THE AMERICAN MAYAPPLE REVISITED—
PODOPHYLLUM PELTATUM—STILL A POTENTIAL CASH CROP?1

RITA M. MORAES, CHARLES BURANDT JR., MARKUS GANZERA, XINGLI LI, IKHLAS KHAN, AND CAMILO CANEL

Moraes, Rita M., Charles Burandt Jr., Markus Ganzera, Xingli Li (National Center for Natural Products Research, Research Institute for Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677), Ikhas Khan (National Center for Natural Products Research, Research Institute for Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677 and Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677) and Camilo Canel (USDA, ARS, NPURU, National Center for Natural Products Research, University, MS 38677).

THE AMERICAN MAYAPPLE REVISITED—PODOPHYLLUM PELTATUM—STILL A POTENTIAL CASH CROP?

Podophyllum peltatum, was reexamined for its potential use in the commercial production of podophyllotoxin, a lignan used in the semisynthesis of important anticancer drugs. A survey of the natural population of the American mayapple, Podophyllum peltatum, was conducted in order to identify high-yielding genotypes. Plants were collected from the eastern and central United States. The lignan content of leaf blades and rhizome material of the collected specimens was characterized by aqueous extraction followed by HPLC analysis. Podophyllotoxin and α-peltatin appeared most prominently among the lignans obtained. Leaf blades were generally richer in podophyllotoxin than rhizomes. Several high-yielding accessions were identified, the blades of which contained 4.0–5.6% podophyllotoxin. A negative correlation was observed between podophyllotoxin and peltatin content in the blades. The combination of high biosynthetic capacity and preferential accumulation of podophyllotoxin in leaves of mayapple makes this plant an excellent candidate for agricultural production of podophyllotoxin.

A MAYAPPLE AMERICANA FOI REAVALIADA—PODOPHYLLUM PELTATUM—SERÁ QUE AINDA TEM POTENCIAL LUCRATIVO COMO CULTURA? O potencial de utilização do Podophyllum peltatum foi reexaminado para a produção comercial de podofilotoxina, um composto usado na semi-síntese de importantes drogas anticancerígenas. Um levantamento da população norte-americana da mayapple, Podophyllum peltatum, foi conduzido com a finalidade de identificar genótipos de alta produtividade. As coletas foram feitas no centroeste dos Estados Unidos e o conteúdo de lignanas dos limbo foliar e dos rizomas foi caracterizado por extração aquosa seguida da análise por HPLC. Podofilotoxina e α-peltatin apareceram mais presentes do que as demais lignanas. Geralmente, o limbo foliar apresentou um conteúdo de podofilotoxina mais rico do que os rizomas. Muitas acessões com alta produtividade foram identificadas e cujo o conteúdo de podofilotoxina no limbo foliar variou entre 4.0–5.6%. Observou-se ainda uma correlação negativa entre a podofilotoxina e o conteúdo de peltatin no limbo foliar. Nas folhas, a combinação entre a capacidade biosintética e o acúmulo preferencial da podofilotoxina, faz com que essa espécie seja considerada uma excelente candidata para a produção agrícola desta lignana.

Key Words: Podophyllum; Berberidaceae; etoposide; teniposide; aryltetralin lignans.

The potential for development of the American mayapple, Podophyllum peltatum L., as a cash crop for rural America was suggested by Meijer (1974) 25 years ago. Interest in this plant derived from its history of medicinal uses and the early, promising medicinal indications for podophyllotoxin, a compound reported in appreciable amounts in the plant's rhizomes. Podophyllotoxin, a natural lignan found in Podophyllum species (Berberidaceae), is the starting material for the semisynthesis of the anticancer compounds etoposide and teniposide (Stähelin

1 Received 7 November 1999; accepted 21 February 2000.

and Wartburg 1991). The main commercial source of podophyllotoxin is *Podophyllum emodi* Wall. (syn. *P. hexandrum Royale*), a species native to alpine and subalpine regions of the Himalayas that was declared in danger of extinction during the 1989 Convention on International Trade in Endangered Species of Wild Fauna and Flora (Foster, 1993). As a result of its endangered status, the export of biomass of *P. emodi* was prohibited. Collection of the plant is still allowed for the preparation and export of the podophyllotoxin-enriched resin that is obtained from its rhizomes and roots. The demand for podophyllotoxin is increasing due to a number of recent developments. The expiration of the patent covering the commercialization of etoposide has made it possible for a number of pharmaceutical companies to start producing this drug. Etoposide is also being tested and formulated for new therapeutic indications (Ajani et al. 1996, 1999; Ekstrom et al. 1998; Wexler et al. 1996). Concurrently, new and more water-soluble analogs of etoposide have been produced, which have shown higher efficacy and are undergoing clinical tests (Imbert 1998; Mross et al. 1996).

The combination of increasing demand and compromised availability of the source material has prompted researchers to look for alternative sources for podophyllotoxin (Choi et al. 1998; Jackson and Dewick 1985; Heyenaga et al. 1990; Uden et al. 1989, 1997). Recent work in our laboratories has demonstrated that, if properly extracted, the leaves of the American mayapple, *P. peltatum* L., could yield greater quantities of podophyllotoxin than the rhizomes of *P. emodi* (Canel et al. 2000). This offers an excellent opportunity for the domestication of the mayapple and its development as a high-value crop for small farmers in the United States. As part of our long-term program to identify high-yielding genotypes of *P. peltatum* and implement their cultivation, we have conducted a survey across the eastern and central United States, the results of which are hereby reported.

**MATERIALS AND METHODS**

Twenty specimens of *P. peltatum* were collected from each of 17 colonies found in 16 localities of the states of Illinois, Indiana, Missouri, North Carolina, Ohio, and West Virginia, during early spring of 1998. Colonies were carefully selected so that they consisted of expanded leaves covering a 10–20 m² area of well-defined boundaries. Colonies were located in shaded woody areas or sunny forest margins areas. Since colonies of mayapple are composed of hundreds of closely spaced shoots that are interconnected through extensive rhizome systems, they are likely to be clones. The specimens consisted of a shoot, having either one or two expanded leaf blades, attached to a segment of rhizome with attached roots. Specimens collected from the same colony were pooled and are collectively referred to as an accession. A second set of 20 leaf blades was collected from three colonies (Table 2).

Shortly after harvest, the leaf blades and underground parts (rhizomes and attached roots referred as rhizomes) were separated and dried at 40–45°C. These were then ground to a fine powder using a KSM 2B grinder (Braun, Lynnfield, MA). The powdered plant materials were stored at room temperature, protected from light and moisture in tightly closed glass bottles. Samples consisting of 500 mg of powdered plant parts were extracted by two methods, using either ethanol at 40°C (Bastos et al. 1996) or an aqueous extraction method recently developed by our group (Canel et al. 2000). Extractions were performed in triplicate. The lignans were separated and quantified by HPLC (Canel et al. 2000). Statistical analysis of data was carried out using SigmaPlot 4.0 software (SPSS Inc., Chicago, IL).

**RESULTS AND DISCUSSION**

Genotypic diversity within mayapple colonies is highly restricted because these colonies are clones, which consist of interconnected rhizomes. Studies of seedling mortality (Rust and Roth 1981), intracolony pollination (Swanson et al. 1976), allelic diversity (Parker 1989), estimations of the costs of sexual reproduction (Sohn and Policanski 1977) and our own experience with seed germination strongly indicate that the establishment of sexual progeny within mayapple colonies is a rare event. Therefore, the accessions described here are assumed to comprise either a single or closely related genotype.

The aqueous extraction of rhizomes and leaves of *P. peltatum* resulted in higher yields of lignans than ethanol extraction and thus more accurately reflected the biosynthetic capacity of these tissues. Therefore, to evaluate the potential of the various accessions for podophyllotoxin