed in duplicate and results expressed as the lowest concentration of plant extract that produced a complete suppression of colony growth, the minimal inhibitory concentration (MIC).

2.5 ANTIFUNGAL ACTIVITY

*C. albicans* inoculum was prepared by diluting the yeast suspension (adjusted to McFarland 0.5 scale) in 0.85% NaCl solution to a final concentration of 105 cell/mL. The filamentous fungi inoculums were prepared by diluting the scraped cells mass in 0.85% NaCl to a final concentration of 106 spore/mL. The diluted inoculums were delivered on the top of the solidified agar with a loop calibrated to deliver 0.005 mL. The plates were incubated at 29°C. Fungal growth was checked both in control plates prepared without any test sample and in experimental plates; the time at which fungal growth was checked was either 24, 48, or 72 h, depending on the incubation time required for a visible growth; 24 h for *C. albicans*, and 72 h for the dermatophytes. Experiments were duplicated and results expressed as MIC. Positive controls were prepared with Myconazol (Sigma) and Nystatin (Merck).

3 RESULTS

A total of 126 interviews of the inhabitants of Xochipala, Guerrero were analyzed. The best-known names for *Galphimia glauca* include “tespancolole” (51.1 %), “flor de estrella” (34.4 %) and “zompancolole” (14.4 %).

The most used parts of the plant are the root and its epidermis (Figure 2), which are used to treat vaginal pimples, pimples with pus, and pimples caused by heat, soil and/or dirty water in any part of the body (Figure 3).
Figure 2: Parts used of *G. glauca* in Xochipala, México

![Graph showing parts used of G. glauca](image)

**Parts of the plant used**

Source: Authors

Figure 3: Main *Galphimia glauca*’s dermal uses reported. VP: vaginal pimples, PP: penile pimples, B: waders’ bites, H/S/D: pimples caused by heat, soil, and/or dirty water, MP: mouth pimples, M: mites, BHP: pimples in babies’ heads, and GwP: pimples with pus.

![Graph showing uses of Galphimia glauca](image)

Source: Authors

Four main plants were reported for the treatment of skin pimples. *G. glauca* or "tespancolole" was the most frequently used (54.9 %), followed by *Hintonia latifolia* or "copalchi" (27.4 %), *Dorstenia drakena* or "gallito" (0.87 %), and *Ziziphus amole* or "corongoro" (7.83 %).

The antifungal effect of the methanolic and dichloromethane extracts on *T. rubrum* and *T. mentagrophytes* was observed at a concentration of 4 µg/mL (Table 1). Growth inhibition was higher when compared to miconazole, the reference drug used, which was evaluated at 8 µg/mL. The minimum inhibitory concentration (MIC) in the other microorganisms was 8 µg/mL or higher in all evaluated extracts compared to the broad-spectrum antibiotic gentamicin (5 µg/mL).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Minimum inhibitory concentration (MIC) in µg/mL</th>
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<tr>
<td></td>
<td><strong>Bacteria</strong></td>
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<td>G</td>
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<tr>
<td>MC</td>
<td>NA</td>
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Source: Authors

4 DISCUSSION

Based on the conducted interviews, *G. glauca* is the most effective plant, followed by *Hintonia latiflora* (*Rubiaceae*), for the treatment of skin diseases and/or skin lesions in the community of Xochipala, Guerrero.

*G. glauca* is mainly known as "Tespancolole", with the root being the most used part of the plant to treat pimples or dermal vaginal eruptions, which may be caused by sexually transmitted diseases.

However, it is difficult to identify the causative agent as skin symptoms can be caused by bacteria, viruses, protozoa, or parasites. Currently, more than 30 pathogens are known to cause about 50 clinical symptoms on the skin (Domingo, 2017). The different dermal lesions for which this plant is used can be caused by factors such as heat, soil, and water, as well as mites (*Trombiculidae* family) and bacterial and/or fungal infections.

This study is the first report on the antimicrobial activity of *G. glauca* root extracts with promising antifungal properties against *T. rubrum* and *T. mentagrophytes*. Fungal skin infections refer to those caused by dermatophytes. Dermatophytes are fungi with the particularity of developing in the keratin, the protein making up stratum corneum, hair, nails and/or mucous membranes (Sánchez-Saldaña *et al.* 2009). Some dermatophytes belong to the genus *Trichophyton*, specifically *T. rubrum* and *T. mentagrophytes*, which
cause athlete's foot and onychomycosis, conditions that are difficult to treat (Brillowska-Dabrowska et al. 2007; Rezusta et al. 1990; Romero et al. 2009). Topical treatment may fail due to a lack of treatment compliance, the presence of other dermatological conditions (psoriasis, contact eczema, etc.) or concomitant bacterial infections, and/or because topical treatment is not possible (Molina, 2011).

The antifungal activity of G. glauca has been reported using the aerial parts of the plant. The hexane extract showed activity for T. rubrum and T. mentagrophytes at a minimum concentration of 4.0 and 8.0 mg/mL, respectively (Navarro et al. 2003), while in the present study, the MIC was observed at 4µg/mL. This discrepancy may be due to the different collection sites since metabolites and their quantities vary according to the place where the plant is collected (García-Granados et al. 2019), as well as the collection date and the plant's reproductive stage at the time of collection, another factor may be the part of the plant use (Sepúlveda-Vázquez et al. 2018). Navarro and collaborators (2003) used aerial parts, whereas the root was used in the present study. The plants used in both studies were subjected to different environmental factors, such as temperature, precipitation, competition for nutrients, and predation, therefore, the type and number of secondary metabolites may vary (Sépulveda-Jímenez et al. 2003). In each organ, tissue, or cell type of the plant, specific synthesis of secondary metabolites can take place. Also, there are metabolites that are synthesized in all organs and tissues of the plant but are stored in organs or tissues different from the place where they were synthetized (Ojito and Portal, 2017).

The effect of the dichloromethanolic and methanolic extracts, which are extracted using medium and high polarity solvents, was observed on T. rubrum and T. mentagrophytes. Cetto (1995) reported the presence of flavonoids in the methanolic extract of the root of G. glauca by colorimetric tests.

According to the literature, flavonoids have anti-inflammatory, antimicrobial, antiallergic, antitumor, anticancer, and antioxidant properties (Escamilla et al. 2009). Some of the flavonoids reported in G. glauca are quercetin, kaempferol, and beta-sitosterol (Prieto and Monturiol, 2016), which could be the cause of the antimicrobial effect.

Bacterial skin infections located in the epidermis, dermis and/or subcutaneous cellular tissue are related to a broad group of clinical conditions of diverse etiology, pathogenesis, and prognosis (Sánchez-Saldaña and Sáenz-Anduaga, 2006). S. aureus growth, associated with pathogenic situations such as pimples, boils, scalded skin
syndrome and pustules, is responsible for 30-90% of wound infections (Madigan, 2001; Mantilla et al. 2009). The hexanic, methanolic, and aqueous extracts of G. glauca root showed activity against S. aureus at an MIC of 8 µg/mL.

5 CONCLUSION

This study supports the traditional knowledge of the community of Xochipala, Guerrero that uses the root of G. glauca, showing antimicrobial activity for the treatment of dermal lesions that may be due to infectious agents such as S. aureus, T. rubrum, and T. mentagrophytes. The doors are open for new research to characterize and quantify the secondary metabolites in the root as well as the antiviral effect of G. glauca on sexually transmitted diseases such as herpes type I and II, which have a prevalence of 18.2% to 29.8% in Mexico (Gayet, 2015). Based on the present research, we can highlight the promising pharmacological use of the root of G. glauca for the treatment of athlete’s foot and onychomycosis caused by T. rubrum and T. mentagrophytes.

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