

## The occurrence of phenylpropanoids in the saps of six *Piper* species (Piperaceae) from Brazil

### La ocurrencia de fenilpropanoides en las savias de seis especies de *Piper* (Piperaceae) de Brasil

RAFAELA F. PAZ<sup>1</sup>, ELSIE F. GUIMARÃES<sup>2</sup> & CLÉCIO S. RAMOS<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, Rural Federal University of Pernambuco, Dom Manoel de Medeiros, s/n, Dois Irmãos, CP 52.171-030, Recife-PE, Brazil.

<sup>2</sup>Rio de Janeiro Botanic Garden Research Institute, Jardim Botânico, 1008, CP 22.460-070, Rio de Janeiro-RJ, Brazil.

\*clecio.ramos@ufrpe.br

#### RESUMEN

Las especies de la familia Piperaceae son ampliamente investigadas debido a su importancia biológica, económica y ecológica. Sin embargo, los estudios químicos con las savias de la familia de las Piperaceae no han recibido tanta atención como la mayoría de otras especies de plantas. El presente estudio demostró la presencia de fenilpropanoides en la savia de seis especies de Piperaceae. Este estudio contribuye significativamente al conocimiento químico de la savia vegetal, hasta el momento poco explorado.

It is well known that phloem and xylem vessels transport a water-based solution with nutrient molecules over long distances in higher plants (Kehr & Rep 2007). Sap phloem contains mainly carbohydrates and other organic molecules, such as amino acids, proteins, hormones, and plant hormone, as well as inorganic ions. Xylem is involved in the transport of water and inorganic nutrients from the soil to the aerial parts of the plant (Krishnan *et al.* 2011). The occurrence of secondary metabolites is not expected in either phloem or xylem tissues. There are rare and isolated reports of the occurrence of secondary metabolites in some types of sap. In the phloem of *Castanospermum australe* A. Cunn. & C. Fraser ex Hook. (Fabaceae), the indolizidine alkaloid castanospermine (Molyneux *et al.* 1990) has been found; in the xylem sap of maize *Zea mays* L. var. *mays* (Poaceae), the phenylpropanoids coumaric, caffeic and ferulic acids (Alvarez *et al.* 2008) were found. An analysis of sap from the *Piper solmsianum* C. DC. stems showed that it belongs to the Piperaceae family, indicating the presence of two secondary metabolites the (-)-grandisin and *E*-isolemicin in the phloem of the plant (Ramos & Kato 2012). Lichen phenolics have been found in the xylem sap of *Quercus pyrenaica* Willd. (Fagaceae) (Avalos *et al.* 1986).

The Piperaceae family and the Chlorantaceae family are considered as basal angiosperms, with approximately 3,000 species described to date (Jaramillo *et al.* 2004). The Piperaceae family is currently divided into five genera: *Macropiper*, *Zippelia*, *Piper*, *Peperomia* and *Manekia*. The

*Piper* genus is considered the most expressive of this family, with more than 1,000 species identified (Nascimento *et al.* 2012). The *Piper* species have been extensively studied and documented due to their medicinal and pesticide properties attributed to the presence of diverse secondary metabolites found in their tissues (Scott *et al.* 2007). Phytochemical studies with the *Piper* species have revealed a variety of secondary metabolite classes such as amides, phenylpropanoids, prenylated benzoic acids, flavonoids, alkaloids, terpenes, chromenes, lignans and neolignans. The pipartine, an amide first isolated from the *Piper longum* L., exhibits diverse pharmacological activities (Bezerra *et al.* 2013). Amides isolated from the *Piper* species, such as piperine, piperiline, pellitorine, piperidine and guineensine, manifest potent insecticidal activity against diverse insect orders (Kwute *et al.* 2013). The *Piper* species have been a model genus for ecological and evolutionary studies. Members of this species of great ecological importance and are considered important because of their association with frugivorous bats (Jaramillo & Paul 2001). Given the economic, ecological, chemical and biological importance of the *Piper* species, and in order to contribute to knowledge of the chemical content of the sap in these plants, we have addressed the identification of secondary metabolites in the sap of six *Piper* species in this study.

The species selected were *Piper aduncum* L. (HST 18177), *P. caldense* C. DC. (HST 18180), *P. arboreum* Aubl. (HST 18179), *P. tuberculatum* Jacq. (HST 18178) and

*P. marginatum* Jacq. (48210). These were collected from a fragment of the Atlantic Forest located on the campus of Federal Rural University of Pernambuco (UFRPE) in Recife, Pernambuco, Brazil. A voucher specimen of each plant was deposited in the Vasconcelos Sobrinho Herbarium of UFRPE. *P. divaricatum* G. Meyer (Kato 1063) was collected in the city of Itabuna, Bahia state, Brazil, and a voucher specimen deposited in the Botanical Institute Herbarium, São Paulo, Brazil. The plants were identified by Dr. Elsie F. Guimarães.

The saps of six *Piper* species were collected between 10:00 AM and 12:00 AM. The sap was obtained 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 were cut from the stem to release the vessel contents. All sap samples were frozen immediately after sampling and stored at -80 °C until analyzed. The saps were extracted with ethyl acetate. The organic fraction was concentrated in a vacuum yielding the crude extracts. The saps were analyzed by GC-MS (60-240 °C at 3 °C min rate) in a Varian 431-GC coupled

to a Varian 220-MS instrument using a fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm.) coated with DB-5. The injector and detector temperatures were 250 and 260 °C, respectively. Hydrogen was used as the carrier gas at a flow rate of 1.5 mL/min; injection was in split mode (1:100) and the injection volume was 1.5 μL of a solution containing ca. 2 mg/mL of each extract in methanol. MS spectra were obtained using electron impact at 70 eV with a scan interval of 0.5 s and mass range from 40 to 550 m/z. Identification of the components was based on GC retention indices with reference to homologous series of C<sub>7</sub>-C<sub>30</sub> n-alkanes calculated using the Van den Dool and Kratz (Van den Dool & Kratz 1963) equation and by computer matching against the mass spectral library of the GC-MS data system as well as other published mass spectra (Adams 2007).

Sap dichloromethane fractions of the six *Piper* species: were analyzed by GC-MS (Fig. 1) and data interpretation indicated the presence of twenty-one secondary metabolites in the six saps analyzed. Phenylpropanoids were the major compounds detected in the six *Piper* species.

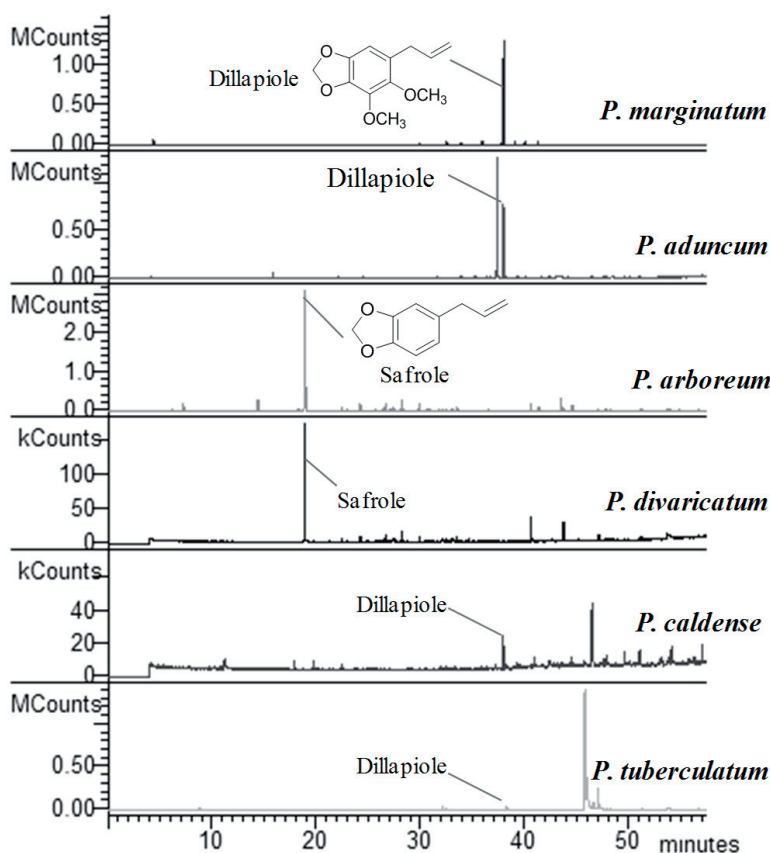


FIGURE 1. Sap chemical profiles from the *Piper* species obtained by GC-MS and the chemical structures of phenylpropanoids, safrrole and dillapiole. / Perfiles químicos de la savia de las especies de *Piper* obtenidos por GC-MS y la estructura química de fenilpropanoides, safról y dillapiole.

Phenylpropanoid dillapiole was identified in the saps from *P. marginatum*, *P. aduncum*, *P. caldense* and *P. tuberculatum* with relative percentages of 76.9%, 34.7%, 8.5% and 1.3%, respectively. Phenylpropanoid safrole was found in the saps of *P. divaricatum* (57.8%) and *P. arboreum* (56.4%). Other phenylpropanoids such as apiole, *E*-azarone, *Z*-azarone, as well as diverse terpenes, were also identified in the saps (Table 1). The largest number of secondary metabolites was identified in the *P. marginatum* sap, including four phenylpropanoids and five sesquiterpenes.

The discovery of phenylpropanoids as chemotypes in the saps of the *Piper* species was unexpected, and the

function of most of these phenylpropanoids in the saps is not yet well understood. The phenylpropanoids dillapiole, *E*-azarone, *Z*-azarone, apiole and safrole found in the *Piper* saps have already been reported as potent natural insecticidal agents, and may be associated with the chemical defense of the plants against sap-sucking insects. Another possible explanation for the occurrence of phenylpropanoids the sap is that they are only translocated to diverse tissues of plant for the biosynthesis of more complex secondary metabolites, thus these phenylpropanoids are precursors of various metabolites such as lignans, lignins and flavonoids, usually found in the *Piper* species.

TABLE 1. Chemical composition of compounds present in the sap content from *Piper* species. / Composición química de compuestos presentes en el contenido de savia de las especies de *Piper*.

Nº	COMPOUNDS <sup>a</sup>	RI <sup>b</sup>	RELATIVE AMOUNT (%)					
			PA	PC	PD	PM	PT	PU
1	3-Methyl-3-buten-1-ol	723		7.5				
2	Isobornyl formate	1235		1.0				
3	<b>Safrole</b>	<b>1285</b>	<b>56.4</b>		<b>57.9</b>			
4	$\alpha$ -Copaene	1374	1.3					
5	Caryophyllene	1417	2.5		3.1			
6	<i>cis</i> -Muurolo-4(14),5-diene	1465	1.1		2.9			
7	Germacrene D	1484			5.4			
8	Farenal	1508						
9	Apofarnesal	1520				0.3		
10	Liguloxide	1536				0.7		
11	Germacrene B	1559				0.4		
12	Guaiol	1600				2.0		
13	<b>Z-Azarone</b>	<b>1616</b>				<b>0.4</b>		<b>0.9</b>
14	Isolongifolan-7- $\alpha$ -ol	1618				0.3		
15	<b>Dillapiole</b>	<b>1620</b>		<b>8.5</b>		<b>76.9</b>	<b>1.3</b>	<b>34.4</b>
16	epi- $\alpha$ -Cadinol	1638				0.5		
17	$\alpha$ -Muurolol	1644	2.5					3.0
18	Cubenol	1645	1.2					
19	7-epi- $\alpha$ -Eudesmol	1662	3.4					
20	<b>E-Azarone</b>	<b>1675</b>				<b>2.5</b>		
21	<b>Apiole</b>	<b>1676</b>	<b>1.0</b>			<b>1.9</b>		

<sup>a</sup>Compounds are listed in order of their elution from an DB-5MS column. <sup>b</sup>Retention indices as determined on non-polar DB-5MS column using the homologous series of n-alkanes. PA: *P. arboreum*; PC: *P. caldense*; PD: *P. divaricatum*; PM: *P. marginatum*; PT: *P. tuberculatum*; PU: *P. aduncum*. / <sup>a</sup>Los compuestos se enumeran en orden de su elución de una columna DB-5MS. <sup>b</sup>Índices de retención determinados en columna DB-5MS no polar usando la serie homóloga de n-alcános. PA: *P. arboreum*; PC: *P. caldense*; PD: *P. divaricatum*; PM: *P. marginatum*; PT: *P. tuberculatum*; PU: *P. aduncum*.

In summary, we found secondary metabolites in the sap of the Piperaceae species in the form of dillapiole and saffrole compounds, distinguished as insecticidal agents because of their allelochemical properties. This indicates that the sap contents may be involved in plant defense against natural enemies.

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