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# Toxicity of medicinal plants used in traditional medicine in Northern Peru

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ABSTRACT

*Ethnopharmacological relevance:* The plant species reported here are traditionally used in Northern Peru for a wide range of illnesses. Most remedies are prepared as ethanol or aqueous extracts and then ingested. The aim of this study was to evaluate the potential toxicity of these extracts.
*Materials and methods:* The toxicity of ethanolic and water extracts of 341 plant species was determined using a brine-shrimp assay.
*Results:* Overall 24% of the species in water extract and 76% of the species in alcoholic extract showed elevated toxicity levels to brine-shrimp. Although in most cases multiple extracts of the same species showed very similar toxicity values, in some cases the toxicity of different extracts of the same species varied from non-toxic to highly toxic.
*Conclusions:* Traditional preparation methods take different toxicity levels in aqueous and ethanol extracts into account when choosing the appropriate solvent for the preparation of a remedy.


## 1. Introduction

In many developing countries, Traditional Medicine (TM) is commonly used as it is an accessible and affordable treatment (WHO, 1999a,b, 2002), while Complementary Alternative Medicine (CAM) is popular in developed countries (WHO, 1998; UNCCD, 2000).

Peru is a prime example for a developing country rich in biodiversity, with a millennia old tradition of curers using the rich flora. Many of same plants are still being used nowadays. Northern Peru is believed to be the center of the Central Andean Health Axis (Camino, 1992), and TM practices in the whole Andean region are part of everyday life (Polia, 1988; De Feo, 1992; Joralemon and Sharon, 1993; Bussmann and Sharon, 2006; Revene et al., 2008). Peru's Social Security System recently implemented a National Program in Complementary Medicine in clinics and hospitals (EsSalud, 2000). The efficacy and potential toxicity of remedies employed in folk medicine do however have to be scientifically evaluated (Farnsworth et al., 1985; Elisabetsky and Castilhos, 1990; Cox and Balick, 1994; Baker et al., 1995; Muñoz and Sauvain, 2002).

A wide variety of studies have reported that some are bioactive (e.g. Perumal Samy and Ignacimuthu, 2000), but potentially active compounds have been isolated only 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). In contrast, crude medicinal activities have been investigated for a wide variety of plants (e.g. Villegas et al., 1997; Hammond et al., 1998; Neto et al., 2002; Bussmann et al., 2008, 2009a,b, 2010). But while toxicity assays are available for many countries, for e.g. Argentina (Bustos et al., 1996; Hernández et al., 2000), Bahrain (Taha and Alsayed, 2000), Bangladesh (Costa-Lotufo et al., 2005), Brazil (Hammer and Johns, 1993; Alves et al., 2000; Hoeltz et al., 2002; Santos Piemanta et al., 2003), Canada (McCuthcheon et al., 1992), Chile (Cuadra et al., 2005), China (Duffy and Power, 2001), Cuba (Martinez et al., 1996; Logarto Parra et al., 2001), Ecuador (Guerrero et al., 2003), Guatemala (Cáceres et al., 1993a,b, 1998; Michel et al., 2007), Honduras (Lentz et al., 1982), India (Padmaja et al., 2002), Kenya (Wanyoike et al., 2004; Kirira et al., 2006), Mexico (Sánchez-Medina et al., 2001), Nicaragua (Coe et al., 2010), Nigeria (Ajaiyeoba et al., 2006), Panama (Sánchez et al., 1993), Papua New Guinea (Nick et al., 1995), Philippines (Horgen et al., 2001), Uruguay (González et al., 1993), USA (Turker and Camper, 2002; Badisa et al., 2007; Van Slambrouck et al., 2007), no data exists on the potential toxicity of Peruvian medicinal species.

In this communication we report on brine-shrimp toxicity assays for 341 plant species ingested for a wide range of traditional uses. The brine shrimp, *Artemia* sp., Artemiidae, are small inverte-

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**Fig. 1.** Location of the study area in Northern Peru.

brates occurring in sea-water and other saline ecosystems. *Artemia* is frequently used as agent in laboratory assays to determine toxicity values by estimating $LC_{50}$ values (median lethal concentration) (Meyer et al., 1982; McLaughlin et al., 1991; Cepleanu et al., 1994; Coe et al., 2010).

## 2. Materials and methods

### 2.1. Plant material

Plants for the presented assays were collected in Northern Peru (Fig. 1) in the field, in markets, and at the homes of traditional healers (*curanderos*) during March–April and June–August 2009, and January–February 2010. The specimens are registered under the collection series "JP", "ACR," "KMM," and AKT". 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 (www.tropicos.org). Species were identified using the available volumes of the *Flora of Peru* (McBride, 1936–1981), as well as Ulloa Ulloa and Jørgensen (1993), Jørgensen and Ulloa Ulloa (1994) and Pestalozzi (1998) and the available volumes of the *Flora of Ecuador* (Sparre and Harling, 1978–2010), and reference material in the herbaria HUT and HAO.

### 2.3. Preparation of extracts

For each species tested, above ground material (in case of trees leaves and) 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.

### 2.4. Brine shrimp hatching

Eggs of brine shrimp (*Artemia* sp., Artemiidae) were purchased from Carolina Biological Supply (Burlington, NC, USA) and were incubated for 48 h in a culture vessel (15 cm × 15 cm × 15 cm) containing saltwater (1% NaCl) prepared from nitrate, phosphate, and silicate-free sea-salt and distilled water (35 g/l) at 25 °C under constant illumination. The saltwater solution was aerated continuously during incubation with an aquarium air pump. After 48 h the Nauplius-larvae were collected from the culture vessel.

### 2.5. Brine shrimp lethality assay

The brine shrimp lethality assay (BSLA) was used to determine if the plant extracts of medicinal species were cytotoxic (Meyer et al., 1982; McLaughlin et al., 1991; Cepleanu et al., 1994; Coe et al., 2010). Plant extracts were diluted to concentrations of 1000; 500; 250; 125; 62.5; 31.25 and 0 (μg/ml). Ten brine shrimp larvae were placed in each vial using a plastic pipette with a 2 mm diameter tip. The larvae were released under the surface of the solution to avoid killing them by trapping air under their carapaces. Survivors were counted under the stereomicroscope after 24 h, and the percent death at each dose and control was determined. All assays were run in triplicate. Vials with brine-shrimp in salt-water, and vials treated with 96% ethanol were used as controls.

### 2.6. Data analysis

The mean results of brine shrimp mortality were plotted against the logarithms of concentrations using the Probit Analysis tool of the NCSS statistical software package from which the median lethal concentration ($LC_{50}$) at 95% confidence intervals (CI) were calculated according to the method of Finney (1971). Biological activity using the brine shrimp assay was recorded as the concentration when 50% of the larvae were killed within 24 h of contact with the extract. $LC_{50}$ values below 249 μg/ml were considered as highly toxic, 250–499 μg/ml as median toxicity and 500–1000 μg/ml