RESEARCH PAPER

Screening of 170 Peruvian plant species for allelopathic activity by using the Sandwich Method

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Peru is one of the 20 botanically extremely diverse countries in the world, with >17 000 flowering plants, of which 30% are endemic. So far, no systematic research has been conducted on the screening of the allelopathic plants. In this study, the allelopathic activity of 170 species from 61 families of Peruvian plants that were collected from the three main regions of Peru – the Costa (Pacific coastline), the Sierra (Andean mountains), and the Selva (Amazonian rainforest) – was evaluated. The allelopathic activity was determined by the Sandwich Method, which can evaluate the activity of leaf leachates. The species that were found to be highly inhibitory in this screening, under the criterion of >90% inhibition of the radicle of lettuce (Lactuca sativa) seedlings, were Aristeguietia ballii and Diplostephium foliosissimum (Asteraceae) and Spondias mombin (Anacardiaceae). All of these species are native plants from Peru. This study gives a strong clue regarding the potential of isolating potent allelochemicals from these plants in the future.

Keywords: allelopathic activity, Aristeguietia ballii, Diplostephium foliosissimum, Sandwich Method, Spondias mombin.

Peru is located in the central part of the western coast of South America, bounded on the north by Ecuador and Colombia, on the south by Chile, on the east by Brazil and Bolivia, and on the west by the Pacific Ocean. The Andes Mountains divide Peru into three geographic regions: the Costa (Pacific coastline), the Sierra (Andean mountains), and the Selva (Amazonian rainforest). The influence of the Andes, the Humboldt Current, and other factors causes great climatic and geographic diversity in Peru (Pulgar 1996; Olson & Dinerstein 2002; Brack & Bravo 2005). The Peruvian flora include many wild species of crops, like potatoes, tomatoes, and peppers, among others (Brack & Bravo 2005). Of the ~20 000 known plant species in Peru, 17 144 are flowering plants and 27.9% of them are endemic (Brack & Mendiola 2004; León et al. 2006). That is one reason why Peru is viewed as a global hotspot for biological diversity (Myers et al. 2000) and is listed as one of the twenty “mega-diverse” countries (Paine et al. 1997).

The term “allelopathy” was coined by Molisch in 1937 from the Greek words αλλελον (of each other) and πάθος (to suffer) to describe beneficial and harmful biochemical interaction between organisms. The secondary metabolites that are related to this phenomenon are called “allelochemicals” and they are released into the environment by the ecological processes of volatilization, leaching, root exudation, or by the decomposition of plant residues (Rizvi et al. 1992). Allelochemicals have been shown to play important roles in the regulation of plant diversity and in sustainable agriculture.
(Chang-Hung 1999; Reigosa et al. 1999). One of the potential areas of applied allelopathy research is in controlling weeds: crop plants with allelopathic potential can be used as a cover, green manure in cultural practices, or in cropping patterns. There is a significant potential for studying allelochemical compounds from the perspective of their application as natural herbicides (Singh et al. 2003; Králová & Masarovičová 2006; Shennan 2008). The Sandwich Method was developed by Yoshiharu Fujii in Japan (Fujii 1994) as a bioassay to determine the allelopathic activity of the leachates from donor plant leaves and it has been used for the screening of a large amount of medicinal plants, both herbal and arbor species (Fujii et al. 2003; 2004; Morita et al. 2005). This method is also used in Pakistan (Khan et al. 2009; Anjum et al. 2010; Gilani et al. 2010) and Brazil (Candido et al. 2010) for the screening of medicinal plants and studying of invasive plant species, respectively.

Peru has become a focus of pharmaceutical research due to its rich biodiversity. This is because, from the phytochemical point of view, there are many species of great interest and several others whose potential has not yet been evaluated (Desmarchelier & Witting Schaus 2000; Palacios 2004; Desmarchelier 2010). However, recently the evaluation of the allelopathic activity of numerous plants has gained importance because of the development of bioherbicides and their application for future weed management. In this research, the screening of large numbers of samples that were collected from the three natural regions of Peru is presented. These results allow us to evaluate the allelopathic potential of various Peruvian species, which can be the first step in the investigation of related allelochemicals and their application in weed management for sustainable agriculture.

MATERIALS AND METHODS

Plant materials

For this study, the allelopathic activity of a total of 170 species (176 samples) from 61 families of Peruvian plants was determined by using the Sandwich Method under control conditions in the laboratory. The plant material consisted mostly of native species, such as medicinal plants, fruit trees, weeds, and native crops, from the three main regions of Peru: (i) 34 species in the Costa; (ii) 53 species in the Sierra; and (iii) 87 species in the Selva.

Identification and preparation of the leaf material

The samples that were collected from the Costa and Sierra were identified in the Herbarium, Department of Biology, Faculty of Science at the National Agrarian University La Molina (UNALM). (La Molina is a province of Lima in Peru.) The remaining samples, which were collected from the Selva, were identified by a specialist at Amazonian Medicinal Plants Laboratory, Peruvian Amazon Research Institute (IIAP), Loreto, Peru.

The leaves of each plant species were freshly collected and placed in a paper bag separately. This material was dried in a drying chamber at 60–70°C for 24 h. The dried leaves were stored in closed plastic bags at room temperature until their use.

Sandwich Method

The Sandwich Method was developed to test the allelopathic activity that is emitted from leaf litter leachates under control conditions in the laboratory. It is a less time-consuming bioassay method that can be used to screen a large number of samples (Shiraishi et al. 2002; Fujii et al. 2003; 2004).

A total of 10 mg of dried leaves (or cortex) was placed into three wells of a six-well (~10 cm² area per well) multi-dish plastic plate (35 mm × 18 mm; Thermo Fisher Scientific, Waltham, MA, USA). Agar powder (Nacalai Tesque, Kyoto, Japan) with a gelling temperature of 30–31°C was used as the growth medium (0.75% w/v). The first layer of agar (5 mL) was applied with a pipette (Gilson, Villiers-le-Bel, France); as a result, the plant material rose to the surface. After gelatinization, the second layer of agar (5 mL) was applied on top. Five seeds of the test plant, lettuce (Lactuca sativa L. cv. Great Lakes no. 366; Takii Company, Kyoto, Japan), were seeded on the gelatinized surface of each well. The multi-dish was covered with plastic tape, labeled, wrapped in aluminum foil, and incubated in the dark at 25°C for 3 days. The length of the hypocotyl and radicle of the lettuce seedlings was measured on the third day; these data were used to calculate the percentage elongation, compared to the control.

Statistical analysis

The radicle and hypocotyl elongation of the lettuce seedlings was measured and the percentage of elongation was calculated with reference to that of the control. For the evaluation of the allelopathic activity, the concept of the “standard deviation value” (SDV) was introduced (Fujii et al. 2003; 2004). For the statistical analysis, the mean and standard deviation were calculated and the criterion of the SDV was evaluated.

RESULTS

The Costa represents 12% of the land area in Peru. Most of the area of Pacific coastline is dry nearly all