Secondary Metabolites from the Phloem of Piper solmsianum (Piperaceae) in the Honeydew of Edessa meditabunda
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ABSTRACT:
Introduction – The phytochemistry of species of the genus Piper has been studied extensively, including Piper solmsianum. However, no studies have addressed the phytochemistry of the sap content of Piper species.
Objective – To evaluate the transferring of secondary compounds from the saps of P. solmsianum to the honeydew of Edessa meditabunda.
Methodology – The honeydew of E. meditabunda and saps of P. solmsianum were analysed by GC-MS, 1H-NMR and LC-MS.
Results – The lignan (−)–grandisin and the phenylpropanoid (E)-isoelemicin were detected in both saps of P. solmsianum and honeydew of E. meditabunda.
Conclusion – Analysis of honeydew secreted by the sap-sucking insect E. meditabunda indicated that (−)–grandisin and (E)-isoelemicin are absorbed from the phloem of Piper solmsianum. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: Lignans; phloem; sap; Edessa meditabunda; Piper solmsianum; Piperaceae

Introduction
The Piperaceae family belongs to the super order Nymphaei florae, order Piperales, and comprises five genera and about 1400 species (Santos et al., 2001). Their species are of pantropical occurrence with species distributed in the Western Hemisphere from Mexico to southeastern Argentina. Brazilian forests harbour 238 species of Piper L., 25 species of Ottonia Sprengel, and two species of Pothonophe Miquel (Guimarães and Carvalho-Silva, 2009).

The phytochemistry of the Piperaceae species, mainly of the Piper genus (Parmar et al., 1997), has been studied extensively due to the variety of biological activities displayed by their secondary metabolites (Alécio et al., 1998; Navickiene et al., 2000; Martins et al., 2003; Lago et al., 2004). Phytochemical studies of Brazilian species of Piperaceae have revealed the presence of amides (Silva et al., 2002; Navickiene et al., 2003), neolignans (Benevides et al., 1999), lignans (Martins et al., 2000, 2003; Ramos et al., 2008) and prenylated benzoic acid and chromene (Baldoqui et al., 1999; Ramos and Kato, 2009; Ramos et al., 2009).

The leaves, roots, stems and inflorescences of P. solmsianum have been investigated previously and are known to contain one dihydrobenzofuran neolignan, one glycosylated flavonoid (Moreira et al., 1995), five phenylpropanoids and four tetrahydrofuran lignans (Martins et al., 2000, 2003), including (E)-isoelemicin and (−)–grandisin (Ramos et al., 2008). In spite of the studies carried out on different organs, no phytochemical investigation has been carried out on the sap content for any of the Piper species. Such analysis may provide evidence for translocation of secondary compounds along the plant and through Piper–insect interactions, such as by the sap-sucking insect Edessa meditabunda. The data may contribute to determining the flow of secondary compounds from the phloem to the honeydew secreted by the insects.

Experimental
General
The 1H-NMR analysis was conducted at 200 MHz (Bruker AC-200) using deuterated chloroform (CDCl3; Aldrich) and tetramethylsilane (TMS; Aldrich) as a solvent reference. Electron impact (EI) MS was measured at 70 eV in a HP 5990/5988A spectrometer. The electrospray ion source (ESI) was measured at 4 kV, on an Agilent Technologies model SL. The HPLC analyses of the extracts obtained were performed in an HP-1050 instrument using a reversed phase column (Supelco, C18; 5 μm i.d., 4 × 250 mm) eluted in a gradient mode starting with acetonitrile:water 3:7 for 8 min; 3:7 to pure acetonitrile in 40 min, and detection at 254 nm. The GC-MS analyses were carried out in a Shimadzu GCMS-QP5050A with a DB-5 column (30 m × 0.25 mm, film thickness 0.25 mm) with the oven temperature programmed from 100 to 280 °C at a rate of 10 °C/min, and a carrier gas (helium) flow rate of 1 mL/min.

Plant material
The leaves of P. solmsianum were collected in the Campus of the University of São Paulo (Brazil) in September 2004. A voucher specimen (329676, P. solmsianum) is deposited in the Herbarium of the Jardim Botânico do Rio de Janeiro.

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(--)-Grandisin and (E)-isoeleminic

The (--)-grandisin and (E)-isoeleminic were isolated from leaves of *P. solmsianum* according to previously reported procedures (Martins et al., 2000, 2003). Dried leaves (2 g) of *P. solmsianum* were milled and extracted with dichloromethane:methanol (2:1, 30 mL × 3), which after concentration in a vacuum yielded 0.12 g of crude extract. From this extract, 2 mg was dissolved in methanol and eluted in a C18 cartridge (Sep-Pak) prior to a HPLC analysis.

Stem sap collection

Sap was collected between 1400 and 1600 hours using a gentle vacuum to suck the vessel content from stem segments. Because a vacuum cannot pull air through the pit fields at the ends of the vessels, successive segments are cut from the stem to release the vessel contents.

Honeydew collection

*Edessa meditabunda* specimens collected from the stems of *P. solmsianum* were maintained in glass flasks, having two individuals each, for 2 days. The honeydew excreted by the insects was collected from the flask walls using methanol. The methanol solution was concentrated under vacuum yielding 1.3 mg of crude extract. The dried extract was dissolved in 1 mL of methanol for HPLC, LC-MS and GC-MS analysis.

Stem sap analysis

The sap extracted from the insects collected in the *P. solmsianum* stems was filtered (0.45 µm) and injected directly into the HPLC. For GC-MS and 1H-NMR analysis, the sap (15 mL) obtained from the stems was extracted with ethyl acetate. The organic fraction was concentrated in a vacuum yielding 0.12 g of crude extract. From this extract, 2 mg was dissolved in methanol and eluted in a C18 cartridge (Sep-Pak) prior to a HPLC analysis.

Insect material

The specimens of *E. meditabunda* were collected on the Campus of the University of São Paulo (Brazil) in September 2004 and identified by Dr Sérgio Antônio Vanin. The specimens were maintained in glass tubes to obtain the honeydew excreted. The dried insects (40 °C) were milled and the honeydew was extracted with dichloromethane:methanol (2:1, 10 mL × 3). The extract was concentrated under vacuum and diluted in methanol for chromatographic and spectrometric analysis. The GC-MS and HPLC analyses of the extracts were carried out using solutions at concentration of 2 mg/mL in methanol.

Results and discussion

The xylem and phloem saps were extracted from the stem of *P. solmsianum* (15 mL) and 10 µL were directly injected into a HPLC. The chromatographic profile of the sap showed two peaks (1 and 2) with similar time of retention to (E)-isoeleminic and (--)-grandisin, respectively, when compared with the chromatographic profile of the extract from *P. solmsianum* leaves (Fig. 1). The sap (15 mL) was fractioned with ethyl acetate and the organic fraction concentrated under vacuum and analysed via GC-MS (Fig. 2), LC-MS and by 1H-NMR. The MS and 1H-NMR data confirmed the identities of 1 and 2 as (E)-isoeleminic and (--)-grandisin, respectively (see Supporting Information). The 1H-NMR spectrum of sap content revealed one singlet at δ 6.67 (H-2/H-2′, H-6/H-6′), two intense singlets at δ 3.87 (4 × OCH3/3,3′,5,5′) and at δ 3.84 (2 × OCH3/4,4′) corresponding to the methoxyl groups. The set of signals corresponding to the tetrahydrofuran moiety included a doublet at δ 1.09 (6H, J = 6.0 Hz, H-9/H-9′), a multiplet at δ 1.78 (2H, H-8/H-8′), a doublet at δ 4.66 (J = 7.9 Hz, H-7/H-7). These data are consistent with those observed for (--)-grandisin. Additionally, minor signals at δ 6.58 (singlet), 6.11 (multiplet) indicated the presence of (E)-isoeleminic in the sap content of *P. solmsianum*. Moreover, the analysis by GC-MS revealed the presence of fatty acids such as oleic acid and palmitic acid in *P. solmsianum* saps.

The crude (xylem) and elaborated (phloem) saps from *P. solmsianum* were collected together and at first it was not possible to determine whether (E)-isoeleminic and (--)-grandisin occurred in the xylem or phloem. Nevertheless, the excretion of honeydew by sap-feeding insects corresponds to the ingestion of phloem sap (Mittler, 1958). Thus, specimens of the sap-sucking insect *E. meditabunda* were collected on leaves of *P. solmsianum* for chemical investigation. The analysis by HPLC and GC-MS of extracts from the body and honeydew excreted by these insect specimens indicated compounds 1 and 2 (Fig. 1) as constituents in the phloem sap from *P. solmsianum*.

The presence of (E)-isoeleminic and (--)-grandisin in the sap and leaves of *P. solmsianum* provides evidence for secondary metabolite transport in the phloem in *Piper* species. Although the transport of secondary metabolites in plants has been investigated previously (Molyneux et al., 1990), this is the first report of secondary metabolites in sap of Piperaceae species. Such a method represents an alternative procedure to study transport and storage of secondary metabolites, which is generally limited to cytochemical techniques, analysis of isolated cell compartments and tracer labelling procedures (Luckner et al., 1980).
Supporting information

Supporting information can be found in the online version of this article.

Acknowledgements

The authors acknowledge Dr Elsie F. Guimarães (Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil) for the identification of plant material and Dr Sérgio A. Vanin for the identification of insect Edessa meditabunda.

References


Figure 2. Chromatographic profiles (GC-MS) of sap content from P. solmsianum.