academicJournals

Vol. 8(45), pp. 1310-1320, 3 December, 2014
DOI: 10.5897/JMPR2014.5656
Article Number: 6C7F7F249284
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Medicinal plants used in South Ecuador for gastrointestinal problems: An evaluation of their antibacterial potential

Lourdes Jerves-Andrade[1], Fabián León-Tamariz[1*], Eugenia Peñaherrera[1], Nancy Cuzco[1], Vladimiro Tobar[2], Raffaella Ansaloni[1], Louis Maes[3] and Isabel Wilches[1]

[1]Faculty of Chemistry, School of Biochemistry and Pharmacy, Universidad de Cuenca, Cuenca, Ecuador.
[2]Faculty of Engineering, Universidad de Cuenca, Cuenca, Ecuador.
[3]Laboratory for Microbiology, Parasitology and Hygiene, Department for Biomedical Sciences, Faculty of Pharmaceutical, Biomedical and Veterinary Science, University of Antwerp, B-2610 Wilrijk, Belgium.

Received 20 October, 2014; Accepted 21 November, 2014

The present study aimed to identify the main medicinal plants used by people in Ecuador South Andes to treat gastrointestinal problems, and to evaluate their antibacterial effect. *Escherichia coli* and *Salmonella spp*., as gram-negative and *Staphylococcus aureus* as gram-positive bacteria were used as *in vivo* models for the antibacterial screening. An ethnobotanical survey about gastrointestinal problems was applied to known healers in the study area. Collected information was contrasted to results of interviews to 13 different yachaks from the same area. The antibacterial potential of chloroform and methanol extracts was evaluated by a micro – dilution technique, and IC50 and IC90 values of the bioactive extracts were estimated. Cytotoxicity of bioactive extracts was assessed using MRC-5 SV2 cells. The ethnomedical approach allowed collecting a total of 132 plants used for the treatment of 9 different gastrointestinal problems described by the consulted experts (yachaks). Among collected plants, 82 are native, 38 are introduced, and 3 are endemic of Ecuadorian Andes. Methanol and chloroform extracts of *Hypericum laricifolium, Otholobium mexicanum* and *Peperomia sp.* showed activity against *S. aureus*, while either chloroform or methanol extracts from other 13 plants were also active against the same bacteria. No activity against gram-negative bacteria was detected. The collected information was verified by yachaks and it agrees with previous ethnomedical reports. The results of antibacterial screening and cytotoxicity assays will contribute to a more rational and safer use of medicinal plants.

Key words: Medicinal plants, ethnobotanical survey, gastrointestinal problems, diarrhea, gastroenteritis, antibacterial effect, Cytotoxicity.

INTRODUCTION

Nowadays, the use of medicinal plants around the world is a frequent health practice for facing relatively common illnesses. For instance in Ecuador, like in other developing countries up to 80% of the population relies

*Corresponding author. E-mail: fabian.leont@ucuenca.edu.ec.

on medicinal plants to treat primary needs of medical care (Bodeker and Ong, 2005). The ethnical, cultural and biological diversities together with other social phenomena have been pointed out as the main reasons to maintain this practice (Tene et al., 2007). Ethnologically, Ecuador is a very diverse country whose people belong to five main ethnical groups: Mestizos, Indians, Whites, Blacks and Mulatos (Chisaguano, 2006). Indians, the main holders of these ancient traditional practices, represent 7% of Ecuadorian population and comprises 27 recognized aboriginal groups that maintain their individual customs and cultures with some or no degree of external influence (Chisaguano, 2006; Instituto Nacional de Estadística y Censos, 2013). In the Southern Andean region of Ecuador, the main groups are the Kichwa de la Sierra, Kañaris and Saraguros (Instituto Nacional de Estadística y Censos, 2013).

Ecuador features great biodiversity, occupying the sixth place in the world on this category (Mittermeier, 1988). Related to plants, ecuadorian territory covers an area of 256.370 km^2 and hosts about 20.000 species of vascular plants, of which five to eight thousand may be medicinal (Neill et al., 1991; Monserrat Ríos, 1995). This natural richness can be explained by its geographical location on the equator, the presence of four natural regions, and the specific biological conditions achieved by the presence of the Andes mountain range (Neill and Jørgensen, 1999). Gastrointestinal (GI) diseases are among the main health problems in Ecuador. According to the Ministry of Public Health official information, gastroenteritis occupies the second place in the general morbidity index at national level with a rate of 21/10.000 inhabitants, and the fifth place with a rate of 125.7/10.000 at children's level (Indicadores Básicos de Salud Ecuador 2011). Ethnomedical national studies show the importance of medicinal plants for the treatment of GI diseases; being that this is the second reported health problem for which medicinal plants are used (Montserrat Ríos et al., 2007; De la Torre et al., 2008).

The main agents of bacterial gastroenteritis are *E. coli* and *Salmonella spp.* Several strains of *E. coli* may produce diahrrea: shiga toxin-producing (STEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC) and enteroaggregative (EAEC). These groups have different pathogenic mechanisms, that is, STEC group inhibits cell protein synthesis. This group is a major cause of acute diarrhea with or without presence of blood, especially in children. There are at least 150 STEC serotypes (Informe de Vigilancia de Escherichia Coli Productora de Toxina Shiga (STEC) 2012; E. Coli Enterohemorrágica 2010). Within the genus Salmonella, *S. enterica* and its sub-specie type I is one of major interest in human and animal pathology. A total of 6 sub-species and over 2500 serotypes within these subspecies are described. Salmonella serotypes cause enteritis, whose symptoms of infection include: digestive disorders, which are relatively common in toddlers (Gil et al., 2002). Although, *S. aureus* is not directly responsible for gastroenteritis, the food poisoning associated to its enterotoxins has an important relation with the disease, and has been introduced in the study as model of gram-positive bacteria in order to complete the biological spectrum for the antibacterial screening.

Despite the importance that medicinal plants have for Ecuadorian population and the high prevalence of GI problems in the country, no specific ethnomedical studies or reports evaluating the pharmacological properties, toxicological risks, and safety attributed to those plants have been carried out. Consequently, many cases of intoxication due to the misuse of medicinal plants have been observed (Montserrat et al., 2007; De et al., 2008). A review of traditional healing practices used for GI problems and their pharmacological evaluation is important to have alternatives in the treatment of these infections.

METHODOLOGY

Study area

The study was carried out in three southern provinces of Ecuador: Azuay, Cañar and Loja based on two factors, the number of indigenous populations and the evidence of strong local traditional medicine practice (Instituto Nacional de Estadística y Censos, 2013).

Ethnobotanical survey

The ethnobotanical study was conducted from March, 2008 to March, 2010. A database provided by the Ministry of Public Health (Zone 6) was used to establish the first contact with different populations. The applied survey was a modification of the formularies reported by Martin (2000) and by the Botanical Garden of Universidad Autónoma de México, strictly oriented to what people use for gastrointestinal problems. It includes among others, questions about GI symptoms and diseases, common names of plants, part of the plant used, recipes, and toxic effects if known. The ethnobotanical data were to be completed with personal information about every informant by means of a technical form obtained from Cunningham (2001).

Non-experimental confirmation

Collected information was compared to the results interviews of 13 shamans or yachaks in the study area. The previously collected information was referred to them in order to confirm its uses because of their longer experience and deeper knowledge on the practice of traditional medicine. Four of those experts belong to the Saraguro community of Loja, while seven shamans live in Azuay and two in Déleg (Cañar). To confirm the described uses of plants, information was also compared with published ethnomedical – ethnobotanical reports developed in the study area or in the nearby region.

Plant material

The whole plant or different plant parts (roots, stems, leaves, etc.) were collected according to what people use, with the help and assistance of traditional healers, in selected places, and according to the World Health Organisation (WHO) guidelines on good agricultural and collection practices (GACP) for medicinal plants (2003). The Herbarium Azuay (HA) at the Universidad del Azuay was used as reference point for the identification of plants and deposition of voucher specimens.

Extract preparation

Plant material was selected, rinsed and dried. Afterwards, plant extracts were obtained by percolation from 10 g of each collected material. Methanol and chloroform were used as extraction solvents. Dried extracts were obtained after lyophilization of methanol extracts using a Labconco Freezone 2.5 lyophilizator (Labconco, Kansas City, Missouri) and vacuum concentration of chloroform extracts using a vacuum concentrator Rapid Vap (Labconco, Kansas City, Missouri). Extracts were kept frozen (-20°C) until analysis of their potential pharmacological activity.

Microorganisms

Bacterial strains *Escherichia coli* ATCC 25922, *Salmonella thyphimurium* ATCC 14028, and *S. aureus* ATCC 25923, were used for the screening of antibacterial activity. Cryo-stocks of each strain at defined concentrations were kept at -80°C on trypticase soy broth with 10% glycerol: *E. coli* ($5x10^6$ CFU/ml), *S. enterica* ($5x10^7$ CFU/ml) and *S. aureus* ($5x10^5$ CFU/ml). The inoculum concentrations for screening were: *E. coli* ($5x10^4$ CFU/ml), *S. enterica* ($5x10^4$ CFU/ml) and *S. aureus* ($5x10^3$ CFU/ml). Those concentrations are between the limits used for micro-dilution techniques to avoid false-positive results due to too low concentrations (example, 10^2 CFU/ml) or false negative because of the use of too high inoculum size (example, 10^7 CFU/ml) (Cos et al., 2006).

Antibacterial assay

The antibacterial potential of plant extracts was tested using a modification of the micro-dilution technique (Eloff, 1998). Briefly, decreasing concentrations (64, 16, 4, 1 and 0.25 µg/ml) of each extract were tested for antibacterial activity on 96-well plates. Trypticase soy broth was used as culture medium, and the microtiter plates with the bacterial inoculums were incubated at 35.5°C for 24 h. The overall process was controlled by serial dilutions of ampicillin. In addition, positive (viability of the bacteria) and negative (sterility of the medium) controls were included in each screening. Once the incubation period was completed, microtiter plates were processed with an ELISA microplate reader (Multiskan-EX, Thermo Scientific, Shanghai, China) at a wavelength of 405 nm. IC50 and IC90 values of the bioactive extracts were calculated from the inhibition readings at different concentrations. Finally, an aliquot (100 µL) of the wells showing bacterial growth inhibition greater than 90% was inoculated in trypticase soy agar and incubated at 35.5°C for 24 h to evaluate either bacteriostatic or bactericidal activity of the bioactive extract. Extracts inhibiting the growth of bacteria after 72 h of incubation were reported as bactericides.

Cytotoxicity

A modification of the method developed by McMillian et al. (2002) was used for the analysis of cytotoxicity of bioactive extracts. Human lung fibroblast cells (MRC-5 SV2) were cultured on MEM supplemented with L-glutamine and fetal bovine serum. The same concentrations of each active extract as the ones used for the determination of the antibacterial activity were considered. After adding the cell suspension, plates were incubated at 37°C in a CO_2 supplied stove for 72 h. Finally, resazurin was added to each well and incubated at 37°C for 4 h. The fluorescence was determined in a microplate reader at a wavelength of 550 nm (excitation) and 590 nm (emission). Astamoxifen and ivermectin were used as reference compounds.

RESULTS

After analyzing the locations with more presence of indigenous population, four different places in Azuay province, one in Cañar and one in Loja were selected: El Pan, Sevilla de Oro, Sígsig, and Nabón in Azuay; Déleg in Cañar, and Saraguro in Loja. Globally, the collections were carried out at altitudes between 2100 until 3200 m above sea level; between the coordinates 2° 30' – 3° 50' south, and 78° 30' – 79° 25' west. The study area is shown in Figure 1 and the different Ecuadorian indigenous nationalities are presented in Table 1. The reported medicinal plants to treat GI symptoms or illnesses are presented in Table 2. One hundred and thirty two plants have been identified as medicinal by the application of the ethnobotanical survey. These plants were collected and identified mostly until species. Among collected plants, 82 are native, 38 are introduced, and 3 are endemic of Ecuadorian Andes, while information about the remaining 9 is incomplete. From the collected plants, 23 correspond to Asteraceae family and 12 belong to Lamiaceae; being these two groups the more representative ones.

According to the 45 traditional healers interviewed, the main GI symptoms were stomachache and diarrhea. Among the reported plants used to treat GI problems (Table 2), 61 were referred for stomachache and 30 for diarrhea. When experts (Yachaks) were interviewed, an association of symptoms, illnesses and the traditional treatment was obtained for some species. Based on the Andean conception of medicine, nine different GI diseases were described: "cólico de frío" (cold colic), whose symptoms are nausea, bad breath, vomit and diarrhea; "cólico de calor" (warmth colic) that exhibits mainly stomachache, fever and chill, and "colerín" showing similar symptoms to cold colic but caused after a rage event. Some external physical factors like falls or sudden movements are pointed out as the ones responsible for the "caída del shungo" and "caída de rabo" diarrheas, while some frightening event was mentioned as the trigger for the called "diarrea por espanto" (panic attack diarrhea). The last three illnesses reported were the diarrhea produced by "empacho" (indigestion), diarrhea produced by "bichos" (parasites infestation), and "diarrea por resfrío" (diarrhea due to cold) present when the patient suffers flu or cold.

In the analysis of methanol extracts action against *S. aureus*, *Clinopodium sp., Hypericum laricifolium* Juss, *Cestrum aff. peruvianum*, *Peperomia sp., Otholobium sp.,*

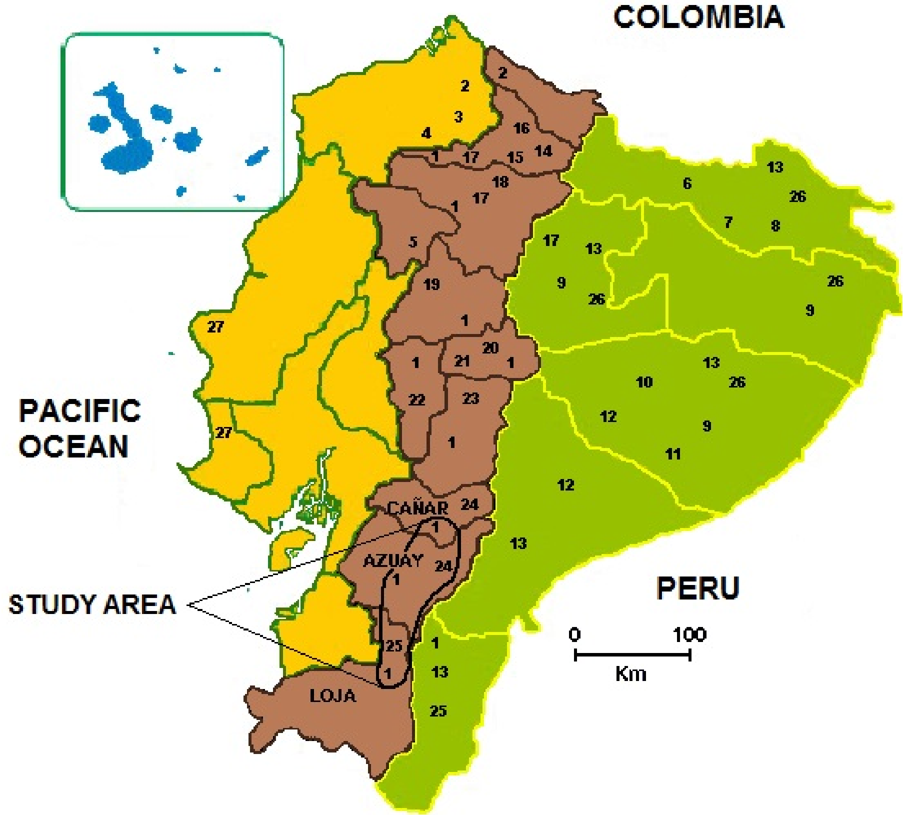

Figure 1. Map of indigenous nationalities and people of Ecuador. The remarked area presents regions where the study has been carried out.
Source: Modified from Ríos et al. (Montserrat Ríos et al., 2007)

and *Otholobium mexicanum* showed antibacterial activity. The activity was assessed by the IC50 values and ranged from slightly to very active, and the results are presented on Table 3. Methanol extracts were also tested against gram-negative bacteria *E. coli* and *S. enterica,* with no activity at any tested concentration. Regarding cytotoxicity, the first three of the above mentioned plant species analyzed showed no effect, and the latter three were cytotoxic. In the case of the chloroform extracts, twelve of them proved to be active against *S. aureus,* and the IC50 values obtained are presented in Table 4. No chloroform extract exerted any effect against *E. coli* and *S. enterica*. Chloroform extracts of *Alloysa triphylla, Apium graveolens, Minthostachis tomentosa, Valeriana hirtella* Kunt*, Commelina sp., Lycopodium sp.,* and *Dianthus caryophyllus* revealed moderate inhibition against *S. aureus* and were non–cytotoxic*.* For these plant species, the reviewed literature also use qualitative techniques to prove its antimicrobial action (Oskay et al., 2005; Bonjar et al., 2004).

DISCUSSION

According to the results, and although the described "illnesses" have not been medically evaluated, they show clear relation to the Ecuadorian morbidity index where diarrhea and gastroenteritis of presumed infectious origin appears at second position (Indicadores Básicos de Salud Ecuador 2011). The described GI problems maintain the traditional Andean duality of warm and cool diseases as reported in other studies (Tene et al., 2007; Scarpa, 2004). People classify them according to its etiology by the presence of symptoms that produce either the warming or cooling of the body (Tene et al., 2007). The medicinal plants also maintain this warm cool classification, although an intermediate group of mild plants are also described (Table 2). The compiled information showed some plants highly used such as *Matricaria recutita* L., *Chenopodium ambrosioides* L., *Malva officinalis* and *Mentha* × *piperita* L. whose medicinal use was also reported in ethnomedical studies developed in the country and around the world; while other species, especially native ones such as *Valeriana tomentosa* Kunth and *Desmodium sp*. are reported for the first time to treat GI problems.

Comparing the reported plants with previous ethnomedical studies related to GI problems, 79 coincidences were found. For example, with the report of Cordero (1950), considered as the first ethnomedical study carried

Table 1. Indigenous nationalities of Ecuador.

S/No	Nationality
1	Kichwa
2	Awa
3	Chachi
4	Epera
5	Tsa Chila
6	Ai Cofan
7	Secoya
8	Siona
9	Waorani
10	Shiwiar
11	Zapara
12	Achuar
13	Shuar
14	Karanki/Kichwa
15	Natabuel/Kichwa
16	Otavalo/Kichwa
17	Kayambi/Kichwa
18	Kitu Karai/Kichwa
19	Panzaleo/Kichwa
20	Chibuleo/Kichwa
21	Salasaca/Kichwa
22	Waranka/Kichwa
23	Puruha/Kivhwa
24	Kanari/Kichwa
25	Sarakuro/Kichwa
26	Amazon Kichwa
27	Mante/Wancavilca/Puna

out in the region, there are 25 coincidences. With White's study there are 42 common plants (White, 1982); while 21 with similar applications for GI problems were also found in the study of Tene et al. (2007). Considering studies conducted at national level, concordance of information and uses is found for 24 plants with Ríos et al. (2007), and 52 with De la Torre et al. (2008). In the Duke's Handbook of Medicinal Plants of Latin America, 17 plant species with similar applications in the treatment of GI problems were found (Duke, 2008). Even though people mainly use plants either by infusion or decoction, the evaluation of antibacterial activity was performed with methanol and chloroform extracts. These solvents will produce extracts with more consistent antibacterial effect than extracts obtained with water due to their higher extraction power of secondary metabolites (Ncube et al., 2008; Parekh and Chanda, 2007).

The activity of methanol and chloroform extracts of *Clinopodium sp.* and *Clinopodium nubigenum* Kuntze against *S. aureus* has also been reported in previous studies with different *Clinopodium* species: a MIC of 2.5 mg/ml against *S. aureus* was described for *Cirsium vulgare L.* (Stefanovic et al., 2011). On previous works developed by Bussmann et al. (2010; 2011), a positive activity of ethanol and water extracts of *Hypericum laricifolium* against *S. aureus* was reported; however, in the present research antibacterial effect against *S. aureus* was detected using methanol (IC50 28.01 µg/ml) and chloroform (IC50 27.5 µg/ml) extracts. Diverse authors also report antibacterial activity of different *Cestrum* species against *S. aureus* and *E. coli*: Bussmann et al. (2010) mention *C. auriculatum*, *C. humboldtii*, and *C. strigilatum*, and Chatterjee et al. (2007) report activity working with aqueous and methanol extracts of *C. nocturnum*; noting that both authors used agar diffusion methods, therefore no quantitative comparison with the current results for *C. aff peruvianum* (IC50 11.1 µg/ml) is possible. Some species similar to *Peperomia sp.* like *Peperomia pellucida* (Mendes et al., 2011) and *Peperomia galioides* (Cortez et al., 2003) have also been reported active against *S. aureus.*

Conclusion

The antibacterial activity of *Otholobium mexicanum* against *S. aureus* evidenced in the present research has also been determined in previous studies (Bussmann et al., 2010). However, despite the antibacterial properties of these plant species, the results obtained from the cytotoxicity assay could restrict their use for gastro-intestinal infections. Among the main results achieved in this work, there are: the correspondence between the yachack's knowledge and ethnomedical studies carried out in the study area; the relation between the ethnomedical perception of GI diseases and morbidity indicators for Ecuador; and the antibacterial effect and cytotoxicity results of the studied plants. This represents an important contribution to promote a more rational and safer use of natural products, and a reference line to deepen the research.

Conflicts of interest

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENTS

The present research was developed under the partner programme for Institutional University Cooperation between Universidad de Cuenca, Ecuador, and Flemish University Council (VLIR), Belgium. All the staff from the VLIR – MedPlan project in the University of Cuenca is highly acknowledged for their support and effort.

Conflict of Interest

Authors have not declared any conflict of interest.

Table 2. Medicinal plants used in traditional medicine of people of Azuay and Loja for the treatment of GI illnesses.

Andean sorting	Family	Genus	Species	Vernacular name(s)	Used parts							Ethnomedical use(s)
					N/I	R	S	L	F	Fr	Other	
Warm	Amaranthaceae	*Althernanthera*	*sp.*	Moradilla	Native				x			Stomach ache
Warm	Amaranthaceae	*Guilleminea*	*densa (Willd.) Moq.*	Hierba de golondrina (Huarmi)	Native		x	x				Stomach ache
Warm	Amaranthaceae	*Amaranthus*	*caudatus*	Ataco	Native	x						Colic
Warm	Apiaceae	*Foeniculum*	*vulgare L.*	Hinojo	Introduced		x	x				Stomach ache, flatulence, "colerín"
Warm	Apiaceae	Incomplete ID	Incomplete ID	Zanahoria de monte II	Incomplete ID	x	x	x	x			Stomach ache
ND	Apiaceae	*Cyclospermum*	*leptophyllum ((Pers.) Sprague ex Britton & P.Wilson)*	Mulalín	Introduced		x	x	x			Stomach ache
Warm	Apiaceae	*Arracacia*	*elata*	Sacha zanahoria	Native	x	x					Stomach ache, diarrhea, vomit
Mild	Apiaceae	*Apium*	*graveolens L.*	Apio	Introduced			x				Diarrhea
ND	Apiaceae	*Conium*	*sp.*	Canutillo	Introduced							Flatulence and colics
Warm	Asteraceae	*Artemisia*	*absinthium L*	Ajenjo	Introduced			x	x			Colic
Warm	Asteraceae	*Ambrosia*	*arborescens Mill.*	Altamisa	Native						young bud	Stomach ache, parasites
Warm	Asteraceae	*Tanacetum*	*parthenium (L.) Sch. Bip.*	Chichira blanca	Introduced		x	x	x			Stomach ache , diarrhea , gastric inflammation
Warm	Asteraceae	Incomplete ID	Incomplete ID	Chichira de huevitos	Incomplete ID		x	x	x			Diarrhea with blood, gastric inflammation
Warm	Asteraceae	*Cotula*	*australis (Sieber ex. Spreng.) Hook. f.*	Chichira sombrerito / chichira huevona	Introduced		x	x	x			Stomach ache , diarrhea , gastric inflammation
ND	Asteraceae	*Werneria*	*nubigena Kunth*	Chicoria de cerro	Native				x			Fever
Warm	Asteraceae	*Chuquiraga*	*jusseuii*	Chuquiragua	Native			x			Inflorescence	Vermifuge, stomachal tonic, fever
ND	Asteraceae	*Pentacalia*	*vaccinioides (Kunth) Cuatrec.*	Cubilán II	Native		x	x	x			Parasites
Mild	Asteraceae	*Taraxacum*	*officinalis*	Diente de León	Introduced		x	x	x			Stomach ache
ND	Asteraceae	*Hypochaeris*	*sessiliflora Kunth*	Guaquer	Native		x	x	x			Stomach ache and vomit
ND	Asteraceae	*Lactuca*	*sativa L.*	Lechuga	Introduced	x						Colics
Cool	Asteraceae	*Gamochaeta*	*americana (Mill.) Wedd.G.*	Lechuguilla	Native	x		x	x			Stomach ache, inflamation and diarrhea (children), infection
Mild	Asteraceae	*Jungia*	*sp.*	Mangapaqui	Native		x	x				Diarrhea and vomit (colerín)
Mild	Asteraceae	*Bidens*	*andicola Kunth*	Ñachac	Native		x	x	x			Stomachal tonic
Cool	Asteraceae	*Sonchus*	*oleraceus L.*	Quin-Quín	Introduced						Bud	Flatulence
Warm	Asteraceae	*Tagetes*	*pusilla*	Sacha anís	Native		x	x			Bud	Colic (colic by cold), flatulence, purgative,stomach ache
ND	Asteraceae	*Bidens*	*alba (L.) DC.*	Shirán	Introduced	x						Gastric infections
ND	Asteraceae	*Diplostephium*	*sp.*	Tushi	Native			x				Stomach ache
ND	Asteraceae	*Gnaphalium*	*sp.*	Vera-vera	Native		x	x				Fever and gastric infection
Warm	Asteraceae	*Baccharis*	*sp.*	Yadán/Sacha Yadán	Native		x	x				Stomach ache
Warm	Asteraceae	*Baccharis*	*sp.*	Yadán negro/Yadán huarmi	Native			x				Stomach ache
Warm	Asteraceae	*Baccharis*	*obtusifolia Kunth.*	Kari Yadán	Native						Bud	Stomach ache, vomit
Warm	Asteraceae	*Matricaria*	*recutita L.*	Manzanilla	Introduced	x	x	x	x			Stomach ache, colic, gastric inflammation
Warm	Betulaceae	*Alnus*	*acuminata Kunth*	Aliso	Native			x				Diarrhea
Warm	Brassicaceae	*Brassica*	*napus L.*	Nabo (Col chaucha)	Introduced			x	x			Stomach ache, nausea, fever and colic
Warm	Brassicaceae	*Lepidium*	*bipinnatifidium (Donn.Sm.)*	Chichira negra	Native		x	x				diarrhea (diarrea de frío)

Table 2. cont'd

Mild	Brassicaceae	*Matthiola*	*incana (L.) R.Br.*	Alelhí	Introduced				x			Stomach ache, colic, diarrhea for frighten
ND	Brassicaceae	*Nasturtium*	*officinale*	Berro	Introduced		x	x	x			Gastric inflamation
ND	Caryophyllaceae	Incomplete ID	Incomplete ID	Yurag pichana	Incomplete ID		x	x	x			diarrhea (diarrea por frío)
ND	Caryophyllaceae	*Cerestium*	*sp.*	Hierba de rocío	Native			x	x			diarrhea
Cool	Caryophyllaceae	*Dianthus*	*caryophyllus*	Claveles	Introduced				x			Vomit, gastric infections
Mild	Chenopodiaceae	*Chenopodium*	*ambrosoides L.*	Paico	Introduced	x		x				Stomach ache, parasites
Warm	Chloranthaceae	*Hedyosmum*	*cumbalense H. Karst.*	Guayusa de cerro	Native			x				Stomach ache
Warm	Clusiaceae	*Hypericum*	*decandrum Turcz.*	Bora pequeño/Romerillo de cerro	Native			x				Stomach ache
Warm	Clusiaceae	*Hypericum*	*laricifolium Juss.*	Bora	Native				x			Stomach ache
Cool	Commelinaceae	*Commelina*	*sp.*	Calceg/Calcio de chacra	Native		x	x				Diarrhea and infections
Cool	Cucurbitaceae	*Cucurbita*	*ficifolia*	Sambo	Introduced						Bud, seed	Stomach ache
Cool	Equisetaceae	*Equisetum*	*bogotense Kunth*	Cola de caballo	Native			x				Stomach ache, colic
ND	Euphorbiaceae	Incomplete ID	Incomplete ID	Preñadilla	Incomplete ID			x				Gastric inflammation
ND	Fabaceae	*Lupinus*	*sp.*	Taure azul pequeño	Native						Bud	Stomach ache
ND	Fabaceae	*Desmodium*	*sp.*	Hierba del Infante	Native	x	x	x	x			Stomach ache, gastric infections, purgative
ND	Fabaceae	*Spartium*	*junceum L.*	Retama	Introduced				x			Diarrhea (diarrea de calor), infections
Mild	Fabaceae	*Otholobium*	*sp.*	Trinitaria, Guallua/ Culín	Native	x	x	x				Purgative, indigestion, colic, diarrhea by parasites
Mild	Fabaceae	*Otholobium*	*mexicanum (L.f.) S.W.*	Trinitaria, Guallua/ Culín	Native	x						Purgative, indigestion, colic, diarrhea by parasites
ND	Fabaceae	*Dalea*	*coeruela*	Shordán	Native			x	x			Stomach ache
Warm	Gentianacea	*Centaurium*	*erythraea Rafn.*	Canchalagua	Introduced		x	x	x			Stomach ache
Mild	Geraniaceae	*Pelargonium*	*sp.*	Esencia de rosa	Introduced			x	x			Stomach ache
Mild	Geraniaceae	*Pelargonium*	*odoratissimum (L.) L' Hér.*	Malva olorosa	Introduced			x				Stomach ache
ND	Geraniaceae	*Erodium*	*cicutarium (L)*	Agujilla	Introduced			x		x		Colic, flatulence
Cool	Iridaceae	*Sisyrinchum*	*sp.*	Latrán pequeño de altura/Totorilla de cerro	Native		x	x				Stomach ache
Warm	Lamiaceae	*Mentha*	*× piperita L.*	Hierba buena	Introduced	x	x	x				Stomach ache, purgative
Warm	Lamiaceae	*Clinopodium*	*sp.*	Huarmipoleo	Native			x	x			Stomach ache, diarrhea for frighten, colic, gastric inflammation
Warm	Lamiaceae	*Salvia*	*ochrantha Epling*	Matico 1	Endemic			x				Gastric infections
ND	Lamiaceae	*Mentha*	*sp.*	Menta	Introduced		x	x				Stomach ache, infection, gastric inflammation
ND	Lamiaceae	*Mentha*	*sp.*	Menta blanca	Introduced						Young bud	Stomach ache, infection, gastric inflammation
Warm	Lamiaceae	*Origanum*	*× majoricum Camb.*	Orégano negro	Introduced			x				Stomach ache, flatulence
Warm	Lamiaceae	*Minthostachys*	*tomentosa (Benth.) Epling*	Poleo grande	Native		x	x				Diarrhea, vomit, stomach ache and gastric infections
Mild	Lamiaceae	*Lepechinia*	*rutocampii Epling & Mathias*	Salvia Gateada	Endemic		x	x				Gastric nfections
Warm	Lamiaceae	*Clinopodium*	*nubigenum (Kunth) Kuntze*	Tipo	Native		x	x	x			Stomach ache, gastric inflamation
Warm	Lamiaceae	*Clinopodium*	*sp.*	Trigo pichana	Native				x			Gastric infections
ND	Lamiaceae	*Stachys*	*sp.*	Pedorrera	Native		x	x	x		Young bud	Stomach ache, gastric infections
Mild	Lamiaceae	*Stachys*	*sp.*	Tripa de cuy (Kuichunshulli)	Native				x			Diarrhea, gastric infections
ND	Liliaceae	*Allium*	cepa (L.)	Cebolla blanca	Introduced	x	x					Stomach ache, diarrhea, gastric inflammation

Table 2 cont'd

ND	Lycopodiaceae	*Lycopodium*	*sp.*	Trenzilla II	Native	x	x	x				Stomach ache
Mild	Lycopodiaceae	*Lycopodium*	*cf.complanatum L.*	Trenzilla	Native	x	x	x				Stomach ache
Mild	Lycopodiaceae	*Lycopodium*	*sp.*	Cubilán I	Native							
ND	Lycopodiaceae	*Lycopodium*	*sp.*	Trenzilla I	Native	x	x	x				Stomach ache
ND	Lythraceae	*Cuphea*	*sp.*	Puca pichana	Native		x	x	x			diarrhea
Mild	Malvaceae	*Malva*	*officinalis*	Malva Blanca	Introduced				x		bark	Gastric infections, diarrhea
ND	Malvaceae	*Sida*	*rhombifolia L.*	Guillo blanco	Native		x	x	x			Gastric infections, diarrhea, vomit
ND	Malvaceae	*Sida*	*poeppigiana (K.Schum) Fryxell*	Guillo negro	Native		x	x	x			Gastric infections, diarrhea, vomit
Warm	Melastomataceae	*Miconia*	*salicifolia (Boonpl.ex Naudin) Naudin*	Kiyuyullo/Romerillo de cerro	Native		x	x				Stomach ache
Warm	Myricaceae	*Morella*	*parviflora (Benth.) Parra-O*	Laurel de cera	Native			x				Stomach ache
ND	Myrsinaceae	*Myrsine*	*dependens (Ruiz & Pav.) Spreng*	Guipi ó Guiqui /Yarac sacha	Native				x			Parasites
Warm	Myrtaceae	*Psidium*	*guajava L.*	Guayaba	Native			x		x		Diarrhea with blood
Cool	Onagraceae	*Oenothera*	*rosea L'Hér. Ex Aiton*	Shuyo/ Shuyo rosado	Native		x	x				Stomach ache
ND	Oxalidaceae	*Oxalis*	*sp.*	Chulco	Native		x					Gastric infections
ND	Oxalidaceae	*Oxalis*	*sp.*	Chulco amarillo	Native	x	x	x				Purgative, indigestion
ND	Oxalidaceae	*Oxalis*	*sp.*	Chulco rojo	Native	x	x	x	x		Bud	Purgative, indigestion
Cool	Passifloraceae	*Passiflora*	*cumbalensis (H. Karst.) Harms*	Gullán/Taxo	Native			x	x			Poultice (topic use)
Cool	Passifloraceae	*Passiflora*	*ligularis Juss.*	Granadilla	Native			x	x			Diarrhea
Warm	Piperaceae	*Peperomia*	*sp.*	Tigreshillo	Native		x	x	x			Stomach ache
Warm	Piperaceae	*Peperomia*	*sp.*	Congona negra	Native		x	x				diarrhea
Cool	Piperaceae	*Peperomia*	*sp.*	Congona blanca	Native		x	x				Diarrhea
ND	Piperaceae	*Piper*	*lineatum Ruiz & Pav.*	Matico 2	Native			x				Gastric infections
ND	Plantaginaceae	*Plantago*	*major*	Llantén	Introduced	x		x				Diarrhea, infections, colic
Cool	Poaceae	*Avena*	*sativa L.*	Avena	Introduced					x		Digestive
Cool	Poaceae	*Zea*	*maiz L.*	Maíz	Native						Corn silk	Colic, gastric inflammation, vomit
Cool	Poaceae	*Bromus*	*pitensis*	Grama dulce	Introduced		x	x				Diarrhea
ND	Poaceae	*Bromus*	*aff. catharticus Vahl*	Pasto mico, hierba de perro	Native			x				Stomach ache
Cool	Poaceae	*Paspalum*	*sp.*	Illín	Native		x	x				Stomach ache
Cool	Polygonaceae	*Rumex*	*crispum L.*	Gula	Native			x				Stomach ache, fever
Warm	Polygonaceae	*Muehlenbeckia*	*tamnifolia (Kunth) Meisn.*	Mollentin	Native				x		Bark	Stomach ache
Cool	Polygonaceae	*Rumex*	*obtusifolius L.*	Sacha gulag	Native	x						Stomach ache
Cool	Polypodiaceae	*Niphydium*	*crassifolium*	Calaguala	Native	x						Vermifuge
Mild	Pteridaceae	*Adianthum*	*poiretii Wikstr.*	Culantrillo pata negra	Native			x				Stomach ache, diarrhea and gastric infections
Warm	Punicaceae	*Punica*	*granatum*	Granado	Introduced			x				Diarrhea
Warm	Rosaceae	*Duchesnea*	*Indica*	Frutilla	Introduced	x						Diarrhea
Warm	Rosaceae	*Cydonia*	*vulgaris*	Membrillo	Introduced			x	x			Diarrhea
Warm	Rosaceae	*Rubus*	*aff. glabratus Kunth.*	Mora del Cerro	Native	x						Parasites
Warm	Rosaceae	*Rubus*	*floribundus*	Mora	Native	x		x	x			Diarrhea
Mild	Rosaceae	*Margycamrpus*	*pinnatus (Lam.) Kuntze*	Piquimuro	Native						Bud	Gastric infections
Cool	Rosaceae	*Lachemilla*	*vulcanica (Schultdl. & Cham.) Ryd*	Shullo blanco	Native							Diarrhea, parasites
Warm	Rutaceae	*Ruta*	*graveolens L.*	Ruda	Introduced				x			Colic

Table 2 cont'd

Warm	Sapindaceae	*Dodonaea*	*viscosa Jacq.*	Chamana	Native			x				Purgative
ND	Scrophulariaceae	*Pedicularis*	*incurva Benth.*	Zanahoria de monte I	Native	x						Parasites
Warm	Scrophulariaceae	*Gentianella*	*sp.*	Uñacushma/Ruda gallinazo	Native		x	x	x			Parasites
Cool	Scrophulariaceae	*Castilleja*	*ecuadorensis N.H. Holmgren*	Mila	Endemic			x				Stomach ache
Mild	Solanaceae	*Solanum*	*sp.*	Mortiño	Native			x	x	x		Gastric infections, "colerín", diarrhea
Warm	Solanaceae	*Cestrum*	*aff. peruvianum (Willd.) ex Roem.&Schult.*	Sauco negro	Native		x	x			Bud	Diarrrhoea with fever and shiver
Mild	Solanaceae	*Solanum*	*sp.*	Mortiño morado	Native							Colerín
Warm	Urticaceae	*Urtica*	*urens L.*	Ortiga	Introduced	x						Parasites, colic
Warm	Urticaceae	*Urtica*	*cf.leptophylla Kunth*	Ortiga macho	Native	x						Stomach ache
Mild	Valerianaceae	*Valeriana*	*tomentosa Kunth*	Chilpalpal	Native			x				Parasites, stomach ache, infections
ND	Valerianaceae	*Valeriana*	*hirtella Kunth.*	Valeriana	Native	x						Parasites, colics
Mild	Verbenaceae	*Verbena*	*litoralis Kunth*	Verbena	Native			x	x			Stomach ache, parasites
Warm	Verbenaceae	*Alloysa*	*triphylla*	Cedrón	Native			x	x			Stomach ache
ND	Verbenaceae	*Lantana*	*camara*	Rosa loca	Introduced							
ND	ND[b]	ND[b]	ND[b]	Calso	Incomplete ID	x	x	x	x	x		Diarrhea, parasites, gastric inflamations
ND	ND[b]	ND[b]	ND[b]	Chinchimanuela	Incomplete ID		x	x	x			Stomach ache
ND	ND[b]	ND[b]	ND[b]	Culantrillo gateador	Incomplete ID							Diarrhea, stomach ache, gastric infections
ND	ND[b]	ND[b]	ND[b]	Guacanguillo	Incomplete ID		x	x	x			Stomach ache
ND	ND[b]	ND[b]	ND[b]	Kanallullo	Incomplete ID			x				Diarrhea, fever and gastric infections

ND No Data available
[a] Part of the plant used: R (root), S (stem), L (leaves), F (flowers), Fr (fruit)
[b] No Data available about botanical characterization

Table 3. Antibacterial activity of methanol extracts against *Staphylococcus aureus.*

Scientific name	IC50 (µg/ml)	IC90 (µg/ml)	Activity	Cytotoxicity
Clinopodium sp.	16.6	>64	slightly active	non-cytotoxic
Hypericum laricifolium Juss	28.0	>64	slightly active	non-cytotoxic
Cestrum aff. peruvianum	11.1	>64	slightly active	non-cytotoxic
Peperomia sp.	20.7	63.5	slightly active	cytotoxic
Otholobium sp	10.6	61.8	slightly active	cytotoxic
Otholobium mexicanum	4.7	34.4	very active	cytotoxic

*A plant extract is considered as: very active when IC50 value is lower than 5 µg/mL or µM; slightly active, IC50 ranges from 30 to 5 µg/mL, and inactive, IC50 greater than 30 µg/mL.

Table 4. Antibacterial activity of chloroform extracts against *Staphylococcus aureus.*

Scientific name	IC_{50} (µg/ml)	IC_{90} (µg/ml)	Activity	Cytotoxicity
Hypericum laricifolium Juss	27.5	>64	Slightly active	Non-cytotoxic
Clinopodium nubigenum Kuntze	25.9	>64	Slightly active	Non-cytotoxic
Alloysa triphylla	29.9	>64	Slightly active	Non-cytotoxic
Apium graveolens	13.2	>64	Slightly active	Non-cytotoxic
Minthostachys tomentosa	22.2	>64	Slightly active	Non-cytotoxic
Valeriana hirtella Kunt	25.9	>64	Slightly active	Non-cytotoxic
Commelina sp.	23.3	>64	Slightly active	Non-cytotoxic
Lycopodium sp.	26	>64	Slightly active	Non-cytotoxic
Dianthus caryophyllus	16.5	>64	Slightly active	Non-cytotoxic
Peperomia sp.	6.6	15.5	Slightly active	Cytotoxic
Otholobium sp	14	>64	Slightly active	Cytotoxic
Otholobium mexicanum	3.3	43.4	Very active	Cytotoxic

*A plant extract is considered as: very active when IC50 value is lower than 5 µg/ml or µM; slightly active, IC50 ranges from 30 to 5 µg/ml, and inactive, IC50 greater than 30 µg/ml.

REFERENCES

Bodeker G, Ong CK (2005). World Health Organization Global Atlas of Traditional, Complementary, and Alternative Medicine. Vol. 1. World Health Organization.

Bonjar GS, Aghighi S, Nik AK (2004). Antibacterial and Antifungal Survey in Plants Used in Indigenous Herbal-Medicine of South East Regions of Iran. J. Biol. Sci. 4(3):405-12.

Bussmann RW, Ashley G, Sharon D, Chait G, Diaz D, Pourmand K, Jonat B, Somogy S, Guardado G, Aguirre C (2011). Proving that traditional knowledge works: the antibacterial activity of Northern Peruvian medicinal plants. Ethnobot. Res. Appl. 9:67-96.

Bussmann RW, Malca-Garcia G, Glenn A, Sharon D, Chait G, Díaz D, Pourmand K, Jonat B, Somogy S, Guardado G (2010). Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. J. Ethnopharmacol. 132(1):101-8.

Chatterjee SK, Bhattacharjee I, Chandra G (2007). Bactericidal Activities of Some Common Herbs in India. Pharm. Biol. 45(5):350-54.

Chisaguano S (2006). La Población Indígena Del Ecuador. Primera. Quito: Instituto Nacional de Estadísticas y Censos.

Cordero L (1950). Enumeración Botánica. Prov. Azuay Cañar Segunda Ed. Afrodisio Aguayo SA Madr.

Cortez C, Arroyo G, Reyes N (2003). Determinación de La Actividad Antimicrobiana de Peperomia Galoides HBK «Congona». Cienc. E Investig. 6(1):25-29.

Cos P, Vlietinck A, Vanden Berghe D, Maes L (2006). Anti-Infective Potential of Natural Products: How to Develop a Stronger in Vitro 'proof-of-Concept.' J. Ethnopharmacol. 106:290-302.

Cunningham A (2001). Applied Ethnobotany:" People, Wild Plant Use and Conservation." Earthscan.

De la Torre L, Navarrete H, Muriel P, Macía MJ, Balslev H (2008). Enciclopedia de Las Plantas Útiles Del Ecuador. Herbario QCA & Herbario AAU.

Duke JA (2008). Duke's Handbook of Medicinal Plants of Latin America. CRC Press.

E. Coli Enterohemorrágica (2010). ECOL_H2009.es10. Iowa State University.

Eloff JN (1998). A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria. Planta Med. 64(8):711-13.

Gil-Setas A, Mazón Ramos A, Martín Salas C, Urtiaga Domínguez M, Inza Elia M (2002). Salmonelosis No Tifoidea En Un Área de Salud de Navarra, España. Rev. Esp. Salud Pública 76(1):49-56.

Indicadores Básicos de Salud Ecuador (2011). Quito: Ministerio de Salud Pública; Organización Panamericana de la Salud.

Informe de Vigilancia de Escherichia Coli Productora de Toxina Shiga (STEC) (2012). DS N° 158/04, Artículo 9°. Ministerio de Salud. Gobierno de Chile.

Instituto Nacional de Estadística y Censos, ed. (2013). Autoidentificación. Accessed December 6. http://www.inec.gob.ec/estadisticas/index.php?option=com_remository&Itemid=&func=startdown&id=1217&lang=es&TB_iframe=true&height=250&width=800.

Martin G (2000). Etnobotánica: Manual de Métodos. Fondo Mundial Para La Naturaleza. UNESCO.

McMillian MK, Li L, Parker JB, Patel L, Zhong Z, Gunnett JW, Powers WJ, Johnson MD (2002). An Improved Resazurin-Based Cytotoxicity Assay for Hepatic Cells. Cell Biol. Toxicol. 18(3):157-73.

Mendes L, Maciel K, Viera A, Mendonça L, Silva R, Rolim-Neto P, Barbosa W, Viera J (2011). Atividade Antimicrobiana de Extratos Etanólicos de Peperomia Pellucida E Portulaca Pilosa. Rev. Ciênc. Farm Básica E Appl. 32(1):121-25.

Mittermeier RA (1988). Primate diversity and the tropical forest: Case studies from Brazil and Madagascar, and the importance of the megadiversity countries. In: E.O. WILSON (Ed.), Biodiversity. National Academy Press, Washington, D.C. pp. 145-154.

Ncube NS, Afolayan AJ, Okoh AI (2008). Assessment Techniques of Antimicrobial Properties of Natural Compounds of Plant Origin: Current Methods and Future Trends. Afr. J. Biotechnol. 7(12):1797-1806.

Neill DA, Jørgensen QM 1999. Climates. In: Jørgensen, QM, Leo´n-Ya´nez S (Eds.), Catalogue of the Vascular Plants of Ecuador. Missouri Botanical Garden Press, St. Louis, 1181. pp. 8-13.

Neill DA, Tapia C, Estrella J (1991). El Rol Del Herbario Nacional Del Ecuador En La Investigación Fitogenética. Memorias. 2 Reunión Nac. Sobre Recur. Fitogenéticos Quito Ecuad. 3-5 Abr 1991.

Oskay AM, Üsame T, Cem A (2005). Antibacterial Activity of Some Actinomycetes Isolated from Farming Soils of Turkey. Afr. J. Biotechnol. 3(9):441-46.

Parekh J, Chanda SV (2007). In Vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turk. J. Biol. 31(1):53-58.

Ríos M (1995). Importancia Y Biodiversidad de Las Plantas Útiles En El Ecuador: Un Estudio de Caso, La Reserva Forestal"ENDESA." Universidad Nacional Autónoma de México.

Ríos M, Koziol MJ, Pedersen HB, Granda G (2007). Plantas Útiles Del Ecuador: Aplicaciones, Retos Y perspectivas/Useful Plants of Ecuador: Applications, Challenges, and Perspectives. Ediciones Abya-Yala.

Scarpa GF (2004). Medicinal Plants Used by the Criollos of Northwestern Argentine Chaco. J. Ethnopharmacol. 91(1):115-35.

Stefanovic O, Stankovic MS, Comic L (2011). In vitro antibacterial efficacy of clinopodium vulgare L. extracts and their synergistic interaction with antibiotics. J. Med. Plants Res. 5(17):4074-4079.
Tene V, Malagón O, Vita-Finzi P, Vidari G, Armijos C, Zaragoza T (2007). An Ethnobotanical Survey of Medicinal Plants Used in Loja and Zamora-Chinchipe, Ecuador. J. Ethnopharmacol. 111:63-81.
White A (1982). Herbs of Ecuador: Medicinal Plants. Quito: Ediciones Libri Mundi 379p.-illus. En, Sp Icones. Geog.
World Health Organization, ed (WHO) (2003). WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants. Geneva: World Health Organization. http://herbalnet.healthrepository.org/handle/123456789/37.