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Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies

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ABSTRACT

Aim: The plant species reported here are traditionally used in Northern Peru to treat bacterial infections, often addressed by the local healers as "inflammation". The aim of this study was to evaluate the minimum inhibitory concentration (MIC) of their antibacterial properties against Gram-positive and Gram-negative bacteria.
Materials and methods: The antimicrobial activity of ethanolic and water extracts of 141 plant species was determined using a deep-well broth microdilution method on commercially available bacterial strains.
Results: The ethanolic extracts of 51 species inhibited *Escherichia coli*, and 114 ethanolic extracts inhibited *Staphylococcus aureus*. In contrast, only 30 aqueous extracts showed activity against *Escherichia coli* and 38 extracts against *Staphylococcus aureus*. The MIC concentrations were mostly very high and ranged from 0.008 to 256 mg/ml, with only 36 species showing inhibitory concentrations of <4 mg/ml. The ethanolic extracts exhibited stronger activity and a much broader spectrum of action than the aqueous extracts. *Hypericum laricifolium*, *Hura crepitans*, *Caesalpinia paipai*, *Cassia fistula*, *Hyptis sidifolia*, *Salvia* sp., *Banisteriopsis caapi*, *Miconia salicifolia* and *Polygonum hydropiperoides* showed the lowest MIC values and would be interesting candidates for future research.
Conclusions: The presence of antibacterial activity could be confirmed in most species used in traditional medicine in Peru which were assayed in this study. However, the MIC for the species employed showed a very large range, and were mostly very high. Nevertheless, traditional knowledge might provide some leads to elucidate potential candidates for future development of new antibiotic agents.

1. Introduction

In developing countries, traditional medicine (TM) is often the only accessible and affordable treatment available. In Latin America, the World Health Organization (WHO) Regional Office for the Americas (AMRO/PAHO) reports that 71% of the population in Chile and 40% of the population in Colombia have used TM. In many Asian countries TM is widely used, even though Western Medicine is often readily available. In the US the number of visits to providers of Complementary Alternative Medicine (CAM) now exceeds by far the number of visits to all primary care physicians (WHO, 1999a,b, 2002), and CAM is becoming increasingly popular in many developed countries (WHO, 1998), and a US survey reported the use of at least one of 16 alternative therapies increased from 34% in 1990 to 42% in 1997 (UNCCD, 2000).

The expense for the use of TM and CAM is growing exponentially in many parts of the world. The 1997 out-of-pocket CAM expenditure was estimated at $ 2.7 billion in the USA. The world market for herbal medicines based on traditional knowledge is now estimated at US$ 60 billion (Breevort, 1998).

Peru is a country rich in biodiversity. For millennia, traditional healers have used the rich flora to cure ailments. The same plants are still being used today. TM continues to be very popular since a large part of the population has either no access to, or no resources to afford Western Medicine. Bacterial infections and inflammation are among the ailments treated by traditional healers. Northern

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Peru is believed to be the center of the Central Andean Health Axis (Camino, 1992), and TM practices in this region remain an important component of everyday life (Sharon, 1978, 1980, 1994, 2000; Polia, 1988; De Feo, 1992; Joralemon and Sharon, 1993; Bussmann and Sharon, 2006; Sharon and Bussmann, 2006; Revene et al., 2008). TM is also gaining more and more respect by national governments and health providers. Peru's National Program in Complementary Medicine and the Pan American Health Organization recently compared Complementary Medicine to allopathic medicine in clinics and hospitals operating within the Peruvian Social Security System (EsSalud, 2000). The WHO has expressed high interest in TM. It is important to demonstrate scientifically that the remedies employed in folk medicine are indeed therapeutically active (Farnsworth et al., 1985; Elisabetsky and Castilhos, 1990; Cox and Balick, 1994; Baker et al., 1995; Muñoz and Sauvain, 2002).

Plants with potential medicinal activity have recently come to the attention of Western scientists, and studies have reported that some are bioactive (e.g. Perumal Samy and Ignacimuthu, 2000). Potentially active compounds have been isolated from a few of the plants tested (Umana and Castro, 1990; Okuyama et al., 1994; Rodriguez et al., 1994; D'Agostino et al., 1995a,b).

Plant species from the Cordillera Blanca in Peru, demonstrated antimicrobial, anti-cancer, and wound-healing activities (Villegas et al., 1997; Hammond et al., 1998; Lee et al., 1999; Neto et al., 2002; Bussmann et al., 2008, 2009a,b). However, despite the fact that the center of healing traditions in Northern Peru is located in the Trujillo/Chiclayo coastal region, no in-depth studies had been undertaken.

In this communication we report on antibacterial assays for 141 plant species with a wide range of traditional uses.

2. Materials and methods

2.1. Plant material

Plants were collected in Northern Peru (Fig. 1) in the field, in markets, and at the homes of traditional healers (*curanderos*) during August–September 2001, July–August 2002, July–August 2003, June–August 2004, July–August 2005, July–August 2006, June–August 2007, June–August 2008, March–April 2009 and June–August 2009. The specimens are registered under the collection series "JULS," "ISA," "GER," "EHCHL," "RBU/PL," "ACR," "KMM," and AKT," depending on the year of fieldwork and collection location. Surveys were conducted in Spanish by fluent speakers. Surveyors would approach healers, collectors and market vendors and explain the premise for the study, including the goal of conservation of medicinal plants in the area. All were asked to participate, but due to expected resistance information could not be recorded from everybody. From those who gave their prior informed consent, information was collected regarding their knowledge and inventory of medicinal plants.

Vouchers of all specimens were deposited at the Herbarium Truxillense (HUT, Universidad Nacional de Trujillo), and Herbario Antenor Orrego (HAO, Universidad Privada Antenor Orrego, Trujillo). In recognition of Peru's rights under the Convention on Biological Diversity, most notably with regard to the conservation of genetic resources in the framework of a study treating medicinal plants, the identification of the plant material was conducted entirely in Peru. No plant material was exported in any form whatsoever.

2.2. Nomenclature

The nomenclature of plant families, genera, and species follows the *Catalogue of the Flowering Plants and Gymnosperms of Peru* (Brako and Zarucchi, 1993) and the *Catalogue of the Vascular Plants of Ecuador* (Jørgensen and León-Yanez, 1999). The nomenclature was compared to the TROPICOS database (Tropicos, 2010). Species were identified using the available volumes of the *Flora of Peru* (McBride, 1936–1981), as well as Jørgensen and Ulloa Ulloa (1994), Pestalozzi (1998) and Ulloa Ulloa and Jørgensen (1993), and the available volumes of the *Flora of Ecuador* (Sparre and Harling, 1978–2009), and reference material in the herbaria HUT, HAO, QCA, LOJA and QCNE.

Fig. 1. Study area: Peruvian Departments of Amazonas, Piura, Lambayeque, La Libertad, Cajamarca, San Martin, and the Ecuadorian Province of Loja.

2.3. Preparation of extracts

For each species tested, above ground material (in case of trees leaves or bark as indicated by the collaborating healers) was collected, and the entire material used for extract preparation. This corroborates with the traditional preparation (Bussmann and Sharon, 2006). Plant material was dried at 35 °C for three days. After drying, the material was ground with an industrial grinder, and 2 samples of 5 g of plant material each were weighted out. One sample was submerged in 100 ml of 96% ethanol and left to macerate for 7 days, while another sample was submerged in 100 ml of boiling distilled water and left to macerate for 24 h. After maceration the plant material was filtered and 100 ml 96% ethanol was added to the water extracts to allow faster solvent removal. The solvent was then evaporated to complete dryness using a standard Buchi rotary-evaporator. The resulting dry extracts were re-suspended in 5 ml distilled water. In order to determine the real concentration of each extract, 1 ml of previous homogenization of the respective extracts was removed and again completely oven-dried and then weighed to determine amount of extract per ml of final solution. The remaining extract was used for MIC assays.