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Volatile organic compounds from *Pachyrhizus ferrugineus* and *Pachyrhizus erosus* (Fabaceae) leaves

[Compuestos orgánicos volátiles de las hojas de *Pachyrhizus ferrugineus* y *Pachyrhizus erosus* (Fabaceae)]

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Abstract: In México, *Pachyrhizus erosus* (Fabaceae) commonly called "jícama", is widely known for its edible tubers. It is cultivated since the pre-Columbian period, and the powdered seeds have been used for the treatment of mange, lice, and fleas, due to their content of rotenone, a well-known insecticidal compound. On the other hand, *P. ferrugineus*, a wild species can only be found in the Tropical Forests, and has no commercial value. It is known that plants release volatile organic compounds (VOCs) showing qualitative and quantitative differences if are wild or cultivated. VOCs are also involved as repelling or attracting chemical signals to insect herbivores, and their natural enemies. Until now, the VOCs of the leaves of *P. erosus* and *P. ferrugineus* have not been investigated. In the present contribution the VOCs of both species were characterized by headspace solid-phase (HS-SPME) extraction and gas chromatography-mass spectrometry (GC-MS-TOF). In *P. erosus* 21 VOCs were found, being the most abundant: cyclohexanone (32.8%), 3-hexen-1-ol (Z) (32.7%), 3-hexenal (Z) (10.5%). The majoritarian compounds were C6 or C5 derivatives In *P. ferrugineus*, the most abundant VOCs were: 5-hexene-1-ol acetate (51.5%), undecanal (22.4%), 2-hepten-1-al (14.5%). The majoritarian compounds were C6, C7 or C11 derivatives.

Keywords: *Pachyrhizus ferrugineus* leaves, *Pachyrhizus erosus* leaves, volatile organic compounds.

Resumen: En México, *Pachyrhizus erosus* (Fabaceae) es llamada comúnmente "jícama" y es conocida por sus tubérculos comestibles. Se ha cultivado desde el período pre-Colombino y las semillas se han utilizado para el tratamiento tópico de la sarna, piojos, pulgas; las semillas contienen rotenona, un compuesto insecticida. Por otra parte, *P. ferrugineus* solo está presente en estado silvestre en los bosques tropicales y carece de valor comercial. Se sabe que las plantas liberan compuestos orgánicos volátiles (COV) y muestran diferencias cualitativas y cuantitativas dependiendo, si son silvestres o cultivadas. Los COV también son señales químicas atrayentes o repelentes de los insectos herbívoros y a sus enemigos naturales. Hasta ahora, los COV en las hojas de *P. erosus* y *P. ferrugineus* no han sido investigados. En el presente trabajo, los COV se identificaron mediante la microextracción (HS-SPME) en fase sólida, e identificados por cromatografía de gases acoplada a espectrometría de masas (GC-MS-TOF). En *P. erosus* se encontraron 21 COV, siendo los más abundantes: ciclohexanona (32.8%), 3-hexen-1-ol (Z) (32.7%) y 3-hexenal (Z) (10.5%). Los compuestos mayoritarios son C6 y C5. En *P. ferrugineus* los más abundantes fueron: 5-hexen-1-ol acetato (51.5%), undecanal (22.4%) y 2-hepten-1-al (14.5%). Los compuestos mayoritarios son C6, C7 o C11.

Palabras clave: Hojas de *Pachyrhizus ferrugineus*, Hojas de *Pachyrhizus erosus*, compuestos volátiles

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INTRODUCTION

The Neotropical genus *Pachyrhizus* is placed taxonomically in the subtribe Diocleinae, tribe Phaseoleae, within the legume family (Fabaceae) (Sørensen *et al.,* 1996). The genus comprises five species found in the American Continent; three of these are cultivated to obtain edible tuberous roots, and the other two only can be found wild. *Pachyrhizus erosus* and *P. ferrugineus* are present in Central America and Mexico, while *P. tuberosus, P. ahipa* and *P. panamensis* are distributed in South America (Sørensen *et al.,* 1996; Ramos *et al.,* 2013).

In México, there are only two *Pachyrhizus* species: P. *erosus* (cultivated) and *P. ferrugineus* (wild) (Sørensen *et al.,* 1996). The first one is commonly called "jícama", it is widely known for its edible tubers. There is evidence that during the pre-Columbian period it was cultivated by the Toltec, Aztec and Maya civilizations (Sørensen *et al.,* 1996). Today, *P. erosus* is a tuber legume crop with high yield potential, high nutritional value, low N_2 fertilizer and low pesticide demand (Castellanos *et al.,* 1997), being ideal for plant growing. The seeds of *P. erosus* have been used in Mexico for the treatment of mange since pre-Columbian times (Bejar *et al.,* 2000), and during the XX century the peasants used the powdered seeds for their insecticidal properties against ectoparasites, such as lice, and fleas. These ethnomedical applications can be explained due to their content of rotenone, a compound well known compound for its insecticidal and acaricidal properties (Reyes-Chilpa et al, 2003), as well as other isoflavonoids.

Pachyrhizus erous is extensively cultivated in Mexico, even for exportation, and has achieved wide acceptance in U.S.A., Southeast Asia, and Western Africa; therefore, it has stimulated agronomic and food science interest (Ramos *et al.,* 2013). It has been estimated that México exported 11,000 tons per year to USA, where it reaches an average price of one dollar per kg (Castañeda, 2000).

Volatile organic compounds (VOCs) are lipophilic compounds with low molecular weight and high vapor pressure at ambient temperatures (Dudareva *et al.,* 2013). The VOCs are emitted as blends from flowers, leaves, fruits, and roots into the surrounding atmosphere (Kigathi *et al.,* 2009). Artificial selection for specific plant traits has often not only changed the appearance of cultivated plants compared to their wild conspecifics, but also their chemistry (Gols *et al.,* 2011). Furthermore, volatile organic compounds have been widely studied due to their contribution to aroma and flavour and as markers for authenticity, for example, biological or geographical origin, cultivar (Socaci *et al.,* 2013). To our best knowledge, volatile constituents of leaves of *P. erosus* (cultivated type) and *P. ferrugineus* (wild type) has not been reported. In the present study volatile compounds of these species were analyzed by HS-SPME/GC-MS-TOF as a starting point of future studies aimed to study their susceptibility to insects, and fungi.

MATERIALS AND METHODS

Plant material

The leaves of two specimens were free of any pesticide. *Pachyrhizus erosus* leaves were collected from farmland to San Andres Tlalquitenango, Morelos, México. A voucher specimen was deposited in MEXU-UNAM (138292). At the time of collecting the plants were in bloom, the flowers were of purple color. *Pachyrhizus ferrugineus* leaves were collected in a Deciduous Tropical Forest in the Natural Reserve “La Sepultura”, Villaflores, Chiapas, México. A Voucher was deposited in MEXU-UNAM (1060890). The plants were collected in the core area of the reserve (wild plants). At the time of collecting the plants had mature fruits, which the contained of 6-8 seeds. The specimens were identified and authenticated by one of us, Alberto Reyes-Garcia from the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM, México).

Collection of VOCs blend by HS-SPME

To identify and quantify the volatile compounds (VOCs) in *P. erosus* and *P. ferrugineus* leaves, the headspace HS-SPME technique was employed. Volatiles were collected twice (N = 2). For this study, the temperature was maintained at 18 ± 1 °C. The VOCs slurry was prepared by blending freshly leaves (5.0 g), 20% sodium chloride solution (2 mL) in a 20 mL flask with a cap and Teflon-faced silicone rubber septa (Supelco, Co., Bellefonte, PA). The flask was placed on a magnetic stirring plate and stirred at 1100 rpm for 15 m. A SPME fiber (DVB/CARBOXEN/PDMS; Supelco, Co., Bellefonte, PA) was then exposed to the headspace of the slurry for 15 m in a water bath at 45° C. The analytical methodology here used was selected after testing 4 different fibers and previous experiences for VOCs analysis (Torres-González *et al.,* 2015).

Leaf volatiles organic compounds analysis by CG-MS-TOF

The analysis of the volatile compounds (VOCs) of *P. erosus* and *P. ferrugineus* leaves was performed with a gas chromatography coupled to a mass spectrometer (GC-MS). The sample analysis was conducted using a Gas Chromatograph (Agilent 6890N) with helium (grade 5; ultra-high purity) as the carrier gas; the outlet of the column (DB-5, 20 m×0.18 mm×0.18 µm film) was coupled to a Mass Spectrometer (LECO Pegasus 4D). Parameters for electron impact sample ionization were as follows. Ionization chamber temperature: 200° C; interface temperature: 250° C; source temperature: 230° C. The gas chromatographic conditions were as follows: split less injection (1:20) at 300° C; initial oven temperature, 40° C for 1 min, increased at 8° C per minute to 300° C and held for 5 min. Desorption time was 4 min. Mass Analyzer: Time of Flight (TOF); Spectral Acquisition: 20 spectra/second; delayed ignition of the filament: 0 min; mass range: 33-400; Ionization chamber temperature: 200° C; calibration compound: perfluoroterbutilamine (PFTBA). To identify the compounds their mass spectra were compared with NIST/EPA/NIH, and reported Kovats Retention Index (KI, **www.pherobase.org** and **www.flavornet**) libraries. The composition was reported as relative percentage of the total peak area. Individual compounds were quantified by calculating the peak area relative to the peak area for the internal standard. A mixture of pure alkanes standards C8 to C24 were used for determining the Kovats index.

RESULTS

In *P. erosus* 21 kind of VOCs were found, being the most abundant: cyclohexanone (32.8%), 3-hexen-1-ol (Z) (32.7%), 3-hexenal (Z) (10.5%), furan-2-ethyl (6.4%), and pentanol (6.4%). Together are 88.99% of the VOCs blend. The majoritarian compounds were C6 or C5 derivatives including ketones, aldehydes, and alcohols (Table 1). Eleven kind of VOCs were detected from *P. ferrugineus*, being the most abundant: 5-hexene-1-ol acetate (51.5%), undecanal (22.4%), 2-hepten-1-al (14.5%), and 2-eicosene (*Z*) (4.76). Together represent 93.24% of the VOCs blend. The majoritarian compounds were C6, C7 or C-11 derivatives including ester, and aldehydes (Table 1).

Tabla 1
Volatile organic compounds (VOCs) contained in *P. erosus* (cultivated plant) and *P ferrugineus* (wild plant) leaves.

	Pachyrhizus erosus					*Pachyrhizus ferrugineus*				
Peak	**RT (min)**	**Compound**	**KIc**	**KIr**	**Abun (%)**	**RT (min)**	**Compound**	**KIc**	**KIr**	**Abun (%)**
1	0.12	Pentanal	733.9	732	1.66	4.11	2-hepten-1-al	946.5	951	14.57
2	0.33	3-methyl-1-butanol	736.0	736	1.66	5.73	5-Hexene-1-ol, acetate	1092.1	1070	51.50
3	0.58	Pentanol	759.4	759	6.43	7.56	Undecanal	1317.6	1310	22.41
4	1.01	Furan 2-ethyl	761.7	730	6.44	8.19	2-dodecanone	1406.3	1401	0.06
5	2.01	3-hexenal, (Z)-	819.1	800	10.57	9.13	2H-Pyran-2-one, tetrahydro-6-pentyl-	1558.1	1525	1.24
6	3.44	Ciclohexanone	901.0	896	32.84	9.99	1-Heptadecene	1709.1	1696	0.95
7	3.47	3-Hexen-1-ol, (Z)-	903.1	882	32.71	10.54	4,14-dimethylheptadecane	1816.4	1816	0.87
|8	3.589	(E,E)-2,4-Hexadienal	910.4	910	0.07	11.22	n-Hexadecanoic acid	1953.3	1951	0.80

9	3.91	Heptanal	932.8	912	0.23	11.57	2-eicosene (Z)	2023.8	2022	4.76
10	4.18	2 heptenal	951.2	951	3.84	11.59	5-Ethylnonadecane	2027.7	2034	0.73
11	4.99	(E,E)-2-4 heptadienal	1009.5	1009	1.96	12.20	Methyl stearate	2161.5	2139	2.11
12	5.21	(E)-3-octen-2-one	1034.1	1034	0.51					
13	5.41	(E)-2-octenal	1056.5	1056	0.04					
14	5.56	1-octanol	1072.7	1072	0.06					
15	6.2	Hexanoic acid, 2-methylpropyl ester	1149.3	1149	0.18					
16	7.14	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	1259.9	1258	0.08					
17	8.09	2-Methylpropanoic acid 3-hydroxy-2,4,4-trimethylpentyl ester	1390.7	1380	0.20					
18	8.25	Unknown	1416.5	ND	0.28					
19	8.57	(E)-β-Ionone sesquiterpene	1467.0	1467	0.06					
20	8.93	3,5,9-Undecatrien-2-one, 6,10-dimethyl-terpene	1526.0	1527	0.10					
21	9.53	2-Methylpropanoic acid, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	1626.4	1594	0.08					
		Total area (%)			100		Total area (%)			100
		Identified total area (%)			99.72		Identified total area (%)			100
		Identified compounds (%)			95.23		Identified compounds (%)			100

KIc: Calculated Kovats retention index;
KIr: Reported Kovats retention Index;
ND: Not determinated. RT: retention time

DISCUSION

It is well known that plants release different VOCs blends according if they belong to the wild or cultivated types (Gols *et al.,* 2011); as well as, if they are attacked or not by herbivores (Kigathi *et al.,* 2009) or are under drought stress Copolovici *et al.,* 2014). Several authors have reported losses of VOCs in cultivated as compared to wild ancestors, for instance in the case of cranberry (*Vaccinium macrocarpon*), caterpillars performed best on the highest-yielding variety which has low induction of volatile sesquiterpenes; therefore it has proposed that breeding in cranberry has compromised plant defenses (Rodríguez *et al*., 2011). Gols *et al.* (2011) have reported not only differences among wild and cultivated cabbages (*Brassica oleracea*), but also that the parasitoid *Cotesia rubecula* and specialized on hosts feeding on brassicaceous plants, was differentially attracted and preferred wild types over cultivated cabbage. VOCs are emitted as a response to insect egg deposition and it also been postulated that their content may drastically diminish as a consequence of crop breeding in mayze (Tamiru *et al.,* 2011). It has also been reported that communication among plants via VOCs is a defense strategy to herbivores (Muroi *et al.,* 2011); thus, plants can reduce the emission of VOCs blend when growing together as an strategy to reduce herbivory attack (Kigathi *et al.,* 2013).

Differences between *P. erosus and P. ferrugineus* VOCs blend, may be due, in first instance that are different species, and second term that are cultivated and wild plants, respectively. It is interesting that *P. erosus* (cultivated) leaves release more VOCs (21) than the wild species *P. ferrugineus* (11). Regarding to the type of VOCs in the species of *Pachrhyzus* here studied, both contain mainly aldehydes, ketones, and alcohols. Only *P. erosus* leaves contained terpenoids such as (E)-β-ionone. Several compounds, such as 3-hexenal (Z), undecanal and 3-hexen-1-ol (Z) have been reported as common constituents of many other plant species (Srinivasa *et al.,* 1989; Kulkarni *et al*., 1998; Kaul *et al*., 1999; Asuming *et al.,* 2005; Rohloff & Bones, 2005; Tayoub *et al.,* 2006). 3-Hexen-1-ol is part of the chemoattractant blend of VOCs to insect natural enemies, when certain plants are invaded or ovopositated by herbivores (Warthen *et al.,* 1997; Han & Chen, 2002; Mumm *et al.,* 2004). Some of the compounds found here in low amounts have been reported to possess insecticidal and antifeedant activity; for instance (E)-β-Ionone Gruber *et al.,* 2009; (Alarcón *et al.,* 2013; Céspedes *et al.,* 2013; Muñoz *et al.,* 2013; Céspedes *et al.,* 2014). Ciclohexanone have only found to date in mango cultivars (Pino *et al*., 2005).

The present research provides the first approach to the study of the role of VOCs blends in *P. erosus* and *P. ferrugineus*. Until now, it has only been described that *P. erosus* seeds contains isoflavonoids with insecticidal activity (Bejar *et al.,* 2000), for instance rotenone (Alavez *et al.,* 1998, Estrella-Parra *et al.,* 2014); however it is unknown if rotenone and other insecticidal isoflavonoids are also synthesized in *P. erosus*, and *P. ferrugineus* leaves.

CONCLUSIONS

This is the first study about the composition of VOCs from *P. erosus* and *P. ferrugineus* leaves and improves the knowledge of the genus *Pachyrhizus*. The VOCs blend from the wild (*P. ferrugineus*) and cultivated plant species (*P. erosus*) showed different composition. *Pachyrhizus erosus* leaves released more VOCs that *P. ferrugineus* leaves. Both species contain ketones, aldehydes, alcohols, and esters.

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