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Antibacterial Activity of Traditional Medicinal Plants Used for the Treatment of Acute Diarrheal Diseases in Chiapas, Mexico

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Abstract: The *Enterobacteriaceae* family poses health risks due to its role in gastrointestinal diseases like acute diarrhea. With rising antibiotic resistance, plants offer promising antibacterial compounds with low toxicity. This study evaluated the antibacterial activity, minimum inhibitory concentration (MIC), and toxicity of ethanolic (EE) and aqueous (AE) extracts from five Mexican medicinal plants traditionally used in Chiapas for treating acute diarrheal diseases (ADD). Antibacterial activity was assessed using disk diffusion assays and MIC determined by macrodilution. Toxicity tests were performed using *Artemia salina*. As a result, EE extracts exhibited higher antibacterial activity than AE extracts. *Byrsonima crassifolia* effectively inhibited *Salmonella enteritidis* (78.26%, MIC 50 mg/mL) and *Shigella dysenteriae* (76.19%, MIC 25 mg/mL). *Solanum torvum* showed efficacy against *Escherichia coli* (55.55%, MIC 12.5 mg/mL) and *Salmonella enteritidis* (73.91%, MIC 25 mg/mL). *Euphorbia maculata* inhibited *Shigella dysenteriae* (104.76%, MIC 25 mg/mL), while *Guazuma ulmifolia* and *Bursera simaruba* exhibited no antibacterial effects. All extracts were non-toxic (LD_{50} > 1000 µg/mL), indicating potential as natural alternatives for ADD treatment.

Keywords: acute diarrhea; ethnobotany; antibacterial activity; antibiogram; *Solanum torvum*; *Guazuma ulmifolia*; *Byrsonima crassifolia*; *Bursera simaruba*; *Euphorbia maculata*

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1. Introduction

Acute infectious diseases still occur in developing countries, predominantly affecting the most vulnerable social and demographic groups such as children under five years old and the elderly [1]. The bacteria of the family *Enterobacteriaceae* are a global health problem because they cause gastrointestinal diseases such as acute diarrhea [2]. Acute diarrhea is one of the most common diseases and the second greatest cause of morbidity and mortality worldwide in developing countries, causing almost two million deaths each year in children

under five [3]. Diarrhea needs to be classified according to the trends over time (acute or chronic, using a limit of 4 weeks to separate the two conditions) and to the characteristics of the stools (watery, fatty, inflammatory) [4]. The duration of diarrhea is important; this is because acute forms are usually due to some infectious agent, intoxication, or food allergy [5]. There are several signs and symptoms associated with infectious diarrhea depending on the bacterial species and age of the patient, such as frequent loose (watery) stools, abdominal cramps, bloating, abdominal pain and fever, bleeding from the rectum, blood in the stool, and light headache/dizziness from dehydration [5,6]. Among the main agents causing acute diarrhea are viruses, bacteria, and parasites/protozoa [7].

Bacterial etiology occurs in 1.5% to 5.6% of cases. The most frequently identified bacteria are *Campylobacter* (2.3%), *Salmonella* (1.8%), *Shigella* (1.1%), or *Escherichia coli* (0.4%). A study from the U.S. reported that *Salmonella*, *Campylobacter*, *Shigella*, and *Escherichia coli* O157:H7 (16.1, 13.4, 10.3, and 1.7 cases per 100,000 persons, respectively) were the pathogens most frequently associated with diarrhea [8]. Symptoms such as fever and bloody diarrhea are due to the presence of *Shigella* spp., *Salmonella* spp., *Campylobacter jejuni*, and *Clostridium difficile* [5,9]. Over the past 15 years, many pathogenic bacteria have exhibited resistance to commercial antimicrobials (antibiotics) [10]. The World Health Organization (WHO) global priority list of pathogens ranked these diarrhea-causing pathogens in the highest priority category (i.e., critical) that require development of novel antibiotics to combat their related infections [11–13]. According to Centers for Disease Control and Prevention's (CDC) Antibiotic Resistance Threats in the United States (AR Threats Report), it is shown that over 2.8 million cases and over 35,000 deaths occur each year due to antibiotic-resistant infections [14]. Moreover, a report showed that diarrheal diseases have caused over 1.4 million deaths every year attributable to antimicrobial resistance compared to other major causes of death [15]. In recent years, natural alternatives to antibiotics have been sought to reduce microbial resistance to commercial antibiotics [16]. Plants are a natural source of bioactive and low toxicity secondary metabolites with potential antibacterial activity [17].

Medicinal plants represent the most ancient form of medication, used for thousands of years in traditional medicine in many countries around the world. Empirical knowledge about their beneficial effects has been transferred from generation to generation within human communities [18]. Traditional medicine in Mexico is deeply rooted in herbalism, relying on the use of medicinal plants as the most accessible and effective form of healthcare for the general population and local communities [19]. In this context, remedies are often prepared using simple and effective methods such as aqueous decoctions and ethanol-based macerations, which are widely valued for their accessibility, low-cost, and efficacy.

Mexico has a 10% of the total global flora. Its southeast region possesses great bio-cultural wealth, being the richest region of the country, with species of vascular and nonvascular, wild and domesticated, and native and exotic plants employed for medicinal purposes [20,21]. The state of Chiapas is one of the most biologically diverse and biocul-turally rich areas in the south of the country [21]. It possesses unique vegetation, such as the Lacandon jungle, where many ethnic groups still reside, such as Choles, Chujes, Lacandones, Mames, Mochos, Jacaltecos, Tzeltales, Tojolabales, Tzotziles, and Zoques [22]. It has been through ethnographies, dictionaries, vocabularies, and project reports that ethnobotanical information of those ethnic groups has been documented, thus maintaining their traditions and folklore along with their ancient herblore [21,23].

Tuxtla Gutiérrez and its valley are home to the Mactumatzá reserve or the ecological center "El Zapotal" and, within its borders, is the "Cañón del Sumidero" National Park. The current city is in Zoque territory and is the capital of the state of Chiapas [24]. Plants are sources of bioactive phytochemicals known as secondary metabolites, and are used in

the medicinal, environmental, and food sectors as well as being widely used in commercial and pharmaceutical products [25,26]. Based on the traditional use of medicinal plants in Tuxtla Gutiérrez, Chiapas, and the reported antimicrobial potential of their extracts, we hypothesize that the ethanolic and aqueous extracts of these plants exhibit antibacterial activity against gastrointestinal pathogens. To test this hypothesis, the study aimed to (1) evaluate the antibacterial activity of ethanolic and aqueous extracts from five medicinal plants (*Solanum torvum, Guazuma ulmifolia, Byrsonima crassifolia, Bursera simaruba*, and *Euphorbia maculata*) frequently used for the treatment of acute diarrheal diseases (ADD), (2) determine the minimum inhibitory concentrations (MIC), and (3) assess the toxicity of the extracts using the Artemia salina model.

2. Materials and Methods

2.1. Ethnobotanical Compilation

To rescue the popular knowledge of medicinal plants and identify the main plant species used in the treatment of diarrheal diseases in Tuxtla Gutiérrez, Chiapas (16° 45′ 11″ N, 93° 06′ 56″ O) and their preparation methods, semi-structured and semi-directed interviews were conducted with 200 people over 18 years old in a simple random manner. The questions of this instrument were not restrictive since the technique used was the semi-open interview. To determine the level of shared knowledge and the uniqueness of this knowledge, the Smith's salience index [27,28] was determined with the Anthropac® program using Equation (1).

$$S = \frac{\sum\left(\frac{nL-nj+1}{nL}\right)}{n} \quad (1)$$

where S is the Smith's salience index, nL is the number of concepts in the list, nj is the position of appearance of concept j within the list, and n is the total number of informants. S scores vary between 0 (no salience) and 1 (full salience), $S \geq 0.5$ indicating cultural importance recognized by all participants. All participants who took part in the survey agreed to the Ethical Clearance and Consent Form for the interview (Figure S1).

2.2. Collection, Treatment of Plant Material and Bacteria

The fresh mature leaves of the plant species used were collected from different places in Tuxtla Gutiérrez, Chiapas (Figure 1); *Solanum torvum* (*ST*) (16° 44′ 41″ N, 93° 03′ 57″ W), *Guazuma ulmifolia* (*GU*) (16° 44′ 38″ N, 93° 04′ 20″ W), *Byrsonima crassifolia* (*BC*) (16° 45′ 35″ N, 93° 05′ 20″ W), *Bursera simaruba* (*BS*) (16° 45′ 08″ N, 93° 05′ 02″ W), and *Euphorbia maculata* (*EM*) (16° 45′ 30″ N, 93° 06′ 12″ W). The mature leaves were collected in January, during the winter season, from 6:00 am to 7:30 am (Table S1). This time frame was chosen to ensure optimal environmental conditions for the preservation of the bioactive compounds. Mature leaves were specifically selected based on visual criteria, including size, uniform color, and the absence of visible damage or disease. All samples collected were cleaned with distilled water, and diseased, stained, or dirty leaves were discarded. Subsequently, the leaves were shade-dried for 5 days at room temperature. The leaves were considered dry when the touch coincides with level three on the dry scale (Table 1) reported by Banchero et al. [29].

The bacterial cultures were obtained from the State Public Health Laboratory of Chiapas (16° 45′ 26″ N, 93° 05′ 03″ W) for use in the present study and these included *Escherichia coli* ATCC-35218 (*EC*), *Salmonella enteritidis* DE-09950 (C.C. ENT-13) (*SE*), and *Shigella dysenteriae* InDRE-LEM-05065 (*SD*).

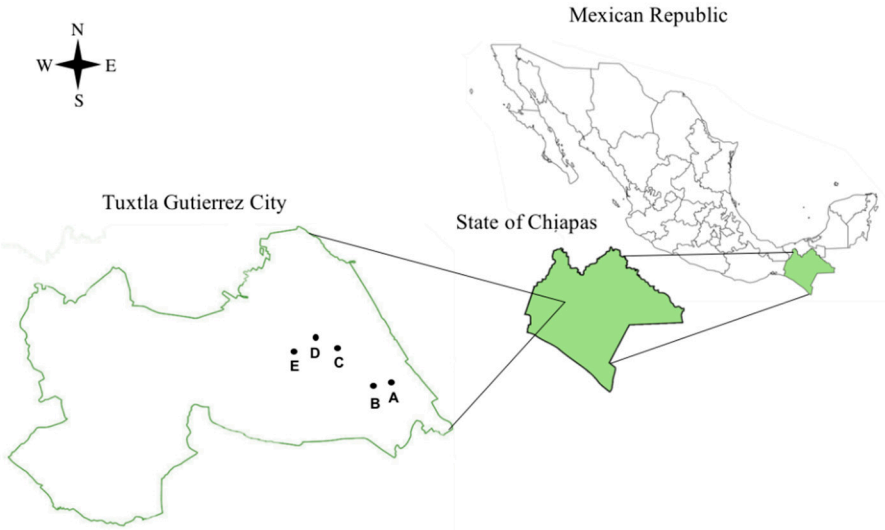
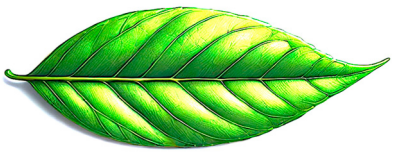
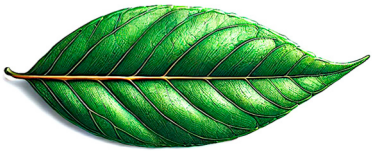
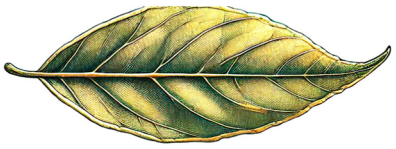


Figure 1. Location of the different collection places for plant species from Tuxtla Gutierrez, Chiapas, Mexico. (A) *Solanum torvum* (ST); (B) *Guazuma ulmifolia* (GU); (C) *Byrsonima crassifolia* (BC); (D) *Bursera simaruba* (BS); (E) *Euphorbia maculata* (EM).

Table 1. Dry scale for leaf characteristics.

Level	Leaves Characteristic	Description	Figure Representation
One	Orear and soft	Leaves still retain significant moisture, showing flexibility to the touch. When bent, they do not show signs of breaking and return to their original shape, like freshly collected leaves that have started to lose a bit of moisture.	
Two	Dry and soft	Leaves have lost most of their moisture and feel dry to the touch, but still maintain some flexibility. If bent, they do not break, although they no longer completely return to their original shape as in level one.	
Three	Dry and semi-brittle	Leaves are considered fully dry at this level. They feel rigid, and when pressure is applied, they begin to crack or break, indicating that dehydration has reached a point where internal structures no longer retain flexibility.	

2.3. Preparation of Ethanolic and Aqueous Extracts

Ethanol and water were selected as extraction solvents based on their alignment with traditional preparation methods of medicinal plants in Mexico. The plant material used for extractions consisted of 100 g of dried powdered leaves (level three) per 700 mL of solvent. The ethanolic extracts (EE) were prepared according to the method reported by Eve et al. [7] with minor modifications. Briefly, extraction was conducted by maceration of the crushed samples in 96% ethanol in amber glass for 5 days at room temperature. Extraction was performed in a magnetic thermo-shaker (Felisa brand, model FE-3111). The extract was filtered using Whatman N°5 (particle retention of 2.5 µm) filter paper (Whatman International Ltd., Maidstone, England) and concentrated in a rotary evaporator (BUCHI brand, model R-300). The aqueous extracts (AE) were prepared according to reported by Torres-Chati et al. [30] with minor modifications. Briefly, the crushed samples were treated

with distilled water at 75 °C for 30 min. Then, the solution was filtered with Whatman Nº5 filter paper (Whatman International Ltd., England), thus obtaining residue-free filtrate; subsequently, it was dried at room temperature for 48 h. All the EE and AE obtained were stored in amber glass vials with airtight lids at 4 °C, protected from direct exposure to air and light until further analysis. The extraction efficiency of all extracts (aqueous and ethanolic) was calculated using Equation (2), where E is the extraction efficiency (%), W_F is the final dry weight of extract, and W_i is initial dry weight of plant material.

$$E(\%) = \left(\frac{W_F}{W_i}\right) \times 100 \quad (2)$$

All extractions were performed in triplicate, and the extraction yields were calculated for each batch independently. The mean and standard deviation (SD) of the extraction efficiency were reported to demonstrate consistency across replicates. Additionally, the extraction process followed standardized protocols [7,30] to minimize variability, including maintaining constant parameters such as temperature, solvent-to-sample ratio, and extraction time.

2.4. Antibacterial Assay

The antibacterial assay was conducted according to the guidelines set by the Clinical and Laboratory Standards Institute described in M100-Ed31 [31] with modifications. Briefly, the trial colonies were taken from a 24 h bacterial cultures on Brain Heart Infusion (BHI) agar. All plates were inoculated with the trial bacteria previously adjusted to 0.5 McFarland standard solution. The surface of the freshly prepared Müller–Hinton Agar plates was inoculated uniformly on the entire surface using sterile cotton swabs. The crude extract was diluted in dimethyl sulfoxide (DMSO) at 5% (p/v) up to 200 mg/mL concentration and was filtered through membranes (Millipore 0.22 µm). Disk impregnated with 10 µL of the crude extract (2 mg) were placed on the previously inoculated Müller–Hinton Agar plates surface. After incubation at 37 °C for 24 h, the measurement of the inhibition halos (mm) was made with a vernier, and percent inhibition was determined using Equation (3). Gentamicin (80 mg/mL) was used as a positive control, and 5% DMSO as negative control. All tests were performed in triplicate.

$$(\%) = \left(\frac{D_E - D_W}{D_C - D_W}\right) \times 100 \quad (3)$$

where I is the inhibition (%), D_E is the extract halo diameter, D_W is the white halo diameter (using DMSO), and D_C is the positive control halo diameter (using antibiotic).

2.5. Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was determined following the macrodilution technique reported by Gregorio-Malbrán [32], with some modifications. Briefly, a stock solution of 200 mg/mL was prepared for each crude extract exhibiting antimicrobial activity. Serial dilutions were performed to create a decreasing concentration gradient of 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL in BHI broth. Each tube containing the diluted extract was inoculated with 1 mL of a bacterial suspension, previously adjusted to 0.5 McFarland standard (1.5×10^8 CFU/mL) using McFarland densitometer (DEN-1B, BIOSAN, Riga, Latvia), as described by Lopardo et al. [33]. The series of tubes were incubated at 35 °C for 24 h under aerobic conditions. Following incubation, bacterial growth was assessed visually by observing turbidity. To verify the complete inhibition of bacterial growth, a sample from the tube showing no visible turbidity was plated onto Blood Agar Base (BAB) and incubated under the same conditions. The MIC was defined as the lowest concentration

of the extract that completely inhibited visible bacterial growth. All tests were performed in triplicate.

2.6. Lethality Bioassay on Artemia Salina

Artemia salina (*AS*) cysts (dormant eggs) were obtained by commercial sale (16° 45′ 08″ N, 93° 06′ 48″ W), and they were hatched for 24 h in synthetic seawater. Synthetic seawater was prepared using reagent grade chemicals as reported by Environmental Protection Agency [34] and subjected to an aeration system for 24 h to achieve the equilibrium of O_2 and CO_2. The median lethal dose (or LD_{50}) was determined according to the methodology reported by Paixao et al. [35] with modifications. Briefly, six mixtures of the crude extract dissolved with seawater were prepared in different concentrations (1000, 500, 100, 10, 5 and 1 mg/L). Synthetic seawater was prepared according to the formula of Dietrich and Kalle [36]. The positive control was $K_2Cr_2O_7$ (5%) and the negative control was synthetic seawater. Subsequently, the number of living and dead organisms was counted to determine the lethal dose at 50% (LD_{50}). All tests were performed in triplicate. The toxicity criterion used was that described in [37–39]: LD_{50} > 1000 µg/mL (non-toxic), 500–1000 µg/mL (moderate toxicity), and <500 µg/mL (toxic).

2.7. Statistical Analysis

The extraction yield was evaluated with a two-way analysis of variance (ANOVA), considering the type of extract (aqueous or ethanolic) and the plant species as factors, followed by Tukey's post hoc test to determine significant differences ($\alpha = 0.05$). The antibacterial activity data, including inhibition zone diameters, were analyzed using a one-way ANOVA, followed by Dunnett's post hoc test to compare the extract treatments against the positive control (gentamicin) and negative control (DMSO). GraphPad Prism version 6.0 (GraphPad Software Inc., La Jolla, CA, USA) was used for these analyses. For the lethality bioassay on AS, the LD_{50} values were calculated using the PROBIT model with SPSS software version 23 (IBM Corp., Armonk, NY, USA), based on six concentrations of each extract tested in triplicate, with 95% confidence intervals reported to ensure statistical reliability. All experiments were conducted in triplicate, and data were expressed as the mean ± standard deviation (SD). A significance level of $p \leq 0.05$ was considered for all analyses.

3. Results

3.1. Social Perception Analysis

The analysis of social perception was of a systematic random type, performed in the center of the city of Tuxtla Gutierrez, Chiapas. The present investigation showed the identification of 15 plant species commonly used as remedies for gastrointestinal ailments in this locality. These medicinal plants were distributed among 15 taxonomic families (Table 2). The Smith's salience index in this study highlighted *Byrsonima crassifolia* (BC, S = 0.766) and *Psidium guajava* (PG, S = 0.667) as the most culturally significant species for treating diarrheal diseases, indicating their prominent role in traditional knowledge and consistent use within the community in Tuxtla Gutiérrez, Chiapas. Following these were *Solanum torvum* (ST, S = 0.593), *Euphorbia maculata* (EM, S = 0.590), *Guazuma ulmifolia* (GU, S = 0.588), and *Bursera simaruba* (BS, S = 0.502), each demonstrating substantial recognition and perceived efficacy. The gradual decline in salience values among these top six plants suggests a clear hierarchy of preference and usage for gastrointestinal diseases (Table 2) and potential therapeutic benefits.

Table 2. Medicinal plants used for the treatment of gastrointestinal diseases in Tuxtla Gutierrez, Chiapas, Mexico.

Plant Species					Smith's Saliency Index
No.	**Mexican Common Name**	**Scientific Name**	**Abbreviations**	**Taxonomic Families**	
1	Nanche	*Byrsonima crassifolia*	BC	*Malpighiaceae*	0.766
2	Guayaba	*Psidium guajava*	PG	*Myrtaceae*	0.667
3	Sosa	*Solanum torvum*	ST	*Solanaceae*	0.593
4	Golondrina	*Euphorbia maculata*	EM	*Euphorbiaceae*	0.590
5	Cuahulote	*Guazuma ulmifolia*	GU	*Malvaceae*	0.588
6	Palo mulato	*Bursera simaruba*	BS	*Burseraceae*	0.502
7	Estafiate	*Artemisia ludoviciana*	AL	*Asteraceae*	0.413
8	Té de zacate	*Cymbopogon citratus*	CC	*Poaceae*	0.358
9	Verbena	*Verbena officinalis*	VO	*Verbenaceae*	0.345
10	Coralillo	*Hamelia patens*	HP	*Rubiaceae*	0.338
11	Hinojo	*Foeniculum vulgare*	FV	*Apiaceae*	0.277
12	Laurel	*Laurus nobilis*	LN	*Lauraceae*	0.255
13	Moringa	*Moringa oleifera*	MO	*Moringaceae*	0.247
14	Orégano	*Origanum vulgare*	OV	*Lamiaceae*	0.191
15	Maguey morado	*Tradescantia spathacea*	TS	*Commelinaceae*	0.180

These plants were selected to evaluate their antibacterial activity. PG was not included in this study due to the large amount of existing research that corroborates its antimicrobial effectiveness against bacteria causing acute diarrheal diseases [40–44]. BC was the plant most used for the treatment of gastrointestinal diseases in the region, followed by ST, EM, GU, and BS. In addition, when analyzing the results of the population studied (200 people), it is observed in Figure 2a that 63% of people who use medicinal plants are women compared to men with 37%. Women's greater knowledge of medicinal plants is due to their traditional role in family healthcare, where they prepare home remedies and care for children and the elderly. Additionally, they are often involved in the collection and cultivation of medicinal plants, which enhances their expertise. In many cultures, women are the primary keepers of ancestral knowledge, passing these practices down through generations. Regarding the population that uses medicinal plants according to the age range, 29% are between a range of 50–59 years, followed by 40–49 (20%) and 30–39 (18%) years (Figure 2b).

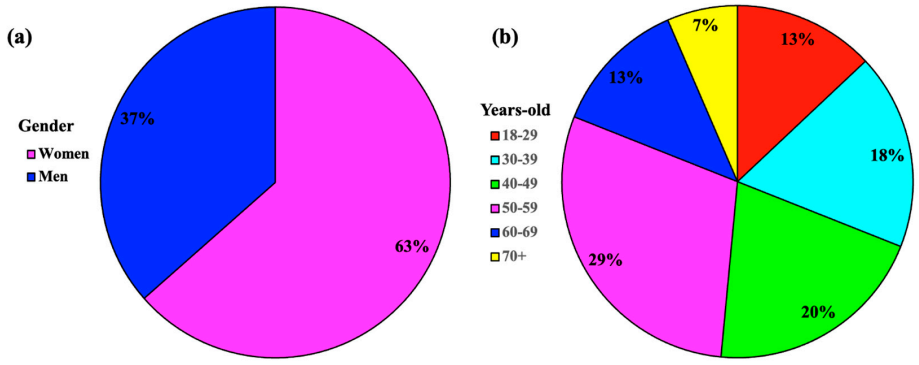


Figure 2. Percentage of the surveyed population using medicinal plants according to (**a**) gender and (**b**) age range.

3.2. Plant Extracts

Figure 3 illustrates the wild forms of the medicinal plants collected in Tuxtla Gutiérrez, Chiapas, showcasing their natural state in the local environment. These images provide a visual context to the study, emphasizing the accessibility of these species within the region and their integration into traditional medicine practices. The presence of these plants in their native habitat not only supports their sustainable use but also highlights the relevance of local biodiversity in addressing community health issues, such as diarrheal diseases.

Figure 4 shows the extraction yields obtained for the different plants studied. According to the results obtained, it was observed that the decoction method using water as a solvent influenced the extraction yield, being higher compared to the extracts obtained by maceration using ethanol as a solvent, where it was lower. The highest yields were obtained by decoction using water as an extraction solvent for both ST and GU, followed by BC, BS, and EM, respectively. These results may be attributed to the hot water used in decoction, which can break down plant cell walls more effectively, facilitating the release of active compounds, a process that is less efficient in ethanol maceration.

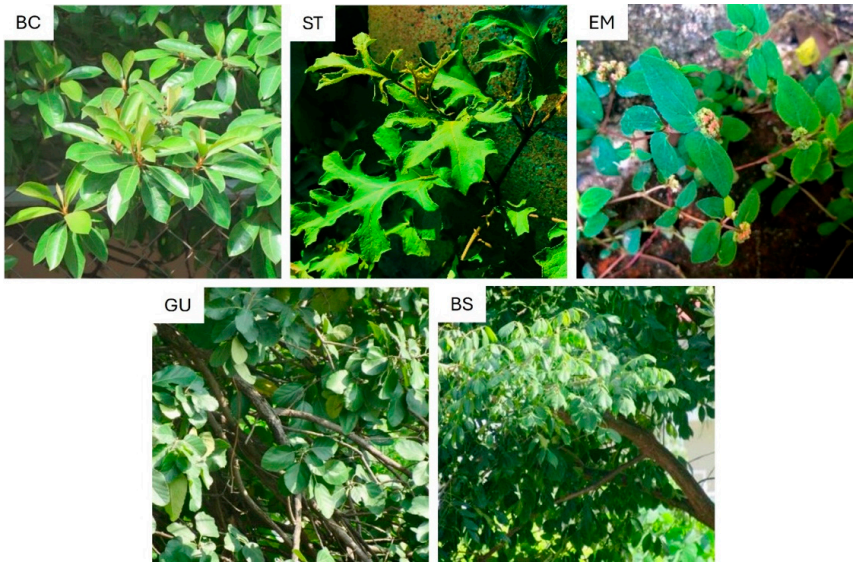


Figure 3. Photos of plants collected in Tuxtla Gutiérrez, Chiapas, Mexico in their wild form (self-authored). *Byrsonima crassifolia* (BC), *Solanum torvum* (ST), *Euphorbia maculate* (EM), *Guazuma ulmifolia* (GU), and *Bursera simaruba* (BS).

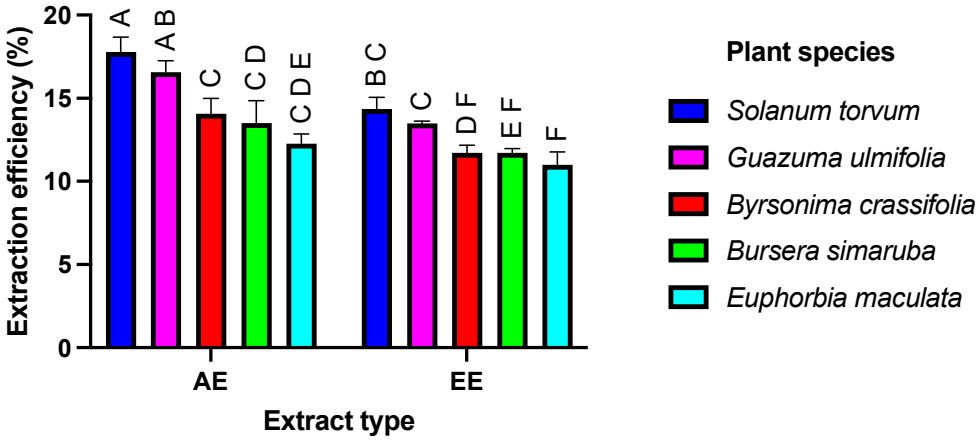


Figure 4. Extraction yield of the most popular medicinal plants used for the treatment of diarrheal diseases in Tuxtla Gutierrez, Chiapas, Mexico. The type of extracts were aqueous extracts (AE) and

ethanolic extracts (EE). Each value of the bars represents the mean ± SD, (n = 3). The letters on the bars (A–F) are significantly different ($p < 0.05$) for the different extraction efficiency as determined by two-way ANOVA followed by Tukey's test.

3.3. Antimicrobial Activity (Assay Disk)

The results of agar disk diffusion assay (or antibiogram) regarding the growth inhibition zones (mean ± standard deviation) of pathogenic bacteria against medicinal plant extracts (200 mg/mL) are summarized in Table 3. The agar disk diffusion test carried out using Gentamicin (G, $C_{17}H_{35}N_5O_7$) (80 mg/mL) showed antibacterial activity on pathogens used in this study. The EE and AE that showed antibacterial activity were BC, EM, and ST. The EE and AE of BC showed antibacterial activity against SE and SD, and the EE and AE of ST against EC and SE. Finally, EM had only antibacterial activity against SD compared to G as a positive control. The ethanolic extract of ST was most effective against EC (55.55%), BC against SE (78.26%), and EM against SD (104.76%). The aqueous and ethanolic extracts of GU and BS showed no antibacterial activity. Figures 5 and 6 show the antibiograms with the presence of the halos of inhibition of aqueous and ethanolic extracts of medicinal plants (BC, ST, and EM).

Table 3. Antimicrobial activity of medicinal plant extracts (200 mg/mL) in pathogenic bacteria by agar disk diffusion test.

Bacteria [b]	**Medicinal Plants** [a]											
	BC		**ST**		**BS**		**GU**		**EM**		**Control** [c]	
	AE	**EE**	**AE**	**EE**	**AE**	**EE**	**AE**	**EE**	**AE**	**EE**	**Positive (G)**	**Negative (DMSO)**
EC	-	-	14 ± 0.57 *** (51.85 ± 2.1%)	15 ± 2 *** (55.55 ± 6.4%)	-	-	-	-	-	-	27 ± 1.53 (100%)	-
SE	16 ± 1.52 *** (69.56 ± 4.8%)	18 ± 1.15 ** (78.26 ± 3.5%)	13 ± 1.52 *** (56.52 ± 4.9%)	17 ± 1.15 *** (73.91 ± 3.6%)	-	-	-	-	-	-	23 ± 0.58 (100%)	-
SD	15 ± 0.57 ** (71.42 ± 2.8%)	16 ± 1.52 ** (76.19 ± 5.1%)	-	-	-	-	-	-	20 ± 1 [ns] (95.23 ± 3.1%)	22 ± 2.08 [ns] (104.76 ± 6.5%)	21 ± 1.15 (100%)	-

[a] Medicinal plants: Byrsonima crassifolia (BC), Solanum torvum (ST), Bursera simaruba (BS), Guazuma ulmifolia (GU), and Euphorbia maculate (EM). [b] Bacteria: Escherichia coli (EC), Salmonella enteritidis (SE), and Shigella dysenteriae (SD). [c] Control: Gentamicin (G, 80 mg/mL) and Dimethyl sulfoxide (DMSO, 5%). Extracts: aqueous extracts (AE) and ethanolic extracts (EE). Data presented are inhibition zone diameter in millimeters (mm) per triplicated expressed as mean ± SD, (n = 3). The values were considered significantly different at $p \leq 0.12$ (ns), $p \leq 0.033$ (*), $p \leq 0.002$ (**), and $p < 0.001$ (***) for the inhibition halos of the extracts compared to the positive control, as determined by one-way ANOVA followed by Dunnett's test. The values in parentheses are the percentages of bacterial growth inhibition with respect to positive control. '-' indicates that the extract exhibited no inhibitory effect on the bacteria.

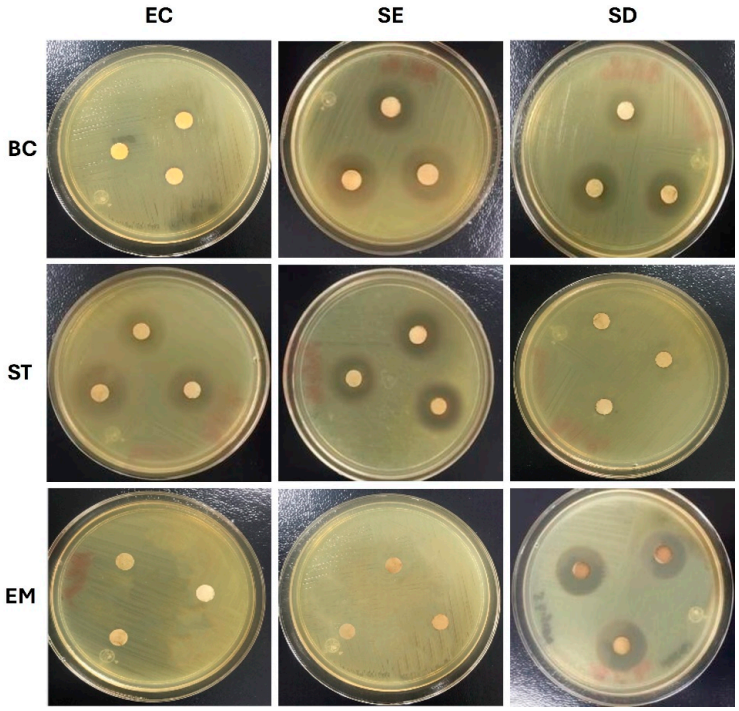


Figure 5. Effect (inhibition halos) of aqueous extracts (AE) of medicinal plants against bacteria causing acute diarrheal diseases. Bacteria: *Escherichia coli* (*EC*), *Salmonella enteritidis* (*SE*), and *Shigella dysenteriae* (*SD*). Medicinal plant extract: *Byrsonima crassifolia* (*BC*), *Solanum torvum* (*ST*), and *Euphorbia maculate* (*EM*).

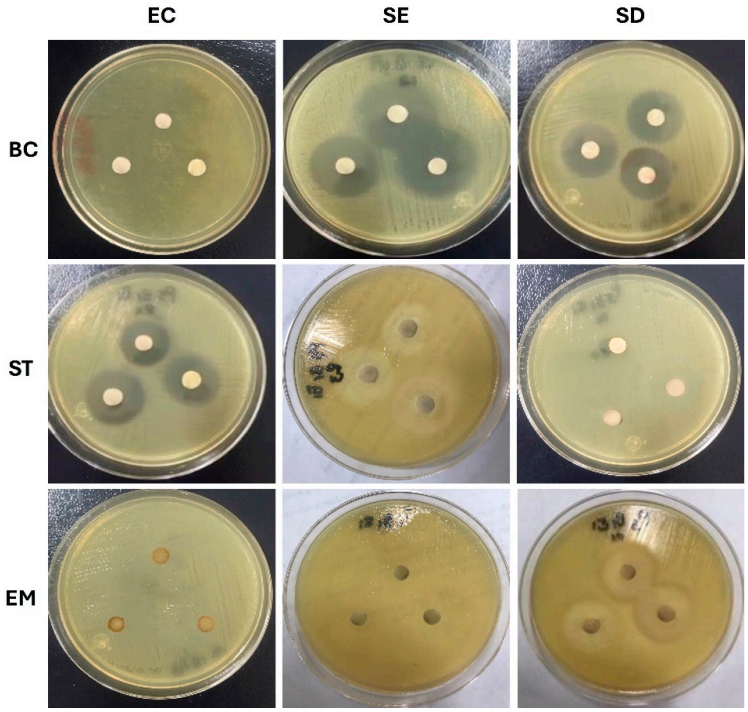


Figure 6. Effect (inhibition halos) of ethanolic extracts (EE) of medicinal plants against bacteria causing acute diarrheal diseases. Bacteria: *Escherichia coli* (*EC*), *Salmonella enteritidis* (*SE*), and *Shigella dysenteriae* (*SD*). Medicinal plant extract: *Byrsonima crassifolia* (*BC*), *Solanum torvum* (*ST*), and *Euphorbia maculate* (*EM*).

3.4. Minimum Inhibitory Concentration

As the extracts of BS and GU had no antibacterial activity, they were discarded for the evaluation of the MIC. Table 4 describes the MIC of BC, ST, and EM extracts, where the

best result obtained was ST versus EC, inhibiting bacterial growth at a concentration of 12.5 mg/mL of ethanolic extract. Overall, the BC ethanolic extracts showed antibacterial activity against SE and SD with a MIC of 50 and 25 mg/mL, respectively. ST against EC and SE had an MIC of 12.5 and 25 mg/mL, respectively, and EM against SD had an MIC of 25 mg/mL. Ethanolic extracts showed a lower MIC compared to aqueous extracts, that is, they more effectively inhibit the growth of bacteria at lower concentrations.

Table 4. Minimum Inhibitory Concentration (MIC) of the aqueous and ethanolic extracts of the leaves of *B. crassifolia*, *S. torvum*, and *E. maculata*.

Plant Species	Bacteria	Extract Type [a]	MIC (mg/mL)
Byrsonima crassifolia	*Salmonella enteritidis*	AE	100
	Shigella dysenteriae		50
	Salmonella enteritidis	EE	50
	Shigella dysenteriae		25
Solanum torvum	*Escherichia coli*	AE	50
	Salmonella enteritidis		50
	Escherichia coli	EE	12.5
	Salmonella enteritidis		25
Euphorbia maculata	*Shigella dysenteriae*	AE	50
		EE	25

[a] AE =aqueous decoction and EE = ethanolic maceration extracts.

3.5. Toxicological Test (LD_{50}) in In Vivo Model (Artemia salina)

According to the rating scheme from the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection [37,39], extracts from evaluated medicinal plants (BC, ST, BS, GU, and EM) showed no toxicity (Table 5) against the AS nauplius. The lethal dose (LD_{50}) of all extracts was >1000 µg/mL with 95% reliability.

Table 5. Lethal dose 50 (LD_{50}) of aqueous and ethanolic extracts against *Artemia salina*.

Plant Species	Popular Name	Extract Type [a]	Concentration (µg/mL) [b]	Classification of Toxicity
Guazuma ulmifolia	Cuahulote	AE	10,603.58	Non-toxic
		EE		
Solanum torvum	Sosa	AE	6224.35	
		EE		
Byrsonima crassifolia	Nanche	AE	1648.11	
		EE		
Euphorbia maculata	Golondrina	AE	5691.21	
		EE		
Bursera simaruba	Palo mulato	AE	3760.39	
		EE		

[a] AE = aqueous decoction and EE = ethanolic maceration extracts. [b] Values above 1000 µg/mL are observed, indicating that none of the extracts result in apparent acute toxicity.

4. Discussion

Khan [18] mentions that medicinal plants are the oldest form of empirical medicine, used for thousands of years worldwide. Salmerón-Manzano et al. [45] affirm that this knowledge is foundational to medicine and pharmacy, with ten percent of vascular plants

used medicinally. According to Vázquez-Medina et al. [46], differences in medicinal plant use between men and women are linked to social roles, labor division, and ancestral knowledge (traditional medicine). Singhal [47] revealed that women are primarily responsible for gathering, processing, storing, and transmitting this knowledge to future generations. Arias-Toledo [48] mentions that older individuals possess greater knowledge of local ethnobotany due to cultural and environmental factors and accumulated life experience compared to younger people. This dynamic interplay of knowledge and tradition underscores the critical role of natural products in modern pharmacotherapy, where Patwardhan et al. [49] affirm that several contemporary drugs have their origins in traditional herbal medicine.

García-de-Alba-García et al. [50] and Ali et al. [51] employed the Smith's salience index to examine people's perceptions of plant resources and their pharmacological potential, providing valuable insights into the interplay between cultural practices and therapeutic applications. This approach not only supports the preservation of ancestral knowledge but also offers a robust framework for identifying plants with pharmacological potential. Building on this methodology, the Smith's salience index was applied in this study to prioritize medicinal plants based on their cultural relevance. Species such as *Byrsonima crassifolia* (S = 0.766), *Solanum torvum* (S = 0.593), and *Euphorbia maculata* (S = 0.590) emerged as culturally significant due to their widespread use in treating gastrointestinal diseases. These plants reflect a cultural consensus rooted in their perceived efficacy and local availability, validating their importance in both traditional knowledge and daily practices. Moreover, the observed correlation between high salience values and antimicrobial activity highlights how cultural preferences can effectively guide the selection of species with therapeutic potential. However, Pires-Sousa et al. [52] emphasized that not all plants perceived as effective by local communities for treating diseases necessarily contain bioactive compounds with therapeutic properties. This observation aligns with our findings for *Guazuma ulmifolia* and *Bursera simaruba*, which, despite their strong cultural acceptance and traditional use for managing diarrheal diseases, exhibited no antibacterial activity in this study.

Nawaz et al. [53] discussed how extraction and purification of phytochemicals are affected by factors like time, temperature, and solvent polarity. Solvent polarity and extraction duration notably influence yield, and the choice of solvent depends on the chemical nature of the desired compounds. Salam et al. [54] noted that biomass extraction yields crude extracts with varied compound diversity, from a few to hundreds or even thousands of unique compounds. However, Bernhoft et al. [55] mention that these compounds, known as secondary metabolites, have pharmacological or toxicological effects in humans and animals. According to the Secretariat of Agriculture and Rural Development through the Technical Accompaniment Strategy of the Production for Welfare Program [56] in Mexico, the form traditional of used of medicinal plants for the obtention of crude extracts is for maceration and decoction methods.

Maceration is a simple process where pulverized biomass soaks in a solvent at room temperature (typically run for at least three days), sometimes with stirring to expedite extraction [57]. Decoction involves boiling plant material in water, generally for 15–20 min, though sometimes longer [58]. According to Li et al. [59], both methods are effective for extracting polyphenolic compounds from medicinal plants. In this study, extraction yields between aqueous and ethanolic extracts were similar, as both solvents are polar [60]. In this work, the performance results between in aqueous and ethanolic extracts are very similar to each other because both solvents used are considered polar protics according to [60]. Considering that the solvents are ethanol and water, it is suggested that, in the extracts of ST and GU, a higher concentration of secondary metabolites with medium-high to high polarities exist.

Fonmboh et al. [61] and Nortjie et al. [62] described the affinity of ethanol and water for extracting polyphenolic compounds, terpenes, and alkaloids. Cowan [63] and Gyawali and Ibrahim [64] observed that these compound classes possess antimicrobial properties, while Pandey and Kumar [65] and Nortjie et al. [62] identified antiviral, anthelmintic, and antidiarrheal activities. Guzmán et al. [66] reported that traditional Mexican medicine primarily uses leaves for extracts, with flowers and occasionally roots or stems also used. Kabera et al. [67] explained that secondary metabolites are distributed throughout plants, produced from biosynthetic pathways like shikimic acid, acetate-malonate, MEP (methylerythritol phosphate pathway), and MVA (mevalonate pathway). The crude extracts from medicinal plant leaves in this study likely contain polyphenols, terpenes, and possibly alkaloids, contributing to their antibacterial properties.

Martínez-Vázquez et al. [68] reported that BC root extracts exhibit strong antibacterial activity against EC, *Salmonella typhi* (18 mm inhibition zone at a dose of 10 mg/mL), *Shiguella flexneri* (25 mm), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, and *Micrococcus luteus*. In this study, the ethanolic leaf extract showed activity only against SE (18 mm at a dose of 200 mg/mL with MIC of 50 mg/mL) and SD (16 mm at dose of 200 mg/mL with a MIC of 25 mg/mL). The preliminary biological screening indicated that methanol (MeOH) extract and MeOH 80% extract, including both the ethyl acetate extract and aqueous fractions of MeOH extract of leaves, showed antimicrobial activity against *Staphylococcus epidermidis*, *Bacillus cereus*, *Bacillus subtilis*, *Salmonella* sp. (MIC of 9, 6, 7.5, and 6 mg/mL, respectively), *Proteus mirabilis*, *Enterococcus faecalis*, *Shigella* sp. (MIC of 3, 7.5, 3, and 7.5 mg/mL, respectively), and *Candida albicans* [69]. In this study, the MIC of aqueous and ethanolic extracts against SE was 100 and 50 mg/mL, respectively, while for SD, it was 50 and 25 mg/mL, respectively.

Gellen and Silva [70] report that aqueous root extracts of BC were effective against *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Pio-León et al. [71] showed that hexane fruit extracts had higher antibacterial efficacy than leaves, supporting its traditional use against EC A011, A019, A055, ATCC 25922, *Salmonella* group A-1, A-2, PDY A-1, B, D, *Salmonella typhi*, *Shigella flexneri*, and SD (MIC of 8 mg/mL). In this study, the MIC of ethanolic leaves extract against SD was 25 mg/mL, but no activity against EC. In leaves, Bonacorsi et al. [72] reported that the methanolic and chloroformic extracts inhibit, in vitro, the growth of *Helicobacter pylori* with MIC value of 1.024 mg/mL. Finally, Michelin et al. [73] reported the antimicrobial activity of methanolic leaves extracts from *Byrsonima fagifolia*, *Byrsonima basiloba* and *Boerhavia intermedia* against *Bacillus subtilis*, *Bacillus cereus*, *Shigella* spp., *Staphylococcus epidermidis*, *Proteus mirabilis*, *Salmonella* spp., *Enterococcus faecalis*, and *Candida albicans* with inhibition zones of 7 to 14 mm at a dose of 50, 75, and 100 mg/mL with MIC values of 1.5 to 12 mg/mL.

In folk medicine, the fruits and leaves of BC have been used as a treatment for gastrointestinal tract-related diseases such as ulcers, diarrhea, and infections caused by bacterial action. Its roots are used for wound healing and mouth and throat infections, such as gingivitis, tonsillitis, pharyngitis, and vaginal infections [74]. Currently, it has been possible to identify secondary metabolites of the polyphenol and terpene type in BC with antibacterial activity such as β-amyrin, betulin, betulinic acid, oleanolic acid, quercetin, (-)-epicatechin, gallic acid, β-sytosterol [75], methyl gallate, (-)-epigallocatechin gallate, and quercetin-3-O-(2″-galloyl)-a-L-arabinopyranoside [69]. Therefore, BC is a plant with potential use against gastrointestinal diseases caused by microorganisms.

For ST, Bari et al. [76] report that methanolic root extract exhibited strong antibacterial effects on *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Streptococcus-β-haemolyticus*, *Salmonella typhi* (7 and 21 mm at a dose of 50 and 200 µg/disc,

respectively, with MIC of 0.064 mg/mL) and SD (7 and 20 mm at a dose of 50 and 200 µg/disc, respectively, with MIC of 0.128 mg/mL) in comparison with leaves extracts. Chah et al. [77] reported that methanolic fruits extract has antimicrobial activity against *Actinomyces pyogenes*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhimurium* (6 mm at a dose of 80 mg/mL), and EC (same, 6 mm at a dose of 80 mg/mL). Also, in ethanolic fruits extracts, Basri et al. [78] observed activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* (6.67 mm at a dose of 10 µg/disc), and EC (8.21 mm at a dose of 10 µg/disc). In addition, Jaabir et al. [79] reported the antimicrobial activity against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, and EC (10 mm at a dose of 25 mg/mL) and Kalita et al. [80] against *Staphylococcus aureus*, *Candida albicans*, *Trichophyton rubrum*, and EC (18.58 mm at a dose of 100 µL).

On the other hand, with respect to the leaves, Kumar et al. [81] observed that methanolic extract (1.2 mm at a dose of 100 µL) was more effective against EC than aqueous extract (0.7 mm at a dose of 100 µL). Naimon et al. [82] reported the effectiveness of ethanolic leaf extract against *Bacillus cereus*, *Staphylococcus*, *Staphylococcus aureus*, *Streptococcus intermedius*, and *Pseudomonas aeruginosa*. Sabarinath et al. [83] reported that petroleum ether extract showed activity against *Staphylococcus aureus*, *Salmonella typhimurium*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and EC. In this study, ethanolic leaves extract showed better antibacterial activity only against EC (15 mm at a dose of 200 mg/mL with MIC of 12.5 mg/mL) and SE (17 mm at a dose of 200 mg/mL with MIC of 25 mg/mL) in comparison with aqueous extract. Finally, Hsu et al. [84] report that aqueous, acetonic, chloroformic, and methanolic extracts inhibited the growth of *Helicobacter pylori*. In Mexico, various parts of this plant are used to treat infections, as well as for its diuretic, antioxidant, anticancer, antiviral, analgesic, antimicrobial, anti-inflammatory, immunostimulatory, antiulcerogenic, nephroprotective, antidiabetic, antidepressant, antimalaria, and as larvicidal properties [85]. Currently, limited information exists, but it has been possible to suggest identification of polyphenols, terpenes, and alkaloids in ST with antimicrobial activity [78,79,86].

EM's antidiarrheal, antibacterial, antifungal, and antioxidant activities have been documented [87,88]; however, information on the in vitro antimicrobial activity is limited. In this regard, Heredia-Castro et al. [89] have reported that ethanolic extract present antibacterial activity against *Listeria monocytogenes*, *Staphylococcus aureus*, EC (8.35 mm at a dose of 3 mg/mL), and *Salmonella enterica* serovar *Typhimurium* (6.55 mm at a dose of 3 mg/mL). In addition, Borchardt et al. [90] reported that hydroalcoholic leaves extract showed antimicrobial activity against *Staphylococcus aureus*. Kirbag et al. [91] referred that methanolic extracts from other plants of the genus *Euphorbia* showed antimicrobial activity against *Staphylococcus aureus*, *Bacillus megaterium*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, EC (8.33 at 12 mm with a dose of 500 µg/disc), *Candida albicans*, *Candida glabrata*, *Epidermophyton* spp., and *Trichophyton* spp. Nagah and Aly [92] observed antimicrobial activity of methanolic extract against *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhimurium*, and EC. Recently, George et al. [93] have studied the green synthesis and antimicrobial activity of silver nanoparticles using leaf extract from EM, showing potent activity against EC and *Staphylococcus aureus*. Previous phytochemical investigations of EM have led to the isolation of several novel compounds, such as polyphenols (flavonoids and tannins) and terpenes (triterpenes) [94–98]. In this work, the ethanolic (22 mm at a dose of 200 mg/mL with MIC of 25 mg/mL) and aqueous (20 mm at a dose of 200 mg/mL with MIC of 50 mg/mL) extract only presented antibacterial activity against SD.

The Brine Shrimp Lethality Assay is an effective preliminary test for plant extract toxicity (in a concentration range of 10, 100, and 1000 µg/mL) [39]. According to Clarkson

et al. [99], extracts with an LD_{50} above 1000 µg/mL are non-toxic, LD_{50} of 500–1000 µg/mL are low toxic, extracts with LD_{50} of 100–500 µg/mL are moderate toxic, while extracts with LD_{50} of 0–100 µg/mL are highly toxic. With respect to the LD_{50} of BC and BS, Barillas-Aragón and De León-Natareno [100] reported that in the *Artemia salina* (AS) assay no toxicity was found in the fractions of hexane, dichloromethane, ethyl acetate, and butanol from leaves and bark (LD_{50} > 1 mg/mL). In this study, we observed that aqueous and alcoholic extracts of leaves from BC and BS did not have toxicity because presented a LD_{50} > 1000 µg/mL. The results obtained of the BS extracts were like those obtained by Villavicencio-Nieto and Pérez-Escandón [101] and Fernández-Calienes et al. [102], who reported the low and moderate toxicity of ethanolic extract. Likewise, in the case of BC extracts, the results obtained were similar with those reported by Blanco-Sierra and Laínez-Zelaya [103], who also found that the ethanolic extract from the bark was not toxic, and by Cáceres et al. [104], who report low toxicity in the fractions of hexane, ethanol, and aqueous from BC leaf and bark. On the other hand, Violante et al. [105] did not observe toxicity of ethanolic extract and the fractions of GU against AS since they showed LD_{50} values higher than 1000 mg/mL. Similar results were reported Navarro et al. [106], where the methanolic and aqueous extracts showed no toxicity. In this study, we observed that aqueous and alcoholic extracts of leaves from GU did not have toxicity against AS (LD_{50} > 1000 µg/mL). However, Assis et al. [107] reported that the methanolic extract of the fruit showed toxicity at a lethal dose of 36.59 µg/mL. In a study of ST, Bari et al. [76] observed that LD_{50} values for crude extracts from leaves, inflorescences, stems, and roots were of 124.29, 119.14, 92.25, and 35.46 µg/mL for chloroform and 497.54, 453.18, 325.71, and 203.59 µg/mL for methanol, respectively. These results indicate that the extracts are both lethal and toxic for AS. In addition, Rahman et al. [108] reported that ethanolic extract of fruits showed moderate toxicity (LD_{50} 478.40 µg/mL). Conversely, Periyanayagam et al. [109] observed that leaves extract had no toxicity on AS. In this study, we observe that aqueous and alcoholic extracts of leaves from ST did not have toxicity against AS (LD_{50} > 1000 µg/mL). Regarding EM, in this study, we observed that aqueous and alcoholic extracts of leaves did not have toxicity against AS (LD_{50} > 1000 µg/mL). There is currently no evidence of toxicity of the extracts from this plant, so it is important to continue with research.

5. Conclusions

The analysis of social perception reveals a strong community recognition of the value and efficacy of medicinal plants, underscoring their cultural importance and trusted role in traditional healthcare practices. This perception aligns with a deep-rooted knowledge base passed down through generations, reflecting both the accessibility and effectiveness attributed to these natural remedies. In this context, our study validates the traditional use of three out of five plants evaluated for treating gastrointestinal diseases associated with bacterial infections. Specifically, the in vitro data demonstrate the antimicrobial activity of BC, ST, and EM extracts against SE, SD, and EC, supporting their potential in preventing and treating bacterial infections.

Additionally, given the limited research on the antimicrobial and toxicological properties of EM, this work significantly contributes to the therapeutic knowledge of this species. Further investigation is required to identify the active compounds, potential synergistic effects, cytotoxicity, and safety profile of these plants, ultimately paving the way for clinical evaluations.

Overall, this study provides valuable insights into the sustainable use of local flora and emphasizes the importance of efficient extraction protocols in advancing phytochemical research and the development of natural therapeutics for gastrointestinal disorders. Future

studies aimed at characterizing specific metabolites will enhance our understanding and application of these traditional medicinal plants in modern healthcare.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres16010010/s1, Figure S1. Ethical clearance and consent form for the interview. Table S1. Plant collection data.

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Abbreviations

ADD	Acute diarrheal diseases
AE	Aqueous extract
ANOVA	Analysis of variance
AS	*Artemia Salina*
BAB	Blood Agar Base
BC	*Byrsonima crassifolia*
BHI	Brain Heart Infusion
BS	*Bursera simaruba*
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
DMSO	Dimethyl sulfoxide
EC	*Escherichia coli*
EE	Ethanolic extract
EM	*Euphorbia maculata*
G	Gentamicin
GESAMP	Group of Experts on the Scientific Aspects of Marine Environmental Protection
GU	*Guazuma ulmifolia*
LD_{50}	Lethal dose 50%
MeOH	Methanol
MEP	Methylerythritol phosphate pathway
MIC	Minimum inhibitory concentration
MVA	Mevalonate pathway
SD	*Shigella dysenteriae*
SE	*Salmonella enteritidis*
ST	*Solanum torvum*
AL	*Artemisia ludoviciana*
CC	*Cymbopogon citratus*

VO	*Verbena officinalis*
HP	*Hamelia patens*
FV	*Foeniculum vulgare*
LN	*Laurus nobilis*
MO	*Moringa oleifera*
OV	*Origanum vulgare*
TS	*Tradescantia spathacea*

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