Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias

Universidad Miguel Hernández de Elche

Tesis Doctoral

Valorización de plantas comestibles silvestres: Estudio de sus propiedades químicas, funcionales y sensoriales

Jesús Clemente Villalba

– 2024 –

Director: **Dr. Ángel A. Carbonell Barrachina**

Codirectora: **Dra. Francisca Hernández García**

La presente Tesis Doctoral, titulada **"Valorización de plantas comestibles silvestres: Estudio de sus propiedades químicas, funcionales y sensoriales"**, se presenta bajo la modalidad de **tesis por compendio** de las siguientes **publicaciones**:

1. **Clemente-Villalba, J.**, Burló, F., Hernández, F., & Carbonell-Barrachina, Á. A. (2023). Valorization of Wild Edible Plants as Food Ingredients and Their Economic Value. *Foods*, 12(5), 1012. https://doi.org/10.3390/foods12051012

2. **Clemente-Villalba, J.**, Burló, F., Hernández, F., & Carbonell-Barrachina, Á. A. (2024). Potential Interest of *Oxalis pes-caprae* L., a Wild Edible Plant, for the Food and Pharmaceutical Industries. *Foods*, 13(6), 858. https://doi.org/10.3390/foods13060858

3. **Clemente-Villalba, J.**, Ariza, D., García-Garví, J. M., Sánchez-Bravo, P., Noguera-Artiaga, L., Issa-Issa, H., Hernández, F., & Carbonell-Barrachina, Á. A. (2020). Characterization and potential use of *Diplotaxis erucoides* as food ingredient for a sustainable modern cuisine and comparison with commercial mustards and wasabis. *European Food Research and Technology*, 246(7), 1429-1438. https://doi.org/10.1007/s00217-020-03501-3

4. **Clemente-Villalba, J.**, Fratianni, A., Issa-Issa, H., Ianiri, G., Hernández, F., Vitone, C., Carbonell-Barrachina, Á. A., & Panfili, G. (2024). *Diplotaxis erucoides* and *Oxalis pes-caprae*: Two Wild Edible Plants as a New and Valuable Source of Carotenoids, Tocols and B1 and B2 Vitamins. Nutrients, 16(14), 2293. https://doi.org/10.3390/nu16142293

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PUBLICACIÓN 1	
Tipo de artículo	Revisión
Título	**Valorization of Wild Edible Plants as Food Ingredients and Their Economic Value**
Autores	Jesús Clemente-Villalba, Francisco Burló, Francisca Hernández, Ángel A. Carbonell-Barrachina
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Revista	Foods
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Categoría JCR	Food Science and Technology
Cuartil	Q1
Ranking	40/173
Factor de impacto	4,700

PUBLICACIÓN 2	
Tipo de Artículo	Artículo Original
Título	**Potential Interest of *Oxalis pes-caprae* L., a Wild Edible Plant, for the Food and Pharmaceutical Industries**
Autores	Jesús Clemente-Villalba, Francisco Burló, Francisca Hernández, Ángel A. Carbonell-Barrachina
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Revista	Foods
Editorial	MDPI
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Categoría JCR	Food Science and Technology
Cuartil	Q1
Ranking	40/173
Factor de impacto	4,700

PUBLICACIÓN 3	
Tipo de artículo	Artículo Original
Título	***Diplotaxis erucoides* and *Oxalis pes-caprae*: Two Wild Edible Plants as a New and Valuable Source of Carotenoids, Tocols and B1 and B2 Vitamins**
Autores	Jesús Clemente-Villalba,Alessandra Fratianni, Hanán Issa-Issa, Giuseppe Ianiri, Francisca Hernández, Caroline Vitone, Ángel A. Carbonell-Barrachina, and Gianfranco Panfili
DOI	https://doi.org/10.3390/nu16142293
Revista	Nutrients
Editorial	MDPI
Fecha de publicación	17 de julio de 2024
Categoría JCR	Nutrition and Dietetics
Cuartil	Q1
Ranking	18/134
Factor de impacto	4,8

PUBLICACIÓN 4	
Tipo de artículo	Artículo Original
Título	**Characterization and potential use of *Diplotaxis erucoides* as food ingredient for a sustainable modern cuisine and comparison with commercial mustards and wasabis**
Autores	Jesús Clemente-Villalba, David Ariza, José Miguel García-Garví, Paola Sánchez-Bravo, Luis Noguera-Artiaga, Hanán Issa-Issa, Francisca Hernández, Ángel A. Carbonell-Barrachina
DOI	https://doi.org/10.1007/s0021 7-020-03501 -3
Revista	European Food Research and Technology
Editorial	Springer
Fecha de publicación	27 de abril de 2020
Categoría JCR	Food Science and Technology
Cuartil	Q2
Ranking	64/143
Factor de impacto	2,998

El Dr. D. Ángel A. Carbonell Barrachina, Catedrático de universidad, director, y **Dña. Francisca Hernández García,** Catedrática de universidad, codirectora de la tesis titulada "**Valorización de plantas comestibles silvestres: Estudio de sus propiedades químicas, funcionales y sensoriales**"

CERTIFICAN:

Que D. Jesús Clemente Villalba, ha realizado bajo nuestra supervisión la Tesis Doctoral titulada **"Valorización de plantas comestibles silvestres: Estudio de sus propiedades químicas, funcionales y sensoriales"**, conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública.

Y para que conste a los efectos oportunos se firma el presente certificado en Orihuela 3 de octubre de 2024.

Firmado:

Dr. Ángel A. Carbonell Barrachina
Director

Dra. Francisca Hernández García
Codirectora

Dr. Dña. Juana Fernández López, Catedrática de Universidad y Coordinadora del Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias (ReTos-AAA) de la Universidad Miguel Hernández de Elche (UMH),

CERTIFICA:

Que la Tesis Doctoral titulada "**Valorización de plantas comestibles silvestres: Estudio de sus propiedades químicas, funcionales y sensoriales**" de la que es autor el titulado en Ingeniería Técnica Agrícola, esp. en Industrias Agrarias y Alimentarias **D. Jesús Clemente Villalba**, ha sido realizada bajo la dirección del **Dr. D. Ángel A. Carbonell Barrachina** y la codirección de la **Dra. Dña. Francisca Hernández García**, actuando como tutor/a de la misma el **Dr. D. Francisco Burló Carbonell**. Considero que la Tesis es conforme, en cuanto a forma y contenido, a los requerimientos del Programa de Doctorado ReTos-AAA, siendo por tanto apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos firmo el presente certificado en Orihuela a 3 de octubre de 2024.

Dra. Dña. Juana Fernández López

Coordinadora del Programa Doctorado ReTos-AAA

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ESTRUCTURA TESIS

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Esta Tesis Doctoral se ha escrito siguiendo el reglamento interno vigente de la Universidad Miguel Hernández de Elche para la presentación de la Tesis Doctoral bajo la modalidad de tesis por compendio de publicaciones. Esta memoria se ha estructurado de la siguiente forma:

- **Resumen y abstract (inglés):** Breve resumen donde se detallan los principales objetivos y los resultados más relevantes.
- **Introducción:** Contextualización del estado del arte de las plantas comestibles silvestres, la importancia de estas plantas en el futuro agroalimentario y el hilo conductor de la Tesis Doctoral.
- **Objetivos:** Se indican el objetivo principal y los objetivos específicos de la investigación.
- **Materiales y métodos:** Se resumen y referencian las plantas comestibles silvestres utilizadas y las muestras comerciales adquiridas. Se detallan los métodos utilizados en las determinaciones llevadas a cabo, así como para el tratamiento de datos.
- **Resultados y discusión:** En esta sección se hace un breve resumen de los principales resultados obtenidos, relacionando cada resultado mostrado con el objetivo específico de la tesis y su publicación correspondiente.
- **Conclusiones y conclusions (inglés):** Se enumeran las principales conclusiones de la Tesis Doctoral.
- **Futuras investigaciones:** En esta sección se estipulan los objetivos y estudios futuros sobre plantas comestibles silvestres.
- **Referencias:** En esta sección se indican las referencias utilizadas para la redacción y justificación de la Tesis Doctoral.
- **Publicaciones científicas:** Se presenta la transcripción literal de las publicaciones científicas incluidas en esta Tesis:

1. "Valorization of Wild Edible Plants as Food Ingredients and Their Economic Value". https://doi.org/10.3390/foods12051012
2. "Potential Interest of *Oxalis pes-caprae* L., a Wild Edible Plant, for the Food and Pharmaceutical Industries". https://doi.org/10.3390/foods13060858

3. "*Diplotaxis erucoides* and *Oxalis pes-caprae*: two wild edible plants as a new and valuable source of carotenoids, tocols and B1 and B2 vitamins". https://doi.org/10.3390/nu16142293
4. "Characterization and potential use of *Diplotaxis erucoides* as food ingredient for a sustainable modern cuisine and comparison with commercial mustards and wasabis". https://doi.org/10.1007/s00217-020-03501-3

RESUMEN

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La superpoblación, el cambio climático, las guerras o el aumento de precios en los alimentos, son factores determinantes para buscar nuevas fuentes de alimentos debido a que en un futuro próximo habrá escasez alimentaria. Una de las alternativas más plausible son las plantas comestibles silvestres. Estas plantas crecen sin necesidad de la ayuda humana, por lo tanto, podrían ser una fuente sostenible para enmendar la situación que esté por llegar. Dentro de las plantas comestibles silvestres, hay dos de ellas que predominan en el clima mediterráneo, *Diplotaxis erucoides* y *Oxalis pes-caprae*. Aparte de la peculiaridad de ser plantas comestibles silvestres, sensorialmente la *Diplotaxis erucoides* tiene sabor picante, muy parecido al wasabi o la mostaza; por parte de la *Oxalis pes-caprae*, su sabor es ácido debido al ácido oxálico que contiene. Sin embargo, actualmente, estas plantas están infravaloradas y suelen ser retiradas de los campos sin cultivar o de las orillas de las carreteras donde crecen. Como antiguamente, se utilizaban para condimentar los platos, la hipótesis de partida era que posiblemente, lo que se llama "malas hierbas" probablemente no lo sea, y puedan tener beneficios para la salud.

Por lo tanto, el objetivo general de esta Tesis Doctoral fue determinar las propiedades químicas, funcionales y sensoriales de dos plantas comestibles silvestres (*Diplotaxis erucoides* DC. y *Oxalis pes-caprae* L.) con la finalidad de poner en valor su uso para la alimentación humana.

Para alcanzar este objetivo general, se plantearon los siguientes objetivos específicos:

- Objetivo 1: Caracterización química (caracterización proximal, azúcares, ácidos orgánicos, minerales, aminoácidos, perfil de ácidos grasos, perfil de compuestos volátiles).
- Objetivo 2: Caracterización funcional (perfil carotenoides, tocoles, tiamina (B1) y riboflavina (B2)).
- Objetivo 3: Desarrollo de léxico sensorial y estudio sensorial descriptivo por un panel experto.
- Objetivo 4: Innovación alimentaria a través de las plantas comestibles silvestres, desarrollando un producto.

Los análisis fueron realizados a tres partes de cada planta para conocer si había diferencias en la composición, por lo tanto, en *Diplotaxis erucoides* las partes seleccionadas fueron las vainas, las hojas y los tallos; y en *Oxalis pes-caprae* fueron las flores, las hojas y los tallos.

Los resultados obtenidos en ambas plantas confirmaron la hipótesis de partida de la tesis doctoral debido a los datos que arrojaron los análisis. La composición de *Oxalis pes-caprae* presentó azúcares como fructosa, glucosa o sacarosa. También, siete minerales diferentes, entre ellos, calcio, potasio, hierro, zinc. Respecto a los aminoácidos, destacaron la leucina, isoleucina, alanina y el ácido glutámico. Los ácidos grasos más representativos en *Oxalis pes-caprae* fueron los ácidos linoleico y linolénico, representando en algunas partes más del 50 % de la concentración de ácidos grasos.

Los resultados mostrados por ambas plantas respecto al perfil de carotenoides fueron coincidentes, siendo los carotenoides más destacados la luteína y el β-caroteno. En el perfil de tocoles, también fueron coincidentes en resultados, siendo por concentración el α-tocoferol el más destacado tanto en *Diplotaxis erucoides* como en *Oxalis pes-caprae*.

Por otro lado, el contenido de vitamina A y E es tan alto que supera el 15% de la cantidad diaria recomendada, dando esto lugar a poder ser considerados legalmente como "fuente natural de vitamina A y E". Aparte de estas vitaminas, también se encontró concentraciones de vitaminas B1(tiamina) y B2 (riboflavina) en ambas plantas.

Las principales conclusiones extraídas tras los resultados fueron, por parte de *Oxalis pes-caprae*, el importante aporte de minerales, azúcares, aminoácidos y ácidos grasos poliinsaturados mostrando una evidencia sobre la importancia nutricional de esta planta. Es posible afirmar que ambas plantas (*Diplotaxis erucoides* y *Oxalis pes-caprae*) son fuente natural de vitaminas A y E. *Diplotaxis erucoides* y *Oxalis pes-caprae* podrían ser consideradas en el futuro como nuevos alimentos, ingredientes o aprovechar los compuestos bioactivos para el beneficio de la salud humana.

ABSTRACT

Overpopulation, climate change, wars or increased food prices are determining factors for seeking new sources of food because in the near future there will be food shortages. One of the most plausible alternatives is wild edible plants. These plants grow without the need for human help; therefore, they could be a sustainable source to amend the situation that is to come. Among the wild edible plants, there are two of them that predominate in the Mediterranean climate, *Diplotaxis erucoides* and *Oxalis pes-caprae*. Apart from the peculiarity of being wild edible plants, *Diplotaxis erucoides* has a spicy flavor, very similar to wasabi or mustard; on the part of *Oxalis pes-caprae*, its flavor is acidic due to the oxalic acid it contains. However, these plants are currently undervalued and are often removed from the uncultivated fields where they grow or from roadsides. As in the past, they were used to season dishes, the starting hypothesis was that possibly, what are called "weeds" probably are not, and may have health benefits.

Therefore, the aim of this Doctoral Thesis was to determine the chemical, functional and sensory properties of two wild edible plants (*Diplotaxis erucoides* DC. and *Oxalis pes-caprae* L.) in order to value their use for food. human.

To achieve this aim, the following specific objectives were set:

- Objective 1: Chemical characterization (proximal characterization, sugars, organic acids, minerals, amino acids, fatty acid profile, volatile compound profile).
- Objective 2: Functional characterization (carotenoid profile, tocols, thiamine (B1) and riboflavin (B2)).
- Objective 3: Development of sensory lexicon and descriptive sensory study by an expert panel.
- Objective 4: Food innovation through wild edible plants, developing a product.

The analyzes were carried out on three parts of each plant to find out if there were differences within the plants themselves, therefore, in *Diplotaxis erucoides* the selected parts were the pods, leaves and stems; and in *Oxalis pes-caprae* they were the flowers, leaves and stems.

The analyzes were carried out on three parts of each plant to find out if there were differences in the composition, therefore, in *Diplotaxis erucoides* the selected parts

were the pods, leaves and stems; and in *Oxalis pes-caprae* they were the flowers, leaves and stems.

The results obtained in both plants confirmed the starting hypothesis of the doctoral thesis due to the data that the analyzes yielded. The composition of *Oxalis pes-caprae* showed sugars such as fructose, glucose or sucrose. Also, seven different minerals such as calcium, potassium, iron, zinc, among others. Regarding amino acids, leucine, isoleucine, alanine and glutamic acid stood out. The most representative fatty acids in *Oxalis pes-caprae* were linoleic and linolenic acids, representing in some parts more than 50% of the fatty acid concentration.

The results shown by both plants regarding the carotenoid profile were coincident, with the most prominent carotenoids being lutein and β-carotene. In the tocol profile, the results were also coincident, with α-Tocopherol being the most prominent by concentration in both *Diplotaxis erucoides* and *Oxalis pes-caprae*.

On the other hand, the content of vitamin A and E is so high that it exceeds 15% of the recommended daily amount, giving rise to it being legally considered a "natural source of vitamin A and E". In addition to these vitamins, concentrations of vitamins B1 (thiamine) and B2 (riboflavin) were also found in both plants.

The main conclusions drawn after the results were, on the part of *Oxalis* pes-caprae, the important contribution of minerals, sugars, amino acids and polyunsaturated fatty acids, showing evidence of the nutritional importance of this plant. It is possible to affirm that both plants (*Diplotaxis erucoides* and *Oxalis pes-caprae*) are natural sources of vitamins A and E. *Diplotaxis erucoides* and *Oxalis pes-caprae* could be considered in the future as new foods, ingredients or taking advantage of bioactive compounds for the benefit of human health.

1. INTRODUCCIÓN

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1.1. Mundo actual y sostenibilidad

Actualmente, la población mundial ha sobrepasado los 8.000 millones de personas, y se estima que en 2050 se alcance los 9.700 millones, e incluso que se superen los 10.000 millones en 2085 (ONU, 2022). Según la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO), hambre es "la sensación física incómoda o dolorosa, causada por un consumo insuficiente de energía alimentaria", sin embargo, hay un término más alarmante, la inseguridad alimentaria, que también ha sido definido por la FAO como, "la persona que carece de acceso regular a suficientes alimentos inocuos y nutritivos para un crecimiento y desarrollo normales, y para llevar una vida activa y saludable" (FAO, 2022a). Las cifras estimativas de la FAO en 2022 indicaban que alrededor del mundo había 731 millones de personas que habían sufrido inseguridad alimentaria (FAO, 2022a). Según el Programa Mundial de Alimentos (PMA) las causas de esta situación son diversas, en primer lugar, los conflictos como la violencia o las guerras, supone la causa principal del 70 % de las personas que pasan hambre; seguido de otra causa fundamental como es la crisis climática, debido a la reducción de superficie de cultivo y consecuentemente la pérdida de forma de vida de muchas personas. Si estas dos principales causas no fueran suficientes, además se añade, el aumento de los precios como los fertilizantes o el gas natural, que ha provocado el aumento del precio de los alimentos, una reducción de las exportaciones y una reducción en la producción de cosechas tan importantes como arroz, soja, maíz o trigo a nivel mundial (PMA, 2022).

A parte de estos datos, se añade que en todo el mundo el volumen de desperdicio alimentario por país no deja de crecer, por ejemplo, anualmente el desperdicio en la Unión Europea es de 88 millones de toneladas de alimento, lo que supone un gasto económico de 143 mil millones. Sin embargo, la situación en Asia no es mejor, siendo las cifras más alarmantes que en la Unión Europea; por ejemplo, en Indonesia el rango de desperdicio oscila entre los 23 y los 48 millones de toneladas anuales. Estas cifras suponen que un solo país supera más del 50 % de desperdicio que toda la Unión Europea en conjunto (Phonthanukitithaworn et al., 2024).

Por lo tanto, la situación actual corrobora la importancia de iniciativas como los Objetivos de Desarrollo Sostenible (ODS) por parte de la ONU, aprobada por todos los

estados miembros en el año 2015. Éste es un plan global basado en 17 objetivos que deberían cumplirse en el año 2030, algunos de ellos tan importantes, como el Fin de la pobreza, Hambre Cero, Educación de calidad o Igualdad de género, entre otros (ONU, 2015). Con la mirada puesta en estas iniciativas, se ha iniciado la búsqueda de fuentes alternativas alimentarias que ayuden a paliar la escasez de alimentos. Una de las alternativas que ha cobrado más fuerza, obteniendo el respaldo de la FAO, es el consumo de insectos comestibles. Estos insectos aportan proteínas, minerales y vitaminas, aparte de ser ecológicamente sostenibles y ofrecer oportunidades económicas a los productores, cumpliendo además con 3 ODS, como serían Hambre Cero (ODS 2), Industria, Innovación e Infraestructura (ODS 9) y Vida de Ecosistemas Terrestres (ODS 15) (FAO, 2022b). Pero no es la única opción que se plantea cara al futuro, también se contempla la carne cultivada en laboratorio, hongos, microalgas o uno de los más emergentes como son las Plantas Comestibles Silvestres (PCS). El futuro depara a la sociedad retos nuevos que se deben solucionar urgentemente de forma sostenible y eficiente, siendo estas alternativas planteadas y otras que vendrán posteriormente, un factor primordial para devolver el equilibrio tanto a la naturaleza como a la sociedad.

1.2. Plantas Comestibles Silvestres (PCS)

El concepto de Plantas Comestibles Silvestres (PCS) proviene de otro global denominado como "Alimento silvestre" que es un término que define desde comer carne de animales silvestres (caza), hasta comer este tipo de plantas. Respecto a este último término, ya en 1999, la FAO describió el término plantas silvestres como “aquellas que crecen espontáneamente en poblaciones automantenidas en ecosistemas naturales o seminaturales y pueden existir independientemente de la acción humana directa” (Heywood, 1999). En tiempos pasados (por ejemplo, en la década de 1960), en Europa, el uso de PCS en la dieta estaba muy extendido debido al estilo de vida de esa época; la agricultura, ganadería o pastoreo fueron actividades que permitieron a la población estar más en contacto directo con la naturaleza, conocer mejor las plantas y saber cuáles de ellas eran comestibles (Łuczaj et al., 2012). La FAO ya estimó en 2016 que alrededor de 100 millones de personas consumieron plantas comestibles silvestres en Europa (Bacchetta et al., 2016); esta cifra pone de relieve el potencial de estas plantas incluso en la actualidad. Hoy día es el sustento para muchas familias debido a que estas plantas son

su medio de vida. Un ejemplo de ello, es el estudio realizado por Mokria et al. (2022), donde se destaca la necesidad de promover planes estratégicos a nivel nacional en Etiopía para el uso sostenible y domesticación de estas plantas. Esto tendría como objetivo la mejora socioeconómica y ayudar a alcanzar una de las metas de los ODS (ODS-2, "Poner fin al hambre y la malnutrición").

Sin embargo, este tipo de plantas no solamente se consumen actualmente, sino que se remonta a la Edad del Bronce como lo demuestran los restos encontrados en un yacimiento de Peñalosa (Jaén, España); en este lugar se identificaron más de 50 especies, entre ellas *Rumex* sp. o *Calendula* sp. La conclusión de este estudio etnobotánico fue que estas plantas se utilizaban como alimento y/o como aditivos aromatizantes (Peña-Chocarro, 2000).

La importancia de estas plantas es evidente a partir de múltiples estudios realizados en todo el mundo, como en Brasil (da Silva et al., 2020), China (Tai et al., 2011), Etiopía (Berihun & Molla, 2017), Guatemala (Turreira-García et al., 2015), India (Dewanjee et al., 2013), Japón (Chen & Qiu, 2012) o Túnez (Salah et al., 2015), entre otros. En Europa, Schulp et al. (2014) escribió una revisión sobre la identificación de PCS en toda Europa, encontrándolas en 17 países. De hecho, hay muchos estudios centrados en la región mediterránea debido a la gran diversidad de este tipo de plantas, especialmente en España, Grecia, Italia y Portugal.

Las familias de plantas con mayor número de PCS dentro de ellas son Asteraceae, Brassicaceae, Fabaceae, Portulacaceae, Oxalidaceae o Polygonaceae, entre otras (Iqbal et al., 2022; Sánchez-Mata & Tardío, 2016).

1.3. ¿Qué son malas hierbas?

Actualmente, a muchas especies de PCS se les denomina "malas hierbas". Este concepto se define como: "planta que crece en un lugar donde no se desea que crezca. Generalmente este concepto se aplica a las especies que crecen en los cultivos". Las "malas hierbas" se caracterizan por su competitividad, su resistencia y su alta capacidad de dispersión, debido a ello, disminuyen el rendimiento de cultivos y dificultan procesos como el cosechado o interfieren en las canalizaciones del agua (Universidad de Navarra, 2023).

1.4. ¿Qué son las especies exóticas invasoras?

El concepto anteriormente mencionado de "mala hierba" y el de especie exótica invasora en muchos casos se relacionan estrechamente, aunque una "mala hierba" no siempre debe ser considerada como especie invasora (Universidad de Navarra, 2023). La legislación española, a través del Real Decreto 623/2013, define las especies exóticas invasoras como: "especie exótica que se introduce o establece en un ecosistema o hábitat natural o seminatural, y que es un agente de cambio y amenaza para la diversidad biológica nativa, ya sea por su comportamiento invasor, o por el riesgo de contaminación genética" (MAPA, 2013). En este mismo real decreto existe un anexo en el cual se enumeran diferentes especies las que cuales se consideran invasoras y algunas de ellas son plantas comestibles silvestres.

1.5. Propiedades beneficiosas para la salud de las PCS

Como anteriormente se ha mencionado, algunas de las plantas comestibles silvestres podrán ser consideradas "malas hierbas" o plantas invasoras, pero independientemente de estos términos, lo que está fuera de toda duda son los beneficios para la salud que aportan muchas de ellas; por ejemplo, *Blumea lacera* DC., *Enhydra fluctuans* Lour., *Erythrina variegata* L., *Sonchus asper* L. o *Sonchus oleraceus* L., entre otras, destacan por su contenido en proteínas, fibra y carbohidratos (Alam et al., 2020; Datta et al., 2019; Guerrero et al., 1999). También el contenido en minerales como el calcio, azúcares como fructosa o glucosa, y ácidos orgánicos como el ácido oxálico fueron significativos en los casos de *Foeniculum vulgare*, *Chondrilla juncea*, *Malva sylvestris o Sonchus oleraceus* (Barros et al., 2010; García-Herrera, Sánchez-Mata, et al., 2014; Petropoulos et al., 2019; Trichopoulou et al., 2000). Además de estos aportes, existen numerosos estudios sobre el poder antioxidante y antifúngico de muchas plantas comestibles silvestres de diversos géneros y situaciones geográficas (Datta et al., 2019; El-Desouky, 2021; García-Herrera, Morales, et al., 2014; Martins et al., 2011; Sanchez-Bel et al., 2015; Trichopoulou et al., 2000). También el poder antiinflamatorio y antidiabético de plantas como *Berberis tinctoria* Lesch (Vignesh et al., 2021), o, incluso el extracto acuoso de *Phlogacanthus thyrsiformis* que suprimió el daño oxidativo al ADN de los linfocitos (Seal et al., 2022).

1.6. Impacto de la valorización económica y social de las PCS

Hoy en día un aspecto fundamental en la sociedad es la vinculación entre economía y sostenibilidad. Por ello, se debe resaltar y estudiar el valor económico de las PCS, en los que las flores comestibles pueden jugar un papel clave; se pueden preparar para su venta en diferentes formatos, como frescos, secos, o incluso confitados. Esta variabilidad junto con el color de estas flores las hace muy atractivas y una buena propuesta de negocio dentro del mercado de las flores. La globalización y el marketing online han creado un mercado emergente para este tipo de productos a nivel mundial. La venta de estas flores suele ser en canastillas de entre 6 y 15 flores, según el tipo de flor y la temporada. El precio de estas canastillas también depende del tipo de flor, pero suele oscilar entre los 8 y los 17 euros, llegando algunas de ellas a aproximadamente los 40 euros (Fine Food Specialist Limited, 2020; Flower Girl NYC, 2021; Innoflower, 2021; Petite Ingredient, 2021). Aparte de esta forma directa de venta de flores comestibles, también existen piruletas con flores comestibles en su interior y cristalizadas. El rango de precios oscila entre 21 y 60 euros (Innoflower, 2021).

Otro sector que en los últimos años se ha interesado y ha valorado positivamente este tipo de flores y plantas es la alta cocina, ya sea como decoración en platos, o en busca de nuevos sabores, aromas o apariencia. Uno de los mejores restaurantes del mundo es "Mugaritz" con dos estrellas Michelín y dirigido por Andoni Luis Aduriz; este restaurante ha sido innovador y pionero a la hora de basar sus platos en plantas silvestres, utilizando también flores comestibles.

A parte de la incorporación en platos a alto nivel, existen otras formas en las que estas plantas aportan su valor económico, siendo a través de la divulgación mediante charlas o libros publicados. En el año 2004 se publicó el libro "Clorofilia" de Andoni Luis Aduriz, donde se recopiló información sobre 50 plantas silvestres, su calendario de floración, información taxonómica y recetas (Andoni Luis Aduriz, 2004). El Basque Culinary Center también publicó otro libro informativo sobre plantas y hierbas silvestres, que contiene información detallada sobre 180 variedades de plantas y hierbas silvestres desde un punto de vista botánico y culinario (Basque Culinary Center, 2022).

Otro ejemplo de la importancia económica de estas plantas, surge en el noreste de la India, las PCS son fundamentales para la supervivencia de las comunidades étnicas. Se realizó una encuesta entre 30 vendedores locales y 550 hogares. Los resultados fueron contundentes, registrándose, en consumo o venta, cinco hongos silvestres comestibles y 158 plantas silvestres (el 78,8 % de ellas comestibles). En la mayoría de los hogares, las plantas silvestres influyeron en los ingresos familiares y representaron entre el 5 y el 75 % de los ingresos familiares. Todos estos resultados demostraron claramente la importancia de estas plantas silvestres para la subsistencia y supervivencia de muchas comunidades rurales (Chaudhury et al., 2021).

Pero estas plantas no sólo son importantes en las comunidades étnicas, sino que el estudio realizado por Matsuura (2021) demostró su importancia para la población japonesa. Este estudio indicó que, en las zonas rurales cercanas a Fukushima, no es posible cosechar PCS u hongos comestibles, lo que impide una dieta completa repleta de todos los productos tradicionales esenciales. Los resultados concluyeron que la tasa de recogida debería permanecer muy baja durante unos años en un radio de entre 12 y 30 km de Fukushima (pueblo de Kawauchi, donde se realizó el estudio) por razones de seguridad. Estos datos muestran la importancia que tienen en estas comunidades para su sustento.

Con todos estos datos queda patente la importancia económica y social de las PCS, siendo esenciales para la supervivencia de muchas familias en todo el mundo.

1.7. Impacto medioambiental de las PCS

El impacto medioambiental de estas plantas, va ligado al impacto económico y social previamente mencionado. La opción en un futuro de considerar a estas plantas dentro del engranaje agroalimentario potenciaría la utilización de lo que actualmente se considera un residuo. Estas plantas legislativamente no pueden ser consideradas subproductos, debido a que deberían cumplir de forma simultánea estas cuatro premisas:

"1. Que se tenga la seguridad de que la sustancia u objeto va a ser utilizado ulteriormente,

2. Que la sustancia u objeto se pueda utilizar directamente sin tener que someterse a una transformación ulterior distinta de la práctica industrial habitual,

3. Que la sustancia u objeto se produzca como parte integrante de un proceso de producción, y

4. Que el uso ulterior cumpla todos los requisitos pertinentes relativos al producto, así como a la protección de la salud humana y del medio ambiente, sin que produzca impactos generales adversos para la salud humana o el medio ambiente." (MITECO, 2022).

Aunque estas circunstancias puedan cambiar en el futuro, por cambios legislativos, o por una domesticación de estas plantas, convirtiéndolas en cultivos como tal, hoy en día, sí pueden ser aprovechadas con fines regenerativos de suelo dañado o compostaje. En el Anexo II de la "Ley 7/2022, de residuos y suelos contaminados para una economía circular", se especifica en las operaciones de valorización, concretamente las R03, "R0301 Compostaje. Instalaciones de compostaje de biorresiduos y otros residuos compostables recogidos separadamente", y las R10 ("Tratamiento de suelos que produzca un beneficio a la agricultura o una mejora ecológica a los mismos"), R1001 "Valorización de residuos en suelos agrícolas y en jardinería" y R1002 "Valorización de residuos para la restauración de suelos degradados" (MITECO, 2022).

Este beneficio medioambiental de regeneración de suelos impacta directamente en el económico y el social, haciendo que la persona encargada de un cultivo no tenga que realizar un desembolso económico para mejorar el suelo de cultivo, y, por lo tanto, tener un sustento familiar con el crecimiento del cultivo en ese periodo del año.

1.8. *Diplotaxis erucoides* y *Oxalis pes-caprae*

Como se comentó en apartados anteriores, la presencia de las plantas comestibles silvestres en el arco mediterráneo es bastante destacado. Existen dos PCS con una representación muy importante en el clima mediterráneo, y que están extendidas en diversos países con este clima como se puede observar en la **Figura 1**. Estas plantas comestibles silvestres son, *Diplotaxis erucoides* DC. y *Oxalis pes-caprae* L.

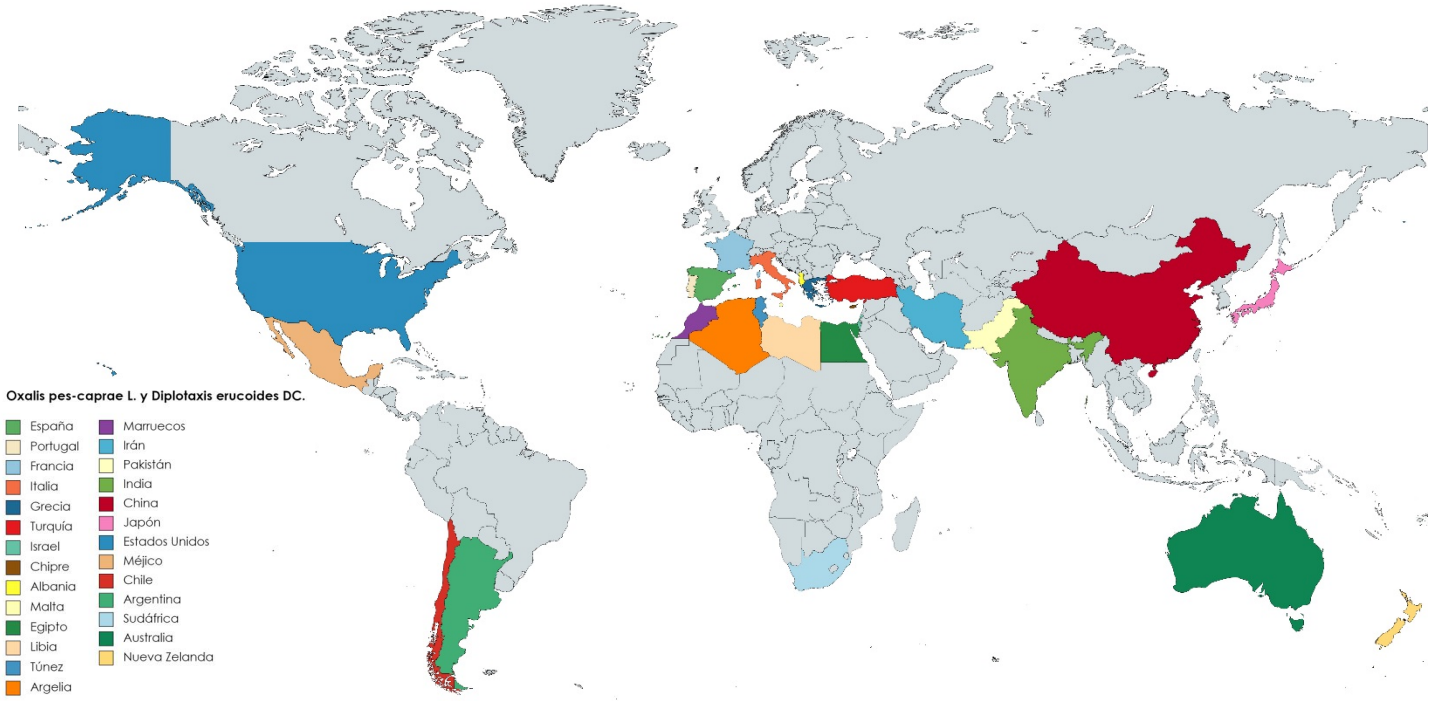

Figura 1. Países con presencia de *Diplotaxis erucoides* y *Oxalis pes-caprae.* Fuente: Elaboración propia.

1.8.1. *Diplotaxis erucoides*

Diplotaxis erucoides DC. (Brassicaceae), siendo su nombre botánico procedente de "diplo" que significa doble y "taxis" que significa orden, esta denominación es debida a que las semillas están dispuestas en orden biserial en la misma bolsa. Respecto a la palabra "erucoides" procede de la palabra "eruca" que significa oruga, dando referencia a su parecido por los suaves tallos de la planta a los pelos de una oruga (Augustin-Pyramus de Candolle, 1821). Esta planta es comúnmente llamada "rabazina blanca, oruga silvestre, jaramago" o "wasabi mediterráneo", y su distribución es enteramente en el clima mediterráneo **(Figura 2)**.

Figura 2. Ladera de montaña con *Diplotaxis erucoides* DC.

Fuente: Elaboración propia.

Diplotaxis erucoides presenta flores con pétalos blancos o ligeramente violáceos; sépalos muy pelosos, erecto-patentes en la floración. Fruto en silícua con valvas convexas, algo comprimidas, cada una con un nervio bien visible, con semillas en dos filas en cada lóculo, y hojas ovaladas u oblongas agudas, dentadas desigualmente o aserradas **(Figura 3)** (Augustin-Pyramus de Candolle, 1821; Universidad de Navarra, 2023).

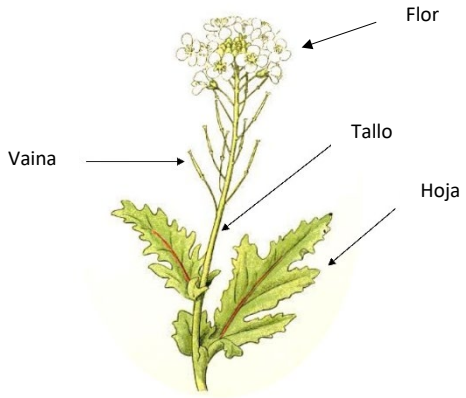

Figura 3. Partes de *Diplotaxis erucoides* DC.

Fuente: Flickr.

La peculiaridad de *Diplotaxis erucoides* es la sensación química "picante" que produce, muy similar a la que se puede notar en la mostaza, el wasabi o el rábano picante debido a que uno de sus componentes responsable de esta sensación (isotiocianatos) es común entre ellas debido a que son todas de la familia Brassicaceae (Bell & Wagstaff, 2019; Tisserand & Young, 2014). Los isotiocianatos son metabolitos secundarios

producidos a partir de glucosinolatos (GSL). Estudios recientes han asociado los GSL con efectos anticancerígenos y antifúngicos, e incluso, ayudan a fortalecer las defensas del organismo y el sistema inmunológico (EFSA, 2011; Traka & Mithen, 2009; Wittstock et al., 2016).

1.8.2. *Oxalis pes-caprae*

Oxalis pes-caprae L. (Oxalidaceae) **(Figura 4)**, siendo su nombre botánico procedente de "Oxys" que en griego significa "afilado, acre" haciendo referencia al sabor ácido de esta planta proveniente del ácido oxálico en su interior (Chuck Griffith, 2019); "pes-caprae" proviene de las palabras latinas "pes" que significa pie, y "caprae" que significa cabra, literalmente sería "pie de cabra", esta denominación es muy probable que provenga por la forma de los foliolos de las hojas (Gaffiot, 1934). Esta planta se conoce comúnmente como "sourgrass"(en inglés) o "vinagrillo, vinagrera o agrio", la cual crece, mayoritariamente, en las zonas de clima mediterráneo o subtropical; aunque esta planta es originaria de Sudáfrica (Costa et al., 2017) y es considerada una especie exótica invasora tipificada en el real decreto 630/2013 por el ministerio de agricultura español (MAPA, 2013).

Figura 4. *Oxalis pes-caprae* L.

Fuente: Elaboración propia.

Oxalis pes-caprae es una geófita bulbífera con flores pentaméricas y pentacíclicas, con cinco sépalos y cinco pétalos fusionados en la base (Signorini et al., 2014). Una de las peculiaridades de *Oxalis pes-caprae* es su sabor ácido debido a su alta concentración del ácido orgánico, ácido oxálico. En cuanto a las propiedades de *Oxalis pes-caprae*, varios estudios reportaron sus propiedades antioxidantes (principalmente por la acción de los polifenoles) y antiinflamatorias, actividad citotóxica y fitotóxica, posibles efectos neuroprotectores, actividad antibacteriana, antifúngica e inhibición de la alfa-amilasa y alfa-glucosidasa; por lo tanto, parece bastante razonable pensar que esta planta puede considerarse una fuente natural de fitoquímicos con potencial aplicación en farmacología (Gaspar et al., 2018; Harumi Iyda et al., 2019; Kabach et al., 2023; Lee et al., 2007; Naila et al., 2020; Nemzer et al., 2020; Vera et al., 2018).

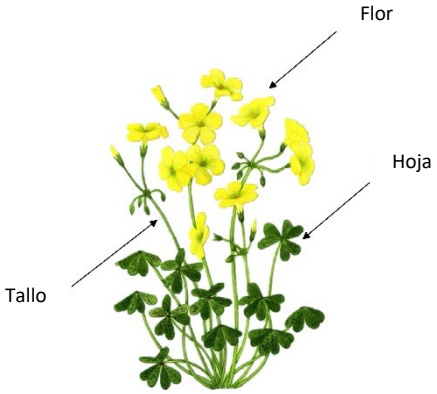

Figura 5. Partes de *Oxalis pes-caprae* L.

Fuente: www.antropocene.it

1.9. Innovación alimentaria

Hoy en día, la innovación alimentaria es una prioridad para la industria agro-alimentaria debido a que los consumidores cada vez son más exigentes con la seguridad alimentaria, sostenibilidad, que el producto sea saludable y que sensorialmente sea aceptado (Bigliardi & Galati, 2013).

Una de las formas más novedosas en la que la industria agroalimentaria está innovando es implementado la Inteligencia Artificial (IA) en sus procesos. Por ejemplo, utilizando una red neuronal sintética para detectar el pH durante el proceso de fermentación de un queso, controlar un proceso de secado evitando secados desiguales en los distintos productos, o detectar instantáneamente una carne podrida de otra en

perfecto estado. Estos avances ahorran energía, evitan el desperdicio alimentario y/o hacen más eficientes los procesos de producción a todos los niveles en la industria agroalimentaria (Nath et al., 2024).

Aunque directamente en los alimentos, la industria alimentaria ha encontrado refugio en su innovación a través de los alimentos funcionales. Estos alimentos fueron ya definidos por primera vez en 1984, en Japón como resultado de un estudio sobre las relaciones entre nutrición, satisfacción sensorial, fortificación y modulación de los sistemas fisiológicos con el fin de definir aquellos productos alimenticios fortificados con componentes especiales que poseen efectos fisiológicos ventajosos; aunque esta definición primaria ha ido cambiando a lo largo de los años, haciéndose cada vez más énfasis en el aspecto de la salud y el bienestar (Bigliardi & Galati, 2013; Vignesh et al., 2021).

Es posible afirmar que, alimentos funcionales podemos encontrar de diversos tipos, como carnes, pescados, cereales, bebidas, vegetales, entre otros; concretamente también pueden encontrarse dentro de lo que denominaríamos hierbas y especias. Ejemplos como la cúrcuma con efectos beneficiosos para la salud, el jengibre con efectos antiinflamatorios o los compuestos bioactivos encontrados en plantas comestibles, evidencian la posibilidad de este tipo de alimento funcional (Azeez & Lunghar, 2021; Kussmann et al., 2023; Vignesh et al., 2021). En esta definición es donde deberían tener cabidas plantas como *Diplotaxis erucoides* u *Oxalis pes-caprae* en un futuro próximo.

2. OBJETIVOS

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El **objetivo general** de esta Tesis Doctoral fue determinar las propiedades químicas, funcionales y sensoriales de dos plantas comestibles silvestres (*Diplotaxis erucoides* DC. y *Oxalis pes-caprae* L.) con la finalidad de poner en valor su uso para la alimentación humana. Los beneficios que se esperan de ambas plantas, contribuirían a mejorar la sostenibilidad, a una mejora nutricional en los seres humanos y a la incorporación futura de nuevas dietas a base de estas plantas.

Para alcanzar este objetivo general, se plantearon los siguientes **objetivos específicos**:

- **Objetivo 1:** Caracterización química (caracterización proximal, azúcares, ácidos orgánicos, minerales, aminoácidos, perfil de ácidos grasos, perfil de compuestos volátiles).
- **Objetivo 2:** Caracterización funcional (perfil carotenoides, tocoles, tiamina (B1) y riboflavina (B2)).
- **Objetivo 3:** Desarrollo de léxico sensorial y estudio sensorial descriptivo por un panel experto.
- **Objetivo 4:** Innovación alimentaria a través de las plantas comestibles silvestres, desarrollando un producto.

3. MATERIALES Y MÉTODOS

3. MATERIALES Y MÉTODOS

3.1. Material vegetal

Las plantas de *Diplotaxis erucoides* DC. y *Oxalis pes-caprae* L. utilizadas en la tesis, fueron recolectadas en el campus de Orihuela de la Universidad Miguel Hernández de Elche (38° 4' 10'' N, 0° 59' 1'' O, Alicante, España), en febrero de 2022. El proceso de recolección de ambas plantas fue manual; las plantas, tras su recolección se llevaron al laboratorio para la separación, de forma manual, de las diferentes partes de la planta. En *Diplotaxis erucoides* se separaron: las vainas, las hojas y los tallos; mientras que de *Oxalis pes-caprae* se separaron: las flores, las hojas y los tallos. Una vez se realizó la separación, se lavaron con agua destilada para posteriormente dejarlas secar a temperatura ambiente. Una vez secas, las diferentes partes fueron congeladas a -80 °C durante 48 horas y posteriormente liofilizadas. Tras la liofilización las muestras fueron trituradas, envasadas al vacío y almacenadas a -20 °C hasta su uso.

3.2. Material comercial

Para el análisis descriptivo, se adquirieron 5 mostazas comerciales de diferentes marcas (M1, M2, M3, M4 y M5) y 3 muestras comerciales de wasabi de diferentes marcas (W1, W2 y W3) en supermercados locales (Alicante, España). A parte de estos productos, se adquirieron de la marca Hacendado® crema batida, leche semi desnatada y aceite de girasol refinado.

3.3. Caracterización proximal

La caracterización proximal fue determinada, analizando los siguientes parámetros: humedad, cenizas, fibra dietética total, grasas y proteínas (método Kjeldahl, utilizando un factor de conversión de 6,25) según la AOAC (AOAC, 1995). Los carbohidratos disponibles se calcularon usando la fórmula:

$$\text{Carbohidratos (\%)} = 100 - (\text{\% humedad} + \text{\% cenizas} + \text{\% grasa} + \text{\% proteína})$$

mientras que la energía o valor calórico total (kJ) se determinó mediante la fórmula en base a una porción de 100 g (AOAC, 1995):

$$\text{Energía (kJ)} = [(\text{\% proteína} \times 4\ \text{kcal/g}) + (\text{\% carbohidratos} \times 4\ \text{kcal/g}) + (\text{\% grasa} \times 9\ \text{kcal/g})] \times 4{,}184$$

3.4. Análisis de Azúcares y Ácidos Orgánicos

Los azúcares y ácidos orgánicos se cuantificaron según Hernández et al. (2016) con algunas modificaciones, se utilizaron 0,150 g de muestra. La determinación del contenido de azúcares y ácidos orgánicos se realizó mediante cromatografía líquida de alta resolución (HPLC-DAD-RID) (serie Hewlett Packard 1100; Willmington, DE, EE. UU.). Una columna SupelcogelTM C-columna 610H (30 cm × 7,8 mm) y una precolumna Supelguard (5 cm × 4,6 mm) (Supelco, Bellefonte, PA, EE. UU.) fueron utilizadas para la separación. La absorbancia se midió utilizando un detector de matriz de diodos (DAD) a 210 nm para la detección de ácidos orgánicos y un detector de índice refracción (RID) para la detección de azúcares. Los estándares de ácidos orgánicos (cítrico, fítico, fumárico, málico, oxálico y tartárico) y azúcares (arabinosa, fructosa, galactosa, glucosa, maltosa y sacarosa) se obtuvieron de Sigma (St. Louis, MO, EE. UU.). Para la cuantificación de ácidos orgánicos y azúcares se utilizaron curvas de calibración, con un rango de concentración entre 1 y 10 g/ L, las cuales mostraron buena linealidad ($r^2 \geq 0,999$). Este análisis fue realizado por triplicado, y los resultados se expresaron como g/ Kg.

Para la extracción del ácido ascórbico se pesaron ~50 mg de las muestras liofilizadas y se disolvió en 1 mL de extractante MeOH:H_2O:HCOOH (75:24:1; v/v/v) usando un baño de ultrasonidos con períodos de 2 a 3 min. Posteriormente, las muestras se centrifugaron a 12.000 × g durante 15 min y se filtró utilizando un filtro de 0,22 μm. Para el análisis de las muestras, se utilizó un equipo de cromatografía líquida de alta resolución (UPLC-QToF-MS) (Agilent, UPLC-QTOF 6550-I-Funnel, Santa Clara, CA, EE. UU.). Las fases móviles utilizadas fueron: Fase móvil A: ácido fórmico acuoso al 0,5%, y Fase móvil B: Metanol/agua (50:50; v/v) que contiene un 0,5% de ácido fórmico.

3.5. Análisis de Minerales

La determinación de minerales se realizó según Cerdá-Bernad et al. (2023) utilizando ~0,100 g de muestras liofilizadas. Las concentraciones totales de macronutrientes (Ca, Mg, Na y K) y micronutrientes (Zn, Cu, Mn y Fe) en las muestras, previamente mineralizadas, se cuantificaron con un espectrómetro de masas de plasma acoplado inductivamente (ICPMS-2030, Shimadzu, Kioto, Japón).

3.6. Análisis de aminoácidos

Los aminoácidos se cuantificaron según Kıvrak et al. (2014) con algunas modificaciones. Aproximadamente 100 mg de cada muestra fueron colocados en un tubo que contenía 1 mL de ácido fórmico al 0,1 % (v/v) en una solución de agua y metanol (80:20) (v/v). Posteriormente, la muestra se inyectó en un cromatógrafo líquido de alta resolución (UPLC-QToF-MS) (Agilent, UPLC-QTOF 6550-I-Funnel, Santa Clara, CA, EE. UU.), con las mismas fases móviles utilizadas para el análisis de ácido ascórbico. Se compraron todos los estándares (arginina, alanina, asparagina, ácido aspártico, cisteína, cistina, fenilalanina, glicina, glutamina, ácido glutámico, histidina, isoleucina, leucina, lisina, metionina, prolina, serina, treonina, tirosina, triptófano y valina) de la marca Sigma-Aldrich (St. Louis, MO, EE. UU.). Se prepararon soluciones estándar madre de aminoácidos (1000 mg/ L) (Kıvrak et al., 2014).

3.7. Análisis de ácidos grasos

Los ácidos grasos se cuantificaron según Park & Goins (1994) con algunas modificaciones. Se pesaron ~0,100 g de muestra liofilizada y se colocaron en un tubo de ensayo; luego se agregaron 100 μL de diclorometano y 1 mL de NaOH 0,5 N en metanol, se cerró el tubo de ensayo y se colocó en un baño de agua caliente a 90 ◦C durante 10 min. A continuación, el tubo de ensayo se enfrió rápidamente en un baño de hielo durante 3 min, donde se añadió 1 mL de BF_3 en metanol y el tubo de ensayo se colocó en oscuridad durante 30 min; posteriormente, se añadió 1 mL de agua ultrapura y 600 μL de hexano. La muestra se agitó vigorosamente durante 1 min en un vórtex (VORTEX 1, IKA, Staufen, Alemania) e inmediatamente después se centrifugó a 4.000 rpm durante 10 min (Eppendorf 5804R, Eppendorf, Hamburgo, Alemania). Posteriormente, el sobrenadante se recuperó cuidadosamente y se colocó en un vial de cromatografía de color ámbar.

Para la separación se utilizó un cromatógrafo de gases (GC) Shimadzu GC-2030 acoplado con un detector de ionización de llama (FID) con un inyector automático AOC-20i (Shimadzu Scientific Instruments, Inc., Columbia, MD, EE. UU.). Se utilizó helio como gas portador y nitrógeno como gas de reposición (24 mL/ min). En el FID se utilizó hidrógeno y aire a velocidades de 32 mL/ min y 200 mL/ min, respectivamente. El sistema

GC utilizó una columna capilar Supelco SP®-2380 (60 m × 0,25 mm × 0,20 μm) (St. Louis, MO, EE. UU.). La temperatura del detector se mantuvo a 260 ◦C y se utilizó una relación de división de 1:20 y una velocidad de flujo lineal total de 28,4 cm/ s. La temperatura del horno comenzó en 70 ◦C y aumentó hasta 250 ◦C a un ritmo de 3 ◦C/ min. Los ácidos grasos metílicos se identificaron en comparación con los tiempos de retención de los estándares FAME Supelco MIX-37 (Supelco Company, Bellefonte, PA, EE. UU.). Los resultados se calcularon como porcentaje de cada ácido graso en el perfil de ácidos grasos totales.

3.8. Perfil de compuesto volátiles

Los compuestos volátiles se cuantificaron según Noguera-Artiaga et al. (2020) con algunas modificaciones. Se pesaron entre 0,150 y 0,300 g de muestras liofilizadas y se colocaron en un vial de 40 mL con tapa de polipropileno y septos de PTFE/silicona. Se añadió 5 μl de acetato de isoamilo (1.000 mg/ L) como estándar interno para la semicuantificación de compuestos. Después de 5 minutos a 45 ◦C (tiempo de equilibrio), se expuso una fibra DVB/CAR/PDMS de 50/30 μm al espacio de cabeza del vial a 40 ◦C con agitación continua (250 rpm) en un agitador magnético (IKA C-MAG HS 4, IKA-Werke GmbH & Co. KG, Staufen, Alemania). Después de 45 minutos de exposición, se extrajo la fibra del vial y se colocó en el inyector GC-MS. La separación e identificación de compuestos se realizó utilizando un Shimadzu GC-MS Nexis GC2030 (Shimadzu Scientific Instruments, Inc., Columbia, MD, EE. UU.), equipado con una columna Sapiens X5MS (30 m × 0,25 mm × 0,25 μm) (Teknokroma, Barcelona, España), y acoplado con un detector de espectrómetro de masas (espectrómetro de masas de triple cuadrupolo TQ8040 NX; Shimadzu Scientific Instruments, Inc., Columbia, MD, EE. UU.). Solo se utilizó el modo de adquisición de cuadrupolo único en el TQ8040 NX (Q3 Scan; velocidad de escaneo 5000 amu/ s; rango de masa 40–400 m/z; tiempo del evento 0,100 s). El programa de temperatura del horno fue el siguiente: (i) temperatura inicial de 35 ◦C, mantenido durante 5 min; (ii) incremento de 5 ◦C/ min hasta 150 ◦C, mantenido durante 1 min; (iii) incremento de 10 ◦C/ min hasta 280 ◦C y mantenido durante 5 min. La presión en la cabeza de la columna de helio fue de 47,6 kPa (modo de velocidad lineal constante de 36 cm/ s). El inyector, la fuente de iones y la interfaz estaban a 250, 230 y 280 ◦C, respectivamente. Se utilizó helio como gas portador, un flujo de columna de 1

mL/ min, con una relación de división de 1:50 y un flujo de purga de 6 mL/ min. Se utilizaron índices de retención de una mezcla estándar de alcanos comercial (Sigma-Aldrich, Steinheim, Alemania) para identificar los compuestos, así como las bibliotecas de índices de retención y espectro de masas NIST 17. La identificación se consideró tentativa cuando se basó únicamente en datos espectrales de masas, y solo los compuestos con una similitud espectral >90 % se consideraron aciertos correctos. El filtro de similitud de retención lineal se fijó en ±10 unidades.

3.9. Determinación de carotenoides

Los carotenoides se extrajeron mediante el método de saponificación de Panfili et al. (2004) y de Fratianni et al. (2021). Brevemente, 5 mL de una solución de pirogalol en etanol de 96° (60 g/ L), 2 mL de etanol de 96°, 2 mL de cloruro de sodio (10 g/ L) y 2 mL de una solución de hidróxido de potasio (600 g/ L) se agregaron secuencialmente a 0,1 g de muestra liofilizada para su análisis. A esta mezcla, se agregaron perlas de vidrio para evitar la ebullición turbulenta y las muestras se lavaron con nitrógeno para evitar la oxidación. Las muestras se colocaron en un baño caliente a 70 °C durante 45 minutos para promover la reacción de hidrólisis. Una vez enfriado, se añadieron 15 mL de cloruro sódico (10 g/ L) y 15 mL de n-hexano:acetato de etilo (9:1; v/v) como disolventes de extracción. Se recogió la capa orgánica y se añadieron 15 mL de n-hexano:acetato de etilo, repitiendo esta operación hasta que quedó incoloro. Posteriormente, se eliminó el disolvente y los residuos se suspendieron en una solución de n-hexano:alcohol isopropílico (90:10; v/v). Los compuestos se analizaron mediante cromatografía líquida de alta resolución (HPLC) de fase normal (para xantofilas) y de fase inversa (carotenos). Se utilizó un sistema analítico HPLC Dionex (Sunnyvale, CA), que consta de un circuito inyector de 50 μL (Rheodyne, Idex Health & Science, Northbrook, IL, EE. UU.) y un sistema de bomba Ultimate 3000. Para la fase inversa, la fase móvil fue metanol:metilterbutiléter:agua, a un caudal de 1 mL/ min, bajo un perfil de gradiente como en Mouly et al. (1999), utilizando una columna de acero inoxidable de 5 μm, C30 YMC (Hampsted, NC, EE. UU.) (250 mm × 4,6 mm de diámetro interno, d.i.). Las muestras se suspendieron en metanol/metilterbutiléter 50:50 (v/v). En condiciones de fase normal, la fase móvil fue n-hexano:alcohol isopropílico en elución en gradiente multilineal del 10 % (A) al 20 % (B) de alcohol isopropílico en n-hexano (Fratianni et al.,

2021). Se utilizó una columna Luna de 5 μm, con una fase estacionaria de sílice (250 mm x 4,6 mm de diámetro interior) (Phenomenex, Torrance, CA). Los datos fueron procesados por un sistema de cromatografía Dionex Chromeleon Versión 6.6 (Sunnyvale, CA). Los carotenoides se detectaron espectrofotométricamente a 450 nm. Para identificar los carotenoides se utilizaron las características espectrales y una comparación de los tiempos de retención con los de los estándares disponibles. Se utilizaron soluciones estándar conocidas para la cuantificación de carotenoides. La actividad de la vitamina A se expresó como Equivalente de Retinol (RE), calculado según el Panel de Productos Dietéticos de la EFSA (2015), considerando las cantidades de carotenos.

3.10. Determinación de tocoles

La extracción y determinación cuali-cuantitativa de tocoles se realizó mediante el método de Panfili et al. (2003), en las mismas condiciones que las utilizadas para los carotenoides. Los residuos se suspendieron en una solución de n-hexano:alcohol isopropílico (99:1 v/v). Se utilizó un sistema HPLC Dionex, equipado con una bomba Ultimate 3000. La separación cromatográfica de compuestos se realizó utilizando una columna Luna de 5 μm, con una fase estacionaria de sílice (250 mm x 4,6 mm de diámetro interior) (Phenomenex, Torrance, CA). Se utilizó como fase móvil una solución de n-hexano:acetato de etilo:ácido acético (97,3:1,8:0,9; v/v/v), a un caudal de 1,6 mL/ min. Los tocoles se detectaron mediante un espectrofluorímetro RF 2000 (Dionex, Sunnyvale, CA, EE. UU.), configurado a una longitud de onda de excitación y emisión de 290 nm y 330 nm, respectivamente, e identificados y cuantificados mediante soluciones estándar conocidas. Los datos fueron procesados por un sistema de cromatografía Dionex Chromeleon Versión 6.6 (Sunnyvale, CA). La actividad de la vitamina E se expresó como Equivalente de Tocoferol (TE) (Sheppard et al., 1992).

3.11. Análisis de tiamina (B1) y riboflavina (B2)

La tiamina y la riboflavina se extrajeron utilizando la metodología de Hasselmann et al. (1989). Las muestras se colocaron en matraces aforados de 100 mL que contenían 20 mL de HCl 0,1 N y se calentaron en un baño de agua a 100 °C durante 30 minutos. Después de enfriar a temperatura ambiente, el pH de las muestras se ajustó a 4,5 con NaOAc 2,5 M. Tras la adición de 0,2 mL de claradiastasa acuosa (50 mg/ mL),

estas muestras se incubaron durante 3 h a 37 °C. Después de enfriar, las muestras se enrasaron a 25 mL con agua destilada. Las muestras se centrifugaron y filtraron a través de un filtro de 0,45 μm. La tiamina se convirtió en tiocromo añadiendo 1,25 mL de ferricianuro de potasio al 1 % en NaOH acuoso al 15 % a 2,5 mL de extracto filtrado. Después de 1 min de oxidación, se agregaron 0,25 mL de H_3PO_4 al 85 %. El extracto se purificó en un cartucho Sep-Pak C18. El cartucho se lavó con 5 mL de MeOH, seguido de 5 mL de NH_4OAc 0,05 M (ajustado a pH 5,0 (ácido) con HOAc). La muestra (5 mL) se cargó en un cartucho Sep-Pak C18 y luego el cartucho se lavó con NH_4OAc 0,05 M y, finalmente, las vitaminas se eluyeron con 5 mL de fase móvil. Los extractos se separaron mediante un cromatógrafo líquido de alta resolución HPLC Dionex (Sunnyvale, CA, EE. UU.), con un sistema de bomba Ultimate 3000. La separación se realizó a un caudal de 0,8 mL/ min con metanol:NaOAc (40:60; v/v) como fase móvil, utilizando una columna de acero inoxidable C18 Luna, Phenomenex (Torrance, CA, EE. UU.) de 5 μm (250 mm × 4,6 mm de diámetro interior). La detección fluorométrica se realizó a una longitud de onda de excitación de 366 nm y una longitud de onda de emisión de 453 nm para tiamina, y una longitud de onda de excitación de 453 nm y una longitud de onda de emisión de 580 nm para riboflavina, mediante un espectrofluorímetro RF 2000 (Dionex, Sunnyvale, California, Estados Unidos). Los datos fueron procesados por un sistema de cromatografía Dionex Chromeleon versión 6.6 (Sunnyvale, CA, EE. UU.). La tiamina y la riboflavina se compararon con estándares disponibles conocidos y se identificaron considerando sus tiempos de retención y orden de elución relativo.

3.12. Elaboración de crema y mayonesa a base de *Diplotaxis*

La elaboración de la crema y de la mayonesa a base de *Diplotaxis* fueron realizadas en las instalaciones de la UMH, con la composición que se muestra en la **Tabla 1**, de la siguiente forma:

(i) Para la elaboración de la crema de *Diplotaxis erucoides* (DeC) se utilizó la máquina Thermomix®, modelo TM6. En primer lugar, la crema batida se calentó a 40 °C hasta espesar; posteriormente se añadió y emulsionó una mezcla de tallos, hojas y vainas de *Diplotaxis erucoides* y sal.

(ii) Para la elaboración de la mayonesa de *Diplotaxis erucoides* (DeM), se mezclaron en una máquina Thermomix®, modelo TM6, leche

semidesnatada, aceite de semillas y, tallos, hojas y vainas de *Diplotaxis erucoides*, emulsionando toda la mezcla a 40 °C.

Tabla 1. Formulación de preparaciones a base de *Diplotaxis erucoides*.

***Diplotaxis erucoides* Crema (DeC)**		***Diplotaxis erucoides* Mayonesa (DeM)**	
Ingredientes	**Cantidad**	**Ingredientes**	**Cantidad**
Crema batida (mL)	250	Aceite de girasol (mL)	100
Hojas (g)	150	Leche semi-desnatada (mL)	250
Vainas (g)	25	Hojas (g)	150
Tallos (g)	25	Vainas (g)	25
Sal (g)	0,1	Tallos (g)	25

3.13. Evaluación sensorial con panel entrenado

Ocho panelistas de gran experiencia (4 hombres y 4 mujeres, con edades comprendidas entre 20 y 52 años), y todos ellos asociados al Grupo de Investigación "Calidad y Seguridad Alimentaria" de la Universidad Miguel Hernández de Elche (Orihuela, Alicante, España) evaluaron las muestras. El panel evaluó muestras de vainas, hojas y tallos de *Diplotaxis erucoides*, 5 muestras de mostaza (comercial), 3 muestras de wasabi (comercial) y 2 nuevos productos (crema y mayonesa) a base de *Diplotaxis erucoides*. El análisis sensorial se realizó en 2 sesiones. En la primera (sesión de orientación), los panelistas se familiarizaron con las partes de la planta *Diplotaxis erucoides* en crudo, y con los productos comerciales, realizando el léxico sensorial. En esta sesión se decidió qué mostaza y wasabi tenían un perfil sensorial más cercano al de *Diplotaxis erucoides*. Finalmente, durante la segunda sesión, el panel realizó una descripción sensorial completa de las muestras seleccionadas de mostaza y wasabi, con la incorporación de los dos nuevos productos a base de *Diplotaxis* (DeC y DeM).

Entre muestras y para la limpieza del paladar, se proporcionó a los panelistas agua y galletas sin sal. Los atributos evaluados fueron: apariencia (brillo), sabor [*Diplotaxis* ID (olor y aroma) y sabor herbáceo-verde], sabores básicos (dulce, salado, ácido y amargo), sensaciones somatosensoriales (pungencia), postgusto y textura (carácter graso y viscosidad). El léxico sensorial utilizado para el análisis sensorial descriptivo de las muestras bajo evaluación se resume en la **Tabla 2**. Los panelistas

utilizaron una escala de 0 a 10 puntos (con incrementos de 0,5) para la evaluación, donde 10 era una intensidad extremadamente alta y 0 era una intensidad extremadamente baja o no perceptible.

Tabla 2. Léxico usado para el análisis descriptivo de mostazas, wasabis y muestras de *Diplotaxis erucoides.*

Atributos	Definición	Referencias e intensidades
Apariencia		
Brillo	El croma del color, que va desde un color opaco y turbio hasta un color puro y brillante.	Pasta de Wasabi Blue Dragon = 2,5; Mostaza amarilla Heinz = 7,5
Flavor		
Diplotaxis-ID (olor y aroma)	Olor/aroma verde y herbáceo asociado con *Diplotaxis erucoides*	25 g triturados de *Diplotaxis erucoides* + 100 mL H_2O = 4; 100 g triturados de *Diplotaxis erucoides* + 25 H_2O = 10
Aroma herbáceo	Aromas frescos, verdes y ligeramente ácidos asociados con vegetales verdes, vides recién cortadas y guisantes.	Habas Kroger (enlatadas) = 3,0; Una ramita pequeña de perejil fresco = 7,0 (aroma); Perejil fresco = 10,0
Dulce	El sabor fundamental asociado con una solución de sacarosa.	Solución Sacarosa 4% = 2,5; Solución Sacarosa 8% = 5,0; Solución Sacarosa 16% = 9,5
Salado	Sensación gustativa fundamental de la que es típico el cloruro de sodio.	Solución NaCl 0,2% = 2,5; Solución NaCl 0,35% = 5,0; Solución NaCl 0,8% = 9,0
Ácido	El sabor estimulado por ácidos como el cítrico y el málico.	Solución Ácido Tartárico 0,05% = 2,5; Solución Ácido Tartárico 0,08% = 4,0; Solución Ácido Tartárico 0,20% = 9,5
Amargo	El gusto estimulado por sustancias como la quinina o la cafeína.	Solución Cafeína 0,05% = 2,5; Solución Cafeína 0,08% = 4,0
Pungencia	Una sensación aguda y físicamente penetrante en la boca y la nariz.	Vinagre blanco Heinz = 8,0 (flavor)
Postgusto	Tiempo en que el sabor específico del sabor de la fruta permanece en la boca después de tragar la muestra.	10 s = 2.0; 30 s = 8,0
Textura		
Carácter graso	Cantidad de aceite que queda en las superficies de la boca.	Mostaza amarilla Heinz = 3,0
Viscosidad	La medida del flujo a medida que el producto se mueve sobre la lengua cuando se presiona entre la lengua y el paladar (2,46 mL de producto).	Crema batida Dillon's = 4,0; Potito Gerber sabor manzana Etapa 1 = 9,0

3.14. Análisis estadístico

Los datos experimentales se sometieron primero a un análisis de varianza unidireccional (ANOVA) y luego a la prueba de rangos múltiples de Tukey para comparar las medias. Las diferencias se consideraron estadísticamente significativas cuando $p<0,05$. Todos los análisis estadísticos se realizaron utilizando el software StatGraphics Plus 5.0 (Manugistics. Inc., Rockville. MD).

4. RESULTADOS

4. RESULTADOS

Esta sección incluye los principales resultados y discusiones de los artículos publicados, los cuales se resumen en cuatro partes agrupadas según cada objetivo específico. Los resultados detallados se pueden consultar en las respectivas publicaciones.

Primera publicación: "*Valorization of Wild Edible Plants as Food Ingredients and Their Economic Value*" tuvo como objetivo principal, dar una visión general de las plantas comestibles silvestres. La revisión bibliográfica fue realizada a través de 93 publicaciones, de las que se extrajeron las siguientes ideas principales:

- Las plantas comestibles silvestres son fuente natural de fibra, proteínas, azúcares, ácidos orgánicos, minerales, ácidos grasos y vitaminas.
- Además, tienen capacidad antioxidante, con altos valores de compuestos fenólicos y de flavonoides entre otras muchas propiedades.
- Las plantas comestibles silvestres es un medio de vida de muchas familias en países en vías de desarrollo.

En conclusión, los datos obtenidos en esta revisión hacen evidente que las plantas comestibles silvestres tienen un alto potencial para su incorporación en un futuro como alimento o ingrediente alimentario. La incorporación de estas plantas dependerá, del tipo de planta, la legislación vigente y también sería necesario más investigación y una estrecha colaboración entre investigadores e industria para poder desarrollar estos productos.

Objetivo específico 1: Caracterización química (caracterización proximal, azúcares, ácidos orgánicos, minerales, aminoácidos, perfil de ácidos grasos, perfil de compuestos volátiles).

Los resultados de este objetivo están reflejados en la siguiente publicación:

Publicación 2: "*Potential Interest of Oxalis pes-caprae L., a Wild Edible Plant, for the Food and Pharmaceutical Industries*". En esta publicación las determinaciones estudiadas en *Oxalis pes-caprae* fueron: caracterización proximal, azúcares, ácidos orgánicos, minerales, aminoácidos, perfil de ácidos grasos y perfil de compuestos volátiles.

4.1. Caracterización proximal

La composición proximal (humedad, cenizas, fibra dietética total, proteínas, grasas y carbohidratos) varió significativamente según la parte de la planta estudiada **(Tabla 3)**, excepto la energía.

Tabla 3. Composición proximal de *Oxalis pes-caprae* L.

Parámetros (%)	*p*-Value	ANOVA[†]	Flor	Hoja	Tallo
Humedad (liofilizada)	0,0000	***	9,05[‡] b	9,17 b	11,10 a
Humedad	0,0000	***	85,86 b	86,07 b	90,95 a
Cenizas	0,0000	***	8,27 b	12,93 a	3,15 c
Fibra dietética total	0,0000	***	30,68 b	28,72 b	36,36 a
Proteína	0,0000	***	13,45 b	19,35 a	8,86 c
Grasa	0,0000	***	6,66 b	12,68 a	3,53 c
Carbohidratos	0,0000	***	62,57 b	45,87 c	73,34 a
Energía (Kcal/100 g)	0,1044	n.s.	364	375	360

*[†]n.s.: no significante al p>0,05; *. **. y ***. significante al p< 0,05, 0,01 y 0,001, respectivamente; [‡]Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey.*

En cuanto a la humedad, los tallos tuvieron el valor más alto, con valores de 11,10 y 90,95 %, respectivamente. Estos valores son mucho más altos que los reportados por Datta et al. (2019), con un 60,28 %, en plantas similares como *Oldenlandia corymbosa*. Sin embargo, en la mayoría de estudios realizados en este tipo de plantas, la humedad varió entre el 80 % y el 95 % de humedad (García-Herrera, Morales, et al., 2014; Pereira et al., 2011; Tardío et al., 2011).

El contenido de cenizas mostró un comportamiento completamente diferente al de la humedad, siendo las hojas las que presentaron el valor más alto (12,93 %) respecto a las flores (8,27 %) y los tallos (3,15 %). El contenido de cenizas encontrado en las hojas de *Oxalis pes-caprae* fue significativamente mayor que el reportado en *Oxalis corymbosa* (3,91 %) por Vera et al. (2018). Los valores de ceniza obtenidos en flores de *Oxalis pes-caprae* fueron similares a los reportados por Datta et al. (2019) en *Oldenlandia corymbosa* (8,34 %). Sin embargo, un estudio realizado en Pakistán muestra resultados de cenizas mucho más altos (28,00 %) en *Oxalis pes-caprae*, por lo que la influencia geográfica en los resultados es importante (Naila et al., 2020).

En cuanto a la fibra dietética, el mayor contenido se obtuvo en los tallos (36,36 %) de *Oxalis pes-caprae* seguido de las flores (30,68 %) y hojas (28,72 %). Los resultados actuales destacan el hecho de que *Oxalis pes-caprae* tiene un mayor contenido de fibra que otras plantas similares, como *Oxalis tuberosa* (0,87–1,69 %) y *Oldenlandia corymbosa* (7,26 %) (Datta et al., 2019; Jimenez et al., 2015).

Por otro lado, tanto en el contenido de proteína como de grasa, las hojas de *Oxalis pes-caprae* presentaron el mayor contenido, mientras que los tallos presentaron el menor contenido para ambas variables. Los valores de proteína obtenidos fueron muy similares a otro estudio sobre las hojas de *Oxalis corniculata* que reportaron un contenido total de nitrógeno de 3,56 %, lo que equivale a 22,25 % de proteína (Jain et al., 2010) luego de la aplicación de un factor de conversión de 6,25 (AOAC, 1995); por tanto, este valor es cercano al encontrado en las hojas de *Oxalis pes-caprae*. En cuanto a la grasa, las hojas tuvieron un 12,68 %, mientras que los tallos solo tuvieron un 3,53 %. Estos valores de grasa fueron más bajos que los encontrados previamente en *Oxalis corniculata*, 23,75 % (Jain et al., 2010), aunque en este estudio, las mismas plantas recolectadas en diferentes áreas tenían contenidos de proteína que oscilaban entre 13,4 y 17,6 %.

El porcentaje de carbohidratos en las partes de *Oxalis pes-caprae* indicó un mayor valor en los tallos (73,34 %), seguido de flores (62,57 %) y hojas (45,87 %); estos valores fueron superiores a los encontrados por Jain et al. (2010) en *Oxalis corniculata* (24,67 %) pero similar a los reportados previamente en plantas de otros géneros como Malvaceae y Lamiaceae, 75–85 % (Barros et al., 2010; Fernandes et al., 2010; Jain et al., 2010).

Finalmente, los contenidos energéticos totales fueron equivalentes en las tres partes de la planta de *Oxalis pes-caprae*. Estos valores son bastante altos en comparación con los reportados previamente para plantas comestibles silvestres de otros géneros, como Fabaceae, Malvaceae y Lamiaceae, ~1548-1694 kJ (Barros et al., 2010; Fernandes et al., 2010; Pinela et al., 2011).

4.2. Azúcares y ácidos orgánicos

Los contenidos de fructosa, sacarosa y ácido oxálico fueron estadísticamente equivalentes en las flores, hojas y tallos de *Oxalis pes-caprae* **(Tabla 4)**.

Tabla 4. Azúcares y ácidos orgánicos en *Oxalis pes-caprae* L.

Compuestos (g/ Kg p.s.[¥])	*p*-Value	ANOVA[†]	Flor	Hoja	Tallo
Fructosa	0,2421	n.s.	107	103	77,9
Glucosa	0,0019	**	75,3 a[‡]	33,2 b	88,9 a
Maltosa	0,0022	**	n.d.	40,35 a	14,04 b
Sacarosa	0,0792	n.s.	193	192	173
Ácido Oxálico	0,4855	n.s.	98,0	98,2	110
Ácido Ascórbico (mg/ 100g p.s.[¥])	0,0000	***	0,42 c	3,17 b	3,50 a

*[¥]p.s. = peso seco; [†]n.s.: no significante al p>0,05; *, **, y *** = significante al p< 0,05, 0,01 y 0,001, respectivamente; n.d. = no detectado; [‡]Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey.*

Las hojas de *Oxalis pes-caprae* eran más ricas en maltosa que el resto de la planta, mientras que presentaban el menor contenido de glucosa; este azúcar (glucosa) era más abundante en los tallos. La fructosa en *Oxalis pes-caprae* fue superior a la encontrada en otras plantas como *Calligonum comosum* L. o *Cynara cardunculus* L. (Gasmi et al., 2019; Mandim et al., 2022); sin embargo, los valores de fructosa encontrados en las flores de *Moringa oleifera* Lam. y *Malva sylvestris* L. (75,6 y 87,2 g/ Kg, respectivamente), fueron muy similares a los encontrados en el presente estudio (Barros et al., 2010; Monteiro et al., 2022).

Respecto a la glucosa, los contenidos encontrados en *Oxalis pes-caprae* fueron cercanos a los reportados previamente en *Malva sylvestris* L., *Cynara cardunculus* L., *Moringa oleifera* Lam., 73,6; 99,5; 120,7 g/ Kg, respectivamente (Barros et al., 2010; Mandim et al., 2022; Monteiro et al., 2022). Finalmente, es importante comentar que la sacarosa es el azúcar predominante en la mayoría de las PCS (Barros et al., 2013; Gasmi et al., 2019; Mandim et al., 2022; Martins et al., 2011); sin embargo, los valores de sacarosa encontrados en estos estudios fueron inferiores a los encontrados para *Oxalis pes-caprae*.

Por otra parte, la concentración de ácido oxálico encontrada en *Oxalis pes-caprae* L. es mucho mayor que la encontrada en otros tipos de PCS, como *Allium ampeloprasum* L. (27,83 mg/ 100 g) (García-Herrera, Morales, et al., 2014). Sin embargo, los valores actuales se acercaron más a los encontrados en las hojas de *Cynara cardunculus* L. var. *altilis* (81 g/ Kg) (Mandim et al., 2022). El ácido oxálico y sus sales, llamadas oxalatos, pueden causar problemas en el cuerpo humano porque eliminan minerales como el

calcio, aunque se han realizado estudios que estiman que los problemas surgirían por encima de 150 mg de ingesta diaria de oxalatos (Noonan & Savage, 1999).

Por otro lado, el ácido ascórbico se acumuló principalmente en los tallos y hojas de las plantas de *Oxalis*, y significativamente menos en las flores **(Tabla 4)**. El contenido de este ácido orgánico fue mayor en otras PCS estudiadas, como *Blumea lacera* (127 mg/ 100 g), *Commelina benghalensis* (23,6 mg/ 100 g), o incluso *Dioscorea praehensilis* con 10 mg/ 100 g (Alam et al., 2020; Pereira et al., 2023; Yimer et al., 2023). En plantas pertenecientes a la misma familia como *Oxalis acetosella*, mostraron valores mucho más altos (3457 μg/ g p.s.) que los encontrados en *Oxalis pes-caprae* (Šircelj et al., 2010).

4.3. Minerales

La composición mineral encontrada en *Oxalis pes-caprae* se muestra en la **Tabla 5**, predominando el potasio y el hierro entre los macro y micronutrientes, respectivamente.

Tabla 5. Composición mineral de *Oxalis pes-caprae* L.

Mineral (mg/ 100 g)		*p*-Value	ANOVA[†]	Flor	Hoja	Tallo
Macro	**Ca**	0,0000	***	104 c[‡]	453 b	620 a
	K	0,0000	***	1247 b	859 c	1399 a
	Na	0,0000	***	35,1 b	69,1 a	71,5 a
	Mg	0,0000	***	95,4 b	129 a	73,2 c
Micro	**Cu**	-	-	n.d.	n.d.	n.d.
	Fe	0,0000	***	7,7 a	3,2 b	1,4 c
	Mn	0,0000	***	0,87 b	1,18 a	0,32 c
	Zn	-	-	0,30	n.d.	n.d.

[†]. **. y ***. significante al p< 0,05, 0,01 y 0,001, respectivamente; n.d. = no detectado; [‡]Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey.*

La concentración de calcio (Ca) fue mayor en los tallos (620 mg/ 100 g), y en las flores fue seis veces menor (104 mg/ 100 g) habiendo un rango bastante amplio entre las diferentes partes de la *Oxalis*. Un estudio realizado por Datta et al. (2019) en seis PCS mostró contenidos de Ca que oscilaban entre 492 y 621 mg/ 100 g; este rango es muy cercano al encontrado en las hojas y tallos de *Oxalis*. En cuanto al potasio (K), los tallos (1399 mg/ 100 g) y las flores (1247 mg/ 100 g) mostraron contenidos similares y fueron significativamente superiores a los de las hojas. El contenido de K encontrado en *Oxalis*

fue mayor que el reportado en *Enhydra fluctuans* (487 mg/ 100 g) pero mucho menor que el reportado en otras 19 PCS, teniendo en cuenta que algunas alcanzaron valores tan altos como 7830 mg/ 100 g en *Smyrnium cordifolium* Boiss (Jalali & Fakhri, 2021). Respecto al sodio (Na), no hubo diferencias entre el contenido de hojas y tallos, pero fueron superiores al contenido de Na en las flores. *Cichorium intybus* L. mostró una mayor concentración de sodio (80,61 mg/ 100 g) en un estudio realizado por Jalali & Fakhri (2021). Las flores de *Oxalis* tenían contenidos de Na similares a los de otros tipos de plantas, como *Allium hirtifolium* Boiss., *Stachys lavandulifolia* Vahl o *Taraxacum vulgar* Hodn. Mzt. (30,28, 30,26, 30,35 mg/ 100 g, respectivamente) (Jalali & Fakhri, 2021). El contenido de magnesio (Mg) siguió el orden hojas > flores > tallos, siendo estos contenidos similares a los encontrados previamente en *Anchusa italica* Retz (120 mg/ 100 g) (Jalali & Fakhri, 2021).

El micronutriente más abundante fue el Fe, especialmente en las flores. No se observaron diferencias biológicamente significativas en los contenidos de Mn y Zn. Los contenidos experimentales de estos tres nutrientes son similares a los informados previamente en otras PCS (Jalali & Fakhri, 2021).

4.4. Aminoácidos

Se encontraron diecinueve aminoácidos en *Oxalis pes-caprae* L. **(Tabla 6)**. En general, los aminoácidos esenciales predominaron en las flores, seguido de las hojas y, finalmente, los tallos, siendo la leucina, isoleucina y valina los compuestos más abundantes. De esta forma, la leucina también predomina en otras plantas silvestres comestibles como *Portulaca oleracea* L. o *Moringa oleifera,* entre otras (Kubmarawa D. et al., 2008; Nemzer et al., 2020; Stadtlander & Becker, 2017). Estos tres aminoácidos (leucina, isoleucina y valina) tienen una función importante en las plantas al contribuir a los compuestos volátiles responsables de su olor y aroma. Estos volátiles producidos por las plantas no sólo tienen funciones aromáticas, sino que también pueden actuar como aromas que atraen a los polinizadores; por tanto, su papel es fundamental en la reproducción de las plantas (Maoz et al., 2022).

Tabla 6. Aminoácidos presentes en *Oxalis pes-caprae* L.

Aminoácidos [mg/ 100 g p.s.¥]		*p*-Value	ANOVA†	Flor	Hoja	Tallo
Esencial	**Arginina**	0,0000	***	13,0 a‡	4,91 b	2,04 c
	Fenilalanina	0,0000	***	48,7 a	50,6 a	29,9 b
	Histidina	0,0000	***	19,9 a	8,98 b	5,57 c
	Isoleucina	0,0000	***	209 a	169 b	170 b
	Leucina	0,0000	***	306 a	244 b	245 b
	Lisina	0,0000	***	5,46 b	6,63 a	2,16 c
	Metionina	0,0000	***	6,01 a	4,82 b	1,83 c
	Treonina	0,0000	***	114 a	13,9 c	20,3 b
	Triptófano	0,0000	***	80,1 a	47,2 b	24,8 c
	Valina	0,0000	***	187 a	189 a	134 b
No esencial	**Alanina**	0,0000	***	302 b	307 b	343 a
	Asparagina	0,0000	***	n.d.	8,42 a	5,94 b
	Aspartato	0,0000	***	73,0 b	91,1 a	56,4 c
	Cisteína	0,0000	***	0,55 b	0,69 a	n.d.
	Ácido Glutámico	0,0000	***	237 b	325 a	220 c
	Glicina	0,0000	***	55,7 a	11,9 c	30,4 b
	Prolina	0,0000	***	102 a	30,5 c	50,7 b
	Serina	0,0000	***	112 a	46,3 b	46,3 b
	Tirosina	0,0000	***	13,8 c	40,1 a	17,0 b
TOTAL				**1885 a**	**1600 b**	**1405 c**

*¥p.s. = peso seco; †n.s.: no significante al p>0,05; *. **. y ***. significante al p< 0,05, 0,01 y 0,001, respectivamente; n.d. = no detectado; ‡Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey.*

En cuanto a los aminoácidos no esenciales, los contenidos de flores y hojas fueron similares y superiores a los de los tallos, siendo la alanina y el ácido glutámico los compuestos predominantes. Sin embargo, la alanina fue el compuesto más abundante en los tallos, mientras que el ácido glutámico predominó en las hojas. En otras PCS como *Sesamum indicum* L., *Balanites aegyptiaca* (L.) Delile y *Portulaca oleracea* L., el compuesto más abundante también fue el ácido glutámico (Kubmarawa D. et al., 2008; Nemzer et al., 2020). La importancia de este aminoácido radica en que es uno de los cuatro ligandos principales del zinc; y este mineral realiza funciones catalíticas o estructurales en las plantas (Barker & Pilbeam, 2015).

4.5. Ácidos grasos

Se identificaron 29 ésteres metílicos de ácidos grasos (FAME) **(Tabla 7)** en las diferentes partes de *Oxalis pes-caprae*; estos FAME están compuestos por 8 ácidos grasos

monoinsaturados (MUFA), 7 ácidos grasos poliinsaturados (PUFA) y 14 ácidos grasos saturados (SFA). Aunque el número de SFA fue mayor, los FAME insaturados predominaron y representaron entre el 70 % y el 80 % del contenido total: flores (MUFA + PUFA) = 73,72 %; hojas (MUFA + PUFA) = 80,37 %; y tallos (MUFA + PUFA) = 77,76 %.

Los tres compuestos más abundantes fueron C18:3n3 (linolénico u omega-3) (AG27), C18:2n6c (linoleico)(AG23) y C16:0 (palmítico)(AG5). En otros estudios previos sobre plantas comestibles silvestres, estos tres mismos compuestos, junto con el ácido oleico, fueron los predominantes (da Silva et al., 2020; Harumi Iyda et al., 2019; Singla et al., 2022). En cuanto al ácido linolénico (AG27), hubo diferencias significativas entre las partes analizadas, siendo las hojas las que presentaron mayor contenido (53,57 %). Estos resultados concuerdan bien con estudios previos que informaron de contenidos cercanos al 50 % de ácido linolénico (Alarcón et al., 2006; Morales et al., 2012; Pereira et al., 2011); este contenido fue reportado en plantas de familias como Amaranthaceae, Asteraceae, Montiaceae, Polygonaceae y Caryophyllaceae y más precisamente en *Beta marítima* (57,80 %), *Chondrilla juncea* (56,27 %), *Montia fontana* (55,57 %), *Rumex acetosella* (51,34 %), *Rumex induratus* (58,84 %) y *Silene vulgaris* (54,5 %). La importancia de este ácido graso proviene de los efectos beneficiosos para la salud, siendo un protector frente a enfermedades cardiovasculares, diabetes tipo II, sistema óseo y enfermedades renales (Kim & Ilich, 2011; Rajaram, 2014).

Por otra parte, la presencia de ácido linoleico (C18:2n6c, AG23) fue mayor en las flores, alcanzando el 47,65 %. Otras PCS con un contenido similar de estos compuestos fueron *Allium ampeloprasum* (53,45 %) y *Tamus communis* (42 %), pertenecientes a las familias Amaryllidaceae y Dioscoreaceae, respectivamente (García-Herrera, Morales, et al., 2014; Martins et al., 2011). La importancia de este ácido graso aparte de también ser cardioprotector y ejercer un control glucémico en los pacientes diabéticos, se ha descubierto que ejerce una influencia positiva junto al ácido oleico frente a las depresiones en mujeres perimenopáusicas (Li et al., 2020; Marangoni et al., 2020).

Tabla 7. Perfil de ácidos grasos de *Oxalis pes-caprae* L.

Código	Ácidos grasos (%)	Tiempo R.	ANOVA[†]	Flor	Hoja	Tallo
AG1	C12:0 (Láurico)	19,330	***	0,31 a[‡]	0,09 b	0,08 b
AG2	C13:0 (Tridecanoico)	21,419	-	n.d.	n.d.	0,02
AG3	C14:0 (Mirístico)	23,420	***	0,51 a	0,31 b	0,20 c
AG4	C15:0 (Pentadecanoico)	25,348	***	0,06 a	0,02 b	0,06 a
AG5	C16:0 (Palmítico)	27,251	***	18,51 a	11,73 c	16,42 b
AG6	C17:0 (Isomargárico)	28,156	***	0,02 b	n.d.	1,41 a
AG7	C17:0 (Margárico)	28,943	***	0,13 a	0,10 b	0,11 b
AG8	C18:0 (Esteárico)	30,649	***	2,05 a	1,22 c	1,46 b
AG9	C19:0 (Nonadecanoico)	32,318	-	n.d.	n.d.	0,18
AG10	C20:0 (Araquidónico)	33,923	***	0,09 c	0,24 a	0,16 b
AG11	C21:0 (Heneicosanoico)	35,336	***	0,15 a	0,15 a	0,04 b
AG12	C22:0 (Behénico)	36,831	***	1,33 a	1,01 b	0,01 c
AG13	C23:0 (Tricosanoico)	38,165	***	0,97 b	2,87 a	0,51 c
AG14	C24:0 (Lignocérico)	39,585	***	0,59 b	0,07 c	0,92 a
	Σ SFA		***	**24,72 a**	**17,81 c**	**21,58 b**
AG15	C15:1 (Pentadecanoico)	26,835	***	0,06 b	0,16 a	0,07 b
AG16	C16:1 (Palmitoleico)	27,856	-	0,01	n.d.	n.d.
AG17	C16:1c9 (Hipogeico)	28,275	***	0,12 b	n.d.	0,89 a
AG18	C18:1t9 (Elaídico)	31,316	***	0,36 c	8,90 a	4,05 b
AG19	C18:1c9 (Oleico)	31,517	***	0,76 c	1,43 b	6,59 c
AG20	C18:1n7 (*cis*-Vacénico)	31,660	***	0,30 c	0,42 b	0,67 a
AG21	C22:1n9 (Erúcico)	37,730	***	1,22 a	0,02 b	n.d.
AG22	C24:1n9 (Nervónico)	40,122	***	0,04 c	0,08 b	0,18 a
	Σ MUFA		***	**2,87 c**	**11,01 b**	**12,45 a**
AG23	C18:2n6c (Linoleico)	32,941	***	47,65 a	10,15 c	29,57 b
AG24	C20:2 (Eicosadienoico)	35,998	***	2,77 a	0,32 c	0,87 b
AG25	C22:2 (Docosadienoico)	38,712	***	1,47 b	2,95 a	n.d.
	Σ n-6 PUFA		***	**51,89 a**	**13,42 c**	**30,44 b**
AG26	C18:3n6 (γ-Linolénico) (omega-6)	33,849	***	0,58 b	1,20 a	0,42 c
AG27	C18:3n3 (α-Linolénico) (omega-3)	34,552	***	18,17 c	53,57 a	34,04 b
AG28	C20:3n3 (Eicosatrienoico)	36,894	***	0,10 b	1,08 a	0,03 b
AG29	C20:3n6 (dihomo- γ-Linoleico)	37,497	***	0,11 b	0,09 b	0,38 a
	Σ n-3 PUFA		***	**18,96 c**	**55,94 a**	**34,87 b**
	Σ PUFA		**n.s.**	**70,85**	**69,36**	**65,31**

*[†]n.s.: no significante al p>0,05; *. **. y ***. significante al p< 0,05, 0,01 y 0,001, respectivamente; n.d. = no detectado; [‡]Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey.*

El tercer ácido graso mayoritario fue el ácido palmítico (AG5) con una presencia del 18,51 % en flores. Respecto al ácido palmítico, varias PCS mostraron resultados similares, como es el caso de *Diplotaxis erucoides* (18,23 %) o *Humulus lupulus* (19,52 %), pertenecientes a las familias Brassicaceae y Cannabaceae, respectivamente (Morales et

al., 2012; Salah et al., 2015). El ácido palmítico no es un ácido graso querido por parte del consumidor, sin embargo, hay estudios que afirman que a dosis bajas puede tener un efecto protector del miocardio disminuyendo el estrés oxidativo, y también mejora la función mitocondrial (Mthembu et al., 2024). En los tallos predominó el ácido oleico (AG19), seguido de las hojas y las flores. El ácido oleico estuvo presente en otras PCS con valores similares a los encontrados en el tallo de *Oxalis*, como *Chenopodium ambrosioides*, *Helichrysum stoechas* y *Scolymus hispanicus*, entre un 6 y un 7 % de contenido de ácido oleico (Barros et al., 2010; Barros et al., 2013; Morales et al., 2012). Sin embargo, las plantas pertenecientes a la familia de las lamiáceas mostraron un mayor contenido de ácido oleico en comparación con otras familias, *Glechoma hederacea*, *Thymus pulegioides* y *Thymus mastichina* mostraron 35,12 %, 11,50 % y 9,82 %, respectivamente (Barros et al., 2011; Fernandes et al., 2010).

4.6. Perfil de compuestos volátiles

Se aislaron, identificaron y cuantificaron un total de 32 compuestos volátiles en las distintas partes de *Oxalis pes-caprae*, así como todos los descriptores aromáticos asociados con los compuestos volátiles **(Tabla 8)** (Bedoukian Research, 2023; FAO/WHO JECFA, 2023; Merck, 2023; National Institute of Standard and Technology (NIST), 2023; Terry & Arn, 2004; The Good Scents Company, 2021). Los compuestos volátiles se pudieron agrupar en siete familias químicas: ésteres (11 compuestos), alcanos (7), terpenos (5), alcoholes (5), aldehídos (2), ácidos (1) y éteres (1); quince de los 32 compuestos volátiles se encontraban en cantidades significativamente diferentes entre las diferentes partes estudiadas de esta planta. Los tres compuestos principales encontrados fueron nerolidol, β-cariofileno y acetato de cis-3-hexenilo.

En general, el nerolidol (V27) fue el compuesto con mayor concentración (21,58 μg/g) en todas las partes de esta planta, siendo las flores la parte más destacada. El nerolidol se asocia típicamente con un olor floral y está presente principalmente en los aceites esenciales de las flores. La presencia de este compuesto en la flor es muy importante porque se ha asociado con potencial antifúngico, antibacteriano y antioxidante (Chan et al., 2016; Lee et al., 2007). Este compuesto puede ser muy importante en el futuro debido a los estudios preliminares realizados en ratas y en los

que se ha concluido que atenúa el síndrome de ovarios poliquísticos (Türkmen et al., 2023).

Respecto al β-cariofileno (V20) también se encontró exclusivamente en las flores (19,86 μg/ g), y sus descriptores sensoriales son dulce, amaderado, especiado y clavo. La presencia de este compuesto volátil también fue respaldada por los resultados de Fukalova-Fukalova et al. (2022), encontrándose β-cariofileno en seis de las siete plantas estudiadas, siendo *Porophyllum ruderale* la que mostró el mayor contenido de este compuesto. El potencial de este compuesto se exhibe en un estudio donde se muestra que produjo un efecto antiinflamatorio a favor de la disminución de la presión arterial en ratones (Espinoza-Gutiérrez et al., 2024).

También se encontraron humuleno (*α*-cariofileno), *α*-terpineol o β-farneseno (1,58, 1,13 y 3,51 μg/ g, respectivamente), pero en concentraciones más bajas, lo que demuestra la importancia del grupo de los terpenos en el perfil volátil de esta PCS en particular.

Se encontró acetato de cis-3-hexenilo (V7) en las tres partes de la planta, encontrándose el mayor contenido en las hojas (16,0 μg/ g). Este compuesto también estaba presente en las hojas de zanahoria, el perejil y las flores de *Nelumbo Nucifera* pertenecientes a la familia Nelumbonaceae (Guijarro-Real et al., 2019; Younis et al., 2023). El contenido de acetato de pentilo (V4) también fue importante, ya que las hojas y los tallos tenían contenidos significativamente mayores que las flores.

Tabla 8. Identificación, concentración y descriptores de olor de compuestos volátiles encontrados en *Oxalis pes-caprae* L.

Código	Compuesto (µg/ g)	FQ	TR (min)	IK (EXP)	IK (LIT)	ANOVA[†]	Flor	Hoja	Tallo	Descriptores[¥]
V1	3-Metilbutanal	Aldehído	3,349	690	686	***	3,02 a	2,95 a	0,08 b	Aldehídico, grasa
V2	3-Hexen-1-ol	Alcohol	10,173	857	857	-	-	-	0,57	Verde, vegetal, herbáceo
V3	Acetateo de 4-Penten-1-ilo	Éster	11,963	901	901	***	1,52 a	1,39 b	1,14 b	Verde, vegetal
V4	Acetato de pentilo	Éster	12,434	914	917	***	4,33 b	6,27 a	6,50 a	Afrutado, banana
V5	Propionato de isoamilo	Éster	14,537	970	969	***	0,98 c	2,15 a	1,52 b	Dulce, Afrutado, banana
V6	Éter diisoamil	Éter	15,728	1002	1002	***	2,03 a	0,66 b	0,36 c	Afrutado
V7	Acetato de cis-3-hexenilo	Éster	15,794	1004	1005	***	2,34 c	16,0 a	7,24 b	Fresco, verde, dulce, afrutado
V8	Acetato de hexilo	Éster	16,062	1011	1011	***	2,14 c	2,64 b	3,14 a	Afrutado, verde, banana, dulce
V9	Butanoato de pentilo	Éster	17,579	1056	1059	-	0,90	-	-	Dulce, Afrutado, banana, cereza
V10	Linalol	Alcohol	19,021	1098	1098	-	3,19	-	-	Floral, cítrico, rosa
V11	Nonanal	Aldehído	19,178	1103	1102	-	0,95	-	-	Cera, aldehídico, cítrico, fresco
V12	Butanoato de isoamilo	Éster	19,231	1104	1104	-	0,95	-	-	Dulce, afrutado, verde
V13	Alcohol feniletílico	Alcohol	19,414	1110	1110	-	0,92	-	-	Floral, rosa
V14	α-terpineol	Terpeno	22,046	1194	1194	-	1,13	-	-	Pino, lila, madera, floral
V15	1,3-bis(1,1-dimetiletil) benceno	Alcano	23,645	1248	1249	***	1,80 a	0,26 c	0,32 b	-
V16	Ácido nonanoico	Ácido	24,005	1261	1267	-	1,59	-	-	Cera, queso, lácteo
V17	4,6-dimetildodecano	Alcano	24,437	1275	1285	***	1,55 b	1,64 a	0,41 c	Afrutado, verde
V18	1,1'-Biciclohexilo	Alcano	25,708	1320	1307	-	2,70	-	-	-
V19	Nonanoato de etilo	Éster	27,686	1290	1294	-	8,15	-	-	Afrutado, rosa, cera
V20	β-cariofileno	Terpeno	28,528	1424	1424	-	19,86	-	-	Dulce, madera, clavo
V21	Benzoato de isoamilo	Éster	28,915	1438	1437	-	1,58	-	-	Dulce, verde, cera
V22	β-farneseno	Terpeno	29,342	1454	1458	-	3,51	-	-	Madera, cítrico, herbáceo, dulce
V23	Humuleno	Terpeno	29,551	1462	1462	-	1,58	-	-	Madera
V24	1-Dodecanol	Alcohol	29,887	1474	1474	***	1,49 b	3,99 a	0,77 c	Tierra, jabón, cera, grasa

V25	Pentadecano	Alcano	30,290	1490	1490	-	-	2,14	-	Cera
V26	2,4-bis(1,1-dimetiletil) fenol	Alcano	30,642	1504	1502	***	1,34 b	1,48 a	0,46 c	-
V27	Nerolidol	Terpeno	31,826	1563	1562	-	21,58	-	-	Floral, verde, cítrico
V28	Dodecanoato de etilo	Éster	32,412	1592	1591	***	3,92 a	0,63 b	-	Dulce, cera, floral, jabón
V29	Hexadecano	Alcano	32,575	1600	1600	***	2,00 a	0,84 b	0,39 c	Alcano
V30	Ciclotetradecano	Alcano	34,045	1691	1679	-	-	2,73	-	Cera
V31	1-Tetradecanol	Alcohol	34,149	1698	1686	***	-	3,06 a	2,14 b	Afrutado, cera
V32	Hexadecanoato de etilo	Éster	37,768	1974	1975	***	1,59 a	0,61 b	0,58 b	Cera, lácteo, oleoso
	TOTAL					*******	**98,64 a**	**49,44 b**	**25,62 c**	

*FC= Familia Química; TR= Tiempo de Retención; IK= Índice Kovats; EXP= Experimental; LIT= Literatura; †*** significante al p<0,001. ‡Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey. ¥Descriptores de olor de los compuestos volátiles.*

Objetivo específico 2: Caracterización funcional (perfil carotenoides, tocoferoles, tiamina (B1) y riboflavina (B2)).

Los resultados de este objetivo están reflejados en la siguiente publicación:

Publicación 3: "*Diplotaxis erucoides and Oxalis pes-caprae: two wild edible plants as a new and valuable source of carotenoids, tocols and B1 and B2 vitamins*". En esta publicación las determinaciones estudiadas fueron: perfil de carotenoides, perfil de tocoles, determinación de tiamina (vitamina B1) y riboflavina (vitamina B2).

4.7. Perfil de carotenoides

Se identificaron diez carotenoides en *Diplotaxis erucoides* y *Oxalis pes-caprae* **(Tablas 9 y 10**, respectivamente**)**. En *Diplotaxis erucoides* los principales carotenoides fueron la luteína y el β-caroteno, siendo ambos compuestos el 70 % del total de carotenoides en cada parte de la planta. En plantas de la misma familia (Brassicaceae), como *Eruca sativa* L., o del mismo género, como *Diplotaxis tenuifolia* L., los principales carotenoides identificados fueron también la luteína y el β-caroteno; en el caso de la luteína, estas dos plantas tienen contenidos más altos (11,10 y 12,99 mg/100 g p.f., respectivamente). Sin embargo, los valores de β-caroteno (3,58 y 4,17 mg/100 g p.f., respectivamente) fueron hasta un 50 % más bajos que los encontrados en *Diplotaxis erucoides* (Reif et al., 2013). Por el contrario, tanto las vainas como los tallos tuvieron contenidos mucho menores (4,75 y 2,70 mg/ 100 g p.f., respectivamente).

El perfil de carotenoides de *Oxalis pes-caprae* fue muy similar al de *Diplotaxis*, encontrándose los valores más altos en las hojas. En este caso, luteína, β-caroteno y β-criptoxantina mostraron los mayores contenidos, alcanzando el 80 % del total de carotenoides en las hojas. En cuanto a la luteína, que era el compuesto principal, su concentración en hojas de *Oxalis pes-caprae* fue de 4,76 mg/100 g p.f., aunque en plantas del mismo género, como *Oxalis corniculata*, este valor fue superior (unos 11,3 mg/100 g p.f.) Estos resultados son esperados porque la luteína es el compuesto responsable del color amarillo y, en el caso de *Oxalis corniculata*, su color amarillo es más intenso que el color amarillo exhibido por las flores de *Oxalis pes-caprae* (Zeb & Imran, 2019). Tanto en flores como en tallos, los valores de carotenoides totales fueron muy similares (1,47 y 1,49 mg/100 g p.f., respectivamente). La zeaxantina estaba presente en contenidos

relativamente altos; sin embargo, este compuesto no estaba presente en *Oxalis corniculata* (Zeb & Imran, 2019). En las hojas de ambas plantas investigadas también se encontraron cantidades bastante elevadas de violaxantina (28% del total de carotenoides) y de neoxantina (con un aporte promedio del 15% del total de carotenoides). A nivel de las plantas, estos carotenoides como luteína, zeaxantina o violaxantina son protectores de la radiación solar B que incide directamente en las plantas que crecen en la Antártida y que daña este tipo de radiación (Singh & Khare, 2021).

Tabla 9. Contenido de carotenoides, en peso fresco y seco, en *Diplotaxis erucoides*.

Carotenoides	ANOVA[ǂ]	*p*-Value	Fresco (mg/ 100 g p.f.[δ])			ANOVA	*p*-Value	Seco (mg/ 100 g p.s.[¥])		
			Vaina	Hoja	Tallo			Vaina	Hoja	Tallo
Luteína	***	0,0000	2,12 ± 0,03 b[ǂ]	8,23 ± 0,96 a	1,53 ± 0,02 b	***	0,0000	9,38 ± 0,14 b	47,38 ± 5,50 a	8,52 ± 0,13 b
Zeaxantina	***	0,0000	0,24 ± 0,01 b	0,37 ± 0,00 a	0,11 ± 0,01 c	***	0,0000	1,04 ± 0,06 b	2,14 ± 0,01 a	0,63 ± 0,08 c
Violaxantina	***	0,0000	0,11 ± 0,01 b	2,28 ± 0,19 a	0,20 ± 0,03 b	***	0,0000	0,50 ± 0,05 b	13,10 ± 1,09 a	1,10 ± 0,15 b
Neoxantina	***	0,0000	n.d.	1,07 ± 0,04 a	0,15 ± 0,02 b	***	0,0000	n.d.	6,14 ± 0,26 a	0,82 ± 0,11 b
β-criptoxantina	n.s.	0,2879	n.d.	0,09 ± 0,01	0,08 ± 0,01	n.s.	0,1846	n.d.	0,53 ± 0,09	0,43 ± 0,06
Antarexantina	***	0,0000	0,15 ± 0,00 b	0,38 ± 0,04 a	0,15 ± 0,02 b	***	0,0000	0,67 ± 0,01 b	2,19 ± 0,20 a	0,39 ± 0,05 b
α-caroteno	***	0,0000	0,18 ± 0,02 b	1,00 ± 0,06 a	0,04 ± 0,00 c	***	0,0000	0,79 ± 0,10 b	5,77 ± 0,35 a	0,23 ± 0,01 c
13-*cis*-β-caroteno	***	0,0000	0,05 ± 0,01 b	1,28 ± 0,08 a	n.d.	***	0,0000	0,24 ± 0,02 b	7,38 ± 0,44 a	n.d.
β-caroteno	***	0,0000	1,32 ± 0,14 b	9,47 ± 0,57 a	0,39 ± 0,01 c	***	0,0000	5,82 ± 0,63 b	54,48 ± 3,27 a	2,18 ± 0,01 b
9-*cis*-β-caroteno	***	0,0000	0,57 ± 0,07 a	0,09 ± 0,01 b	0,13 ± 0,01 b	***	0,0000	2,54 ± 0,32 a	0,51 ± 0,03 b	0,72 ± 0,01 b
Total carotenoides	***	**0,0000**	**4,75 ± 0,28 b**	**24,26 ± 1,85 a**	**2,70 ± 0,05 b**	***	**0,0000**	**20,97 ± 1,25 b**	**139,63 ± 10,62 a**	**15,04 ± 0,33 b**

*[δ]p.f. = peso fresco; [¥]p.s. = peso seco; [ǂ]n.s.: no significante al p>0,05; *. **. y ***. significante al p< 0,05, 0,01 y 0,001, respectivamente; n.d. = no detectado; [ǂ]Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey.*

Tabla 10. Contenido de carotenoides, en peso fresco y seco, en *Oxalis pes-caprae*.

Carotenoides	ANOVA[†]	*p*-Value	Fresco (mg/ 100 g p.f.[δ])			ANOVA	*p*-Value	Seco (mg/ 100 g p.s.[¥])		
			Flor	Hoja	Tallo			Flor	Hoja	Tallo
Luteína	***	0,0000	0,10 ± 0,02 b[‡]	4,76 ± 0,29 a	0,38 ± 0,05 b	***	0,0000	0,72 ± 0,12 c	34,20 ± 2,07 a	4,25 ± 0,59 b
Zeaxantina	**	0,0085	0,17 ± 0,01 a	0,15 ± 0,03 a	0,10 ± 0,01 b	n.s.	0,6089	1,21 ± 0,08	1,12± 0,21	1,11 ± 0,01
Violaxantina	-	-	n.d.	n.d.	0,04 ± 0,01	-	-	n.d.	n.d.	0,49 ± 0,03
Neoxantina	-	-	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.
β- criptoxantina	***	0,0001	0,16 ± 0,03 b	1,35 ± 0,24 a	0,32 ± 0,05 b	***	0,0000	1,11 ± 0,23 c	9,74 ± 1,74 a	3,53 ± 0,06 b
Anteraxantina	***	0,0003	n.d.	0,43 ± 0,06	0,02 ± 0,01	***	0,0004	n.d.	3,12 ± 0,46 a	0,24 ± 0,02 b
α-caroteno	***	0,0004	0,05 ± 0,01 b	0,34 ± 0,08 a	0,05 ± 0,01 b	***	0,0006	0,37 ± 0,01 b	2,45 ± 0,61 a	0,58 ± 0,01 b
13-*cis*- β-caroteno	***	0,0000	0,05 ± 0,01 a	0,01 ± 0,01 b	0,01 ± 0,01 b	***	0,0000	0,34 ± 0,01 a	0,10 ± 0,01 c	0,13 ± 0,01 b
β-caroteno	***	0,0005	0,49 ± 0,01 b	4,50 ± 1,19 a	0,44 ± 0,05 b	***	0,0006	3,48 ± 0,03 b	32,23 ± 8,55 a	4,90 ± 0,56 b
9-*cis*-β-caroteno	**	0,0014	0,12 ± 0,01 b	0,93 ± 0,29 a	0,11 ± 0,01 b	***	0,0000	0,88 ± 0,01 c	6,69 ± 0,22 a	1,26 ± 0,08 b
Total carotenoides	***	**0,0000**	**1,47 ± 0,07 b**	**12,50 ± 1,71 a**	**1,49 ± 0,11 b**	***	**0,0000**	**8,11 ± 0,47 b**	**89,73 ± 10,23 a**	**16,49 ± 1,21 b**

*[δ]p.f. = peso fresco; [¥]p.s. = peso seco; [†]n.s.: no significante al p>0,05; *. **. y ***. significante al p< 0,05, 0,01 y 0,001, respectivamente; n.d. = no detectado; [‡]Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey.*

Es difícil comparar los datos de la literatura sobre carotenoides, debido a los diferentes métodos analíticos utilizados para su determinación y a que estos pigmentos pueden variar dependiendo del genotipo, condiciones climáticas, estado de madurez, ubicación o estacionalidad, entre otros (Walsh et al., 2015); a pesar de las diferencias, se han obtenido en diversos estudios que diferentes vegetales de hojas verdes son fuentes ricas en luteína y buenas fuentes de β-caroteno (Fratianni et al., 2024; Panfili et al., 2020; Sánchez-Mata & Tardío, 2016; Žnidarčič et al., 2011).

En cuanto al β-caroteno, en comparación con otras PCS, cabe destacar plantas como *Amaranthus spinosus* (6,94±5,13 mg/100 g p.f.) de Brasil o *Vigna gallinacea* A. Rich., *Trilepisium madagascariense* DC. y *Cleome gynandra* L. (34,49, 24,92 y 28,67 mg/100 g p.s., respectivamente) de Etiopía, que tuvieron valores muy similares a *Oxalis pes-caprae* (4,50±1,19 mg/100 g p.f. correspondientes a 32,23±8,55 mg/100 g p.s.) (Pereira et al., 2023; Yimer et al., 2023). Otro ejemplo son las hojas de *Cichorium intybus*, que pertenece a una de las familias de PCS más representativas, Asteraceae. Tienen un perfil similar a *Diplotaxis erucoides*, excepto por dos carotenoides que no están presentes en las muestras analizadas como son la β-criptoxantina y la anteraxantina (Delfine et al., 2022).

La **Figura 6** muestra la actividad de vitamina A de *Diplotaxis erucoides* y *Oxalis pes-caprae*, expresada como Equivalentes de Retinol (ER). Centrándonos en *Diplotaxis erucoides*, la mayor concentración de ER se obtuvo en las hojas, alcanzando un valor de 1783 μg/ 100 g. En el Anexo XIII del Reglamento de 2011, publicado por la Unión Europea (Reglamento UE n° 1169/2011), basado en la opinión de expertos de la EFSA sobre la Cantidad Diaria Recomendada (CDR) para adultos de vitaminas y minerales (EFSA, 2015; European Union, 2011), los valores recomendados de vitamina A son 800 μg/ día de ER. Según esta referencia, al ingerir 100 g de hojas frescas de *Diplotaxis erucoides* se puede conseguir más del doble (223%) de la dosis diaria recomendada de vitamina A. Respecto a *Oxalis pes-caprae*, el mayor contenido también se presentó en las hojas (970 μg/ 100 g p.f.) (121 % de la CDR), por lo que 100 g de hojas frescas de *Oxalis* cubrirían la cantidad diaria recomendada de vitamina A. Por el contrario, tanto la flor como el tallo tuvieron valores muy bajos (14 μg/ 100 g p.f.). El Reglamento de la Unión Europea especifica que cualquier fuente de alimento que alcance un valor superior al 15 % de la CDR puede declararse en la etiqueta como "fuente de vitamina A" (European

Union, 2011). Así pues, tanto en el caso de *Diplotaxis erucoides* como de *Oxalis pes-caprae* se puede afirmar que 100 g de sus hojas son fuente de vitamina A.

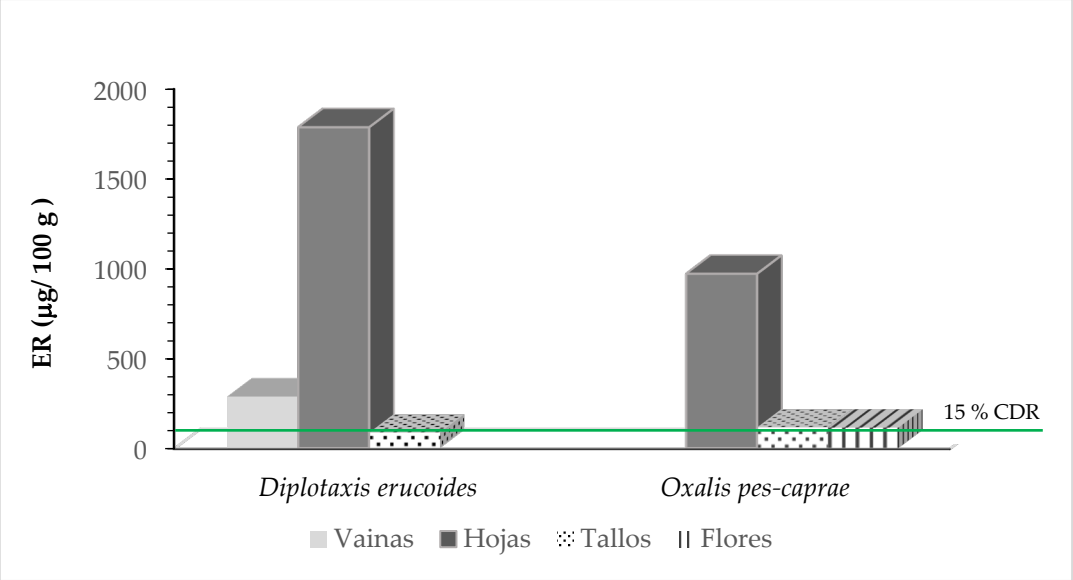

Figura 6. Actividad de Vitamina A en *Diplotaxis erucoides* and *Oxalis pes-caprae* (Equivalentes de Retinol expresado en µg/ 100 g)

4.8. Perfil de tocoles

Se realizó un análisis de tocoferoles y tocotrienoles (tocoles), aunque no se detectaron tocotrienoles. Sin embargo, sí se encontraron tres tocoferoles diferentes en ambas plantas α, β y γ **(Tabla 11)**, entre los cuales, α y γ fueron los más representativos en *Diplotaxis erucoides*. Los valores de α-tocoferol encontrados en hojas frescas de *Diplotaxis* y *Oxalis* (4,13 y 6,81 mg/ 100 g p.f., respectivamente; correspondientes a 23,79 y 48,9 mg/ 100 g p.s., respectivamente) fueron similares a los encontrados en otras especies de PCS como *Sonchus asper*, *Sonchus oleraceus*, *Spinacia oleracea* y *Cichorium intybus* (19, 20, 32 y 33 mg/ 100g ps, respectivamente) (Fratianni et al., 2024; Fratianni et al., 2021; Panfili et al., 2020). Los valores de α-tocoferol en *Oxalis pes-caprae* fueron más altos que los encontrados en hojas jóvenes de *Oxalis acetosella* (18,6 mg/ 100 g de peso seco) (Šircelj et al., 2010). Los contenidos de γ-tocoferol de las hojas frescas de *Oxalis* (3,91 mg/100 g p.f.) fueron muy similares a los encontrados en *Sonchus asper*, *Sonchus oleraceus* y *Spinacia oleracea* (3,3, 2,7 y 4,7 mg/ 100 g p.f., respectivamente) (Fratianni et al., 2024; Fratianni et al., 2021). A diferencia de *Oxalis* y *Diplotaxis*, otras PCS de Brasil, como "caruru" (*Amaranthus spinosus* L.) y "trapoeraba" (*Commelina benghalensis*) reportaron contenidos de δ-tocoferol que alcanzaron valores de 3,27 y 0,71 µg/ 100 g p.f.,

respectivamente (Pereira et al., 2023). En *Oxalis pes-caprae* no se encontraron β y δ-tocoferol; de igual manera, en otro estudio tampoco se mostraron resultados para el β-tocoferol en *Oxalis acetosella*, que es del mismo género. Sin embargo, sí se encontró δ-tocoferol en hojas de *Oxalis acetosella* con una concentración de 140 mg/ 100 g de peso seco (Šircelj et al., 2010).

La **Figura 7** muestra la actividad de la vitamina E de *Diplotaxis erucoides* y *Oxalis pes-caprae*, expresada como Equivalentes de Tocoferol (ET), en mg por 100 g de peso fresco. Centrándonos en *Diplotaxis erucoides*, el mayor valor de ET se presentó en hojas, alcanzando un valor de 4,24 mg/ 100 g. Como se comentó anteriormente en el apartado de carotenoides, según el Reglamento de 2011, al ingerir 100 g de hojas frescas de *Diplotaxis erucoides*, un consumidor puede alcanzar el 35% de la CDR de vitamina E (European Union, 2011).

Respecto a *Oxalis pes-caprae*, el mayor contenido también se mostró en las hojas (7,20 mg/ 100 g p.f.), por lo que el 60% de la dosis diaria recomendada de vitamina E se puede cubrir con 100 g de hojas frescas de *Oxalis*. Tanto las flores como los tallos tuvieron valores muy bajos de actividad de vitamina E (de 0,6 a 1,5 mg/100 g p.f.). El Reglamento de la Unión Europea especifica que cualquier fuente de alimento que alcance un valor superior al 15% de la CDR puede declararse en la etiqueta como "fuente de vitamina E" (EFSA, 2015; European Union, 2011). Por tanto, tanto en el caso de *Diplotaxis erucoides* como de *Oxalis pes-caprae*, se puede afirmar que 100 g de sus hojas son fuente de vitamina E.

Tabla 11. Perfil de tocoles, en peso fresco y seco, de *Diplotaxis erucoides* y *Oxalis pes-caprae*.

Diplotaxis erucoides	ANOVA[†]	*p*-Value	Fresco (mg/ 100 g p.f.[δ])			ANOVA[†]	*p*-Value	Seco (mg/ 100 g p.s.[¥])		
			Vaina	Hoja	Tallo			Vaina	Hoja	Tallo
α-Tocoferol	***	0,0000	1,81 ± 0,13 b[‡]	4,13 ± 0,44 a	0,65 ± 0,06 c	***	0,0000	7,97 ± 0,57 b[‡]	23,79 ± 2,53 a	3,62 ± 0,33 c
β-Tocoferol	***	0,0000	n.d.	0,10 ± 0,01 a	0,01 ± 0,01 b	***	0,0000	n.d.	0,60 ± 0,02 a	0,06 ± 0,01 b
γ-Tocoferol	***	0,0000	0,56 ± 0,03 a	0,50 ± 0,07 a	0,04 ± 0,01 b	***	0,0000	2,47 ± 0,12 a	2,89 ± 0,40 a	0,21 ± 0,02 b
Total tocoferoles	***	**0,0000**	**2,37 ± 0,16 b**	**4,74 ± 0,51 a**	**0,70 ± 0,05 c**	***	**0,0000**	**10,44 ± 0,70 b**	**27,28 ± 2,94 a**	**3,89 ± 0,25 c**

Oxalis pes-caprae	ANOVA[†]	*p*-Value	Fresco (mg/ 100 g p.f.)			ANOVA[†]	*p*-Value	Seco (mg/ 100 g p.s.)		
			Flor	Hoja	Tallo			Flor	Hoja	Tallo
α-Tocoferol	***	0,0000	1,17 ± 0,13 b[‡]	6,81 ± 0,44 a	1,41 ± 0,01 b	***	0,0000	8,25 ± 0,92 c[‡]	48,89 ± 3,16 a	15,55 ± 0,02 b
β-Tocoferol	-	-	n.d.	n.d.	n.d.	-	-	n.d.	n.d.	n.d.
γ-Tocoferol	***	0,0003	3,05 ± 0,56 a	3,91 ± 0,28 a	1,11 ± 0,16 b	***	0,0002	21,59 ± 2,96 b	28,09 ± 1,99 a	12,23 ± 1,81 c
Total tocoferoles	***	**0,0000**	**4,22 ± 0,33 b**	**10,72 ± 0,16 a**	**2,51 ± 0,16 c**	***	**0,0000**	**29,84 ± 1,89 b**	**76,98 ± 1,17 a**	**27,78 ± 1,83 b**

*[δ]p.f. = peso fresco; [¥]p.s. = peso seco; [†]n.s.: no significante al p>0,05; *. **. y ***. significante al p< 0,05, 0,01 y 0,001, respectivamente; n.d. = no detectado; [‡]Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey.*

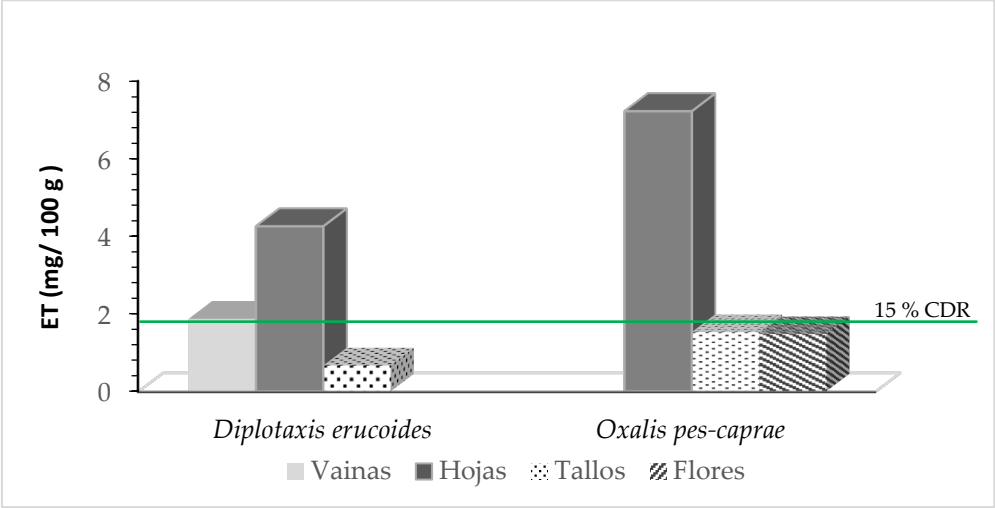

Figura 7. Actividad de Vitamina E en *Diplotaxis erucoides* y *Oxalis pes-caprae* (Equivalentes de Tocoferol expresados en mg/ 100 g).

4.9. Contenido de tiamina (B1) y riboflavina (B2)

Los tallos de *Diplotaxis erucoides* mostraron el mayor contenido de tiamina, con una concentración de 1.46 mg/ Kg p.f., siendo superior a la encontrada en las hojas (0.64 mg/ Kg p.f.) **(Tabla 12)**. Sin embargo, este compuesto no se encontró en las vainas. La comparación con otras PCS mostró valores similares a los reportados en *Sonchus asper* (0,09 mg/ 100 g p.f.), *Sonchus oleraceus* (0,10 mg/ 100 g p.f.) y *Crepis vesicaria* (0,13 mg/ 100 g p.f.), todos ellos pertenecientes a la familia Asteraceae (Panfili et al., 2020).

Por otro lado, las flores de *Oxalis pes-caprae* mostraron el mayor contenido de tiamina, 1,17 mg/ Kg p.f. (correspondiente a 8,25 mg/ Kg p.s.), seguidas de las hojas, con 0,33 mg/ Kg p.f. (correspondientes a 2,36 mg/Kg p.s.), mientras que este compuesto no se encontró en los tallos. En comparación con otras plantas, como las hojas de *Ipomoea acuática* Forssk. (0,45 mg/100 g p.s.), *Achyranthes aspera* L. (0,13 mg/ 100 g p.s.) o *Enhydra fluctuans* Lour. (0,40 mg/100 g p.s.), se obtuvieron valores similares (Datta et al., 2019).

Respecto a la riboflavina, fue encontrada en todas las partes de ambas plantas, en niveles bajos. En *Diplotaxis erucoides*, las vainas presentaron el mayor contenido, 0,23 mg/ Kg p.f. (correspondiente a 1,02 mg/ Kg p.s.), seguidas de las hojas, con 0,14 mg/Kg p.f. (correspondientes a 0,83 mg/ Kg p.s.), y, finalmente, por los tallos. En el caso de *Oxalis pes-caprae*, las hojas presentaron las mayores cantidades, 0,16 mg/ Kg p.f. (correspondiente a 1,14 mg/ Kg p.s.), seguidas de las flores, con 0,10 mg/ Kg p.f., (correspondientes a 0,69 mg/ Kg p.s.) y por último los tallos. Tanto en *Diplotaxis* como en *Oxalis* los valores fueron similares a los de tres plantas pertenecientes a la familia Asteraceae (*Sonchus asper* (0,01 mg/100 g p.f.), *Sonchus oleraceus* (0,01 mg/100 g p.f.) y *Crepis vesicaria* (0,02 mg/100 g p.f.) (Fratianni et al., 2024; Panfili et al., 2020). En comparación con otras PCS, como las hojas de *Ipomoea acuática* Forssk (0,710 mg/100 g p.s.), *Achyranthes aspera* L. (0,384 mg/ 100 g p.s.) o *Enhydra fluctuans* Lour. (1,043/ 100 g de peso seco), tanto *Diplotaxis* como *Oxalis* tuvieron contenidos significativamente más bajos (Datta et al., 2019).

Tabla 12. Contenido de tiamina y riboflavina, en peso fresco y seco, en *Diplotaxis erucoides* y *Oxalis pes-caprae*.

Diplotaxis erucoides	ANOVA[†]	*p*-Value	Fresco (mg/ Kg p.f.[δ])			ANOVA[†]	*p*-Value	Seco (mg/ Kg p.s.[¥])		
			Vaina	Hoja	Tallo			Vaina	Hoja	Tallo
Tiamina	***	0,0000	n.d.	0,64 ± 0,06 b	1,46 ± 0,04 a	***	0,0000	n.d.	3,72 ± 0,15 b[‡]	8,13 ± 0,21 a
Riboflavina	***	0,0000	0,23 ± 0,02 a	0,14 ± 0,01 b	0,03 ± 0,01 c	***	0,0000	1,02 ± 0,10 a	0,83 ± 0,05 b	0,18 ± 0,06 c

Oxalis pes-caprae	ANOVA[†]	*p*-Value	Fresco (mg/ Kg p.f.)			ANOVA[†]	*p*-Value	Seco (mg/ Kg p.s.)		
			Flor	Hoja	Tallo			Flor	Hoja	Tallo
Tiamina	***	0,0000	1,17 ± 0,05 a	0,33 ± 0,02 b	n.d.	***	0,0000	8,25 ± 0,33 a[‡]	2,36 ± 0,16 b	n.d.
Riboflavina	***	0,0000	0,10 ± 0,01 b	0,16 ± 0,01 a	0,02 ± 0,01 c	***	0,0000	0,69 ± 0,01 b	1,14 ± 0,06 a	0,26 ± 0,01 c

*[δ]p.f. = peso fresco; [¥]p.s. = peso seco; [†]n.s.: no significante al p>0,05; *. **. y ***. significante al p< 0,05, 0,01 y 0,001, respectivamente; n.d. = no detectado; [‡]Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey.*

Objetivo específico 3: Desarrollo de léxico sensorial y estudio sensorial descriptivo por un panel experto.

Objetivo específico 4: Innovación alimentaria a través de las plantas comestibles silvestres, desarrollando un producto.

Los resultados de estos objetivos están reflejados en la siguiente publicación:

Publicación 4: "*Characterization and potential use of Diplotaxis erucoides as food ingredient for a sustainable modern cuisine and comparison with commercial mustards and wasabis*". En esta publicación se evaluaron: el perfil volátil de *Diplotaxis erucoides* y los productos comerciales de referencia, ácidos orgánicos y azúcares de *Diplotaxis erucoides* y productos comerciales de referencia, análisis descriptivo sensorial y desarrollo de léxico con los productos desarrollados a base de *Diplotaxis* y dos referencias comerciales.

4.10. Desarrollo de productos

Para el desarrollo de dos productos alimentarios (crema y mayonesa) se utilizó la planta *Diplotaxis erucoides*. Se realizó una comparativa con 5 mostazas y 3 wasabis comerciales aprovechando el sabor picante tan parecido que tiene esta planta a estos productos. Se determinó el perfil volátil, los ácidos orgánicos y azúcares, y un análisis descriptivo de dichas salsas y los nuevos productos, utilizando también la planta fresca como control.

4.10.1. Perfil de compuestos volátiles de la crema y la mayonesa a base de *Diplo taxis*

Se aislaron, identificaron y semicuantificaron un total de 37 compuestos volátiles en todas las muestras estudiadas **(Tabla 13)** y pudiendo agruparse en 10 familias químicas: terpenos (6 compuestos), terpenoides (5), aldehídos (5), isotiocianatos (4), alcoholes (3), compuestos de azufre (3), ácidos (3), ésteres (2), compuestos de nitrógeno (2), cetonas (1) y 3 compuestos que se incluyeron en la clase "otros". En las mostazas estaban presentes todas las familias químicas, mientras que en el wasabi sólo se encontraron 5 de las 10 familias químicas; se encontró una tendencia similar en la planta fresca de *Diplotaxis erucoides* y en las salsas (DeC y DeM), donde predominaron solamente 4 y 3 familias químicas, respectivamente.

En todas las muestras analizadas, la familia química "isotiocianatos" (ITC), con solo 4 compuestos (isotiocianato de alilo, isotiocianato de isobutilo, isotiocianato de 3-butenilo e isotiocianato de fenetilo) fue la predominante (~80,6 %, media de vainas, hojas y tallos), seguidas de lejos por las familias de compuestos azufrados (S) (~6,9 %) y ácidos orgánicos (~5,9 %); finalmente, todas las demás familias tuvieron contenidos inferiores al 2,5 % **(Tabla 13)**. El predominio de esta misma familia química (ITC) se ha informado previamente en otras plantas (Tisserand & Young, 2014), y también para especies similares a la *Diplotaxis erucoides* como es *Eruca sativa* (Kala et al., 2018). Tres isotiocianatos eran compuestos alifáticos: isotiocianato de alilo, isotiocianato de 3-butenilo (se forman a partir de metionina) e isotiocianato de isobutilo (formado a partir de leucina); mientras que el compuesto final, isotiocianato de fenetilo, es un compuesto aromático procedente de la fenilalanina.

Tabla 13. Compuestos volátiles (% total del área identificada) de *Diplotaxis erucoides*, mostazas y wasabis comerciales, y productos a base de *Diplotaxis*.

Cod.	Compuestos	TR (min)	IK	ANOVA†	*Diplotaxis erucoides* (%)			Mostazas (%)					Wasabis (%)			Platos (%)	
					Vaina	Hoja	Tallo	M1	M2	M3	M4	M5	W1	W2	W3	DeC	DeM
V1	Sulfuro de óxido de carbono	1,933	496	***	n.d.	n.d.	n.d.‡	n.d.	26,70 a	n.d.	0,07 b	n.d.	n.d.	0,04 b	0,05 b	n.d.	n.d.
V2	Etanol	2,269	517	*	n.d.	n.d.	n.d.	0,78 a	n.d.	0,41 b	0,18 c	0,16 c	n.d.	0,02 d	0,02 d	n.d.	n.d.
V3	Sulfuro de dimetilo	2,429	527	*	1,79 b	1,47 b	2,93 a	n.d.	n.d.	n.d.	n.d.	n.d.	0,06 c	0,07 c	0,02 c	n.d.	n.d.
V4	Disulfuro de carbono	2,585	537	**	n.d.	n.d.	n.d.	0,98 d	n.d.	3,33 c	9,47 b	19,44 a	8,84 b	11,63 b	3,05 c	n.d.	n.d.
V5	Acetato de etilo	3,094	568	*	n.d.	n.d.	n.d.	5,17 a	n.d.	0,51 b	0,19 b	0,42 b	n.d.	n.d.	n.d.	n.d.	n.d.
V6	Ácido acético	3,212	576	***	n.d.	n.d.	n.d.	30,16 a	n.d.	11,02 b	15,04 b	16,00 b	0,08 c	0,15 c	0,10 c	n.d.	n.d.
V7	Metilacrilonitrilo	3,919	619	*	n.d.	n.d.	n.d.	n.d.	1,03 b	2,34 a	1,69 ab	2,19 a	n.d.	0,05 c	0,03 c	n.d.	n.d.
V8	Hexanal	6,502	756	*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2,92 a
V9	Isotiocianato de alilo	8,248	828	***	96,34 a	95,07 a	83,63 c	26,51 f	67,11 d	70,09 d	69,10 d	58,33 e	86,5 b	83,85 c	94,15 a	90,02 b	87,27 b
V10	trans-2-Hexenal	8,921	820	*	0,26 b	0,80 b	0,22 b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8,00 a	9,41 a
V11	Isotiocianato de isobutilo	9,539	874	*	n.d.	n.d.	n.d.	n.d.	0,33 b	0,62 a	0,16 c	0,31 b	0,27 b	0,33 b	0,03 d	n.d.	n.d.
V12	Isotiocianato de 3-butenilo	11,327	935	*	0,39 e	0,29 e	0,29 e	0,98 d	2,41 b	4,26 a	1,58 c	1,71 c	0,17 e	0,17 e	0,04 e	n.d.	n.d.
V13	1.8-Cineol	12,099	960	*	n.d.	n.d.	0,10 b	1,13 a	n.d.	0,9 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V14	α-felandreno	13,366	1001	*	n.d.	n.d.	n.d.	0,77 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V15	ácido hexanoico	14,350	1032	*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,61 a	n.d.
V16	Limoneno	14,394	1033	*	n.d.	n.d.	n.d.	0,51 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V17	Felandreno	14,548	1038	*	n.d.	n.d.	n.d.	0,55 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V18	γ-terpineno	15,694	1074	*	n.d.	n.d.	n.d.	0,33 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V19	Nonanal	16,033	1085	*	n.d.	0,12 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V20	Alcanfor	16,939	1114	*	n.d.	n.d.	n.d.	n.d.	n.d.	0,21 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V21	Fenilacetaldehído	17,710	1139	*	n.d.	n.d.	0,59 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V22	Estragol	17,760	1141	*	n.d.	n.d.	n.d.	1,24 a	0,13 b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V23	α-terpineol	17,976	1147	*	n.d.	n.d.	n.d.	0,37 a	n.d.	0,20 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V24	Linalol	18,915	1178	*	n.d.	n.d.	n.d.	0,36 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V25	Decanal	19,112	1184	**	0,19 b	0,18 b	0,22 ab	0,43 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V26	Bencenopropanonitrilo	20,766	1239	*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,13 ab	n.d.	0,09 b	0,24 a	0,03 c	n.d.	n.d.
V27	Bencilacetona	20,777	1239	*	n.d.	n.d.	n.d.	n.d.	0,31 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V28	2-Pirrolidinetiona	22,437	1295	*	n.d.	n.d.	n.d.	n.d.	0,77 c	1,89 a	1,36 b	0,62 c	n.d.	n.d.	n.d.	n.d.	n.d.
V29	Triacetina	22,751	1305	*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,13 a	n.d.	n.d.	n.d.	n.d.	n.d.
V30	Octanoato de etilo	22,859	1309	*	n.d.	n.d.	n.d.	0,20 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V31	Terpinen-4-ol	23,061	1316	*	n.d.	n.d.	n.d.	4,34 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V32	Eugenol	23,313	1325	***	n.d.	n.d.	n.d.	22,17 a	n.d.	1,94 b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V33	Ar-curcumeno	25,770	1412	*	n.d.	n.d.	n.d.	n.d.	0,23 a	0,24 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V34	β-ciclocitral	26,250	1430	*	n.d.	0,12 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V35	Isotiocianato de fenetilo	26,967	1457	***	0,66 e	1,55 d	11,04 a	n.d.	0,63 e	1,49 d	0,61 e	0,34 e	3,64 b	3,14 b	2,14 c	n.d.	n.d.
V36	trans-Anetol	28,443	1516	**	n.d.	n.d.	n.d.	1,99 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
V37	trans-Cinamaldehído	29,452	1568	*	n.d.	n.d.	n.d.	0,52 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Cod = Código; TR= Tiempo de Retención; IK= Índice Kovats; M = Mostaza; W = Wasabi; DeC = Crema Diplotaxis erucoides; DeM = Mayonesa Diplotaxis erucoides; n.d. = no detectado; †, **, y ***, significante al p< 0,05, 0,01 and 0,001, respectivamente; ‡Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey.*

En la planta fresca de *Diplotaxis erucoides* el mayor contenido fue el de la familia ITC (~96,4 %) **(Figura 8)**, mientras que las demás familias no alcanzaron ni el 3,0 %. Esta misma tendencia se ha informado previamente para diversas especies dentro del género Brassicaceae (Raffo et al., 2018; Shahidi, 1994; Spadafora et al., 2016). En mostazas, la familia ITC siguió siendo predominante (~61,3 %); aunque en este caso, otras familias tenían contenidos relativamente altos, incluidos ácidos orgánicos (~14,4 %), compuestos S (~12,0 %) y terpenoides (5,4 %). En cuanto a las muestras de wasabi, la familia principal fue nuevamente ITC (~91,5 %), y los compuestos S representaron ~8,0 %. Finalmente, en los productos basados en *Diplotaxis*, la familia ITC tuvo el mayor contenido (~89,0 %); sin embargo, el segundo contenido más alto fue el de aldehídos (~9,0 %), los cuales no jugaron un papel predominante en las muestras anteriores. La tercera familia química fueron los ácidos orgánicos (~2,3 %); sólo estas tres familias estaban presentes en los productos de nuevo desarrollo.

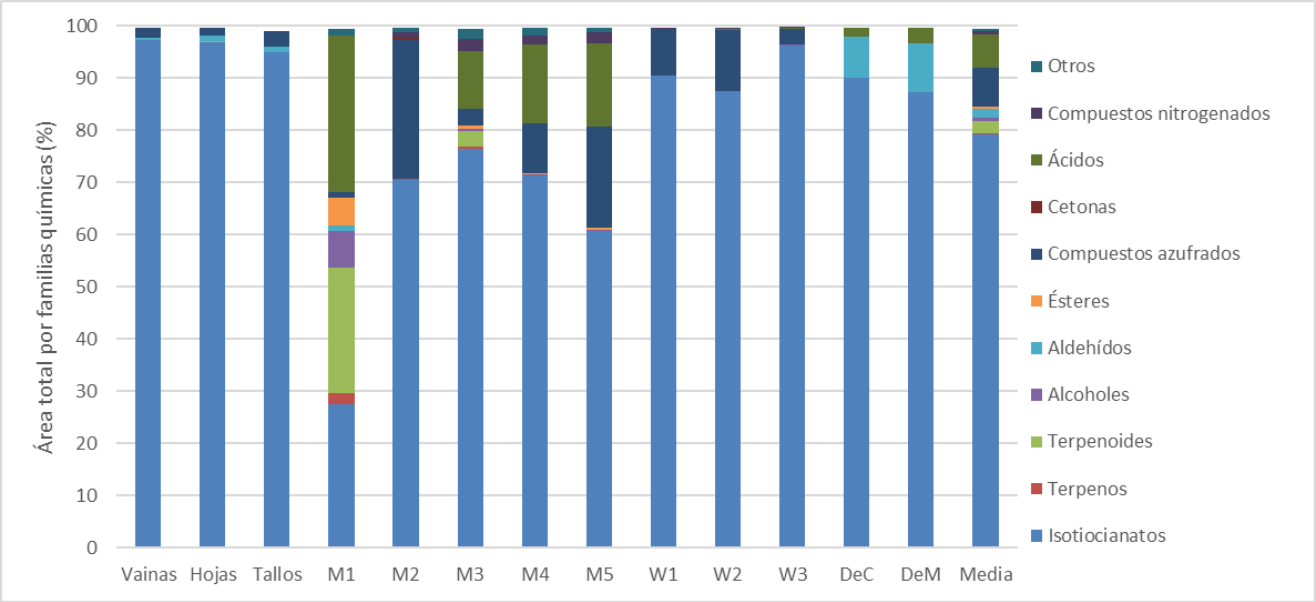

Figura 8. Familias químicas (% del área total) de los compuestos volátiles encontrados en la planta *Diplotaxis erucoides*, muestras comerciales de mostaza y wasabi y productos a base de *Diplotaxis*.

El isotiocianato de alilo fue el principal compuesto volátil, representando el 77,5 % del total de compuestos identificados en todas las muestras analizadas. Dentro de la familia ITC, este compuesto representó ~95,0 % del contenido total, y fue el predominante en todas las muestras excepto en M1. El orden general para el contenido de isotiocianato de alilo fue *Diplotaxis erucoides* > Productos a base de *Diplotaxis* > wasabi > mostaza. Otro compuesto importante fue el ácido acético, que estuvo presente en 7 de

las 13 muestras analizadas. Fue el segundo compuesto volátil más abundante en M3 y M4 (11 y 15 %, respectivamente), y en el caso de la muestra M1, el ácido acético (~30 %) predominó incluso por encima del isotiocianato de alilo **(Tabla 13)**. Normalmente, las recetas de mostaza contienen vinagre (ácido acético) y su contenido depende de las marcas y mercados (Cools & Terry, 2018). Cabe destacar también el caso del eugenol, que predominó en dos de las muestras de mostaza (M1 y M3), alcanzando un contenido de hasta 22 % en la muestra M1. El eugenol es el compuesto volátil predominante del clavo, y esta especia es bastante habitual como aromatizante de mostazas (Nurdjannah & Bermawie, 2012).

La degradación de los glucosinolatos conduce a la presencia de isotiocianatos intermedios, que a su vez producen compuestos como el sulfuro de óxido de carbono (COS) y el disulfuro de carbono (CS2) (Brown et al., 1991; Pecháček et al., 1997). En muestras frescas de *Diplotaxis erucoides* no se encontró COS, así como en los productos a base de *Diplotaxis*. Sin embargo, aparecieron cantidades apreciables de estos compuestos (COS y CS2) en todas las muestras comerciales.

En cuanto a la complejidad de las muestras (aquellas que tienen un alto número de compuestos volátiles), las mostazas tuvieron compuestos de todas las familias, especialmente la muestra M1 que tuvo hasta 21 compuestos; por el contrario, solo se encontraron 4 compuestos (hexanal, isotiocianato de alilo, trans-2-hexenal, ácido hexanoico) en los dos productos basados en *Diplotaxis* (DeC y DeM). Anteriormente se informó una tendencia y composición similares en plantas del mismo género como es el caso de *Diplotaxis tenuifolia* (Raffo et al., 2018; Spadafora et al., 2016).

4.10.2. Ácidos orgánicos y azúcares de la crema y mayonesa a base de *Diplotaxis*

Se identificaron seis ácidos orgánicos **(Tabla 14)**, siendo los ácidos fítico y cítrico los predominantes. Este perfil de ácidos orgánicos es típico de la familia Brassicaceae (Ghnaya et al., 2013; Vale et al., 2015). El ácido cítrico fue el único compuesto encontrado en todas las muestras y su contenido osciló entre 0,018 y 11,32 g/ Kg (W2 y *Diplotaxis*-tallos, respectivamente). Normalmente, el ácido fítico está presente en la familia Brassicaceae (Frank, 2013); sin embargo, no estuvo presente ni en la planta de *Diplotaxis erucoides* ni, por consiguiente, en los productos basados en *Diplotaxis* (DeC y DeM). El ácido fítico alcanzó contenidos muy altos tanto en muestras de mostaza como de wasabi,

oscilando entre 318 y 2085 g/ Kg (M3 y W3, respectivamente). Los otros cuatro ácidos orgánicos (fumárico, málico, oxálico y tartárico) sólo se encontraron en *Diplotaxis erucoides* en contenidos bajos o incluso en trazas.

Además, se identificaron 6 azúcares entre todas las muestras **(Tabla 14)**, siendo la fructosa, la glucosa y la sacarosa los predominantes, estando presentes los dos primeros compuestos en todas las muestras. La fructosa era el azúcar más abundante en el wasabi, mientras que, tanto la glucosa como la sacarosa desempeñaban un papel importante tanto en la mostaza como en el wasabi. Además, el contenido de maltosa fue relativamente alto en dos muestras de mostaza (M1 y M2). Finalmente, *Diplotaxis* y los productos basados en *Diplotaxis* tenían un contenido de azúcar muy bajo y en su perfil predominó la glucosa. En otras plantas Brassicaceae, como *Eruca sativa*, los contenidos de azúcar fueron superiores a los de *Diplotaxis erucoides*, pero nunca alcanzaron los de las mostazas y wasabis comerciales (Bell et al., 2017).

Tabla 14. Ácidos orgánicos y azúcares encontrados en *Diplotaxis erucoides* (vainas, hojas, y tallos), mostazas y wasabis comerciales, y en productos a base de *Diplotaxis*.

Código	Compuesto	ANOVA†	*Diplotaxis erucoides* (g/ Kg)			Mostazas (g/ Kg)					Wasabis (g/ Kg)			Platos (g/ Kg)	
			Vaina	Hoja	Tallo	M1	M2	M3	M4	M5	W1	W2	W3	DeC	DeM
AO1	Cítrico	**	0,020 d‡	0,038 d	0,018 d	9,77 ab	8,24 b	6,22 c	7,92 b	7,44 bc	7,66 bc	11,32 a	7,41 bc	6,20 c	5,91 c
AO2	Fumário	n.s.	Traza	Traza	Traza	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AO3	Málico	n.s.	Traza	0.023	Traza	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AO4	Fítico	n.s.	n.d.	n.d.	n.d.	1501	431	2085	825	915	751	710	318	n.d.	n.d.
AO5	Oxálico	n.s.	Traza	Traza	Traza	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AO6	Tartárico	*	0,026 a	0,028 a	0,003 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A1	Arabinosa	*	0,027 a	0,028 a	0,042 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A2	Fructosa	**	Traza	Traza	Traza	Traza	18,66 b	Traza	Traza	Traza	262 a	303 a	205 a	Traza	Traza
A3	Galactosa	*	0,038 a	Traza	0,050 a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A4	Glucosa	***	0,102 f	Traza	0,100 f	Traza	50,80 c	82,04 b	102 a	94,35 a	40,92 d	27,14 e	35,32 d	0,262 f	0,230 f
A5	Maltosa	*	n.d.	n.d.	n.d.	23,31 b	54,49 a	n.d.	n.d.	n.d.	23,12 b	19,13 b	5,27 c	n.d.	n.d.
A6	Sacarosa	***	0,015 g	0,002 g	0,010 g	66,83 d	101 b	3,99 e	7,47 e	n.d.	82,74 c	137 a	93,16 b	0,070 f	0,043 f

AO = ácido Orgánico; A = azúcar; M = Mostaza; W = Wasabi; DeC = Crema Diplotaxis erucoides; DeM = Mayonesa Diplotaxis erucoides; †, **, y ***, significante al p< 0,05, 0,01 and 0,001, respectivamente; n.s. = no signficante; traza = por debajo del límite de cuantificación; n.d. = no detectado; ‡Valores (media de 3 repeticiones) seguidas por la misma letra, dentro de la misma fila y factor, no fueron significativamente diferentes (p>0,05). Prueba de rangos múltiples de Tukey.*

4.10.3. Evaluación sensorial

El panel describió el perfil sensorial de 4 muestras bajo análisis: (i) crema a base de *Diplotaxis* (DeC), (ii) mayonesa a base de *Diplotaxis* (DeM), (iii) mostaza M3 y (iv) wasabi W2 **(Figura 9)**. Las dos últimas muestras fueron seleccionadas previamente por el panel como las que tenían el sabor más cercano al de *Diplotaxis erucoides*, y fueron descritas completamente.

El olor y aroma de "*Diplotaxis*-ID" son la percepción (con el producto fuera o dentro de la boca, respectivamente) de aromáticos comúnmente asociados o identificados como *Diplotaxis erucoides* recién cortado; estos compuestos aromáticos pueden describirse como verdes, picantes, y sulfurosos. Las muestras W2 y DeM mostraron la mayor intensidad de los atributos "*Diplotaxis*-ID", seguidas de M3 y DeC. Ambos productos a base de *Diplotaxis*, presentaron alta intensidad del atributo herbáceo-verde; lo que puede estar relacionado con el alto contenido de trans-2-hexenal, cuyo principal descriptor sensorial es verde/herbáceo (Sigma-Aldrich, 2014). El alto dulzor del DeC probablemente se debió a la crema batida utilizada en su formulación. Las mostazas y el wasabi tenían un alto nivel de sal y acidez debido a la adición de NaCl y ácidos cítrico/acético (Ho et al., 2017; Joye, 2019). Finalmente, DeM tuvo el mayor amargor debido a su contenido relativamente alto de hexanal, entre otros factores (Kesen et al., 2018; Sigma-Aldrich, 2014).

Por otra parte, uno de los atributos más importantes en este tipo de productos es el picante. En este caso, W2 tuvo la mayor intensidad, seguido de M3 y los productos basados en *Diplotaxis* tuvieron la menor intensidad. El picante de la planta fresca de *Diplotaxis erucoides* fue alto, pero la enzima mirosinasa se inactivó durante la preparación de los nuevos productos debido a la temperatura a la que se prepararon (40 °C) y, por lo tanto, no fueron tan picantes como se esperaba (Niu et al., 2015). Recientemente se ha demostrado que cocinar puede afectar significativamente las concentraciones de glucosinolato (Baenas et al., 2019; Soares et al., 2017). Sin embargo, ambos productos basados en *Diplotaxis* mostraron un postgusto prolongado, especialmente DeM, y esto está relacionado con la alta intensidad de atributos clave como el olor/aroma de "*Diplotaxis*-ID" y las notas herbáceas y verdes. Por último, el largo postgusto de los productos *Diplotaxis* está relacionado con su alto contenido en trans-2-hexenal.

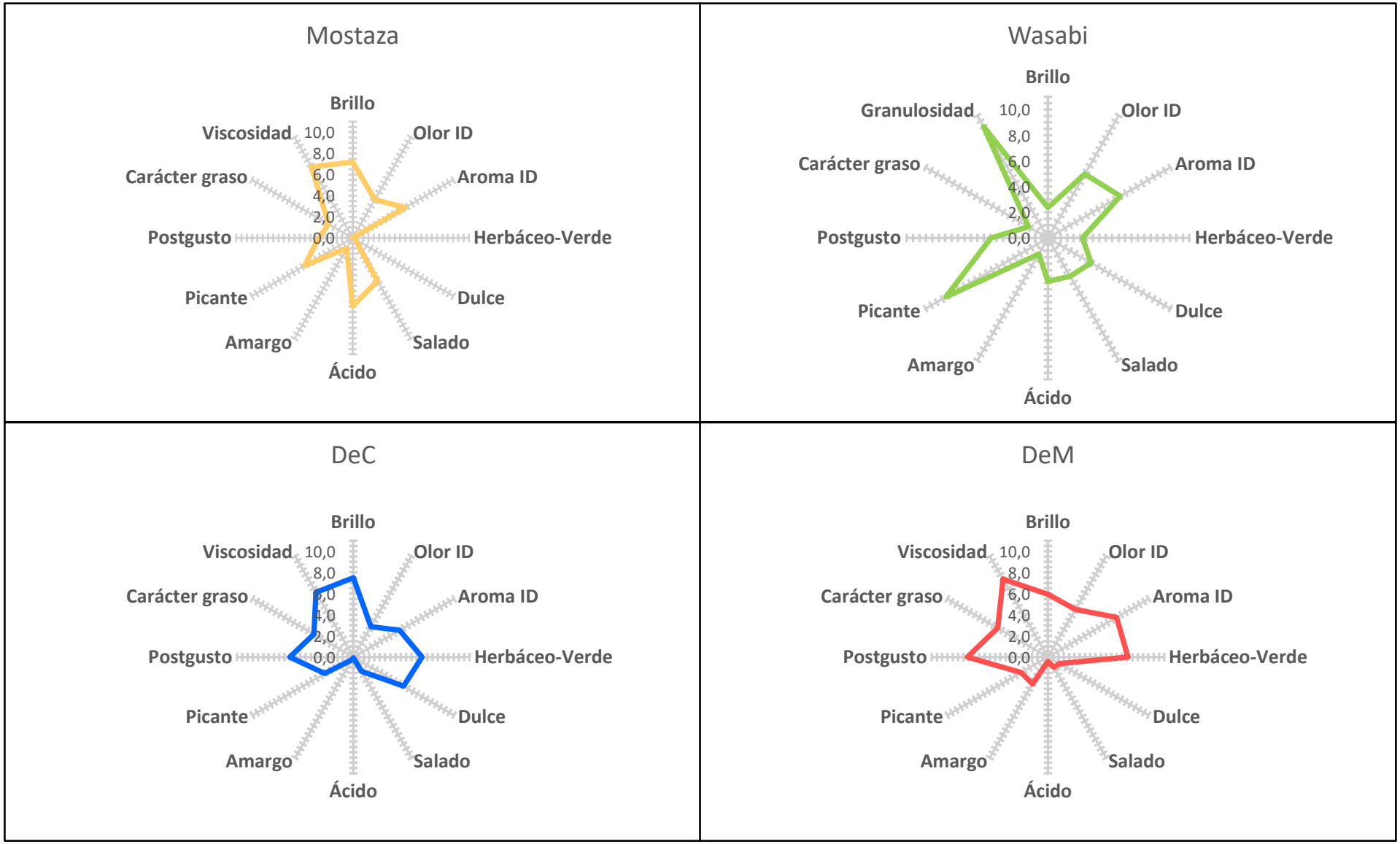

Figura 9. Análisis sensorial descriptivo de la mostaza y wasabi seleccionados, y de la crema y mayonesa a base de *Diplotaxis*.

5. CONCLUSIONES

5. CONCLUSIONES

- Las plantas comestibles silvestres constituyen un medio de vida para muchas familias en países en vías de desarrollo.

- De las tres partes estudiadas de *Oxalis pes-caprae*, la flor fue la parte que destacó por sus mayores niveles en minerales, azúcares, aminoácidos y ácidos grasos poliinsaturados.

- Las hojas de *Diplotaxis erucoides* y de *Oxalis pes-caprae* mostraron los mayores contenidos de carotenoides y tocoferoles.

- *Diplotaxis erucoides* y *Oxalis pes-caprae* pueden aportar más de un 15 % (Cantidad Diaria Recomendada) de vitamina A y E, por lo tanto, se las puede considerar fuente natural de ambas vitaminas.

- Los productos a base de *Diplotaxis erucoides* a nivel de ingredientes fueron más naturales que las mostazas y wasabis comerciales.

- El sabor picante de los productos formulados a base *Diplotaxis erucoides*, asociado típicamente a los isotiocianatos, no fue el esperado debido a que la temperatura de los platos inactivó la mirosinasa, que es la enzima responsable de la liberación de los isotiocianatos.

- Las plantas comestibles silvestres ofrecen un importante valor nutricional para el ser humano y tienen un impacto social positivo en términos de sostenibilidad. Estas cualidades las posicionan como opciones prometedoras para el desarrollo futuro de la industria agroalimentaria.

5. CONCLUSIONS

- Wild edible plants serve as a livelihood for many families in developing countries.

- Of the three parts of *Oxalis pes-caprae* studied, the flower stood out for its higher levels of minerals, sugars, amino acids, and polyunsaturated fatty acids.

- The leaves of *Diplotaxis erucoides* and *Oxalis pes-caprae* showed the highest contents of carotenoids and tocopherols.

- *Diplotaxis erucoides* and *Oxalis pes-caprae* can provide more than 15 % of the Recommended Daily Allowance (RDA) of vitamins A and E, therefore, they can be considered a natural source of both vitamins.

- Products based on *Diplotaxis erucoides* at the ingredient level were more natural than commercial mustards and wasabi.

- The spicy flavor of products formulated with *Diplotaxis erucoides*, typically associated with isothiocyanates, was not as expected because the temperature of the dishes inactivated myrosinase, the enzyme responsible for the release of isothiocyanates.

- Wild edible plants offer significant nutritional value to humans and have a positive social impact in terms of sustainability. These qualities make them promising options for the future development of the agro-food industry.

6. FUTURAS INVESTIGACIONES

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Como se ha comprobado, los resultados y las conclusiones evidencian un futuro prometedor para las plantas comestibles silvestres. Por ello, los objetivos establecidos a medio-largo plazo serían los siguientes:

- Estudio de los compuestos bioactivos a través de un perfil fenólico.
- Obtención de extractos mediante técnicas sostenibles como por ejemplo, Fluidos Supercríticos con CO_2.
- Estudio de toxicidad y alérgenos para determinar inocuidad o cantidad recomendable a ingerir.
- Obtención de extractos mediante solventes de extracción sostenible como, NaDES (Natural Deep Eutectic Solvents).
- Microencapsulación de compuestos bioactivos para la incoporación en matrices alimentarias.
- Estudios enzimáticos y de actividades biológicas.
- Estudios *in-silico* para modelar, simular y visualizar procesos biológicos.
- Estudios pre-clínicos con digestiones *in-vitro* (bioaccesibilidad), y fermentaciones colónicas (efecto prebiótico y su posible efecto en el eje microbiota intestino-cerebro) y estudios de biodisponibilidad.
- Desarrollo de un producto alimentario (propiedades tecnofuncionales, análisis sensorial, etc.).

7. REFERENCIAS

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8. PUBLICACIONES

PUBLICACIÓN 1

foods

MDPI

Review

Valorization of Wild Edible Plants as Food Ingredients and Their Economic Value

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Abstract: (1) Background: Wild Edible Plants (WEPs) are plants that grow without human help, by simply using the available resources. These types of plants are undervalued, because there is a lack of knowledge about their bioactive composition and nutritional/functional potential. (2) Scope and Approach: The main aim of this review is to fully identify the potential uses and importance of WEPs in certain regions based on (i) their sustainability, because they grow with their own resources, (ii) their content of bioactive compounds and consequently nutritional and functional value, (iii) their socio-economic relevance, and (iv) their ability to be useful in the agri-food industry in the short term. (3) Results: This review found evidence that a consumption of between 100 and 200 g of some of these WEPs can cover up to 50% of the recommended daily intake of proteins and fiber, being also a natural source of macro- and micro-minerals. Regarding their bioactive composition, most of these plants contain phenolic compounds and flavonoids, which determine their antioxidant capacity. (4) Conclusions: These reported results clearly demonstrate the high potential of the WEPs from a nutritional, economic and social point of view; although further studies are needed to gather deeper scientific information about their potential role in the socio-economic sustainability of specific groups of farmers worldwide.

Keywords: antioxidants; edible flowers; economic value; minerals; principal component analysis; sugars; total phenol content; flavonoids

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1. Introduction

"Wild Edibles" is a term used to describe both plants and animals consumed by humans. In 1999, the Food and Agriculture Organization of the United Nations (FAO) described the term wild plants as "those that grow spontaneously in self-maintaining populations in natural or semi-natural ecosystems and can exist independently of direct human action" [1]. The FAO estimated in 2016 that as much as ~100 million people consumed wild edible plants (WEPs) in Europe [2]; this figure highlights the potential of these plants even currently. The consumption of this type of plants dates back to the Bronze Age as shown by the remains found in a site in Peñalosa (Jaén, Spain); more than 50 species were identified in this site, including *Rumex* sp. and *Calendula* sp. The conclusion of this ethnobotanical study was that these plants were used as food and/or as flavoring additives [3]. The importance of these plants is evident from multiple studies conducted worldwide, such as in Brazil [4], China [5], Ethiopia [6], Guatemala [7], Iceland [8], India [9], Japan [10], and Tunisia [11]. In Europe, Schulp et al. [12] wrote a review on the identification of WEPs throughout Europe, finding them in 17 countries. In fact, there are many studies focusing on the Mediterranean region because it has a great diversity of WEPs, especially in Greece, Italy, Portugal and Spain. In all of these studies, nutritional potential or bioactive profiles were reported [2,13–17]. Despite all the studies carried out over the last 10 years, the full potential of WEPs has still not been fully reached and this is a hot topic that deserves

deeper attention by the scientific community considering especially the economy and their role in the sustainability of rural areas.

These plants may play an important role in environmental sustainability as they grow wildly and can be used as a functional ingredient to develop new food products. This sustainable character is persuading more and more consumers, chefs and nutritionists to introduce WEPs in their dishes and the diets they prescribe. The relevance of this review is supported by the growing global demand for a change in eating habits, where key new trends are essential and include: (i) reduction in gasses' emissions, (ii) growth of sustainable crops, and (iii) greater environmental awareness. An example of this change and this new trend is the guide that FAO published in 2017 on wild food plants, or current campaigns for the consumption of edible insects as a new and sustainable source of protein [18]. The future must be more sustainable and WEPs can make a significant contribution to this change.

2. Scientific Literature Review

To identify interesting scientific publications dealing with the composition and relevance of WEPs, this review was based on the 2020 update of the PRISMA approach [19]. The literature was searched in different databases: (i) Scopus, (ii) FSTA and (iii) ScienceDirect; the keywords used were the following: "wild edible plants", "WEP", "edible plants", "ruderal plants", and "wild edible plants food". Most of the articles that were selected (1999 to 2022) were included in the Journal Citation Reports (JCR). The selection process is shown in Figure 1. The review is structured in different sections: (i) proximate characterization, (ii) sugars and organic acids, (iii) mineral content, (iv) fatty acids, (v) phenolic content and flavonoids, and (vi) economic value of WEPs.

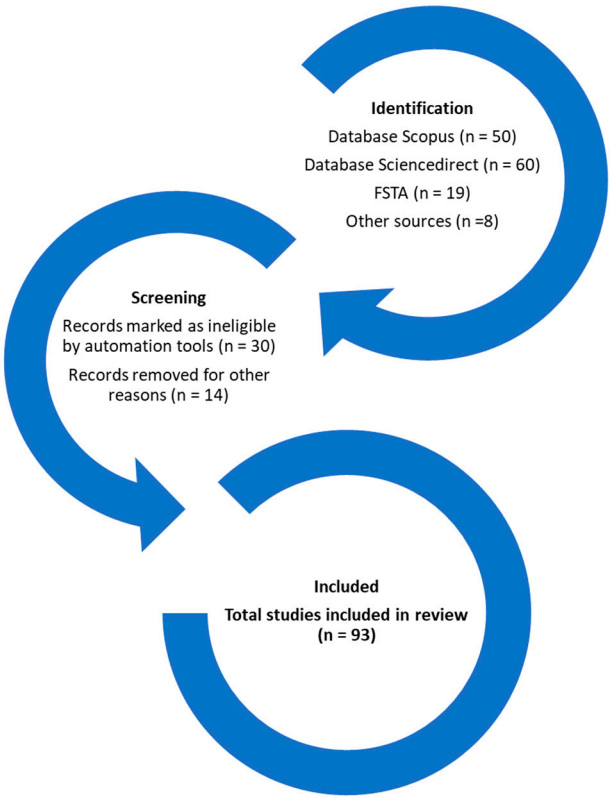

Figure 1. Diagram of selection of scientific publications.

3. Wild Edible Plants, WEPs

This review collects information on 115 WEPs (Table S1, Supplementary Material) located around the world and belonging to 47 different families. The families with the highest representation were Asteraceae > Brassicaceae > Fabaceae > Lamiaceae.

4. Proximate Characterization

Proximate analysis is used in foods to estimate the values of energy, moisture, protein, lipids, water, ash, and carbohydrate in the samples under study. Proteins, lipids and carbohydrates contribute to the total energy content in an organism, while ash and water also contribute to the organism mass [20]. Therefore, these determinations are essential for correct nutritional labelling, whose main objective is to provide data on macro- and micro-nutrients [21]. Nutritional labelling is regulated in the United States by the Food and Drugs Administration (FDA) and in the European Union by the European Food Safety Authority (EFSA). The main values reflected on food labels are: energy, protein, total fat, saturated fat, total carbohydrate, total sugars and sodium; all are expressed in g, mg or μg per 100 g [22].

The different parameters of the proximate characterization were compiled and summarized for 47 WEPs. The families with the highest representation in the proximate characterization were Asteraceae (9) > Lamiaceae (5) > Polygonaceae (4) (Table 1).

The water content or **moisture** is one of the most important parameters in a plant. For a plant to produce 1 kg of organic matter, it needs to absorb 500 kg of water, which is subsequently eliminated by different processes (e.g., transpiration or evaporation) [23]. In herbaceous plants, the moisture content usually reaches ~90% of the fresh weight, and only on rare occasions (such as intense water stress conditions) it is below 70%. The functions of the water content in the plant are essential, because it maintains cell turgor, facilitates the transport of solutes through the plant, participates in the reduction of CO_2 through photosynthesis, and even in the cooling of the leaves during hot hours [24]. The average moisture content in the WEPs averaged ~80%. It should be noted that, out of the 115 WEP reviewed, the highest content was found in *Silybum marianum* (Asteraceae family) when it reached 93.4 g per 100 g [25,26]; on the contrary, the plant with the lowest moisture content was *Thymus pulegioides* (Lamiaceae family) with 47.6 g per 100 g [27]. In WEPs of the Lamiaceae family, it was observed that moisture values remained between 47.6 and 73.0 g per 100 g; these values were below the average (Table 1).

Studies dealing with 45 out of the 115 studied WEPs (from 24 families), provided results on ash contents. The two WEPs that showed the highest ash contents were: *Blumea lacera* (Asteraceae family) and *Hygrophila schulli* (Amaranthaceae family) with values of 24.05% and 23.36%, respectively [28]. On the contrary, the family that showed the lowest ash values was the Polygonaceae, which included four plants (*Rumex acetosella*, *Rumex induratus*, *Rumex papillaris*, and *Rumex pulcher*) with values of 1.2, 1.0, 1.0 and 1.9 g per 100 g, respectively [29,30].

Table 1. Proximate composition reported in wild edible plants, WEPs.

Plant Species	Part of Plant	Unit	Moisture	Ash	Proteins	Fat	Carbohydrates	Fibre	Energy [§]	Reference
Asystasia gangetica (L.) T. Anderson	-	%	70.21 ± 0.98	17.35 ± 0.26	7.84 ± 0.12	2.04 ± 0.03	10.63 ± 0.23	8.14 ± 0.55	92.27 ± 0.27	[31]
Achyranthes aspera L.	-	%	53.34 ± 0.58	23.26 ± 0.65	12.60 ± 0.11	1.196 ± 0.01	14.35 ± 0.14	16.89 ± 0.34	118.62 ± 0.06	[31]
Allium ampeloprasum L.	Bulbs	g/100 g	78.3 (76.0–81.5)	0.8 (0.5–1.0)	1.7 (1.2–2.0)	0.18 (0.12–0.23)	16.6 (12.0–20.9)	4.2 (3.6–4.7)	-	[29]
Allium ampeloprasum L.	Bulbs	g/100 g	78.3 (76.3–80.3)	0.79 (0.59–0.99)	1.67 (1.31–2.03)	0.34 (0.13–0.61)	16.6 (12.8–19.7)	4.23 (3.72–4.74)	85 (65–103)	[17]
Amaranthus viridis L.	-	%	55.80 ± 0.23	13.31 ± 0.40	13.99 ± 0.12	1.40 ± 0.02	19.84 ± 0.07	6.54 ± 0.28	148.02 ± 0.28	[31]
Anchusa azurea Mill.	Leaves	g/100 g	91.2 (88.9–92.7)	1.9 (1.8–2.1)	1.9 (1.1–2.8)	0.15 (0.07–0.23)	1.3 (0.9–1.8)	3.9 (3.5–4.4)	-	[29]
Apium nodiflorum (L.) *Lag*	Stems	g/100 g	92.0 (90.0–94.0)	1.7 (1.0–3.3)	1.6 (1.1–2.1)	0.10 (0.07–0.14)	1.2 (0.7–2.1)	2.7 (1.9–3.4)	-	[29]
Asparagus acutifolius L.	Stems	g/100 g	84.6 ± 3.8	12.3 ± 0.0	22.4 ± 0.1	3.99 ± 0.33	61.3 ± 0.3	-	371 ± 1 [‡]	[32]
Asparagus acutifolius L.	Shoots	g/100 g	85.4 (81.2–88.5)	2.23 (0.93–3.70)	2.40 (1.69–3.25)	0.61 (0.32–0.99)	3.56 (1.03–4.67)	4.83 (4.71–6.63)	40 (23–56)	[17]
Beta maritima L.	Leaves	g/100 g	87.3 (75.4–91.4)	2.68 (2.00–5.60)	3.10 (1.80–3.91)	0.34 (0.18–0.70)	1.71 (0.75–4.30)	4.38 (3.29–9.50)	31 (16–59)	[17]
Beta vulgaris subsp. *maritima*	Leaves	g/100 g	84.5 (75.4–89.1)	3.4 (2.0–5.6)	2.6 (1.8–3.6)	0.24 (0.16–0.40)	3.6 (2.9–4.3)	5.9 (3.9–9.5)	-	[29]
Berberis aristata DC.	Leaves	g/100 g dw [ρ]	87.44 ± 2.22	15.46 ± 0.35	19.11 ± 0.78	2.14 ± 0.32	45.19 ± 0.56	18.10 ± 2.03	-	[28]
Blumea lacera (Burm. f.) DC.	Leaves	g/100 g dw	77.78 ± 2.68	24.05 ± 0.69	22.52 ± 0.97	0.93 ± 0.09	31.82 ± 1.26	20.68 ± 2.55	-	[28]
Borago officinalis L.	Leaves	g/100 g	86.9 (86.5–87.3)	2.4 (2.2–2.5)	1.2 (1.0–1.4)	0.16 (0.13–0.19)	9.5 (9.2–9.7)	-	44 [‡]	[30]
Borago officinalis L.	Leaves	g/100 g	87.2 (86.4–88.8)	2.35 1.93–2.91	2.35 (1.93–2.91)	0.16 (0.13–0.19)	9.45 (7.23–10.7)	-	44 (34–50)	[17]
Bryonia dioica Jacq.	Stems	g/100 g dw	82.9 ± 2.3	8.79 ± 0.01	16.6 ± 0.4	15.1 ± 1.9	59.5 ± 1.2	-	440 [‡]	[32]
Bryonia dioica Jacq.	Shoots	g/100 g	85.9 (70.9–90.8)	1.48 (1.00–3.30)	3.97 (1.00–11.9)	1.12 (0.10–2.90)	4.21 (0.80–10.37)	4.60 (3.40–10.7)	55 (14–141)	[17]
Cichorium intybus L.	Leaves	g/100 g fw [γ]	86.4 (84.8–87.9)	1.8 (1.7–2.1)	2.9 (1.5–4.3)	0.13 (tr-0.25)	3.5 (1.8–4.7)	6.1 (5.1–6.7)	157 (137–180)	[25]
Cichorium intybus L.	Leaves	g/100 g	87.9 (75.0–94.5)	1.65 (1.25–2.10)	1.83 (0.20–4.30)	0.46 (Traces–0.92)	3.50 (1.80–4.7)	3.6 (1.20–6.70)	33 (10–58)	[17]
Chondrilla juncea L.	Leaves	g/100 g	87.8 ± 0.88	1.8 ± 0.11	1.9 ± 0.07	0.5 ± 0.04	2.0 ± 0.08	5.8 ± 0.32	19.6	[33]
Chondrilla juncea L.	Leaves	g/100 g	83.4 (65.9–89.7)	2.41 (1.39–4.35)	2.50 (1.83–6.13)	0.80 (0.09–1.50)	3.58 (1.49–9.69)	7.70 (4.10–13.4)	44 (22–104)	[17]
Cynara cardunculus L.	Flowers	g/100 g fw	84.94	1.13	3.27	0.15	10.51	5.4	47	[34]
Enhydra fluctuans Lour.	-	%	67.69 ± 0.78	15.15 ± 0.44	8.00 ± 0.06	1.10 ± 0.01	9.64 ± 0.06	15.37 ± 0.21	80.53 ± 0.16	[31]
Erythrina variegata L.	Leaves	g/100 g dw	87.44 ± 2.22	20.15 ± 0.53	21.12 ± 1.58	1.55 ± 0.15	39.63 ± 1.11	17.55 ± 1.98	-	[28]

Table 1. *Cont.*

Plant Species	Part of Plant	Unit	Moisture	Ash	Proteins	Fat	Carbohydrates	Fibre	Energy §	Reference
Foeniculum vulgare Mill.	Leaves	g/100 g	86.7	-	3.8	-	4.9	3.5	48	[35]
Foeniculum vulgare Mill.	Leaves	g/100 g	82.4 (72.9–90.1)	2.34 (1.50–2.41)	2.76 (0.60–4.20)	0.42 (0.08–0.80)	9.67 (1.40–22.4)	3.87 (2.70–6.20)	63 (14–130)	[17]
Glechoma hederacea L.	Leaves	g/100 g	73.1 ± 8.05	3.47 ± 0.1	1.34 ± 0.00	1.18 ± 0.23	21.0 ± 0.17	-	99.96 ± 0.80	[27]
Humulus lupulus L.	Leaves	g/100 g	85.5 (85.2–93.2)	1.4 (0.9–2.0)	4.3 (3.1–5.1)	0.20 (0.11–0.26)	1.6 (1.4–1.8)	5.2 (4.3–6.4)	-	[29]
Humulus lupus L.	Shoots	g/100 g	85.8 (85.0–93.4)	1.35 (0.90–2.01)	4.25 (3.13–5.10)	0.37 (0.10–1.08)	1.85 (1.40–2.20)	4.85 (4.35–6.42)	39 (29–55)	[17]
Hygrophilla schulli (Hamilt.) M.R. Almeida & S.M. Almeida	Leaves	g/100 g dw	91.23 ± 1.01	23.36 ± 0.66	17.19 ± 1.49	1.92 ± 0.18	43.46 ± 0.42	14.07 ± 1.21	-	[28]
Ipomoea aquatica Forssk.	-	%	69.11 ± 0.72	16.37 ± 0.67	13.82 ± 0.08	2.19 ± 0.08	10.51 ± 0.08	7.44 ± 0.27	117.27 ± 0.24	[31]
Malva sylvestris L.	Flowers	g/100 g	72.49 ± 1.89	10.54 ± 0.30	8.50 ± 0.51	2.84 ± 0.37	78.12 ± 0.44	-	372.02 ± 2.13 ‡	[36]
Malva sylvestris L.	Leaves	g/100 g	81.0 (75.7–86.9)	3.21 (2.32–5.44)	3.00 (0.83–5.70)	0.56 (0.40–0.76)	2.23 (1.93–2.44)	4.76 (4.18–5.34)	35 (23–50)	[17]
Mentha pulegium L.	Inflorescences	g/100 g	59.47 ± 9.22	5.92 ± 0.09	7.12 ± 0.49	2.22 ± 0.22	84.74 ± 0.59	-	387.44 ± 0.53 ‡	[37]
Montia fontana subsp. *amporitana* Sennen	Leaves	g/100 g	91.47 ± 1.18	1.13 ± 0.13	1.76 ± 0.14	1.94 ± 0.13	1.81 ± 0.55	4.44 ± 0.34	31.48 ± 1.18	[38]
Nasturtium officinale R. Br.	Leaves	g/Kg	931 ± 10	9.4 ±0.9	22.4 ± 0.7	1.43 ± 0.08	35.6 ± 0.9	-	1023 ± 15 ¤	[39]
Oldenlandia corymbose Aiton.	-	%	60.28 ± 0.40	8.34 ± 0.39	10.52 ± 0.10	2.16 ± 0.06	9.08 ± 0.37	7.26 ± 0.30	97.94 ± 0.04	[31]
Origanum vulgare L.	Leaves	g/100 g	51.82 ± 5.11	2.87 ± 0.07	2.28 ± 0.03	2.81 ± 0.33	40.22 ± 0.28	-	195.31 ± 0.96	[27]
Papaver rhoeas L.	Leaves	g/100 g	91.0	-	2.9	-	3.1	2.5	36	[35]
Papaver rhoeas L.	Leaves	g/100 g	88.3 (68.5–91.2)	2.50 (1.45–5.20)	3.50 (1.50–5.90)	0.64 (0.15–1.03)	3.35 (2.90–5.30)	4.40 (2.50–11.10)	42 (24–78)	[17]
Portulaca oleracea L.	Leaves	%	81.5	28.9	27.8	0.141	-	10.0	-	[40]
Portulaca oleracea L.	Leaves	g/100 g	92.6 (90.0–94.3)	1.88 (1.25–2.95)	3.00 (2.50–3.50)	0.35 (0.30–0.40)	1.98 (1.11–2.70)	1.20 (0.90–1.80)	25 (19–32)	[17]
Pterospartum tridentatum (L.) Willk.	Flowers	g/100 g	60.8 ± 0.16	2.36 ± 0.00	15.92 ± 0.60	2.69 ± 0.51	79.03 ± 0.74	-	404.01 ± 4.05	[41]
Rumex acetosella L.	Leaves	g/100 g	89.9 ± 1.01	10.93 ± 1.06	7.85 ± 1.86	2.35 ± 0.28	78.87 ± 1.50	-	368.03 ± 3.98 ‡	[30]
Rumex induratus Boiss. & Reut	Leaves	g/100 g	90.29 ± 0.53	11.07 ± 0.30	13.54 ± 0.28	3.97 ± 0.14	71.42 ± 0.28	-	375.55 ± 0.36 ‡	[30]

Table 1. *Cont.*

Plant Species	Part of Plant	Unit	Moisture	Ash	Proteins	Fat	Carbohydrates	Fibre	Energy [§]	Reference
Rumex papillaris Boiss. & Reut	Leaves	g/100 g	89.1 (87.8–90.7)	1.0 (0.4–1.3)	2.4 (1.6–3.5)	0.22 (0.26–0.28)	2.0 (1.6–2.7)	4.4 (4.0–5.0)	-	[29]
Rumex pulcher L.	Leaves	g/100 g	86.6 (87.4–89.2)	1.9 (1.1–3.1)	3.2 (1.9–5.5)	0.20 (0.10–0.32)	3.3 (1.5–4.5)	4.7 (4–5.2)	-	[29]
Raphanus raphanistrum L.	Leaves	g/100 g fw	89.9 ± 0.6	1.58 ± 0.08	4.04 ± 0.01	0.23 ± 0.03	4.22 ± 0.08	-	35.1 ± 0.1	[42]
Scolymus hispanicus L.	Leaves	g/100 g fw	84.1 (81.8–92.7)	3.19 (1.7–5.2)	1.8 (0.3–5.3)	0.09 (0.08–0.11)	3.4 (1.1–9.2)	7.0 (3.1–12.)	167 (53–280) [¥]	[25]
Sesbania sesban (L.) Merr.	Leaves	g/100 g dw	90.13 ± 1.55	18.68 ± 0.22	15.65 ± 1.10	0.97 ± 0.05	49.51 ± 0.72	15.19 ± 1.79	-	[28]
Silene vulgaris (Moench) Garcke	Leaves	g/100 g	87.1 (80.4–88.5)	0.3	3.3 (3.0–3.6)	0.70	3.4 (2.9–3.9)	2.8 (2.6–3.1)	-	[43]
Silene vulgaris (Moench) Garcke	Leaves	g/100 g	85.9 (86.6–88.5)	1.53 (0.20–4.33)	2.47 (1.31–3.60)	0.67 (0.31–1.31)	2.32 (1.03–3.90)	4.36 (2.60–6.63)	34 (17–56)	[17]
Silybum marianum (L.) Gaertn.	Leaves	g/100 g fw	93.4 (92.9–93.8)	1.5 (1.0–1.9)	0.6 (0.5–0.8)	0.01 (tr-0.03)	1.1 (0.5–1.7)	2.6 (2.3–2.9)	51.8 (42.2–61.4) [¥]	[25]
Sonchus asper L.	Leaves	g/Kg	864.3 ± 11.2	30.4 ± 3.0	32.5 ± 3.2	6.8 ± 0.7	19.8 ± 1.1	35.6 ± 2.2	1110 ± 120	[44]
Sonchus oleraceus L.	Leaves	g/Kg	872.4 ± 14.0	29.9 ± 1.8	31.7 ± 1.5	7.5 ± 0.9	18.2 ± 1.4	32.5 ± 2.4	1110 ± 120	[44]
Sonchus oleraceus L.	Leaves	g/100 g	87.6 (83.0–91.9)	2.17 (1.58–3.00)	2.22 (1.11–3.48)	0.60 (0.20–1.28)	2.29 (0.94–4.20)	3.37 (2.60–5.57)	33 (16–56)	[17]
Sonchus oleraceus L.	Leaves	g/100 g fw	88.25 (83.2–91.0)	2.04 (1.6–2.7)	2.22 (1.3–3.5)	0.29 (0.20–0.41)	2.51 (0.9–4.2)	4.3 (3.5–5.6)	127 (91–163) [¥]	[25]
Sonchus oleraceus L.	Leaves	g/100 g wb [ø]	89.3 ± 3.04	1.5 ± 0.01	3.0 ± 0.13	0.4 ± 0.01	-	5.5 ± 0.35	-	[45]
Sonchus tenerrimus L.	Leaves	g/Kg	877.3 ± 20.8	30.2 ± 1.9	31.8 ± 2.0	5.2 ± 0.4	13.2 ± 1.8	31. 2 ± 1.6	935 ± 105	[44]
Tamus communis L.	Leaves	g/100 g	86.2 (84.6–89.0)	1.4 (0.9–2.4)	3.2 (2.5–3.8)	0.17 (0.10–0.22)	2.2 (1.9–2.7)	4.7 (3.5–6.0)	-	[29]
Tamus communis L.	Shoots	g/100 g	85.2 (82.0–89.0)	1.25 (0.90–2.40)	3.13 (2.52–3.85)	0.49 (0.10–1.28)	5.20 (1.80–11.7)	4.35 (3.50–6.00)	46 (25–85)	[17]
Taraxacum obovatum (Willd.) DC.	Leaves	g/100 g fw	83.3 (79.2–86.7)	2.13 (1.8–2.5)	1.57 (1.02–2.09)	0.22 (0.19–0.27)	3.34 (1.63–5.39)	7.01 (5.4–8.7)	152 (114–205) [¥]	[25]
Thymus mastichina L.	Leaves	g/100 g	54.67 ± 7.03	2.67 ± 0.08	2.2 ± 0.5	3.80 ± 0.10	36.64 ± 0.08	-	189.65 ± 0.44	[27]
Thymus pulegioides L.	Inflorescences	g/100 g	47.6 ± 12.60	4.94 ± 0.62	5.53 ± 1.40	0.18 ± 0.02	89.35 ± 1.54	-	381.14 ± 1.76 [‡]	[37]
Viola x Wittrockiana	Flowers	g/100 g dw	87.76 (wet matter)	7.92	10.14	1.67	80.27	-	376.67 [‡]	[4]
Umbilicus rupestris (Salisb.) Dandy	Leaves	g/100 g fw	93 ± 1	0.91 ± 0.01	1.83 ± 0.06	0.255 ± 0.002	3.90 ± 0.03	-	25.2 ± 0.1	[46]

[§] kcal/100 g; [‡] kcal/100 g dw; [¤] kJ/kg; [¥] kJ/100 g; Mean value (minimum-maximum); [ρ] dw = dry weight; [ϒ] fw = fresh weight; [ø] wb = wet bases.

With respect to protein, three WEPs stood out for their high protein content, each belonging to a different family (Portulacaceae, Asteraceae, and Asparagaceae): *Portulaca oleracea* with 27.8 g per 100 g [40], followed by *Blumea lacera* and *Asparagus acutifolius* with 22.52 and 22.40 g per 100 g, respectively [28,32]. On the contrary, the plant with the lowest protein content was *Silybum marianum* (Asteraceae family), with a protein content as low as 0.6 g per 100 g [25,26].

Fat content was only reported in 47 of the available 115 WEPs. *Bryonia dioica* (Cucurbitaceae family) showed the highest content at 15.1 per 100 g dry weight (Martins et al., 2011). The family with the highest number of plants (Asteraceae) had, in general, low contents, reaching its maximum with *Silybum marianum* and *Enhydra fluctuans* (1.1 g per 100 g), and its minimum with *Scolymus hispanicus* (0.09 g per 100 g) [25,26,31].

The scientific literature only provides the carbohydrates content for 46 WEPs out of the 115 reviewed plants. In this regard, *Thymus pulegioides* and *Mentha pulegium* had the highest content, reaching 89.35 and 84.74 per 100 g, respectively [37]; both plants belong to the Lamiaceae family. Within this family, plants showed a wide variability in carbohydrate content, with *Thymus pulegioides* having the highest value (89.35 g per 100 g), while *Glechoma hederacea* had the lowest with 21 g per 100 g [27].

The content of dietary fiber was only found for 36 WEPs. The WEPs that stood out for their high fiber content were *Blumea lacera* (20.68 g per 100 g dw) and *Berberis aristata* (18.10 g per 100 g dw), belonging to the Asteraceae and Berberaceae families, respectively [28]. In general, the fiber content of the Laminaceae and Brassicaceae plants was not analyzed and thus was not reported [27,37,39,42].7

Energy is calculated from the determination of food macro-nutrients including protein, fiber, carbohydrates, fat and alcohol [47]. Nowadays, part of society is willing to have a balanced diet, but unfortunately, most consumers, due to their lifestyle, replace traditional diets with diets high in sugars and refined fats, which leads to large caloric intakes; these diets result in increased incidence of coronary heart disease, strokes, type II diabetes, and obesity [48,49]. However, in the USA the actual daily intake for men is 2800 kcal per day and for women 2000–2200 kcal per day, which are comparable to the 2030 kcal that a person 2 m tall and 88 kilos should ingest, this value being the highest of those established for men and women by the FAO [50]. This difference in daily kilocalories shows a gap between the ideal intake and that which is averaged in countries such as the USA. Energy data was only available for 28 WEPs out of the 115 plants reviewed. The plant with the highest energy value was *Bryonia dioica* with 440 kcal per 100 g dw [32]. Regarding families, the Polygonaceae showed high contents, for instance, *Rumex acetosella* and *Rumex induratus* with 368 and 376 kcal per 100 g dw, respectively [30].

5. Sugars and Organic Acids

5.1. Sugars

The role of the sugars, which are generated through the photosynthesis process, in plants is fundamental; they are the main source of carbon and energy for the plants, and participate in the plant metabolism control, for example participating in multiple biological processes, from embryogenesis to plant senescence [51,52]. Sugars can be classified into monosaccharides, disaccharides, and polysaccharides. Apart from the biological importance of sugars in plants, it is necessary to highlight their importance in the health of humans. Sugars are the main source of energy for multiple metabolic processes, as well as making a necessary contribution for cells to stay alive [53].

Sugar contents of 22 WEPs (from 16 families) are summarized in Table 2. In general, the predominant sugars were fructose, glucose and sucrose. It should be noted that, of the three main sugars, glucose was that with the highest total content in all 22 plants, followed by fructose and subsequently sucrose.

Table 2. Sugars reported in wild edible plants, WEPs.

Plant Species	Part of Plant	Unit	Sugars			Reference
			Fructose	Glucose	Sucrose	
Asparagus acutifolius L.	Stems	g/100 g dw	2.49 ± 0.13	1.98 ± 0.04	4.27 ± 0.12	[32]
Borago officinalis L.	Leaves	g/100 g dw	0.14 ± 0.03	0.58 ± 0.06	1.52 ± 0.13	[30]
Bryonia dioica Jacq.	Stems	g/100 g dw	3.45 ± 0.08	2.97 ± 0.09	0.572 ± 0.014	[32]
Chenopodium ambrosioides L.	Leaves	g/100 g dw	0.24 ± 0.01	0.46 ± 0.01	1.43 ± 0.12	[54]
Foeniculum vulgare Mill.	Leaves	g/100 g fw	0.49 ± 0.05	0.76 ± 0.12	0.04 ± 0.00	[55]
Glechoma hederacea L.	Leaves	g/100 g fw	0.15 ± 0.01	0.08 ± 0.02	0.40 ± 0.06	[27]
Helichrysum stoechas (L.) Moench	Stems	g/100 g dw	1.02 ± 0.04	0.59 ± 0.02	1.84 ± 0.09	[56]
Malva sylvestris L.	Flowers	g/100 g dw	8.72 ± 0.14	7.36 ± 0.13	2.74 ± 0.05	[36]
Mentha pulegium L.	Inflorescences	g/100 g dw	2.39 ± 0.11	3.37 ± 0.22	4.62 ± 0.28	[37]
Montia fontana subsp. *amporitana* Sennen	Leaves	g/100 g dw	0.76 ± 0.17	1.00 ± 0.02	0.44 ± 0.05	[30]
Nasturtium officinale R. Br.	Leaves	mg/kg	1104 ± 31	696 ± 20	233 ± 51	[39]
Origanum vulgare L.	Leaves	g/100 g fw	0.19 ± 0.01	0.58 ± 0.01	0.30 ± 0.00	[27]
Portulaca oleracea L.	Leaves	mg/100 g fw	290 (202–352) ‡	81.25 (59–118)	161.5 (75–271)	[57]
Pterospartum tridentatum (L.) Willk.	Flowers	g/100 g dw	3.49 ± 0.11	1.19 ± 0.05	0.58 ± 0.03	[41]
Rubus ulmifolius Schott	Flowers	g/100 g dw	1.66 ± 0.21	2.23 ± 0.19	1.34 ± 0.15	[56]
Rumex acetosella L.	Leaves	g/100 g dw	0.60 ± 0.00	0.73 ± 0.01	0.21 ± 0.07	[30]
Rumex induratus Boiss. & Reut	Leaves	g/100 g dw	1.71 ± 0.09	1.26 ± 0.20	1.25 ± 0.31	[30]
Raphanus raphanistrum L.	Leaves	g/100 g fw	0.153 ± 0.004	0.348 ± 0.003	-	[42]
Tamus communis L.	Leaves	g/100 g dw	3.83 ± 0.13	1.80 ± 0.14	0.695 ± 0.05	[32]
Thymus mastichina L.	Leaves	g/100 g fw	0.45 ± 0.01	0.97 ± 0.11	0.02 ± 0.00	[27]
Thymus pulegioides L.	Inflorescences	g/100 g dw	0.22 ± 0.00	0.33 ± 0.03	1.06 ± 0.02	[37]
Umbilicus rupestris (Salisb.) Dandy	Leaves	g/100 g fw	-	-	0.082 ± 0.002	[46]

‡ Mean value (minimum-maximum).

The most commonly studied plant family regarding sugars was Lamiaceae with five plants. Regarding fructose, the WEPs that had the highest content were *Malva sylvestris* (Malvaceae family) and *Tamus communis* (Dioscoraceae family), with 8.72 and 3.83 g/100 g dw, respectively [32,36]. *Malva sylvestris* also showed the highest glucose content at 7.35 g/100 g dw [36]. The sucrose concentration in these 22 WEPs was mainly dominated by two plants: *Mentha pulegium*, which had 4.62 g/100 g dw, and *Asparagus acutifolius* with a sucrose concentration of 4.27 g/100 g dw [32,37].

5.2. Organic Acids

At a general level, organic acids are weak acids, which can be classified mainly by four criteria: (i) nature of the carbon chain (aliphatic, aromatic, etc.); (ii) saturation or unsaturation properties; (iii) substituted or unsubstituted characteristics; and (iv) number of functional groups. Acids play an essential role in the physiology of plants, participating in processes such as pH regulation, balancing the redox potential cells, the Krebs cycle, or even in organoleptic properties such as color, taste and aroma of both fruits and vegetables [58–60].

Three organic acids (oxalic, malic and shikimic) were mainly found in WEPs, and especially in four families: Asteraceae, Brassicaceae, Crassulaceae, and Portulacaceae (Table S2).

One of the most representative organic acids in the Asteraceae family is oxalic acid [25,26], which show a high content in six plants of this family [61]. Being a small group of plants in which organic acids were determined, a PCA (Principal Component Analysis) was carried out to understand these results in a visual way (Figure 2). The values can be found in Table S2. PCA showed a high percentage of correlation (92.08%) among organic acids and WEPs, being able to differentiate three groups. In the first cluster, malic acid was

associated with *Raphanus raphanistrum* (Brassicaceae family); the second cluster was based on the association of oxalic acid with *Sonchus oleraceus* (Asteraceae family); while in the third group both oxalic acid and shikimic acid were associated with *Hymenonema graecum* (Asteraceae family).

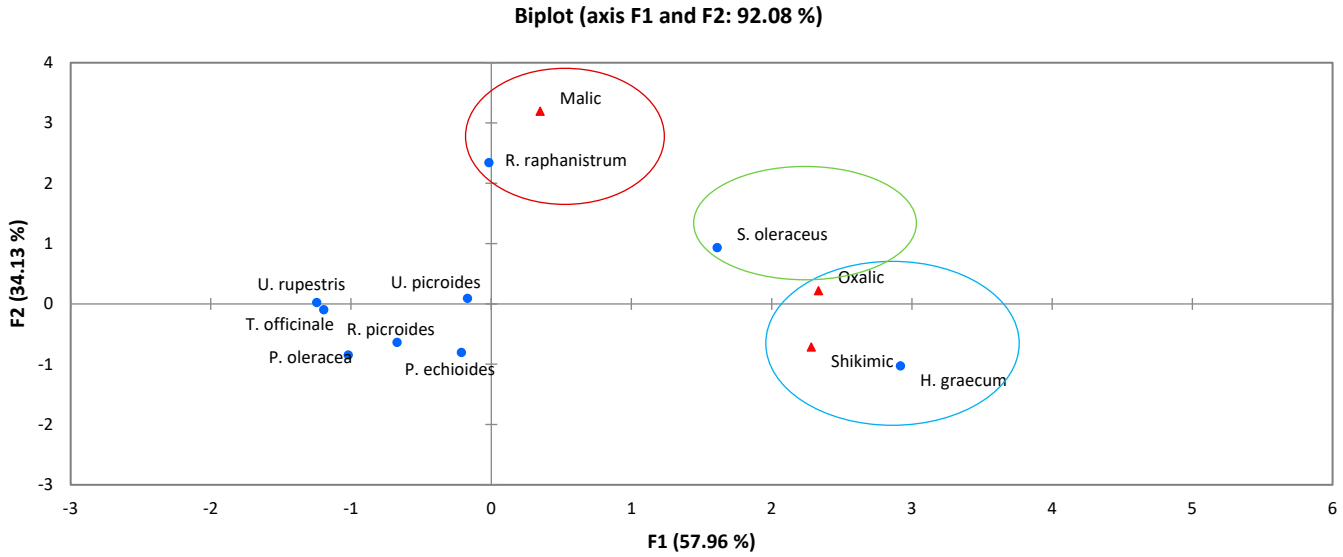

Figure 2. Principal Component Analysis (PCA) of organic acids present in wild edible plants, WEPs.

In this way, the highest contents of malic acid were reported in *Raphanus raphanistrum* and *Sonchus oleraceus* (580 and 415 mg/100 g, respectively) [42,61]. The WEPs in which the highest amounts of oxalic acid were found, in decreasing order were: *Hymenonema graecum* > *Sonchus oleraceus* > *Raphanus raphanistrum*, with concentrations between 972 and 706 mg/100 g [42,61]. Regarding shikimic acid, two plants stood out for their contents: *Hymenonema graecum* and *Sonchus oleraceus*, with 244 and 166 mg/100 g, respectively; the other WEPs where this acid was found had values of below 100 mg/100 g [61].

6. Mineral Elements

The Mediterranean diet is considered by many experts as one of the best in the world at a nutritional level, and one of its main strengths is the contribution of minerals and vitamins [62]. In body composition, minerals represent fourth place in abundance, reaching values of up to 6.1% of the body weight for a person of 65 kg [50]. This importance of minerals for the human body determines health problems due to mineral deficiency; in this way, key elements which are often linked to deficiency are iron, zinc, and iodine. Iron deficiency can affect as many as 18% of the world's children under 5 years of age, along with pregnant women [63]. In addition, zinc and iodine affect 17% and 28% of the world's population, respectively [63]. According to Zeece [64], the main source of minerals is the soil, because from there it passes to plants and through the food chain to humans. The daily intake of minerals establishes the recommended intake for each of these elements; in Table S3, it is possible to find these values according to the Food & Drug Administration (FDA) [65].

Principal Component Analysis (PCA) (Figure 3) was carried out after adjusting all values in a common unit, mg/100 g fw. PCA showed a total percentage of correlation (70.13%) among minerals contents and WEPs. Mineral composition was reported only for 17 of the 115 plants (from 10 families) covered in this review. In this way, most of the plants which had their mineral profile analyzed (n = 7) belonged to the Asteraceae family. The macro-elements identified were calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na); while the micro-elements were copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). In Table S4 [25,34,35,38,57,66–68], values for the contents of these minerals can be found.

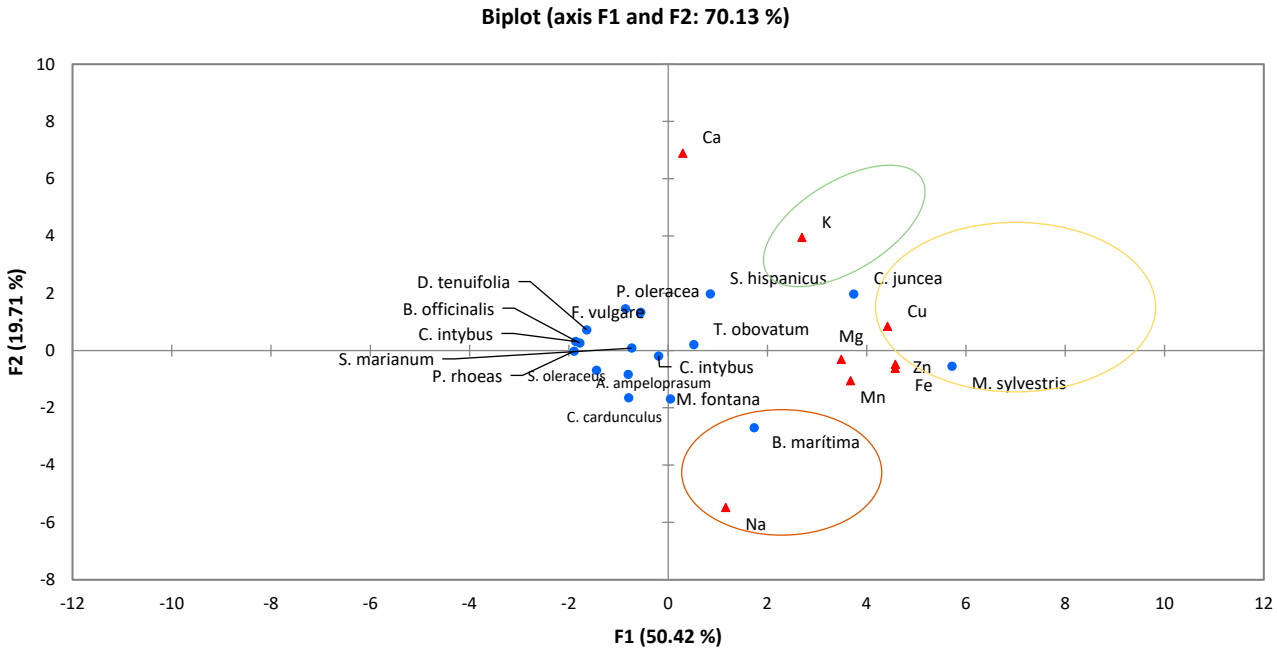

Figure 3. Principal Component Analysis of minerals present in wild edible plants, WEPs.

Regarding Figure 3, three different groups can be easily observed. On the one hand, Ca was isolated from the entire group of minerals as not being associated with any plant. The first group contained the highest number of minerals (Cu, Fe, Mn, Mn, and Zn). The second and third groups were linked to K and Na, respectively.

Calcium was not associated with any specific WEPs, although *Foeniculum vulgare* was that with the highest content of this mineral, followed by *Chondrilla juncea* (341 and 301 mg/100 g, respectively) [25,35].

The first group was mainly associated with two plants: *Chondrilla juncea* and *Malva sylvestris*. Regarding Cu and Mn, the WEPs that had the highest simultaneous contents, were *Chondrilla juncea* (0.43 and 0.97 mg/100 g, respectively) followed by *Malva sylvestris* (0.33 and 0.76 mg/100 g, respectively); although the WEP that had the highest Mn content was *Montia fontana* with 1.08 mg/100 g [38]. Regarding Fe, Mg, and Zn, *Malva sylvestris* had the highest contents (5.82, 715 and 1.98 mg/100 g, respectively) compared to *Chondrilla juncea* (3.97, 40.80 and 1.63 mg/100 g, respectively) [25]. In the second group, K was exclusively associated with *Scolymus hispanicus*, the content of which was much higher than in the rest of the WEPs (1040 mg/100 g) [25,26]. In the third group, Na was exclusively associated with *Beta maritima*, containing the highest concentration of this mineral, 171 mg/100 g) [66].

The mineral values clearly demonstrated that many WEPs have a high nutritional value, for example, *Chondrilla juncea*, which can provide almost 50% of the recommended daily intake of Cu after consumption of 200 g of the plant. *Montia fontana*, with its Mn content, could also cover approximately 50% of the recommended daily amount after consumption of 200 g (Table S3) [65].

7. Fatty Acids

Of the 115 WEPs evaluated in this review, only 43 (from 21 families) had FA profiles published; the most commonly studied families regarding FAs were: Asteraceae (8) > Lamiaceae (5) > Polygonaceae (4) > Brassicaceae (3). As can be seen in Table 3, the most representative FAs in WEPs were palmitic, oleic, linoleic, and linolenic acids.

Table 3. Fatty acids reported in wild edible plants, WEPs.

Plant Species	Part of Plant	Unit	C16:0 (Palmitic Acid)	C18:1 (Oleic Acid)	C18:2 (Linoleic Acid)	C18:3 (Linolenic Acid)	Reference
Allium ampeloprasum L.	Bulbs	%	26.42 ± 0.30	7.39 ± 0.42	53.45 ± 0.27	nd	[67]
Anchusa azurea Mill.	Leaves	%	10.45 ± 0.62	2.20 ± 0.00	12.16 ± 0.11	64.74 ± 0.23	[69]
Apium nodiflorum (L.) Lag	Stems	%	16.29 ± 0.96	3.33 ± 0.02	24.60 ± 0.77	43.46 ± 0.08	[70]
Asparagus acutifolius L.	Stems	%	17.5 ± 0.2	4.94 ± 0.35	44.5 ± 1.3	23.7 ± 0.9	[32]
Bellis perennis L.	Aerial parts	%	1.5	-	5.1	13.6	[71]
Beta maritima L.	Leaves	%	11.03 ± 0.15	3.51 ± 0.01	21.28 ± 0.04	57.80 ± 0.03	[70]
Borago officinalis L.	Leaves	%	12.03 ± 0.70	2.08 ± 0.20	9.50 ± 1.25	12.26 ± 1.90	[30]
Bryonia dioica Jacq.	Stems	%	13.5 ± 0.3	1.52 ± 0.09	6.39 ± 0.16	70.3 ± 0.1	[32]
Cichorium intybus L.	Leaves	%	10.64 ± 0.63	1.61 ± 0.04	21.14 ± 0.06	60.45 ± 0.41	[70]
Chenopodium ambrosioides L.	Leaves	%	14.16 ± 0.03	6.90 ± 0.12	19.23 ± 0.12	48.54 ± 0.13	[54]
Chondrilla juncea L.	Leaves	%	12.96 ± 0.47	1.91 ± 0.01	19.92 ± 0.17	56.27 ± 0.13	[70]
Daucus carota L.	Roots	%	tr	-	1.0	-	[71]
Diplotaxis erucoides (L.) DC	Aerial parts	%	18.23	3.42	39.31 ‡	-	[11]
Diplotaxis virgata (Cav.) DC	Flowers	%	14.35	-	36.01 ‡	-	[11]
Foeniculum vulgare Mill.	Leaves	%	20.15 ± 0.09	4.35 ± 0.37	23.25 ± 0.07	43.55 ± 0.40	[55]
Glechoma hederacea L.	Leaves	%	12.23 ± 0.23	35.12 ± 0.27	8.15 ± 0.08	27.87 ± 0.20	[27]
Helichrysum stoechas (L.) Moench	Stems	%	13.24 ± 0.16	6.15 ± 0.79	25.67 ± 0.08	22.79 ± 1.86	[56]
Humulus lupulus L.	Leaves	%	19.52 ± 0.61	1.88 ± 0.10	29.72 ± 0.85	38.16 ± 0.02	[70]
Malva sylvestris L.	Flowers	%	9.79 ± 1.07	3.31 ± 0.42	11.96 ± 0.42	67.79 ± 0.96	[36]
Mentha pulegium L.	Inflorescences	%	14.82 ± 0.09	5.77 ± 0.20	16.27 ± 0.33	37.00 ± 0.35	[37]
Montia fontana subsp. *amporitana* Sennen	Leaves	%	17.22 ± 1.06	2.37 ± 0.38	18.71 ± 0.45	55.57 ± 0.80	[30]
Origanum vulgare L.	Leaves	%	4.95 ± 0.10	5.08 ± 0.01	23.22 ± 0.14	62.34 ± 0.04	[27]
Papaver rhoeas L.	Leaves	%	9.66 ± 0.39	1.36 ± 0.00	16.53 ± 0.01	64.98 ± 0.07	[70]
Portulaca oleracea L.	Leaves	%	24.7 (23.4–26.9) ¥	12.4 (9.7–15.1)	28.8 (25.1–32.9)	23.6 (17.9–28.4)	[57]
Pterospartum tridentatum (L.) Willk.	Flowers	%	14.84 ± 0.83	9.22 ± 1.09	19.59 ± 0.67	29.50 ± 1.98	[41]
Rumex acetosella L.	Leaves	%	11.23 ± 0.73	3.43 ± 0.32	20.18 ± 0.48	51.34 ± 1.41	[30]
Rumex induratus Boiss. & Reut	Leaves	%	9.36 ± 0.71	2.20 ± 0.05	13.76 ± 0.01	58.84 ± 1.03	[30]
Rumex papillaris Boiss. and Reut.	Leaves	%	11.20 ± 0.32	5.80 ± 0.14	22.79 ± 0.19	51.77 ± 0.14	[70]
Rumex pulcher L.	Leaves	%	9.30 ± 0.11	4.22 ± 0.01	17.03 ± 0.16	62.97 ± 0.03	[70]
Raphanus raphanistrum L.	Leaves	mg/100 g fw ¶	25.2 ± 2.3	2.4 ± 0.3	24 ± 2	171 ± 16	[42]
Sambucus nigra L.	Aerial parts	%	tr	-	16.1	2.6	[71]
Scolymus hispanicus L.	Leaves	%	20.65 ± 0.85	6.41 ± 0.07	26.44 ± 0.26	30.55 ± 0.23	[70]
Silene vulgaris (Moench) Garcke	Leaves	%	13.5 (13.1–15.1)	2.4 (2.1–2.7)	22.4 (18.9–24.4)	54.5 (51.2–56.9)	[43]
Silybum marianum (L.) Gaertn.	Leaves	%	28.69 ± 1.60	3.86 ± 0.10	31.01 ± 0.63	21.60 ± 0.25	[70]
Sonchus oleraceus L.	Leaves	%	10.43 ± 0.70	0.92 ± 0.10	13.78 ± 0.61	nd	[70]
Tamus communis L.	Leaves	%	17.0 ± 0.7	7.51 ± 0.18	42.0 ± 0.3	27.5 ± 0.4	[32]
Taraxacum obovatum (Willd.) DC.	Leaves	%	11.83 ± 0.09	3.24 ± 0.01	17.64 ± 0.08	58.53 ± 0.23	[70]
Thymus mastichina L.	Leaves	%	10.22 ± 0.20	9.82 ± 0.18	11.83 ± 0.06	45.65 ± 0.55	[27]
Thymus pulegioides L.	Inflorescences	%	16.70 ± 0.22	11.40 ± 0.10	12.98 ± 0.52	36.69 ± 0.25	[37]
V. x Wittrockiana	Flowers	g/100 g dw £	36.41	8.27	32.30	nd	[4]
Umbilicus rupestris (Salisb.) Dandy	Leaves	%	10.6 ± 0.8	0.641 ± 0.002	18.3 ± 0.6	62 ±2	[46]

‡ Linoleic acid C18: 2 y Linolelaidic acid C18:2; nd = not detected; tr = trace; ¥ Mean value (minimum-maximum); £ dw = dry weight; ¶ fw = fresh weight.

Linolenic acid was by far the FA with the highest percentage in most plants, showing in eight of these 42 plants a percentage above 60%; the list of plants in decreasing order of abundance was as follows: *Bryonia dioica* Jacq. (70.3%) > *Malva sylvestris* (67.8%) > *Papaver rhoeas* (65.0%) > *Anchusa azurea* (64.7%) > *Rumex pulcher* (63.0%) > *Origanum vulgare* (62.3%) > *Umbilicus rupestris* (62.0%) > *Cichorium intybus* (62.0%) [27,32,46,56,70]. It is important to highlight that this compound was present in a wide range of families.

Linoleic acid was the second most abundant FA, highlighting its importance in WEPs, especially in *Allium ampeloprasum* (53.5%) > *Asparagus acutifolius* (44.5%) > *Tamus communis* (42.0%) [32,67,72]; these plants belong to the Amaryllidaceae, Asparagaceae and Dioscoreaceae families, respectively.

Another of the most representative FAs, palmitic acid, was present in all the WEPs analyzed. The WEP that obtained the highest value in palmitic acid was *Viola x wittrockiana* (Violaceae family) with 36.41 g/100 g dw [4]. *Silybum marianum* (Asteraceae), *Allium ampeloprasum* (Amaryllidaceae) and *Portulaca oleracea* (Portulaceae) had values of 28.7%, 26.4% and 24.7%, respectively [57,67,70,72].

Regarding oleic acid, the highest content was found in *Glechoma hederacea* with 35.1% [27], followed by *Portulaca oleracea* with 12% [57]; whilst, the WEP with the lowest content of oleic acid was *Umbilicus rupestris* (0.6%) [46].

In general, the main FAs identified in WEPs were linolenic and linoleic acids. On the other hand, it should be noted that palmitic acid was identified in all plants, although at lower concentrations.

8. Phenolic Content and Flavonoids

Phenolic compounds are the most abundant secondary metabolites in plants, helping in various functions of great importance, such as pigmentation, growth, and/or resistance to pathogens, also playing a fundamental role in maintaining redox homeostasis of cells [73–75]. Apart from the importance in the physiology of plants themselves, it has been clearly demonstrated that these compounds have antioxidant activity, anti-inflammatory effects, help in the reduction of oxidative stress, and can even help in the prevention of tumors [76–79].

In the references evaluated in this review, instudying up to 115 WEPs (from 47 families), the total phenolic contents (TPC) were provided for 100 plants; for 51 plants, the total flavonoids content (TFC) was also provided (Table 4) [5,30,32,36,39,42,46,54,56,57,67,69,72,80,81]. Regarding TPC and TFC, the most commonly studied families were Asteraceae (17) > Brassicaceae (7) > Fabaceae (6) = Lamiaceae (6). These analyses were performed by HPLC [31,54,81–84] and spectrophotometric method [4,5,28,30,32,36,37,39,41,56,57,67,69,72,80].

Table 4. Total phenolic content (TPC) and total flavonoids content (TFC) in wild edible plants.

Plant Species	Part of Plants	Unit TPC	TPC	Unit TFC	TFC	Reference
Asystasia gangetica (L.) T.Anderson	-	mg GAE/g de ‡	91.797 ± 0.295	mg RE/g de §	20.132 ± 0.093	[31]
Achyranthes aspera L.	-	mg GAE/g de	74.831 ± 0.243	mg RE/g de	20.793 ± 0.122	[31]
Ageratum conyzoides (L.) L.	Flowers	mg GAE/g	4.63 ± 0.52	-	-	[81]
Allamanda cathartica L.	Flowers	mg GAE/g	4.16 ± 0.11	-	-	[81]
Allium ampeloprasum L.	Bulbs	mg GAE/g	5.70 ± 0.62	mg CE/g ø	0.86 ± 0.5	[67]
Amaranthus viridis L.	-	mg GAE/g de	50.700 ± 0.079	mg RE/g de	19.970 ± 0.252	[31]
Anagallis arvensis (L.)	Aerial parts	mg GAE/g	27.54 ± 0.92	mg QE/g £	26.15 ± 0.85	[84]
Anchusa azurea Mill.	Leaves	mg GAE/g extract.	148.62 (146.62–150.62) ¥	mg CE/g extract.	84.81 (80.78–88.84)	[72]
Apium nodiflorum (L.) Lag.	Leaves	mg GAE/g extract.	80.47 ± 4.41	mg CE/g extract.	45.48 ± 1.61	[69]
Asparagus acutifolius L.	Stems	mg GAE/g extract.	624 ± 28	mg CE/g extract.	57.8 ± 2.4	[32]

Table 4. *Cont.*

Plant Species	Part of Plants	Unit TPC	TPC	Unit TFC	TFC	Reference
Asparagus acutifolius L.	Leaves	mg CAE/kg ww ¶	43.1	mg QE/kg ww	2262.9	[83]
Bahuinia purpurea L.	Flowers	mg GAE/g	6.14 ± 0.30	-	-	[81]
Beta marítima L.	Leaves	mg GAE/g extract.	61.91 ± 7.51	mg CE/g extract.	21.55 ± 0.87	[72]
Berberis aristata DC.	Leaves	mg GAE/g dw	135.56 ± 3.26	mg QE/g dw	82.05 ± 0.78	[28]
Bidens pilosa L.	Flowers	mg GAE/g	8.12 ± 0.41		-	[81]
Blumea lacera (Burm. f.) DC.	Leaves	mg GAE/g dw	95.23 ± 1.35	mg QE/g dw	59.87 ± 0.93	[28]
Bombax malabaricum DC.	Flowers	mg GAE/g	3.88 ± 0.17	-	-	[81]
Borago officinalis L.	Leaves	mg CAE/kg ww	64.0	mg QE/kg ww	189.4	[83]
Bougainvillea spectabilis Willd.	Flowers	mg GAE/g	6.87 ± 0.23	-	-	[81]
Brassica campestris L.	Flowers	mg GAE/g	3.32 ± 0.09	-	-	[81]
Brunfelsia acuminata (Pohl) Benth.	Flowers	mg GAE/g	4.08 ± 0.25	-	-	[81]
Bryonia dioica Jacq.	Stems	mg GAE/g extract.	258 ± 22	mg CE/g extract.	18.1 ± 1.2	[32]
Calliandra haematocephala Hassk.	Flowers	mg GAE/g	14.43 ± 0.71	-	-	[81]
Camellia japonica L.	Flowers	mg GAE/g	5.14 ± 0.29	-	-	[81]
Chaenomeles sinensis (Thouin) Koehne	Flowers	mg GAE/g	13.93 ± 0.34	-	-	[81]
Chrysanthemum coronarium L.	Flowers	mg GAE/g	3.76 ± 0.29	-	-	[81]
Chrysanthemum morifolium Ramat	Flowers	mg GAE/g	3.75 ± 0.11	-	-	[81]
Cichorium intybus L.	Leaves	mg CAE/kg ww	158.6	mg QE/kg ww	1066.0	[83]
Chenopodium ambrosioides L.	Leaves	mg GAE/100 g dw	822.33 ± 12.25	mg CE/g extract.	768.27 ± 10.70	[54]
Chondrilla juncea L.	Leaves	mg GAE/g extract.	37.66 ± 2.44	mg CE/g extract.	7.43 ± 0.28	[72]
Dianthus caryophyllus L.	Flowers	mg GAE/g	5.50 ± 0.28	-	-	[81]
Dianthus chinensis L.	Flowers	mg GAE/g	5.27 ± 0.25	-	-	[81]
Diplotaxis erucoides DC.	Leaves	mg CAE/kg ww	48.7	mg QE/kg ww	2876.7	[83]
Enhydra fluctuans Lour.	-	mg GAE/g de	70.338 ± 0.103	mg RE/g de	21.759 ± 0.039	[31]
Erythrina variegata L.	Leaves	mg GAE/g dw	170.33 ± 2.18	mg QE/g dw	53.42 ± 1.23	[28]
Erythrina variegata L.	Flowers	mg GAE/g	3.90 ± 0.29	-	-	[81]
Foeniculum vulgare Mill.	Leaves	mg GAE/g meth. extract.	42.16 ± 0.98	mg CE/g extract.	9.72 ± 0.70	[69]
Gerbera jamosenii Bolus ex Hook.f.	Flowers	mg GAE/g	4.89 ± 0.15	-	-	[81]
Gladiolus x hybridus C.Morren	Flowers	mg GAE/g	2.30 ± 0.23	-	-	[81]
Glechoma hederacea L.	Leaves	mg GAE/g	196.61 ± 6.09	mg CE/g	95.02 ± 2.73	[80]
Helianthus annuus L.	Flowers	mg GAE/g	1.86 ± 0.22	-	-	[81]
Helichrysum stoechas (L.) Moench	Inflorescences	mg GAE/g	184.42 ± 0.35	mg CE/g	34.75 ± 0.83	[56]
Hibiscus rosa-sinensis L.	Flowers	mg GAE/g	6.80 ± 0.63	-	-	[81]
Humulus lupulus L.	Leaves	mg GAE/g	55.83 ± 1.34	mg CE/g	9.56 ± 0.65	[69]
Hygrophilla schulli (Hamilt.) M.R.Almeida & S.M.Almeida	Leaves	mg GAE/g dw	148.05 ± 3.21	mg QE/g dw	87.12 ± 0.86	[28]

Table 4. *Cont.*

Plant Species	Part of Plants	Unit TPC	TPC	Unit TFC	TFC	Reference
Impatiens walleriana Hook.f.	Flowers	mg GAE/g	7.62 ± 0.16	-	-	[81]
Ipomoea aquatica Forssk.	-	mg GAE/g de	45.449 ± 0.130	mg RE/g de	13.941 ± 0.040	[31]
Ipomoea cairica (L.) Sweet	Flowers	mg GAE/g	1.77 ± 0.13	-	-	[81]
Iris japonica Thunb.	Flowers	mg GAE/g	0.63 ± 0.03	-	-	[81]
Jasminum nudiflorum Lindl.	Flowers	mg GAE/g	3.08 ± 0.09	-	-	[81]
Jatropha integerrima Jacq.	Flowers	mg GAE/g	17.22 ± 0.77	-	-	[81]
Lantana camara L.	Flowers	mg GAE/g	3.50 ± 0.08	-	-	[81]
Ligustrum sinense Lour.	Flowers	mg GAE/g	6.22 ± 0.11	-	-	[81]
Lilium brownii F.E.Br. ex Miellez	Flowers	mg GAE/g	1.27 ± 0.13	-	-	[81]
Limonium sinuatum (L.) Mill.	Flowers	mg GAE/g	34.17 ± 1.17	-	-	[81]
Loropetalum chinense var. *rubrum* Yieh	Flowers	mg GAE/g	11.46 ± 0.26	-	-	[81]
Magnolia soulangeana Soul.-Bod.	Flowers	mg GAE/g	5.30 ± 0.22	-	-	[81]
Malva sylvestris L.	Flowers	mg GAE/g extract.	386.45 ± 8.54	mg CE/g extract.	210.81 ± 7.99	[36]
Malvaviscus arboreus Cav.	Flowers	mg GAE/g	3.12 ± 0.41	-	-	[81]
Matthiola incana (L.) R.Br.	Flowers	mg GAE/g	1.70 ± 0.08	-	-	[81]
Mentha pulegium L.	Inflorescences	mg GAE/g extract.	331.69 ± 19.63	mg CE/g extract.	139.85 ± 1.27	[37]
Montia fontana L.	Leaves	mg GAE/g extract.	75.53 ± 7.05	mg CE/g extract.	16.67 ± 0.62	[69]
Nasturtium officinale R. Br.	Aerial parts	g GAE/kg extract	87 ± 2	g CE/kg extract	36 ± 1	[39]
Oldenlandia corymbosa Aiton	-	mg GAE/g de	47.184 ± 0.060	mg RE/g de	15.848 ± 0.125	[31]
Oncidium varicosum Lindl.	Flowers	mg GAE/g	4.46 ± 0.40	-	-	[81]
Origanum vulgare subsp. *virens*	Inflorencences	mg GAE/g	368.58 ± 18.18	mg CE/g	224.15 ± 0.96	[80]
Orostachys fimbriata (Turcz.) A. Berger	Flowers	mg GAE/g	12.36 ± 0.43	-	-	[81]
Osmanthus fragrans Lour.	Flowers	mg GAE/g	16.00 ± 0.57	-	-	[81]
papver corymbosa DC.	Flowers	mg GAE/g	2.20 ± 0.07	-	-	[81]
Papaver rhoeas L.	Leaves	mg GAE/g extract.	25.86 ± 3.52	mg CE/g extract.	12.00 ± 0.46	[72]
Pelargonium hortorum L.H. Bailey	Flowers	mg GAE/g	25.68 ± 1.02	-	-	[81]
Phaseolus vulgaris L.	Flowers	mg GAE/g	1.86 ± 0.10	-	-	[81]
Platydocon grandiflorus (Jacq.) A.DC.	Flowers	mg GAE/g	4.57 ± 0.28	-	-	[81]
Portulaca oleracea L.	Leaves	mg GAE/g extract.	12.89 (7.65–20.1)	mg CE/g extract.	1.76 (0.12–5.30)	[57]
Pterospartum tridentatum (L.) Willk.	Flowers	mg ClAE/g extract.	523.42 ± 36.09	mg QE/g extract.	58.12 ± 5.78	[41]
Rhapniolepis indica (L.) Lindl.	Flowers	mg GAE/g	7.97 ± 0.29	-	-	[81]
Rhododendron simsii Planch	Flowers	mg GAE/g	6.75 ± 0.22	-	-	[81]

Table 4. *Cont.*

Plant Species	Part of Plants	Unit TPC	TPC	Unit TFC	TFC	Reference
Rhoeo discolor (L'Hér.) Hance	Flowers	mg GAE/g	2.56 ± 0.05	-	-	[81]
Rubus ulmifolius Schott	Flowers	mg GAE/g extract.	257.89 ± 3.28	mg CE/g extract.	172.45 ± 3.42	[56]
Rumex acetosella L.	Leaves	mg GAE/g extract.	141.58 ± 3.67	mg CE/g extract.	67.91 ± 3.02	[30]
Rumex induratus Boiss. & Reut.	Leaves	mg GAE/g extract.	117.08 ± 2.54	mg CE/g extract.	89.78 ± 2.81	[30]
Rumex papillaris Boiss. & Reut.	Leaves	mg GAE/g extract.	104.18 ± 4.17	mg CE/g extract.	39.49 ± 3.26	[72]
Rumex pulcher L.	Leaves	mg GAE/g extract.	73.44 ± 5.32	mg CE/g extract.	26.14 ± 0.87	[72]
Salvia splendens Sellow ex Roem. & Schult.	Flowers	mg GAE/g	2.57 ± 0.07	-	-	[81]
Sesbania sesban (L.) Merr.	Leaves	mg GAE/g dw	167.66 ± 2.37	mg QE/g dw	97.16 ± 1.38	[28]
Silene vulgaris (Moench) Garcke	Leaves	mg GAE/g extract.	26.72 ± 1.63	mg CE/g extract.	21.65 ± 5.53	[69]
Silybum marianum (L.) Gaertn.	Leaves	mg GAE/g extract.	3.72 ± 0.36	mg CE/g extract.	1.13 ± 0.27	[72]
Sinapis incana (L.) Maly	Leaves	mg CAE/kg ww	92.2	mg QE/kg ww	1364.7	[83]
Sinapis nigra (L.) W.D.J.Koch	Leaves	mg CAE/kg ww	44.3	mg QE/kg ww	1545.6	[83]
Sonchus oleraceus L.	Leaves	mg GAE/g extract.	51.33 ± 1.75	mg CE/g extract.	14.83 ± 0.98	[72]
Sophora viciifolia Hance	Flowers	mg GAE/g dry extract	143.8 ± 8.7	mg RE/g dry extract	237.2 ± 10.3	[5]
Strelitzia reginae Banks ex Aiton	Flowers	mg GAE/g	9.40 ± 0.58	-	-	[81]
Tamus communis L.	Leaves	mg GAE/g extract.	49.51 ± 4.07	mg CE/g extract.	9.33 ± 1.44	[69]
Taraxacum obovatum (Willd.) DC.	Leaves	mg GAE/g extract.	58.26 ± 0.90	mg CE/g extract.	30.03 ± 0.66	[72]
Thymus mastichina L.	Inflorescences	mg GAE/g	165.29 ± 1.11	mg CE/g	83.85 ± 1.42	[80]
Thymus pulegioides L.	Inflorescences	mg GAE/g extract.	210.49 ± 21.16	mg CE/g extract.	128.24 ± 6.00	[37]
Viola x Wittrockiana	Flowers	mg GAE/g	6.08	-	-	[4]
Wedelia trilobata (L.) Hitchc.	Flowers	mg GAE/g	3.85 ± 0.03	-	-	[81]
Youngia japonica (L.) DC.	Flowers	mg GAE/g	1.11 ± 0.03	-	-	[81]
Zantedeschia aethiopica (L.) Spreng	Flowers	mg GAE/g	3.07 ± 0.07	-	-	[81]

[¥] Mean value (minimum-maximum); [‡] GAE = gallic acid equivalents; [§] RE = rutin equivalents; [ø] CE = catequin equivalents; [£] QE = quercetin equivalent; [¶] CAE = caffeic acid equivalent; ClAE = chlorogenic acid equivalents.

The WEP with the highest TPC was *Tamus communis* reaching 404 mg GAE/g, this plant being the only from the Diocoreaceae family [82]. Regarding the Asteraceae family (17 WEPs), the TPC ranged between 1.1 mg GAE/g (*Youngia japonica*) to 184 mg GAE/g (*Helichrysum stoechas*) [81,82]. It is necessary to highlight that the Polygonaceae family, although only data for four plants was available, showed values above the average, ranging between 73.44 mg GAE/g (*Rumex pulcher*) and 142 mg GAE/g (*Rumex acetosella*) [30,72].

Data regarding TFC (mg EC/g) were found for 31 WEPs, with *Origanum vulgare* (Laminaceae family) and *Malva sylvestris* (Malvaceae family) having the highest values at 224 and 211 mg CE/g, respectively [36,80]. Regarding the 7 WEPs whose flavonoids content was expressed in mg RE/g, the highest flavonoids content was shown by *Sophora viciifolia*

(Fabaceae family) with values of 237 mg rutin/g dry extract [5]. On the other hand, of the 6 WEPs whose flavonoids content was expressed in mg of quercetin, three stood out for their high content: *Sesbania sesban* (Fabaceae family) > *Hygrophila schulli* (Acanthaceae family) > *Berberis aristata* (Berberidaceae family) (97.16, 87.12 and 82.05 mg QE/g, respectively) [28]. *Diplotaxis erucoides* (Brassicaceae family) and *Asparagus acutifolius* (Asparagaceae family) showed the highest content of flavonoids expressed in mg QE/kg WW, at 2877 and 2263, respectively [83].

These data show the great importance of the WEPs due to their bioactive composition, and also their great diversity, with so many families represented.

9. Economic Value of Wild Edible Plants (WEPs)

Nowadays, a fundamental aspect in society is the economy and its linkage with sustainability is essential. Therefore, the economic value of WEPs must be highlighted and studied, in which edible flowers can play a key role; they can be prepared for sale in different formats, such as fresh, dried, or even candied. This variability together with the color of these flowers makes them highly attractive and a good business proposition within the flowers market. Globalization and online marketing have created an emerging market for this type of product worldwide. The sale of these flowers is usually in punnets of between 6 and 15 flowers, depending on the type of flower and the season. The price of these punnets also depends on the type of flower, but is usually between 8 and 17 euros, some of them reaching approximately 40 euros [85–88]. In recent years, haute cuisine has valued this type of flowers, either as a decoration on dishes, or in search of new flavors, aromas or appearance. One of the best restaurants in the world is "Mugaritz" with two Michelin stars and directed by Andoni Luis Aduriz; this restaurant has been innovative and pioneering when it comes to basing its dishes on wild plants, also using edible flowers.

Apart from this direct way of selling edible flowers, there are also lollipops with edible flowers inside, or crystallized. The price range is between 21 and 60 euros [85].

Other ways in which these plants contribute their economic value is through disclosure through books. In 2004, the book "Clorofilia" by Andoni Luis Aduriz was released, where information was collected on 50 wild plants, their flowering calendar, taxonomic information and recipes [89]. Another informative book on wild plants and herbs was published by the Basque Culinary Center, which contains detailed information on 180 varieties of wild plants/herbs from a botanical and culinary point of view [90].

So far, we have studied the entire market for WEPs, either directly or through other sources; however, for many families these plants are their livelihood. In the study by Mokria et al. [91], the need to promote strategic plans at the national level in Ethiopia for the sustainable use and domestication of these plants is highlighted. This would aim at socio-economic improvement and to help in achieving one of the SDG targets (2. End hunger and malnutrition). In northeast India, WEPs are critical to the survival of ethnic communities. A survey was conducted among 30 local vendors and 550 households. The results were overwhelming, registering, in consumption or sale, five wild edible mushrooms and 158 wild plants (78.8% of them being edible). In most households, wild plants influenced family income, accounting for between 5 and 75% of family income. All these results clearly demonstrated the importance of these wild plants for the subsistence and survival of many rural communities [92].

Not only are these plants important in ethnic communities, but the study carried out by Matsuura [93] showed their importance for the Japanese population. This study indicated that, in rural areas near Fukushima, it is not possible to harvest WEPs or edible mushrooms, which precludes a complete diet full of all the essential traditional products. The results concluded that the collection rate should remain very low for a few years within a radius of between 12 and 30 km from Fukushima (Kawauchi village, where the study was carried out) due to safety reasons. These data show the importance in these communities for their livelihood.

With all these data, the economic importance of WEPs is evident, being essential for many families around the world to survive.

10. Conclusions

The topic of this review is a step into the future, because developing countries are facing a serious problem, the fast growth of their population and the consequent increase food needs/availability. Furthermore, in many rural communities it is a necessity, because WEPs are not only a food but a way of earning a living. WEPs are a natural source of minerals, vitamins, fiber, and antioxidants, and at the same time they are inexpensive, as they are not cultivated. Therefore, they are a real alternative in trying to reduce this gap between food production and demand, especially to produce natural and sustainable food additives, such as flavorings and aromas. In addition, the food industry could take advantage of the properties of WEPs to develop nutritional, organic, and sustainable foods. Nevertheless, more research is needed to go beyond just their composition information (which is the most commonly found in the scientific databases for this type of plant); information on their technological and functional properties is needed to include them in industrial processes. Toxicological studies are also needed to determine their activities (e.g., anti,-mutagenic, etc.). Thus, a deep investigation into WEPs is needed to start developing commercial products based on these inexpensive and sustainable plants/ingredients. Sustainability, nutrition, and the agri-food industry converge around these plants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods12051012/s1, Table S1: List of Wild Edible Plants, WEPs; Table S2: Organic acids content in Wild Edible Plants, WEPs; Table S3: Daily value of minerals recommended by Food & Drug Administration (FDA); Table S4: Mineral elements in Wild Edible Plants, WEP's. [25,34,35,38,42,46,57,61,66–68]

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Potential Interest of *Oxalis pes-caprae* L., a Wild Edible Plant, for the Food and Pharmaceutical Industries

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Abstract: (1) Background: *Oxalis pes-caprae* L. is a plant considered within the group of so-called Wild Edible Plants (WEPs). The particularity of these plants is that they grow only with the natural resources at their disposal. Unfortunately, these types of plants are undervalued, being regularly uprooted from the fields. (2) Methods: Therefore, this study aimed to valorize the *Oxalis pes-caprae* plant, analyzing the proximate composition (sugars, organic acids, minerals, amino acids profile, fatty acids content, and volatile profile) of the plant shoots (flower, leaves, and stem) to demonstrate the full potential of this WEP. (3) Results: The results showed that *Oxalis pes-caprae* can be considered a natural source of minerals; furthermore, 19 essential and non-essential amino acids were found. Regarding the fatty acid profile, flowers are an important source of linoleic acid, and leaves present a high amount of α-linolenic acid. (4) Conclusions: Therefore, this research provides new information that reaffirms the capacity of *Oxalis pes-caprae* L. (WEP) to be a plant with great future progression due to its nutritional quality since it could be used in the food, nutritional, or pharmaceutical fields. Further research must be conducted to assay the biomass production and the costs of recommending farmers not to destroy this plant in their fields.

Keywords: amino acids; fatty acids; minerals; sugars; volatile profile

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1. Introduction

It is estimated that in 2050, the world's population will be approximately 9 billion people; this increase will create a global problem linked to the lack of enough food to feed the entire population [1]. This fact together with the health problems associated with food (e.g., obesity, allergies, etc.), are challenges that the world will face in the future, although this last problem has already started. In the 1970s, the first warnings about harmful diets with a high content of sugars and/or too high fat intake, were reported. However, it was not until the 1990s when these tendencies normally linked to sedentarism began to show their negative effects on health, such as diabetes, hypertension, or obesity [2]. These consumption trends can also have a significant impact on the environment; for instance, by 2050, if these diets are not controlled, they will result in an 80% increase in greenhouse gas emissions [3].

The homogenization of diets and the standardization of crops (basically looking for high yields) have led to 94 cultivated species representing 90% of the world's food supply, although only 9 represent 66% of total crop production [4,5]. Furthermore, currently, there are 866 domesticated species; therefore, wild edible plants (WEPs) could be used to further expand the number of species used to produce global food [5,6]. It must be considered that this action could also help the Sustainable Development Goals (SDGs), specifically numbers 2 and 12. Goal number 2 is "Zero Hunger" and focuses mainly on ending world

hunger, achieving food security and improving nutrition, and promoting sustainable agriculture by 2030; in this sense, wild edible plants (WEPs) will be ideal for combating these inequalities [7]. In addition, SDG 12, "Responsible Production and Consumption", evaluates the impact on resources such as water, energy, and food, exposing the production phase (agriculture) as one of the most serious stages with an environmental impact. The concept of WEP comes from the term wild plants described in 1999, which refers to plants that grow spontaneously without direct human intervention [8]. The families with the highest number of WEP species are Asteraceae, Brassicaceae, Fabaceae, Portulacaceae, and Oxalidaceae, among others [9,10].

The WEP *Oxalis pes-caprae* (Oxalidaceae), commonly called "sourgrass" or "vinagrillo o agrio" (in Spanish), normally grows in the areas of Mediterranean or subtropical climates; although this plant is native to South Africa [11], and it is an invasive species. This plant is a bulbiferous geophyte with pentameric and pentacyclic flowers, with five sepals and five petals fused at the base [12]. Regarding the properties of Oxalis pes-caprae, several studies reported its antioxidant (mainly due to the action of polyphenols) and anti-inflammatory properties, cytotoxic and phytotoxic activity, possible neuroprotective effects, antibacterial, antifungal activity and inhibition of alpha-amylase and alpha-glucosidase; therefore, it seems quite reasonable to think that this plant can be considered an interesting natural source of phytochemicals with potential application in pharmacological applications [13–19]. To our knowledge, no studies have been carried out about the content of amino acids, sugars, volatile composition, or even the proximal composition of the different parts (flowers, leaves, and stems) of *Oxalis pes-caprae* L. Consequently, the aim of this study was to determine the nutritional and chemical composition of the different parts of the *Oxalis pes-caprae* L. plant. This information will provide a basis for the selection of the most suitable WEPs for use as a functional ingredient and to be able to develop new food products in particular. It would determine whether the aerial portions of this WEP are of interest for further domestication and/or use in the food or pharmaceutical industries.

2. Materials and Methods

2.1. Plant Material

Oxalis pes-caprae plants used were collected at the Orihuela campus of the Universidad Miguel Hernández de Elche (38°4′10″ N, 0°59′1″ O, Alicante, Spain) in February 2022. Harvested plants of *Oxalis pes-caprae* were washed with tap and distilled water and separated into flowers, leaves, and stems. Samples were lyophilized before storage at room temperature.

2.2. Proximate Characterization

In the three parts (flowers, leaves, and stems) of *Oxalis pes-caprae*, the following parameters were analyzed: moisture, ash, total dietary fiber, fat, and protein (Kjeldahl method using a conversion factor of 6.25) according to the AOAC [20]. Available carbohydrates were calculated using the formula:

$$\text{Carbohydrates (\%)} = 100 - (\text{\% moisture} + \text{\% ash} + \text{\% fat} + \text{\% protein})$$

while the energy or total caloric value (kcal) was determined by the formula on the basis of a 100 g portion [20]:

$$\text{Energy (kJ)} = [(\text{\% protein} \times 4\ \text{kcal/g}) + (\text{\% carbohydrates} \times 4\ \text{kcal/g}) + (\text{\% fat} \times 9\ \text{kcal/g})] \times 4184$$

2.3. Analysis of Sugars, Organic Acids

Sugars and organic acids were quantified according to Hernández et al. [21] with some modifications using 0.150 g of sample. The determination of the content of sugars and organic acids was conducted using high-performance liquid chromatography (HPLC–DAD-RID) (Hewlett Packard 1100 series; Willmington, DE, USA). A Supelcogel TM C-610H column (30 cm × 7.8 mm) and a Supelguard precolumn (5 cm × 4.6 mm) (Supelco,

Bellefonte, PA, USA) were used for separation. The absorbance was measured using a diode-array detector (DAD) at 210 nm for the organic acids detection and a refractive index detector (RID) was used for the detection of sugars. Standards of organic acids (citric, fumaric, malic, oxalic, phytic, and tartaric) and sugars (arabinose, fructose, galactose, glucose, maltose, and sucrose) were obtained from Sigma (St. Louis, MO, USA). Calibration curves, with a concentration range between 1 and 10 g L^{-1}, were used for the quantification of organic acids and sugars and showed good linearity ($r^2 \geq 0.999$). This analysis was run in triplicate, and the results were expressed as g kg^{-1}.

2.4. Analysis of Minerals and Ascorbic Acid (Vitamin C)

The determination of minerals was carried out according to Cerdá-Bernad et al. [22] using ~0.100 g of freeze-dried samples. Total concentrations of macronutrients (Ca, Mg, Na, and K) and micronutrients (Zn, Cu, Mn, and Fe) in the previously mineralized samples were quantified with an Inductively Coupled Plasma Mass Spectrometer (ICPMS-2030, Shimadzu, Kyoto, Japan).

For the extraction of the ascorbic acid, ~50 mg of the lyophilized samples were weighed and dissolved in 1 mL of extractant MeOH:H_2O:HCOOH (75:24:1) ($v/v/v$) using an ultrasonic bath for short periods of 2–3 min. The samples were then centrifuged at 12,000× g for 15 min and filtered using a filter of 0.22 µm. For the analysis of the samples, the liquid chromatography equipment UPLC-QToF-MS (Agilent, UPLC-QTOF 6550-I-Funnel, Santa Clara, CA, USA) was used, with the mobile phases: Mobile phase A: 0.5% aqueous formic acid, and Mobile phase B: Methanol/water (50:50, v/v) containing 0.5% formic acid.

2.5. Analysis Amino Acids

Amino acids were quantified according to Kıvrak et al. [23] with some modifications. Approximately 100 mg of each sample was placed into a tube containing 1 mL of 0.1% (v/v) formic acid in water–methanol (80:20) (v/v) solution. Then, the sample was injected into a UPLC-QToF-MS (Agilent, UPLC-QTOF 6550-I-Funnel, Santa Clara, CA, USA) with the same mobile phases as for the analysis of ascorbic acid. All standards (arginine, alanine, asparagine, aspartic acid, cysteine, cystine, glutamine, glutamic acid, glycine, histidine, isoleucine, serine, proline, valine, threonine, leucine, lysine, methionine, phenylalanine, tyrosine, and tryptophan) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Stock standard solutions of amino acids (1000 mg L^{-1}) were prepared in distilled water.

2.6. Analysis Fatty Acids

Fatty acids were quantified according to Park and Goins [24] with some modifications. Approximately ~0.100 g of freeze-dried sample was weighed and placed into a test tube; then, 100 µL of dichloromethane and 1 mL of 0.5 N NaOH in methanol were added, and the test tube was closed and placed in a hot water bath at 90 °C for 10 min. Then, the test tube was rapidly cooled in an ice bath for 3 min. One mL of BF3 in methanol was added, and the test tube was placed in the dark for 30 min; later, 1 mL of ultrapure water and 600 µL of hexane were added. The sample was vigorously shaken for 1 min in a vortex (VORTEX 1, IKA, Staufen, Germany) and immediately afterward, centrifuged at 4000 rpm for 10 min (Eppendorf 5804R, Eppendorf, Hamburg, Germany). Subsequently, the supernatant was carefully recovered and placed into an amber chromatography vial.

For separation, a gas chromatograph (GC) Shimadzu GC-2030 coupled with a flame ionization detector (FID) with an automatic injector AOC-20i was used (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). Helium was used as a carrier gas, and nitrogen was used as a make-up gas (24 mL min^{-1}). FID used hydrogen and air at rates of 32 mL min^{-1} and 200 mL min^{-1}, respectively. The GC system used a Supelco SP®-2380 capillary column (60 m × 0.25 mm × 0.20 µm) (St. Louis, MO, USA). The detector temperature was kept at 260 °C, and a 1:20 split ratio and a total lineal flow velocity of 28.4 cm s^{-1} were used. The oven temperature started at 70 °C and increased up to 250 °C at a rate of 3 °C min^{-1}. Methyl fatty acids were identified by comparison with the retention times of the FAME Supelco

MIX-37 standards (Supelco Company, Bellefonte, PA, USA). Results were calculated as a percentage of each fatty acid in the total fatty acids profile.

2.7. Volatile Profile

Volatile compounds were quantified according to Noguera-Artiaga et al. [25] with some modifications. Between 0.150–0.300 g of freeze-dried samples were weighed and placed into a 40 mL vial with polypropylene cap and PTFE/silicone septa; isoamyl acetate (1000 mg/L) (5 µL) was added as the internal standard for semi-quantification of compounds. After 5 min at 45 °C (equilibration time), a 50/30 µm DVB/CAR/PDMS fiber was exposed to the vial headspace at 40 °C with continuous agitation (250 rpm) in a magnetic stirrer (IKA C-MAG HS 4, IKA-Werke GmbH & Co. KG, Staufen, Germany). After 45 min of exposure, the fiber was extracted from the vial and placed into the GC-MS injector. The separation and identification of compounds was performed using a Shimadzu GC-MS Nexis GC2030 (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA), equipped with a Sapiens X5MS column (30 m × 0.25 mm × 0.25 µm) (Teknokroma, Barcelona, Spain), and coupled with a mass spectrometer detector (TQ8040 NX triple quadrupole mass spectrometer; Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). Only the single quadrupole acquisition mode was used on the TQ8040 NX (Q3 Scan; scan speed 5000 amu s^{-1}; mass range 40–400 *m/z*; event time 0.100 s). The oven temperature program was as follows: (i) initial temperature of 35 °C, hold for 5 min; (ii) increment of 5 °C min^{-1} up to 150 °C, hold for 1 min; (iii) increment of 10 °C min^{-1} up to 280 °C and hold for 5 min. Helium column head pressure was 47.6 kPa (constant linear velocity mode of 36 cm s^{-1}). The injector, ion source, and interface were at 250, 230, and 280 °C, respectively. Helium was used as the carrier gas, column flow of 1 mL min^{-1}, with a split ratio of 1:50, and a purge flow of 6 mL min^{-1}.

Retention indexes of a commercial alkane standard mixture (Sigma-Aldrich, Steinheim, Germany) were used to identify the compounds, as well as the NIST 17 Mass Spectral and Retention Index Libraries. The identification was considered tentative when it was based only on mass spectral data, and only compounds with spectra similarity > 90% were considered correct hits. The linear retention similarity filter was set at ±10 units.

2.8. Statistical Analysis

In general, experimental data were subjected first to one-way analysis of variance (ANOVA) and later to Tukey's multiple range test to compare the means. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics. Inc., Rockville, MD, USA).

3. Results and Discussion

3.1. Proximate Composition

The proximate composition (moisture, ash, total dietary fiber, protein, fat, and carbohydrates) was significantly affected by the part of the plant under study (Table 1), except energy.

Regarding moisture, the stems had the highest value in both the fresh and freeze-dried samples, with values of 11.10 and 90.95%, respectively. These values are far higher than those reported by Datta et al. (2019), 60.28%, in similar plants such as *Oldenlandia corymbosa*. However, in most of the previous studies, the moisture varied in the range of 80–95% moisture [26–28].

The ash content showed a completely different behavior from that of moisture, with leaves showing the highest value (12.93%) as compared to flowers (8.27%) and stems (3.15%). The ash content found in the *Oxalis pes-caprae* leaves is significantly higher than those reported in *Oxalis corymbosa* (3.91%) by Vera, Espino Manzano, and Hernandez [19]. Ash values obtained in *Oxalis pes-caprae* flowers were similar to those reported by Datta et al. [29] in *Oldenlandia corymbosa* (8.34%). However, a study conducted in Pakistan shows much higher ash results (28.00%) therefore the geographical influence on the results is important [17].

Table 1. Proximate composition of *Oxalis pes-caprae* L.

Parameters (%)	Flowers	Leaves	Stems	*p*-Value	ANOVA †
Moisture (freeze-dried)	9.05 ± 0.78 b ‡	9.17 ± 1.22 b	11.10 ± 0.54 a	0.0000	***
Moisture	85.86 ± 0.26 b	86.07 ± 0.36 b	90.95 ± 0.57 a	0.0000	***
Ash	8.27 ± 0.20 b	12.93 ± 0.72 a	3.15 ± 1.01 c	0.0000	***
Total dietary fiber	30.68 ± 1.32 b	28.72 ± 1.50 b	36.36 ± 3.69 a	0.0000	***
Protein	13.45 ± 0.28 b	19.35 ± 0.54 a	8.86 ± 1.78 c	0.0000	***
Fat	6.66 ± 2.16 b	12.68 ± 0.10 a	3.53 ± 0.40 c	0.0000	***
Carbohydrates	62.57 ± 0.55 b	45.87 ± 0.80 c	73.34 ± 1.15 a	0.0000	***
Energy (kJ)	1522 ± 16.90	1569 ± 23.04	1506 ± 18.57	0.1044	NS

† NS: not significant at $p > 0.05$; *** significant at $p < 0.001$. ‡ Values (mean of 3 replications) followed by the same letter within the same row were not significantly different ($p > 0.05$) according to Tukey's least significant difference test.

Regarding the total dietary fiber, the highest content was obtained in the stems (36.36%) of *Oxalis pes-caprae* followed by the flowers (30.68%) and leaves (28.72%). Current results highlight the fact that *Oxalis pes-caprae* has higher fiber content than other similar plants, such as *Oxalis tuberosa* (0.87–1.69%) and *Oldenlandia corymbosa* (7.26%) [29,30].

On the other hand, for both protein and fat content, *Oxalis pes-caprae* leaves showed the highest content, while stems showed the lowest content for both variables. Very similar protein contents were obtained for *Oldenlandia corymbosa* (10.52%) [29]. Another study on *Oxalis corniculata* leaves reported a total nitrogen content of 3.56%, which is equivalent to 22.25% [31] after the application of a 6.25 conversion factor [20]; thus, this value is close to that found in the leaves of *Oxalis pes-caprae*. Regarding fat, the leaves had 12.68%, while the stems only had 3.53%. These fat values were lower than those found previously reported in *Oxalis corniculata*, 23.75% [31], although in this study, the same plants collected in different areas had protein contents ranging between 13.4–17.6%. The percentage of carbohydrates in the parts of *Oxalis pes-caprae* indicated a higher value in the stems (73.34%), followed by flowers (62.57%) and leaves (45.87%); these values were higher than those found by Jain et al. (2010) in *Oxalis corniculate* (24.67%) but similar to those previously reported in plants of other genera such as Malvaceae and Lamiaceae, 75–85% [31–33]. Finally, the total energy contents were equivalent in the three parts of the *Oxalis pes-caprae* plant. These values are quite high as those previously reported for plants of similar genera, such as Fabaceae, Malvaceae, and Lamiaceae, ~1548–1694 kJ [32–34].

3.2. Sugars, Organic Acids and Ascorbic Acid (Vitamin C)

The contents of fructose, sucrose, and oxalic acid were statistically equivalent in the flowers, leaves, and stems of *Oxalis pes-caprae* (Table 2).

Table 2. Sugars and organic acids found in *Oxalis pes-caprae* L.

Compound (g kg^{-1} dw)	Flowers	Leaves	Stems	*p*-Value	ANOVA †
Fructose	107 ± 33.77	103 ± 4.65	77.9 ± 8.90	0.2421	NS
Glucose	75.3 ± 18.59 a ‡	33.2 ± 3.43 b	88.9 ± 1.09 a	0.0019	**
Maltose	n.d.	40.35 ± 6.22 a	14.04 ± 1.87 b	0.0022	**
Sucrose	193 ± 14.76	192 ± 4.20	173 ± 3.03	0.0792	NS
Oxalic acid	98.0 ± 21.51	98.2 ± 6.55	110 ± 7.56	0.4855	NS
Ascorbic Acid (mg 100 g^{-1} dw)	0.42 ± 0.006 c	3.17 ± 0.007 b	3.50 ± 0.004 a	0.0000	***

† NS: not significant at $p > 0.05$; ** significant at $p < 0.01$, *** significant at $p < 0.001$. ‡ Values (mean of 3 replications) followed by the same letter within the same row were not significantly different ($p > 0.05$) according to Tukey's least significant difference test. n.d.: not detected.

The leaves of *Oxalis pes-caprae* were richer in maltose than the rest of the plant, while they had the lowest content of glucose; this sugar (glucose) was more abundant in stems. The fructose in *Oxalis pes-caprae* was higher than those found in other plants such as *Calligonum comosum* L. or *Cynara cardunculus* L. [35,36]; however, in the flowers of *Moringa*

oleifera Lam. and *Malva sylvestris* L., 75.6 and 87.2 g kg^{-1}, respectively, [32,37] the values were very similar to those found in the current study. The contents of glucose found here were close to those previously reported in *Cynara cardunculus* L., *Moringa oleifera* Lam., or *Malva sylvestris* L., 99.5, 120.7, 73.6 g kg^{-1}, respectively [32,36,37]. Finally, it is important to comment that sucrose is the predominant sugar in most WEPs [35,36,38,39]; however, the values of sucrose found in these studies were lower than those reported here for *Oxalis pes-caprae*.

The concentration of oxalic acid found in *Oxalis pes-caprae* L. is much higher than those found in other types of WEPs, such as *Allium ampeloprasum* L. (27.83 mg/100 g) [26]. However, the current values were closer to those found in the leaves of *Cynara cardunculus* L. var. *altilis* (81 g kg^{-1}) [36]. These high values of oxalic acid were expected because the genus *Oxalis* took the name after the high content of oxalic acid found in these plants. Oxalic acid and its salts, called oxalates, can cause problems in the human body because they scavenge minerals such as calcium, although it has been calculated that the problems would arise above 150 mg of daily intake of oxalates [40].

On the other hand, the ascorbic acid accumulated mainly in the stems and leaves of *Oxalis* plants, and significantly less in the flowers (Table 2). The content of this organic acid was higher in other studied WEPs, such as *Blumea lacera* (127 mg/100 g), *Commelina benghalensis* (23.6 mg/100 g), or even *Dioscorea praehensilis* with 10 mg/100 g [41–43]. In plants belonging to the same family, another study carried out by Šircelj et al. [44] with *Oxalis acetosella* showed much higher values (3457 µg/g dw) than those found in *Oxalis pes-caprae*.

3.3. Minerals

The mineral composition found in *Oxalis pes-caprae* is shown in Table 3, with potassium and Fe predominating among the macro- and micro-nutrients, respectively.

Table 3. Mineral composition of *Oxalis Pes-caprae* L.

Mineral [mg (100 g)$^{-1}$ dw]		Flowers	Leaves	Stems	*p*-Value	ANOVA †
Macro	Ca	104 ± 16.97 c ‡	620 ± 20.21 b	453 ± 10.60 a	0.0000	***
	K	1247 ± 3.53 b	859 ± 20.56 c	1399 ± 21.21 a	0.0000	***
	Na	35.1 ± 12.47 b	69.1 ± 1.20 a	71.5 ± 3.88 a	0.0000	***
	Mg	95.4 ± 2.47 b	129 ± 2.80 a	73.2 ± 0.00 c	0.0000	***
Micro	Fe	7.7 ± 0.28 a	3.2 ± 0.16 b	1.4 ± 0.03 c	0.0000	***
	Mn	0.87 ± 0.00 b	1.18 ± 0.01 a	0.32 ± 0.00 c	0.0000	***
	Zn	0.30	n.d.	n.d.	-	-

† *** significant at $p < 0.001$. ‡ Values (mean of 3 replications) followed by the same letter within the same row were not significantly different ($p > 0.05$) according to Tukey's least significant difference test. n.d.: not detected.

Calcium (Ca) concentration was higher in the stems (620 mg/100 g), and is six times lower in the flowers (104 mg/100 g). A study conducted by Datta, Sinha, Bhattacharjee, and Seal [29] on six WEPs showed Ca contents ranging from 492 to 621 mg/100 g; this range is very close to that found in *Oxalis* leaves and stems. Regarding potassium (P), stems (1399 mg/100 g) and flowers (1247 mg/100 g) showed similar contents and were significantly higher than leaves. K content found here was higher than those reported in *Enhydra fluctuans* (487 mg/100 g) but much lower than those reported in other 19 WEPs, reaching a value as high as 7830 mg/100 g in *Smyrnium cordifolium* Boiss [45]. Regarding sodium (Na), there were no differences between the contents of leaves and stems, but they were higher than the Na content in the flowers. *Cichorium intybus* L. showed a higher sodium concentration (80.61 mg/100 g) in a study carried out by Jalali and Fakhri [45]. The *Oxalis* flowers had similar Na contents to those of other types of plants, such as *Allium hirtifolium* Boiss., *Stachys lavandulifolia* Vahl, or *Taraxacum vulgar* Hodn. Mzt. (30.28, 30.26, 30.35 mg/100 g, respectively) [45]. The content of magnesium (Mg) followed the order

leaves > flowers > stems, with these contents being similar to those previously found in *Anchusa italica* Retz (120 mg/100 g) [45].

The most abundant micro-nutrient was Fe, especially in the flowers. No biologically significant differences were observed in the contents of Mn and Zn. The experimental contents of these three nutrients are similar to those previously reported in other WEPs [45].

3.4. Amino Acids

Nineteen amino acids were found in *Oxalis pes-caprae* L. (Table 4). In general, the essential amino acids predominated in the flowers, followed by leaves and, finally, stems, with leucine, isoleucine, and valine being the most abundant compounds of this chemical family. In this way, leucine also predominates in other plants [18,46,47]. These three amino acids (leucine, isoleucine, and valine) have an important function in plants by contributing to the volatile compounds responsible for their odor and aroma. These volatiles produced by plants not only have aromatic functions but can also act as aromas that attract pollinators; therefore, their role is fundamental [48].

Table 4. Amino acids found in *Oxalis pes-caprae* L.

Amino Acids [mg (100 g)$^{-1}$ dw]		Flowers	Leaves	Stems	*p*-Value	ANOVA †
Essential	Arginine	13.0 ± 0.37 a ‡	4.91 ± 0.02 b	2.04 ± 0.08 c	0.0000	***
	Histidine	19.9 ± 7.32 a	8.98 ± 0.03 b	5.57 ± 0.04 c	0.0000	***
	Isoleucine	209 ± 1.67 a	169 ± 3.45 b	170 ± 6.63 b	0.0000	***
	Leucine	306 ± 4.24 a	244 ± 9.65 b	245 ± 12.30 b	0.0000	***
	Lysine	5.46 ± 0.70 b	6.63 ± 0.14 a	2.16 ± 0.08 c	0.0000	***
	Methionine	6.01 ± 0.24 a	4.82 ± 0.08 b	1.83 ± 0.17 c	0.0000	***
	Phenylalanine	48.7 ± 3.20 a	50.6 ± 1.17 a	29.9 ± 2.60 b	0.0000	***
	Threonine	114 ± 3.49 a	13.9 ± 0.22 c	20.3 ± 0.25 b	0.0000	***
	Tryptophan	80.1 ± 6.07 a	47.2 ± 1.00 b	24.8 ± 3.91 c	0.0000	***
	Valine	187 ± 9.47 a	189 ± 7.02 a	134 ± 1.72 b	0.0000	***
Non-essential	Alanine	302 ± 12.44 b	307 ± 7.40 b	343 ± 11.17 a	0.0000	***
	Asparagine	n.d.	8.42 ± 0.22 a	5.94 ± 0.29 b	0.0000	***
	Aspartate	73.0 ± 3.27 b	91.1 ± 3.72 a	56.4 ± 6.03 c	0.0000	***
	Cysteine	0.55 ± 0.03 b	0.69 ± 0.02 a	n.d.	0.0000	***
	Glutamic acid	237 ± 11.93 b	325 ± 20.9 a	220 ± 4.57 c	0.0000	***
	Glycine	55.7 ± 6.84 a	11.9 ± 1.41 c	30.4 ± 4.72 b	0.0000	***
	Proline	102 ± 15.32 a	30.5 ± 0.30 c	50.7 ± 4.01 b	0.0000	***
	Serine	112 ± 22.49 a	46.3 ± 2.30 b	46.3 ± 1.42 b	0.0000	***
	Tyrosine	13.8 ± 0.82 c	40.1 ± 2.84 a	17.0 ± 0.80 b	0.0000	***
TOTAL		**1885 a**	**1600 b**	**1405 c**	**0.0000**	***

† *** significant at $p < 0.001$. ‡ Values (mean of 3 replications) followed by the same letter within the same row were not significantly different ($p > 0.05$) according to Tukey's least significant difference test. n.d.: not detected.

Regarding the non-essential amino acids, the contents of flowers and leaves were similar and higher than those of the stems, with alanine and glutamic acid being the predominant compounds. However, alanine was the most abundant compound in the stems, while glutamic acid predominated in the leaves. In other WEPs such as *Sesamum indicum* L., *Balanites aegyptiaca* (L.) Delile [46] and *Portulaca oleracea* L. [18], the most abundant compound was glutamic acid. Glutamic acid is one of the four main ligands of zinc; this mineral performs catalytic or structural functions in plants [49].

3.5. Fatty Acids

In the present study, 29 fatty acid methyl esters (FAMEs) were identified (Table 5) in different tissues of *Oxalis pes-caprae*; these FAMEs consisted of are composed of 8 monounsaturated fatty acids (MUFAs), 7 polyunsaturated fatty acids (PUFAs) and 14 saturated fatty acids (SFAs). Although the number of SFAs was higher, the unsaturated FAMEs predominated

and represented as much as 70–80% of the total content: flowers (MUFA + PUFA) = 73.72%; leaves (MUFA + PUFA) = 80.37%; and stems (MUFA + PUFA) = 77.76%.

The three most abundant compounds were C18:3n3 (-linolenic), C18:2n6c (linoleic), and C16:0 (palmitic). In previous studies on wild edible plants, these three same compounds, along with oleic acid, were the predominant ones [14,50,51]. Regarding linolenic acid (FA27), there were significant differences among the tissues under analysis, with leaves having the highest content (53.57%). These results agree well with previous studies reporting contents of ~50% of linolenic acid [27,52,53]; this content was reported in Amaranthaceae, Asteraceae, Montiaceae, Polygonaceae and Caryophyllaceae plants and more precisely in *Beta maritime* (57.80%), *Chondrilla juncea* (56.27%), *Montia fontana* (55.57%), *Rumex acetosella* (51.34%), *Rumex induratus* (58.84%), and *Silene vulgaris* (54.5%). However, the presence of linoleic acid (C18:2n6c, FA23) was higher in the flowers, reaching 47.65%. Other WEPs with a similar content of these compounds were *Allium ampeloprasum* (53.45%) and *Tamus communis* (42%), belonging to the Amaryllidaceae and Dioscoreaceae families, respectively [26,39].

The third major fatty acid was palmitic acid (FA5) with a presence of 18.51% in flowers. Regarding palmitic acid, several WEPs showed similar results, as is the case of *Diplotaxis erucoides* (18.23%) or *Humulus lupulus* (19.52%), belonging to the Brassicaceae and Cannabaceae families, respectively [53,54]. Oleic acid (FA19) predominated in the stems, followed by leaves and flowers. Oleic acid was present in other WEPs with values similar to those found in the stem of *Oxalis*. *Chenopodium ambrosioides*, *Helichrysum stoechas*, and *Scolymus hispanicus* have between 6 and 7% oleic acid content [32,38,53]. However, plants belonging to the lamiaceae family showed a higher content of oleic acid compared to other families. *Glechoma hederacea*, *Thymus pulegioides*, and *Thymus mastichina* showed 35.12%, 11.50%, and 9.82%, respectively [33,55].

Table 5. Fatty acid profile (main groups and ratios) of *Oxalis pes-caprae* L.

Code	FA (%) [Υ]	R. Time	ANOVA [†]	Flower	Leaf	Stem
FA1	C12:0 (Lauric)	19.330	***	0.31 ± 0.06 a [‡]	0.09 ± 0.05 b	0.08 ± 0.019 b
FA2	C13:0 (Tridecanoic)	21.419	-	n.d.	n.d.	0.02 ± 0.002
FA3	C14:0 (Myristic)	23.420	***	0.51 ± 0.06 a	0.31 ± 0.02 b	0.20 ± 0.08 c
FA4	C15:0 (Pentadecanoic)	25.348	***	0.06 ± 0.001 a	0.02 ± 0.001 b	0.06 ± 0.004 a
FA5	C16:0 (Palmitic)	27.251	***	18.51 ± 0.47 a	11.73 ± 0.74 c	16.42 ± 0.43 b
FA6	C17:0 (Isomargaric)	28.156	***	0.02 ± 0.001 b	n.d.	1.41 ± 0.32 a
FA7	C17:0 (Margaric)	28.943	***	0.13 ± 0.006 a	0.10 ± 0.005 b	0.11 ± 0.01 b
FA8	C18:0 (Stearic)	30.649	***	2.05 ± 0.11 a	1.22 ± 0.07 c	1.46 ± 0.01 b
FA9	C19:0 (Nonadecanoic)	32.318	-	n.d	n.d.	0.18 ± 0.009
FA10	C20:0 (Arachidic)	33.923	***	0.09 ± 0.005 c	0.24 ± 0.03 a	0.16 ± 0.04 b
FA11	C21:0 (Heneicosanoic)	35.336	***	0.15 ± 0.01 a	0.15 ± 0.05 a	0.04 ± 0.003 b
FA12	C22:0 (Behenic)	36.831	***	1.33 ± 0.30 a	1.01 ± 0.20 b	0.01 ± 0.0001 c
FA13	C23:0 (Tricosylic)	38.165	***	0.97 ± 0.20 b	2.87 ± 0.76 a	0.51 ± 0.44 c
FA14	C24:0 (Lignoceric)	39.585	***	0.59 ± 0.04 b	0.07 ± 0.01 c	0.92 ± 0.07 a
	Σ SFA		***	**24.72 a**	**17.81 c**	**21.58 b**
FA15	C15:1 (Pentadecenoic)	26.835	***	0.06 ± 0.003 b	0.16 ± 0.01 a	0.07 ± 0.006 b
FA16	C16:1 (Palmitoleic)	27.856	-	0.01	n.d.	n.d.
FA17	C16:1c9 (Hypogeic)	28.275	***	0.12 ± 0.002 b	n.d.	0.89 ± 0.09 a
FA18	C18:1t9 (Elaidic)	31.316	***	0.36 ± 0.009 c	8.90 ± 0.45 a	4.05 ± 0.05 b
FA19	C18:1c9 (Oleic)	31.517	***	0.76 ± 0.009 c	1.43 ± 0.09 b	6.59 ± 0.74 c
FA20	C18:1n7 (*cis*-Vaccenic)	31.660	***	0.30 ± 0.008 c	0.42 ± 0.01 b	0.67 ± 0.009 a
FA21	C22:1n9 (Erucic)	37.730	***	1.22 ± 0.26 a	0.02 ± 0.001 b	n.d.
FA22	C24:1n9 (Nervonic)	40.122	***	0.04 ± 0.007 c	0.08 ± 0.01 b	0.18 ± 0.07 a
	Σ MUFA		***	**2.87 c**	**11.01 b**	**12.45 a**
FA23	C18:2n6c (Linoleic)	32.941	***	47.65 ± 0.37 a	10.15 ± 0.56 c	29.57 ± 1.44 b
FA24	C20:2 (Eicosadienoic)	35.998	***	2.77 ± 0.04 a	0.32 ± 0.04 c	0.87 ± 0.04 b
FA25	C22:2 (Docosadienoic)	38.712	***	1.47 ± 0.59 b	2.95 ± 0.37 a	n.d.
	Σ n-6 PUFA		***	**51.89 a**	**13.42 c**	**30.44 b**

Table 5. *Cont.*

Code	FA (%) [ϒ]	R. Time	ANOVA [†]	Flower	Leaf	Stem
FA26	C18:3n6 (γ-Linolenic)	33.849	***	0.58 ± 0.03 b	1.20 ± 0.07 a	0.42 ± 0.07 c
FA27	C18:3n3 (α-Linolenic)	34.552	***	18.17 ± 0.03 c	53.57 ± 2.45 a	34.04 ± 2.05 b
FA28	C20:3n3 (Eicosatrienoic)	36.894	***	0.10 ± 0.06 b	1.08 ± 0.13 a	0.03 ± 0.02 b
FA29	C20:3n6 (dihomo-γ-Linoleic)	37.497	***	0.11 ± 0.007 b	0.09 ± 0.008 b	0.38 ± 0.02 a
	Σ n-3 PUFA		***	**18.96 c**	**55.94 a**	**34.87 b**
	Σ PUFA		**NS**	**70.85**	**69.36**	**65.31**

[†] *** significant at $p < 0.001$. [‡] Values (mean of 3 replications) followed by the same letter within the same row were not significantly different ($p > 0.05$) according to Tukey's least significant difference test. [ϒ] FA = Fatty acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. n.d.: not detected. NS: Not Significant.

3.6. Volatile Profile

A total of 32 volatile compounds were isolated, identified, and quantified in the aerial tissues of *Oxalis pes-caprae*, as well as all the aromatic descriptors associated with the volatile compounds (Table 6) [56–61]. The volatile compounds can be grouped into six chemical families: esters (11 compounds), alkanes (7), terpenes (5), alcohols (5), aldehydes (2), acids (1), and ethers (1). Fifteen out of the 32 volatile compounds were found to be significantly different amounts in the studied tissues of this plant. The three main compounds found were nerolidol, caryophyllene, and 3-hexen-1-ol acetate.

In general, nerolidol (V27) was the compound with the highest concentration (21.58 μg/g) in all the tissues of this plant, more precisely in the flowers. Nerolidol is typically associated with a floral odor and is present mainly in the essential oils of flowers. The presence of this compound in flower is very important because it has been associated with antifungal, antibacterial, and antioxidant potential [16,62]. Regarding β-caryophyllene (V20) was also found exclusively in the flowers, and its sensory descriptors are sweet, woody, spicy, and clove. Humulene (α-caryophyllene), α-terpineol or β-farnesene (1.58, 1.13, and 3.51 μg/g, respectively) were also found but in lower concentrations, demonstrating the importance of the terpenes group in the volatile profile of this particular WEP. This statement was also supported by the results Fukalova Fukalova et al. [63], with β-caryophyllene being found in six out of the seven plants studied, with Porophyllum ruderale showing the highest content of this compound.

3-Hexenyl acetate (V7) was found in all three parts of the plant, with the highest content being found in the leaves (16.0 μg/g). This compound was also present in carrot leaves, parsley, and the Nelumbo Nucifera flowers belonging to the Nelumbonaceae family [64,65]. The content of pentyl acetate (V4) was also of importance, with leaves and stems having significantly higher contents than flowers.

The total content of volatile compounds was significantly higher in the *Oxalis* flowers, followed by leaves and stems. This behavior is quite logical as normally flowers smell more intensely than leaves, and these more than stems.

Table 6. Identification, concentration, and odor descriptors of volatile compound found in *Oxalis pes-caprae* L.

Code	Compound (μg g^{-1})	CF	RT (min)	KI (EXP)	KI (LIT)	Flowers	Leaves	Stems	ANOVA †	Odor Descriptors ¥
V1	3-Methyl butanal	Aldehyde	3.349	690	686	3.02 ± 0.2 ‡ a	2.95 ± 0.1 a	0.08 ± 0.001 b	***	Aldehydic, fatty
V2	3-Hexen-1-ol	Alcohol	10.173	857	857	-	-	0.57 ± 0.06	-	Green, vegetable, herbal
V3	4-Penten-1-yl acetate	Ester	11.963	901	901	1.52 ± 0.42 a	1.39 ± 0.17 b	1.14 ± 0.24 b	***	Green, vegetable
V4	Pentyl acetate	Ester	12.434	914	917	4.33 ± 0.47 b	6.27 ± 0.35 a	6.50 ± 0.50 a	***	Fruity, banana
V5	Isoamyl propionate	Ester	14.537	970	969	0.98 ± 0.20 c	2.15 ± 0.33 a	1.52 ± 0.17 b	***	Sweet, fruity, banana
V6	Diisoamyl ether	Ether	15.728	1002	1002	2.03 ± 0.61 a	0.66 ± 0.06 b	0.36 ± 0.04 c	***	Fruity
V7	3-Hexenyl acetate	Ester	15.794	1004	1005	2.34 ± 0.30 c	16.0 ± 2.19 a	7.24 ± 1.16 b	***	Fresh, green, sweet, fruity
V8	Hexyl acetate	Ester	16.062	1011	1011	2.14 ± 0.16 c	2.64 ± 0.22 b	3.14 ± 0.29 a	***	Fruity, green, banana, sweet
V9	Pentyl butanoate	Ester	17.579	1056	1059	0.90 ± 0.08	-	-	-	Sweet, fruity, banana, cherry
V10	Linalool	Alcohol	19.021	1098	1098	3.19 ± 0.56	-	-	-	Floral, citrus, rose
V11	Nonanal	Aldehyde	19.178	1103	1102	0.95 ± 0.10	-	-	-	Waxy, aldehydic, citrus, fresh
V12	Isoamyl butanoate	Ester	19.231	1104	1104	0.95 ± 0.05	-	-	-	Sweet, fruity, green
V13	Phenylethyl alcohol	Alcohol	19.414	1110	1110	0.92 ± 0.13	-	-	-	Floral, rose
V14	α-Terpineol	Terpene	22.046	1194	1194	1.13 ± 0.21	-	-	-	Pine, lilac, woody, floral
V15	1,3-bis(1,1-dimethylethyl)benzene	Alkane	23.645	1248	1249	1.80 ± 0.25 a	0.26 ± 0.01 c	0.32 ± 0.007 b	***	-
V16	Nonanoic acid	Acid	24.005	1261	1267	1.59 ± 0.45	-	-	-	Waxy, dirty, cheese, dairy
V17	4,6-Dimethyl dodecane	Alkane	24.437	1275	1285	1.55 ± 0.02 b	1.64 ± 0.04 a	0.41 ± 0.01 c	***	Fruity, green
V18	1,1′-Bicyclohexyl	Alkane	25.708	1320	1307	2.70 ± 0.64	-	-	-	-
V19	Ethyl nonanoate	Ester	27.686	1290	1294	8.15 ± 1.67	-	-	-	Fruity, rose, waxy
V20	β-Caryophyllene	Terpene	28.528	1424	1424	19.86 ± 3.01	-	-	-	Sweet, woody, spicy, clove
V21	Isoamyl benzoate	Ester	28.915	1438	1437	1.58 ± 0.32	-	-	-	Sweet, balsamic, green, waxy
V22	β-Farnesene	Terpene	29.342	1454	1458	3.51 ± 0.89	-	-	-	Woody, citrus, herbal, sweet
V23	Humulene	Terpene	29.551	1462	1462	1.58 ± 0.44	-	-	-	Woody
V24	1-Dodecanol	Alcohol	29.887	1474	1474	1.49 ± 0.39 b	3.99 ± 0.85 a	0.77 ± 0.11 c	***	Earthy, soapy, waxy, fatty
V25	Pentadecane	Alkane	30.290	1490	1490	-	2.14 ± 0.57	-	-	Waxy

Table 6. *Cont.*

Code	Compound (μg g^{-1})	CF	RT (min)	KI (EXP)	KI (LIT)	Flowers	Leaves	Stems	ANOVA †	Odor Descriptors ¥
V26	2,4-bis(1,1-dimethylethyl)phenol	Alkane	30.642	1504	1502	1.34 ± 0.14 b	1.48 ± 0.06 a	0.46 ± 0.09 c	***	-
V27	Nerolidol	Terpene	31.826	1563	1562	21.58 ± 4.05	-	-	-	Floral, green, citrus
V28	Ethyl dodecanoate	Ester	32.412	1592	1591	3.92 ± 0.91 a	0.63 ± 0.14 b	-	***	Sweet, waxy, floral, soapy
V29	Hexadecane	Alkane	32.575	1600	1600	2.00 ± 0.31 a	0.84 ± 0.05 b	0.39 ± 0.02 c	***	Alkane
V30	Cyclotetradecane	Alkane	34.045	1691	1679	-	2.73 ± 0.77	-	-	Waxy
V31	1-Tetradecanol	Alcohol	34.149	1698	1686	-	3.06 ± 0.28 a	2.14 ± 0.08 b	***	Fruity, waxy
V32	Ethyl hexadecanoate	Ester	37.768	1974	1975	1.59 ± 0.15 a	0.61 ± 0.10 b	0.58 ± 0.04 b	***	Waxy, creamy, milky, oily
TOTAL						98.64 a	49.44 b	25.62 c	***	

CF = Chemical Family; RT = Retention Time; KI = Kovats Index; EXP = Experimental; LIT = Literature; † *** significant at $p < 0.001$. ‡ Values (mean of 3 replications) followed by the same letter within the same row were not significantly different ($p > 0.05$) according to Tukey's least significant difference test. ¥ Odour descriptors of the volatile compounds.

4. Conclusions

Three parts of *Oxalis pes-caprae* L. (flowers, leaves, and stems) were analyzed to deepen their composition. The flower stood out in its sugar content, such as fructose and sucrose. Regarding minerals, it was the part of *Oxalis* that had the highest iron content, and the only one that had a zinc concentration. This part also stood out for having the highest concentration of amino acids in the entire plant. Apart from these values, the results obtained in the fatty acid profile were more than 50% in the Σ n-6 PUFA acids in the whole plant. In the leaves, its protein content stood out compared to the flowers and stems; also, it stood out in its concentration of maltose. Regarding minerals, the flowers stood out in their magnesium and manganese content. In the fatty acid profile, alpha-linolenic acid had the highest content compared to the rest of the acids. The stems showed in the proximal composition the highest total dietary fiber content, and the lowest fat value. It also showed the highest concentrations of calcium, potassium, and sodium. In the fatty acid profile, it was the most balanced, obtaining around 30% in both Σ n-6 PUFA and Σ n-3 PUFA. For the introduction of *Oxalis pes-caprae* as food, it should be domesticated, obtaining growing conditions that could allow lowering the levels of oxalic acid, and therefore, ingesting it directly without limitations. This fact is independent of taking advantage of the antibacterial, antifungal properties and cytotoxic inhibition capacity that the plant has. The interesting insight about the results shown by *Oxalis pes-caprae* L., is that all parts can be considered high-value biomass. This allows us to affirm that the plant is valid as a whole because each part of it adds a different type of nutritional contribution after its intake. The results make evident the promising future and the potential of this plant for industries such as agri-food or pharmaceutical, being an undervalued and discarded plant.

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Diplotaxis erucoides and *Oxalis pes-caprae*: Two Wild Edible Plants as a New and Valuable Source of Carotenoids, Tocols and B1 and B2 Vitamins

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Abstract: The aim of this study was to determine the profile and contents of carotenoids, tocols and B1 and B2 vitamins in different parts of two wild edible plants (WEPs), *Diplotaxis erucoides* and *Oxalis pes-caprae*. Results showed interesting amounts of these bioactive compounds in the leaves, with intakes higher than the Recommended Daily Allowance (RDA) for vitamin A and vitamin E after consumption of 100 g. *Diplotaxis erucoides* and *Oxalis pes-caprae* leaves evidenced high amounts of carotenoids, such as lutein (about 8 mg/100 g and 5 mg, respectively) and β-carotene (about 8 mg/100 g and 4 mg/100 g, respectively). Even when not present at high amounts, the investigated plants can also contribute to the daily intake of thiamine and riboflavin. The rich profile and high contents of bioactive compounds in these WEPs clearly justify their potential use as food ingredients in a healthy and sustainable modern cuisine and in the development of new functional foods.

Keywords: WEPs; carotenoids; tocols; riboflavin; thiamine

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1. Introduction

The Millennium Ecosystem Assessment is a study promoted by the United Nations where more than 1300 researchers from 95 different countries evaluated the consequences of the change in ecosystems for human well-being. This study determined that wild edible plants (WEPs) suffered a decrease in both consumption and collection, due to rural exodus, modernization of lifestyle and industrialization, among other causes [1]. WEPs are plants that grow without the help of humans, suffering undervaluation over years [2]. However, in many developing countries, due to food shortages, these plants have become a fundamental support to meet the daily food needs of many families [3], also providing minerals, antioxidants, antifungal power, and even, for some of them, preventive activity against different health diseases [4–6].

Among bioactive compounds found in WEPs, carotenoids are fat-soluble compounds responsible for the color of many vegetables and fruits. They are present in plants, fungi, algae, animals and bacteria. Approximately one thousand two hundred carotenoids were identified, but only six of them (lycopene, α-carotene, lutein, β-carotene, β-cryptoxanthin and zeaxanthin) represent more than 90% in human plasma [7]. Several studies have linked carotenoids with a better response to cataracts, lower risk of cardiovascular diseases,

osteoporosis, prevention and treatment of age-related macular degeneration (AMD) and improvement in the control of hypertension [8].

Other interesting compounds found in WEPs are tocols, among which, eight compounds are the most common: tocopherols (α, β, γ and δ) and tocotrienols (α, β, γ and δ). Recent evidences suggest that only α-tocopherol shows vitamin E activity and it still unknown if other tocols are able to prevent vitamin E avitaminosis in humans like α-T [9]. Tocols are commonly found in oils of plant origin [10]. The incorporation of tocopherols into the diet or through deficiency supplements is essential for the proper functioning of the body. Vitamin E, due to its high antioxidant power, was shown to have effect against obesity. Moreover, it helps at improving or preventing health problems such as cataracts and HIV-AIDS, stimulates the immune system and has therapeutic potential against several degenerative diseases [11]. In plants, tocol's functions range from maintaining the membrane integrity, photo-protection of chloroplasts, to the regulation of the electron transport [11].

The main culinary use of WEPs is in dishes (e.g., soups, salads), where the associated oil ingredient increases the rate of absorption of tocols and carotenoids.

The importance of B1 (thiamine) and B2 (riboflavin) vitamins comes from their essential functions in the human body and problems associated with their deficiency. Thiamine is essential in energy generation, amino acid interconnections and neurological functions. Its deficiency is responsible of a disease called "beriberi" [12]. Riboflavin is essential for the metabolism of lipids, carbohydrates and amino acids and exerts antioxidant protection of cells. Its deficiency leads to skeletal deformities, anemia, ataxia, photophobia or inflammation at the gastrointestinal level, among other symptoms. Its supplementation has been reported to be effective in combating migraines and improving neurological motor disability associated with multiple sclerosis [13].

Within WEPs, the most widespread family is that of Asteraceae. Outside this family, plants, such as *Diplotaxis erucoides* and *Oxalis pes-caprae*, are of importance due to their presence in many countries around the world, especially those of the Mediterranean area. *Diplotaxis* is a therophyte plant belonging to the Brassicaceae family, with a flower with four white and violet petals. It is commonly called "Mediterranean wasabi" for its characteristic spicy flavor. *Oxalis* is a geophyte plant belonging to the Oxalidaceae family, from South Africa, with five-petal yellow flowers and a characteristic acidic flavor. In the past, the importance of these plants was much greater due to their use as ingredients in typical dishes, such as "paella", as an aromatic plant. In 2022, the Basque Culinary Center published a book called "*Silvestre*" about the botany of these plants, with recipes using wild edible plants [14]. Currently, for both plants, flowers are used as a decoration, while leaves are used in salads.

Considering the importance of these plants and their compounds in human physiology, in this study, the profiles and contents of carotenoids, tocols, thiamine and riboflavin of *Diplotaxis erucoides* and *Oxalis pes-caprae* were investigated. These compounds were analyzed in different botanical parts of the two wild edible plants. Results obtained will contribute to the knowledge of the distribution of these bioactives along the plant and will allow the development of new strategies focused on the valorization of the phytochemical profile of these WEPs.

2. Materials and Methods

2.1. Plant Material

The investigated *Diplotaxis erucoides* DC. and *Oxalis pes-caprae* L. plants were collected at the Miguel Hernández University, Orihuela campus (38°4′10″ N, 0°59′1″ O, Alicante, Spain) during February 2022. Flowers, leaves and stems of *Oxalis pes-caprae* were investigated; for *Diplotaxis erucoides*, the parts were pods, leaves and stems (Supplementary Figures S1 and S2). Fifty grams of pods of *Diplotaxis erucoides* and fifty grams of flowers of *Oxalis pes-caprae*, two hundred grams of leaves and stems of both plants, chosen randomly, were manually collected. The collection of both plants was carried out, in phenological

terms, at the height of flowering. The non-edible parts were discarded (roots) Some aliquots were freeze-dried through a Christ Alpha 2–4 apparatus (B. Braun Biotech International, Melsungen, Germany) for 48 h, to a constant weight, and grounded by means of a refrigerated mill (Taurus Aromatic, Oliana, Spain), mixed and stored at −20 °C until analysis. Some aliquots were immediately stored at –20 °C as a reserve material to be eventually freeze dried and processed as above. The AOAC method was used to determine moisture [15].

2.2. Chemicals

Solvents and other analytical grade reagents, all-trans-β-carotene, thiamine and riboflavin standards were from Sigma (Sigma Aldrich, St. Luis, MO, USA). α-Carotene, 9-*cis*-β-carotene and antheraxanthin, 13-*cis*-β-carotene, neoxanthin, violaxanthin standards were from CaroteNature (Lupsingen, Switzerland). Lutein, zeaxanthin, and β-cryptoxanthin were provided by Extrasynthese (Z.I. Lyon-Nord, Genay, France); α, β, γ and δ-tocopherol standards were from Merck (Darmstadt, Germany); α, β, γ and δ-tocotrienol standards were obtained as in Panfili et al. [16].

2.3. Determination of Carotenoids

Carotenoids were extracted by using the saponification and solvent extraction method of Panfili et al. [17] and Fratianni et al. [18] on 0.1 g of freeze dried samples. The residues were suspended in a *n*-hexane:isopropyl alcohol solution (90:10 *v*/*v*) and analyzed through a normal (for xanthophylls) and a reverse phase (for carotenes) HPLC. A Dionex HPLC (Sunnyvale, CA, USA) analytical system, with an Ultimate 3000 pump system, was used. For reverse phase, the mobile phase was methanol:methylterbutylether:water, at a flow rate of 1 mL/min, under a gradient profile as in Mouly et al. [19], by using a 5 µm, C30 YMC (Hampsted, NC, USA) stainless steel column (250 mm × 4.6 mm internal diameter, id). Samples were suspended in methanol/methylterbutylether 50:50 (*v*/*v*). Under normal phase conditions, the mobile phase was *n*-hexane:isopropyl alcohol in multilinear gradient elution from 10% (A) to 20% (B) of isopropyl alcohol in n-hexane as reported elsewhere [18]. A 5 µm Luna column, with a silica stationary phase (250 mm × 4.6 mm id), was used (Phenomenex, Torrance, CA, USA). Data were processed through a Dionex Chromeleon Version 6.6 chromatography system (Sunnyvale, CA, USA). Carotenoids were spectrophotometrically detected at 450 nm, identified and quantified by means of available standard solutions. Vitamin A activity was expressed as Retinol Equivalent (RE) as in [20].

2.4. Determination of Tocols

The extraction and determination of tocols were carried out using the method of Panfili et al. [16]. The extraction conditions, the used silica column and the HPLC system were the same reported for carotenoids. The residues were suspended in a n-hexane:isopropyl alcohol solution (99:1 *v*/*v*). A n-hexane:ethyl acetate:acetic acid solution (97.3:1.8:0.9 *v*/*v*/*v*) was used as the mobile phase, at a flow rate of 1.6 mL/min. Tocols were detected through a RF 2000 spectrofluorimeter (Dionex, Sunnyvale, CA, USA), set at an excitation and emission wavelength of 290 nm and 330 nm, respectively, and identified and quantified through known standard solutions. Data were processed by a Dionex Chromeleon Version 6.6 chromatography system (Sunnyvale, CA, USA). Vitamin E activity was expressed as Tocopherol Equivalent (TE), as in Sheppard et al. [21].

2.5. Thiamine and Riboflavin Analysis

Thiamine and riboflavin were extracted as in Panfili et al. [22] on 0.4 g of freeze dried samples. Extracts were separated by a Dionex HPLC (Sunnyvale, CA, USA), with an Ultimate 3000 pump system, at a flow rate of 0.8 mL/min, using methanol:NaOAc (40:60 *v*/*v*) as the mobile phase. A 5 µm C18 Luna Phenomenex (Torrance, CA, USA) stainless steel column (250 mm × 4.6 mm i.d.) was used. Fluorimetric detection was performed (RF 2000 spectrofluorimeter; Dionex, Sunnyvale, CA, USA), at an excitation wavelength of 366 nm and an emission wavelength of 453 nm for thiamine, and at an

excitation wavelength of 453 nm and an emission wavelength of 580 nm for riboflavin. A Dionex Chromeleon Version 6.6 chromatography system (Sunnyvale, CA, USA) was used to process data. Thiamine and riboflavin were identified and quantified through known available standards.

2.6. Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) and to Tukey's multiple range test to compare means. Differences were statistically significant at $p < 0.05$. All statistical analyses were performed using a StatGraphics Plus 5.0 software (Manugistics. Inc., Rockville, MD, USA).

3. Results and Discussion

3.1. Profile of Carotenoids and Tocols

Ten carotenoids were identified in *Diplotaxis erucoides* and *Oxalis pes-caprae*: lutein, zeaxanthin, violaxanthin, neoxanthin, β-cryptoxanthin, antheraxanthin, α-carotene, 13-*cis*-β-carotene, β-carotene and 9-*cis*-β-carotene (Table 1).

In *Diplotaxis erucoides*, the main carotenoids were lutein and β-carotene (70% of total carotenoids) in each part of the plant. Leaves of plants from the same family (Brassicaceae), such as *Eruca sativa* L., or from the same genus, such as *Diplotaxis tenuifolia* L., had similar values of lutein, about 11 and 13 mg/100 g f.w. (fresh weight), respectively, and β-carotene (about 3.6 and 4.2 mg/100 g f.w., respectively) [23]. Compared to the leaves (about 24 mg/100 g f.w.), both pods and stems had about five–ten-fold lower carotenoid contents (about 5 and 3 mg/100 g f.w., respectively).

The carotenoid profile of *Oxalis pes-caprae* was very similar to that of *Diplotaxis*, with the highest values found in leaves. In this case, lutein, β-carotene and β-cryptoxanthin showed the highest contents, reaching 80% of total carotenoids (TC) in leaves. Lutein concentration in leaves was about 4.8 mg/100 g f.w. In plants of the same genus, such as *Oxalis corniculata*, this value was slightly higher (about 11 mg/100 g f.w.), while no zeaxanthin was detected [24]. In flowers and stems, the total carotenoid values were very similar (about 1.5 mg/100 g f.w). In both the investigated plant leaves, quite high amounts of violaxanthin (28% of TC) and neoxanthin (about 15% of TC) were also found. As for β-carotene, plants, such as *Amaranthus spinosus* (about 7 mg/100 g f.w.), or *Vigna gallinacea* A. Rich., *Trilepisium madagascariense* DC. and *Cleome gynandra* L. (about 35, 25 and 29 mg/100 g d.w. dry weight, respectively) had values very similar to *Oxalis pes-caprae* (about 32 mg/100 g d.w.) [25,26].

Several green leafy vegetables were reported as good sources of β-carotene and rich sources of lutein [27,28]. Contents on carotenoids may vary due to the different analytical methods used, genotype, weather conditions, maturity stage, location and seasonality [29].

Only α (α-T), β (β-T) and γ (γ-T) tocopherols were found in both plants (Table 2). In *Diplotaxis erucoides* α-T and γ-T were the most representative. While α-T was mainly present in leaves, the same amounts of γ-T were found in leaves and pods. Values of α-T found in leaves of both *Diplotaxis* and *Oxalis* (about 24 and 49 mg/100 g d.w., respectively) were similar to those of the leaves of *Sonchus asper*, *Sonchus oleraceus*, *Spinacia oleracea* and *Cichorium intybus* (19, 20, 32 and 33 mg/100 g d.w., respectively) [18,22,27].

Table 1. Content of carotenoids in *Diplotaxis erucoides* and *Oxalis pes-caprae* (mg/100 g f.w., d.w.).

	ANOVA	***p*-Value**	**Fresh (mg/100 g f.w.)**			**ANOVA**	***p*-Value**	**Dry (mg/100 g d.w.)**		
	Diplotaxis erucoides									
			Pods	Leaves	Stems			Pods	Leaves	Stems
Lutein	***	0.0000	2.12 ± 0.03 ^{b}	8.23 ± 0.96 ^{a}	1.53 ± 0.02 ^{b}	***	0.0000	9.38 ± 0.14 ^{b}	47.38 ± 5.50 ^{a}	8.52 ± 0.13 ^{b}
Zeaxanthin	***	0.0000	0.24 ± 0.01 ^{b}	0.37 ± 0.00 ^{a}	0.11 ± 0.01 ^{c}	***	0.0000	1.04 ± 0.06 ^{b}	2.14 ± 0.01 ^{a}	0.63 ± 0.08 ^{c}
Violaxanthin	***	0.0000	0.11 ± 0.01 ^{b}	2.28 ± 0.19 ^{a}	0.20 ± 0.03 ^{b}	***	0.0000	0.50 ± 0.05 ^{b}	13.10 ± 1.09 ^{a}	1.10 ± 0.15 ^{b}
Neoxanthin	***	0.0000	nd	1.07 ± 0.04 ^{a}	0.15 ± 0.02 ^{b}	***	0.0000	nd	6.14 ± 0.26 ^{a}	0.82 ± 0.11 ^{b}
β-Cryptoxanthin	ns	0.2879	nd	0.09 ± 0.01 ^{a}	0.08 ± 0.01 ^{a}	ns	0.1846	nd	0.53 ± 0.09 ^{a}	0.43 ± 0.06 ^{a}
Antheraxanthin	***	0.0000	0.15 ± 0.00 ^{b}	0.38 ± 0.04 ^{a}	0.15 ± 0.02 ^{b}	***	0.0000	0.67 ± 0.01 ^{b}	2.19 ± 0.20 ^{a}	0.39 ± 0.05 ^{b}
α-Carotene	***	0.0000	0.18 ± 0.02 ^{b}	1.00 ± 0.06 ^{a}	0.04 ± 0.00 ^{c}	***	0.0000	0.79 ± 0.10 ^{b}	5.77 ± 0.35 ^{a}	0.23 ± 0.01 ^{c}
13-*cis*-β-Carotene	***	0.0000	0.05 ± 0.01 ^{b}	1.28 ± 0.08 ^{a}	nd	***	0.0000	0.24 ± 0.02 ^{b}	7.38 ± 0.44 ^{a}	nd
β-Carotene	***	0.0000	1.32 ± 0.14 ^{b}	9.47 ± 0.57 ^{a}	0.39 ± 0.01 ^{c}	***	0.0000	5.82 ± 0.63 ^{b}	54.48 ± 3.27 ^{a}	2.18 ± 0.01 ^{b}
9-*cis*-β-Carotene	***	0.0000	0.57 ± 0.07 ^{a}	0.09 ± 0.01 ^{b}	0.13 ± 0.01 ^{b}	***	0.0000	2.54 ± 0.32 ^{a}	0.51 ± 0.03 ^{b}	0.72 ± 0.01 ^{b}
Totals	***	0.0000	4.75 ± 0.28 ^{b}	24.26 ± 1.85 ^{a}	2.70 ± 0.05 ^{b}	***	0.0000	20.97 ± 1.25 ^{b}	139.63 ± 10.62 ^{a}	15.04 ± 0.33 ^{b}
	Oxalis pes-caprae									
			Flowers	Leaves	Stems			Flowers	Leaves	Stems
Lutein	***	0.0000	0.10 ± 0.02 ^{b}	4.76 ± 0.29 ^{a}	0.38 ± 0.05 ^{b}	***	0.0000	0.72 ± 0.12 ^{c}	34.20 ± 2.07 ^{a}	4.25 ± 0.59 ^{b}
Zeaxanthin	**	0.0085	0.17 ± 0.01 ^{a}	0.15 ± 0.03 ^{a}	0.10 ± 0.01 ^{b}	ns	0.6089	1.21 ± 0.08 ^{a}	1.12± 0.21 ^{a}	1.11 ± 0.01 ^{a}
Violaxanthin			nd	nd	0.04 ± 0.01			nd	nd	0.49 ± 0.03
Neoxanthin			nd	nd	nd			nd	nd	nd
β-Cryptoxanthin	***	0.0001	0.16 ± 0.03 ^{b}	1.35 ± 0.24 ^{a}	0.32 ± 0.05 ^{b}	***	0.0000	1.11 ± 0.23 ^{c}	9.74 ± 1.74 ^{a}	3.53 ± 0.06 ^{b}
Antheraxanthin	***	0.0001	nd	0.43 ± 0.06 ^{a}	0.02 ± 0.01 ^{b}	***	0.0004	nd	3.12 ± 0.46 ^{a}	0.24 ± 0.02 ^{b}
α-Carotene	***	0.0004	0.05 ± 0.01 ^{b}	0.34 ± 0.08 ^{a}	0.05 ± 0.01 ^{b}	***	0.0006	0.37 ± 0.01 ^{b}	2.45 ± 0.61 ^{a}	0.58 ± 0.01 ^{b}
13-*cis*- β-Carotene	***	0.0000	0.05 ± 0.01 ^{a}	0.01 ± 0.01 ^{b}	0.01 ± 0.01 ^{b}	***	0.0000	0.34 ± 0.01 ^{a}	0.10 ± 0.01 ^{c}	0.13 ± 0.01 ^{b}
β-Carotene	***	0.0005	0.49 ± 0.01 ^{b}	4.50 ± 1.19 ^{a}	0.44 ± 0.05 ^{b}	***	0.0006	3.48 ± 0.03 ^{b}	32.23 ± 8.55 ^{a}	4.90 ± 0.56 ^{b}
9-*cis*-β-Carotene	**	0.0014	0.12 ± 0.01 ^{b}	0.93 ± 0.29 ^{a}	0.11 ± 0.01 ^{b}	***	0.0000	0.88 ± 0.01 ^{c}	6.69 ± 0.22 ^{a}	1.26 ± 0.08 ^{b}
Totals	***	0.0000	1.47 ± 0.07 ^{b}	12.50 ± 1.71 ^{a}	1.49 ± 0.11 ^{b}	***	0.0000	8.11 ± 0.47 ^{c}	89.73 ± 10.23 ^{a}	16.49 ± 1.21 ^{b}

Data are shown as mean of 3 replicates ± standard deviation. **, *** significant at $p < 0.01$ and 0.001, respectively. nd (not detected). ns (not significant at $p < 0.05$). Values followed by the same letter, within the same row, were not significantly different.

Table 2. Tocol content in *Diplotaxis erucoides* and *Oxalis pes-caprae* (mg/100 g f.w., d.w.).

	ANOVA	***p*-Value**	**Fresh (mg/100 g f.w.)**			**ANOVA**	***p*-Value**	**Dry (mg/100 g d.w.)**		
	Diplotaxis erucoides									
			Pods	Leaves	Stems			Pods	Leaves	Stems
α-Tocopherol	***	0.0000	1.81 ± 0.13 ^{b}	4.13 ± 0.44 ^{a}	0.65 ± 0.06 ^{c}	***	0.0000	7.97 ± 0.57 ^{b}	23.79 ± 2.53 ^{a}	3.62 ± 0.33 ^{c}
β-Tocopherol	***	0.0000	nd	0.10 ± 0.01 ^{a}	0.01 ± 0.01 ^{b}	***	0.0000	nd	0.60 ± 0.02 ^{a}	0.06 ± 0.01 ^{b}
γ-Tocopherol	***	0.0000	0.56 ± 0.03 ^{a}	0.50 ± 0.07 ^{a}	0.04 ± 0.01 ^{b}	***	0.0000	2.47 ± 0.12 ^{a}	2.89 ± 0.40 ^{a}	0.21 ± 0.02 ^{b}
Totals	***	0.0000	2.37 ± 0.16 ^{b}	4.74 ± 0.51 ^{a}	0.70 ± 0.05 ^{c}	***	0.0000	10.44 ± 0.70 ^{b}	27.28 ± 2.94 ^{a}	3.89 ± 0.25 ^{c}
	Oxalis pes-caprae									
			Flowers	Leaves	Stems			Flowers	Leaves	Stems
α-Tocopherol	***	0.0000	1.17 ± 0.13 ^{b}	6.81 ± 0.44 ^{a}	1.41 ± 0.01 ^{b}	***	0.0000	8.25 ± 0.92 ^{c}	48.89 ± 3.16 ^{a}	15.55 ± 0.02 ^{b}
β-Tocopherol			nd	nd	nd			nd	nd	nd
γ-Tocopherol	***	0.0003	3.05 ± 0.56 ^{a}	3.91 ± 0.28 ^{a}	1.11 ± 0.16 ^{b}	***	0.0002	21.59 ± 2.96 ^{b}	28.09 ± 1.99 ^{a}	12.23 ± 1.81 ^{c}
Totals	***	0.0000	4.22 ± 0.33 ^{b}	10.72 ± 0.11 ^{a}	2.51 ± 0.16 ^{c}	***	0.0000	29.84 ± 1.89 ^{b}	76.98 ± 1.17 ^{a}	27.78 ± 1.83 ^{b}

Data are shown as mean of 3 replicates ± standard deviation. *** significant at $p < 0.001$, respectively. nd (not detected). Values followed by the same letter, within the same row, were not significantly different.

The same tocol profile of *Diplotaxis* was found in *Oxalis pes-caprae*, with α-T values in leaves (about 50 mg/100 g d.w.) higher than those of leaves of *Oxalis acetosella* (18.6 mg/100 g d.w.) [30], while γ-T contents (about 4.0 mg/100 g f.w.) were close to those found in *Sonchus asper*, *Sonchus oleraceus* and *Spinacia oleracea* (about 3.3, 2.7 and 4.7 mg/100 g f.w., respectively) [22,27]. Gamma tocopherol was also found in Brazilian WEPs, such as *Amaranthus spinosus* L. and *Commelina benghalensis*, at 3.3 and 0.7 µg/100 g f.w., respectively [25]. β-T and δ-T were not found in *Oxalis pes-caprae*, while δ-T was reported in *Oxalis acetosella* (140 mg/100 g d.w.) [30].

3.2. Contents of Thiamine and Riboflavin

Stems of *Diplotaxis erucoides* showed the highest content of thiamine (about 1.5 mg/kg f.w.), followed by leaves, while pods had no contents (Table 3). Similar values were found in the leaves of *Sonchus asper* and *oleraceus* (1.0 mg/kg f.w.) and *Crepis vesicaria* (1.3 mg/kg f.w.) [22].

Table 3. Contents of thiamine and riboflavin in *Diplotaxis erucoides* and *Oxalis pes-caprae* (mg/kg f.w., d.w.).

	ANOVA	*p*-Value	Fresh (mg/kg f.w.)			ANOVA	*p*-Value	Dry (mg/kg d.w.)		
			Diplotaxis erucoides							
			Pods	Leaves	Stems			Pods	Leaves	Stems
Thiamine	***	0.0000	nd	0.64 ± 0.06 ^{b}	1.46 ± 0.04 ^{a}	***	0.0000	nd	3.72 ± 0.15 ^{b}	8.13 ± 0.21 ^{a}
Riboflavin	***	0.0000	0.23 ± 0.02 ^{a}	0.14 ± 0.01 ^{b}	0.03 ± 0.01 ^{c}	***	0.0000	1.02 ± 0.10 ^{a}	0.83 ± 0.05 ^{b}	0.18 ± 0.06 ^{c}
			Oxalis pes-caprae							
			Flowers	Leaves	Stems			Flowers	Leaves	Stems
Thiamine	***	0.0000	1.17 ± 0.05 ^{a}	0.33 ± 0.02 ^{b}	nd	***	0.0000	8.25 ± 0.33 ^{a}	2.36 ± 0.16 ^{b}	nd
Riboflavin	***	0.0000	0.10 ± 0.01 ^{b}	0.16 ± 0.01 ^{a}	0.02 ± 0.01 ^{c}	***	0.0000	0.69 ± 0.01 ^{b}	1.14 ± 0.06 ^{a}	0.26 ± 0.01 ^{c}

Data are shown as mean of 3 replicates ± standard deviation. *** significant at $p < 0.001$, respectively. nd (not detected). Values followed by the same letter, within the same row, were not significantly different.

Oxalis pes-caprae flowers had the highest thiamine content, followed by leaves, with no amounts in stems. Levels close to those detected in leaves (about 4 mg/kg d.w.), were reported in *Ipomoea aquatic* Forssk. (4.5 mg/kg d.w.), *Achyranthes aspera* L. (1.3 mg/kg d.w.) or *Enhydra fluctuans* Lou. (4.0 mg/kg d.w.) [31].

All parts of both plants showed low riboflavin levels. In *Diplotaxis erucoides*, pods had the highest content, followed by leaves and stems. In *Oxalis pes-caprae* the highest amounts were in leaves, about 0.2 mg/Kg f.w., followed by flowers and stems. Both values in leaves of *Diplotaxis* and *Oxalis* were similar to those of *Sonchus asper*, *Sonchus oleraceus* (0.1 mg/kg f.w.) and *Crepis vesicaria* (0.2 mg/kg f.w.) [22,27]. Higher contents were found in *Ipomoea aquatic* Forssk. (7.0 mg/kg d.w.), *Achyranthes aspera* L. (about 4 mg/kg d.w.) and *Enhydra fluctuans* Lour. (about 10 mg/kg d.w.) [31].

3.3. Vitamin A and Vitamin E Activity

Figure 1 shows the vitamin A activity of *Diplotaxis erucoides* and *Oxalis pes-caprae*, expressed as Retinol Equivalents (RE). In *Diplotaxis erucoides*, the highest values of RE were reached in leaves, 1783 µg/100 g. Lower values were found in pods and stems (about 290 and 86 µg/100 g, respectively). In the Annex XIII of the Regulation (EU) No 1169/2011, published by the European Union [32], the Recommended Daily Amounts (RDA) for vitamin A, expressed as RE, are 800 µg/day. By ingesting 100 g of fresh *Diplotaxis erucoides* leaves, more than double (223%) of the RDA for vitamin A can be achieved. One hundred grams of pods, instead, covered the 35% of the RDA. Similarly, in *Oxalis pes-caprae*, the highest vitamin A activity was shown in leaves (970 µg/100 g f.w.), so to cover, with 100 g, about 120 % of the RDA for this vitamin. On the contrary, both flowers and stems had lower RE values (about 115 µg/100 g f.w.). The European Union Regulation specifies that any food source that reaches the 15% of the RDA can be declared on the label as a

"source of vitamin A" [32]. Therefore, for both *Diplotaxis erucoides* and *Oxalis pes-caprae* it can be stated that 100 g of their leaves are sources of vitamin A.

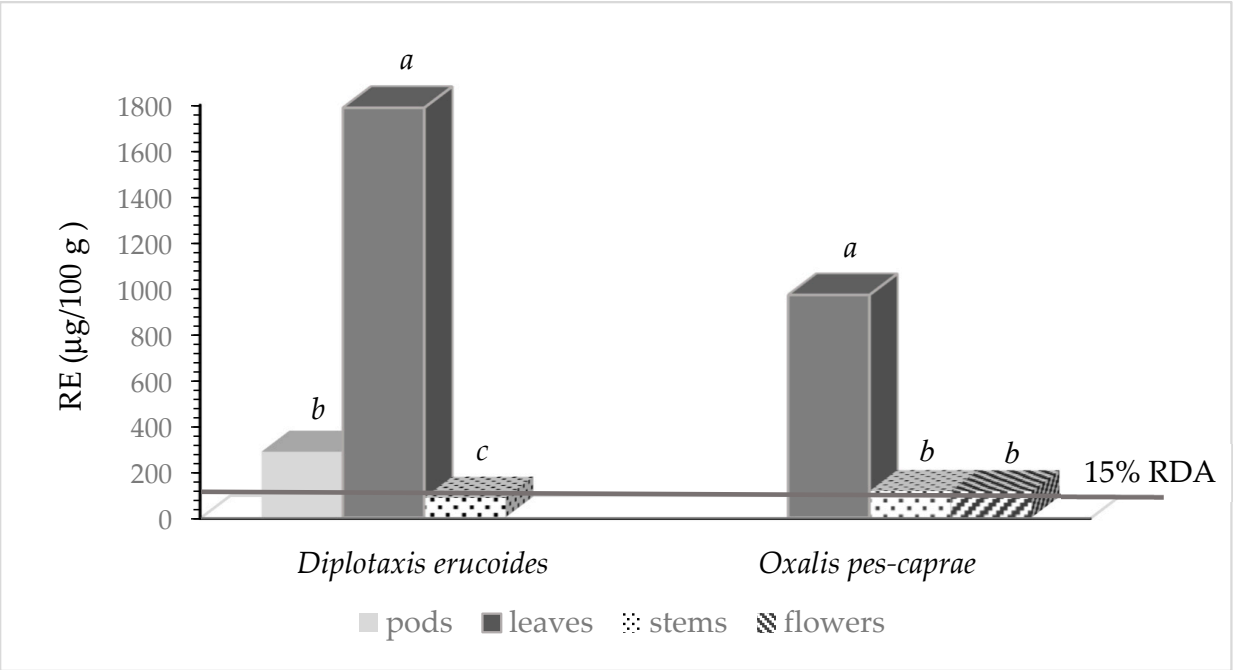

Figure 1. Vitamin A activity (RE) of *Diplotaxis erucoides* and *Oxalis pes-caprae* (µg/100 g). Bars marked with different letters are statistically different at $p < 0.05$.

The found vitamin A levels are far below the Tolerable Upper Intake Levels (ULs) for preformed vitamin A, which are applied only to products from animal sources and supplements whose vitamin A comes entirely from retinol or its ester forms. Nevertheless, there are no upper limits for beta-carotene and other forms of provitamin A supplementation because of the lack of relevance of studies for human risk assessment [33].

Figure 2 shows the vitamin E activity of *Diplotaxis erucoides* and *Oxalis pes-caprae*, expressed as Tocopherol Equivalents (TE), as mg per 100 g of fresh weight. In *Diplotaxis erucoides* the highest value of TE was shown in leaves, reaching 4.2 mg/100 g f.w., while it was 1.9 mg/100 g f.w. in pods and 0.7 mg/100 g f.w. in stems. By ingesting 100 g of leaves of *Diplotaxis erucoides*, a consumer can achieve 35% of 12 mg, which is the RDA for vitamin E [32]. Similarly to what was found in *Diplotaxis erucoides*, in *Oxalis pes-caprae*, the highest TE value was reached in leaves (7.2 mg/100 g f.w.), so 60% of the RDA for vitamin E can be covered by 100 g of *Oxalis* leaves. Both flowers and stems had lower values of vitamin E activity (about 1.5 mg/100 g f.w.). The European Union Regulation [32] specifies that any food source that reaches a value greater than 15% of the RDA can be declared on the label as a "source of vitamin E". Therefore, 100 g of leaves of both *Diplotaxis erucoides* and *Oxalis pes-caprae*, can be stated as sources of vitamin E.

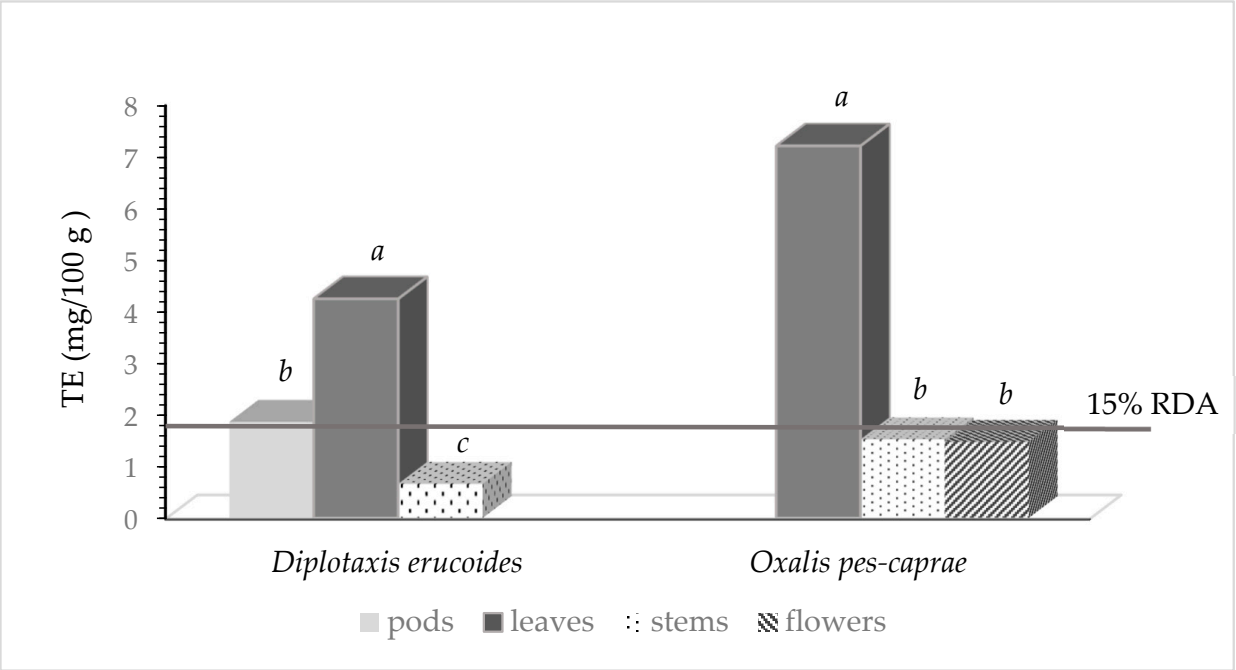

Figure 2. Vitamin E activity (TE) of *Diplotaxis erucoides* and *Oxalis pes-caprae* (mg/100 g). Bars marked with different letters are statistically different at $p < 0.05$.

4. Conclusions

Results on carotenoids, tocols, thiamine and riboflavin of different parts of *Diplotaxis erucoides* and *Oxalis pes-caprae* show that the profiles and contents of these bioactive compounds are of interest. The highest levels of almost all compounds were mainly found in the leaves. In both plants the major carotenoids were lutein and β-carotene. Among tocols, α-tocopherol was the main compound in all analyzed parts, with the exception of the flowers of *Oxalis*, where γ-tocopherol was predominant. One hundred grams of leaves of *Diplotaxis erucoides* and *Oxalis pes-caprae* provide over the 15% of the Recommended Daily Allowance for vitamin E and vitamin A, so to be considered as a source of these vitamins. These plants can also contribute to the daily intake of thiamine and riboflavin.

Finding results could be used to improve the nutritional databases and evidence a promising future for WEPs in consumer demand for healthy foods, produced and processed with sustainable methods.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu16142293/s1. Figure S1. Parts of *Oxalis pes-caprae* L. Source: www.antropocene.it; Figure S2. Parts of *Diplotaxis erucoides* DC. Source: Flickr.

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PUBLICACIÓN 4

Characterization and potential use of *Diplotaxis erucoides* as food ingredient for a sustainable modern cuisine and comparison with commercial mustards and wasabis

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Abstract

Diplotaxis erucoides is a wild edible plant (WEP) of the Brassicaceae family, growing as a weed in Mediterranean countries. It contains glucosinolates, which are hydrolyzed by myrosinase rendering isothiocyanates; these compounds are responsible for its characteristic aroma and flavor, similar to that of mustard or wasabi. The aim of this study was to characterize the *Diplotaxis erucoides* plant by analyzing its volatile, sugar, organic acid, and sensory profiles. This information will be essential to decide whether it is possible to develop sustainable and healthy alternatives to commercial mustard and wasabi using this WEP. Two *Diplotaxis*-based products were prepared one cream and one mayonnaise. Allyl isothiocyanate represented more than 80% of total content of volatile compounds in the leaves, pods and stems. The sugar and organic acid content of *Diplotaxis* and its based products were very low. The *Diplotaxis* products were characterized by having intense *Diplotaxis* odor/aroma, herbaceous flavor and long aftertaste, but lacked some pungency. *Diplotaxis erucoides* is a sustainable and healthy option to produce herbaceous and pungent sauces.

Keywords Allyl isothiocyanate · Glucosinolate · Mustard · Pungency · Wasabi

Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), 100 million people in Europe consumed wild foods in 2016 [1]. However, wild food (WF) is a global concept going from eating bushmeat (hunting) to eating wild edible plants (WEP) [2]. The term WEP refers to plants growing without human action and using only natural resources [3]. In former times (e.g. 1960s) in Europe, the use of WEPs in the diet was widespread due to the lifestyle of that era [4]. The agriculture, livestock farming, shepherding were activities that allowed the population to be more in direct contact with nature, to know better the plants, and to know which of them were edible. In fact, as an example, Brassicaceae is one of the most important families in WEPs and includes between 11–20 species [5]. This family includes several genera, such as *Brassica* (such as mustards, broccoli and cauliflower), *Raphanus* (radish and horseradish), *wasabia* (wasabi) [6], and the genus *Diplotaxis* (e. g. *tenuifolia* and *erucoides*); as can be easily guessed, not all of these genera are WEPs and have been domesticated and are now widely used nowadays.

Typically, the chemical feeling "spiciness" in mustards, wasabis, horseradish, and *Diplotaxis erucoides* is similar because their most relevant flavor components are isothiocyanates [7, 8]. This chemical family (isothiocyanates) are secondary metabolites produced from glucosinolates (GSLs);

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around 130 GSLs have been identified, and are hydrolyzed by the enzyme myrosinase rendering isothiocyanates, thiocyanates, nitrile, sulfates and goitrins [9–11]. In recent studies, GSLs have been associated by European Food Safety Authority (EFSA) with anti-cancer and anti-fungical effects, and even, to help strengthen body defenses and the immune system [12–14].

This study focused on *Diplotaxis erucoides* (Brassicaceae), which is one of the most frequent WEPs in the Mediterranean area of, at least, Spain [5, 7]. Up to 11 glucosinolates have been identified in *Diplotaxis erucoides* [9, 15], and their hydrolyzation leads, mainly, to the formation of allyl isothiocyanate (AITC) [11]. Besides, *Diplotaxis erucoides* contains high nutritional values, such as low Na content (12.5 mg Na *per* 100 g) [5], high Ca (400–800 mg Ca *per* 100 g [3], and important antioxidant capacity [16, 17].

Considering all the above, the aim of this study was to fully characterize the volatile composition and the profiles of sugars and organic acids of *Diplotaxis erucoides* because these compounds will determine its odor and flavor. The final goal is to replace commercial sauces (e.g. mustard and wasabi), which are not native of the Mediterranean countries and are expensive, by healthy (low in Na and sugar, and high in Ca and isothiocyanates) and local alternatives based on *Diplotaxis erucoides*. This paper would be the first at determining the volatile and sensory profiles of the different edible parts of the plant *Diplotaxis erucoides* and can be used as a model to demonstrate that there are underutilized wild edible plants which are appropriate for sustainable use in modern kitchens.

Material and methods

Plant material and commercial products

Stems (S), leaves (L) and pods (P) of *Diplotaxis erucoides* were studied. This WEP is being used locally as an ingredient for modern cuisine dishes and was harvested in Alcoy (Alicante, Spain) in May of 2019. On the other hand, 5 commercial mustards (M1, M2, M3, M4, and M5) and 3 commercial wasabi samples (W1, W2, and W3) were purchased from local supermarkets (Alicante, Spain). The wild plants were processed at the UMH facilities and two products were prepared (1) *Diplotaxis erucoides* cream (DeC) and (2) *Diplotaxis erucoides* mayonnaise (DeM).

Preparation of *Diplotaxis*-based dishes

The preparation of the cream and mayonnaise was carried out, using the Thermomix® machine, model TM6. The composition of these two *Diplotaxis*-based sauces (cream DeC and mayonnaise DeM) is shown in Table 1. For the first one, whipped cream heated to 40 ºC until thickened; after that, a mixture of *Diplotaxis erucoides* stems, leaves, pods and salt was added and emulsified. For mayonnaise, semi-skimmed milk, seed oil and *Diplotaxis erucoides* stems, leaves, pods were mixed in a Thermomix® machine, and emulsified at 40 ºC.

Table 1 Formulation of *Diplotaxis*-based products

Diplotaxis erucoides cream (DeC)		*Diplotaxis erucoides* mayonnaise (DeM)	
Ingredients	Amount	Ingredients	Amount
Whipped cream (mL)	250	Seed oil (mL)	100
Leaves (g)	150	Semi-skimmed milk (mL)	250
Pods (g)	25	Leaves (g)	150
Stem (g)	25	Pods (g)	25
Salt (g)	0.1	Stems (g)	25

Extraction of volatile aroma compounds

Volatile compounds were extracted using the protocol by Andreu-Sevilla et al. [18], with slight modifications. Volatile compounds of the leaves, stems and pods of the *Diplotaxis erucoides* plant, mustards, wasabis and *Diplotaxis*-based products were extracted using the headspace solid-phase micro-extraction method (HS-SPME). Briefly, 5 g of each sample were mixed with 1 g of NaCl, placed in 50 mL vials with polypropylene caps and PTFE/silicone septa, and constantly stirred. Then, a 50/30 pm DVB/CAR/PDMS fiber (length of 2 cm) was exposed to the sample headspace for 50 min at 40 °C (to simulate the mouth temperature). Volatile extractions were run in triplicate.

Chromatographic analysis of volatile compounds by GC–MS

The isolation and identification of the volatile compounds were done according to El-Zaeddi et al. [19], on a gas chromatograph (GC), Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector (MS) QP-5050A. The GC–MS system was equipped with a Rxi-1301Sil MS column (Restek Corporation, Bellefonte, USA). The volatile compounds were identified using 3 analytical methods: (1) retention indices, (2) GCMS retention indices (authentic chemicals), and (3) mass spectra (NIST05 spectral library collection). The semi-quantification of the volatile compounds was performed on a gas chromatograph (Shimadzu, model 2010) with a flame ionization detector (FID). The column and chromatographic conditions were those previously reported by El-Zaeddi et al. [19]. This analysis was run in triplicate and results were expressed as a percentage of the total area represented by

each one of the volatile compounds; this semi-quantitative approach was good enough to identify the predominant compounds in the *Diplotaxis* plant.

Analysis of organic acids and sugars

Organic acids and sugars were quantified according to Hernández et al. [20], using high-performance liquid chromatography (HPLC–DAD-RID) (Hewlett Packard 1100 series; Willmington, DE, USA). A supelcogel TM C-610H column 30 cm × 7.8 mm and Supelguard 5 cm × 4.6 mm; precolumn (Supelco, Bellefonte, PA) were used for separation: the absorbance was measured using a diode-array detector (DAD) at 210 nm for organic acids detection. A refractive index detector (RID) was used for the detection of sugars. Standards of organic acids (citric, fumaric, malic, oxalic, phytic, and tartaric) and sugars (arabinose, fructose, galactose, glucose, maltose, and sucrose) were obtained from Sigma (St. Louis, MO). Calibration curves, with a concentration range between 1 and 10 g L^{-1}, were used for the quantification of organic acids and sugars and showed good linearity ($R^2 \geq 0.999$). This analysis was run in triplicate and results were expressed as g kg^{-1}.

Sensory evaluation with trained panel

Eight highly experienced panelists (four males and four females, aged between 20 and 52 years), and all associated with the Research Group "Food Quality and Safety" of the Universidad Miguel Hernández de Elche (Orihuela, Alicante, Spain). The panel evaluated samples of stems, leaves and pods of *Diplotaxis erucoides*, five mustard samples, three wasabi samples, and two new dished (cream and mayonnaise) based on *Diplotaxis erucoides*. The sensory analyses were conducted in two sessions. In the first one (orientation session), the panelists got used to the products to be evaluated and decided which commercial mustard and wasabi had a sensory profile closer to that of *Diplotaxis erucoides*. Finally, during the second session, the panel made a full sensory description selected mustard and wasabi samples and the two new *Diplotaxis*-based products (DeC and DeM).

Between samples and for palate cleansing, water and unsalted crackers were provided to panelists. The attributes under evaluation were: appearance (brightness) flavor [*Diplotaxis* ID (odor and aroma) and herbaceous-green flavor], basic tastes (sweet, salty, sour and bitter), chemical sensations (pungency), and texture (fatty character and viscosity); the sensory lexicon used for the descriptive sensory analysis of the samples under evaluation is summarized in Table 2. Panelists used a scale of 0–10 points (with 0.5 increments) for the evaluation, where ten was extremely high intensity and 0 was extremely low intensity or not noticeable.

Statistical analysis

Experimental data were subjected first to one-way analysis of variance (ANOVA) and later to Tukey's multiple range test to compare the means. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics. Inc., Rockville. MD).

Results and discussion

Volatile compounds

A total of 37 volatile compounds were isolated, identified and semi-quantified in all studied samples (Table 3) and can be ground in 10 chemical families: terpenes (6 compounds), terpenoids (5), aldehydes (5), isothiocyanates (4), alcohols (3), sulfur compounds (3), acids (3), esters (2), nitrogen compounds (2), ketones (1), and three compounds were included in the class "others". All chemical families were present in mustards, while in wasabi only 5 out of the 11 chemical families were found; a similar trend was found in fresh *Diplotaxis erucoides* plant and sauces (DeC and DeM) were only 4 and 3 chemical families were found, respectively.

In all samples under analyses, the chemical family "isothiocyanates" (ITCs), with only four compounds (allyl isothiocyanate, isobutyl isothiocyanate, 3-butenyl isothiocyanate and phenethyl isothiocyanate) was the predominating one (~ 80.6%, mean of stems, leaves and pods), followed by far by the families S-compounds (~ 6.9%) and organic acids (~ 5.9%); finally, all other families had contents below 2.5% (Table 3). The predominance of this same chemical family has been previously reported for this specific herb [8], and also for similar species such as *Eruca sativa* [21]. Three isothiocyanates were aliphatic compounds: ally isothiocyanate, 3-butenyl isothiocyanate (they are formed from methionine) and isobutyl isothiocyanate (formed from leucine); while the final compound, phenethyl isothiocyanate, is an aromatic compound coming from phenylalanine.

In *Diplotaxis erucoides*, the highest content was that of the ITC family (~ 96.4%) (Fig. 1), while the other families did not even reach 3.0%. This same trend has been previously reported for other Brassicaceae genus [22–24]. In mustards, the ITC family remained the predominant one (~ 61.3%); although in this case, other families had relatively high contents, including organic acids (~ 14.4%), S-compounds (~ 12.0%), and terpenoids (5.4%). Regarding wasabi samples, the main family was again ITC (~ 91.5%),

Table 2 Lexicon used for the descriptive analysis of mustards, wasabis and *Diplotaxis erucoides* samples

Attributes	Definition	References and intensities
Appearance		
Brightness	The chroma of the color, ranging for dull, muddied to pure, bright color	Wasabi paste Blue Dragon = 2.5; Yellow Mustard Heinz = 7.5
Flavor		
Diplotaxis-ID (odor and aroma)	Green and herbaceous odor/aroma associated with *Diplotaxis erucoides*	25 g of grinded *Diplotaxis erucoides* + 100 mL H_2O = 4; 100 g of grinded *Diplotaxis erucoides* + 25 H_2O = 10
Herbaceous-green flavor	Fresh, green, slightly sour aromatics associated with green vegetables, newly cut vines, snap peas	Kroger lima beans (canned) = 3.0; Small sprig fresh parsley = 7.0 (aroma); Fresh parsley = 10.0
Sweet	The fundamental taste factor associated with a sucrose solution	Sucrose solution 4% = 2.5; sucrose solution 8% = 5.0; sucrose solution 16% = 9.5
Salty	Fundamental taste sensation of which sodium chloride is typical	0.2% NaCl solution = 2.5; 0.35% NaCl solution = 5.0; 0.8% NaCl solution = 9.0
Sour	The taste stimulated by acids, such as citric and malic	Tartaric acid solution 0.05% = 2.5; tartaric acid solution 0.08% = 4.0; tartaric acid solution 0.20% = 9.5
Bitter	The taste stimulated by substances such as quinine or caffeine	Caffeine solution 0.05% = 2.5; caffeine solution 0.08% = 4.0
Pungent	A sharp physically penetrating sensation in the mouth and nose	Heinz white vinegar = 8.0 (flavor)
Aftertaste	Time in which the specific flavor of the fruit flavor remains in the mouth after swallowing the sample	10 s = 2.0; 30 s = 8.0
Texture		
Fatty Character	Amount of oil left on the mouth surfaces	Yellow Mustard Heinz = 3.0
Viscosity	The measure of flow as the product moves on the tongue when pressed between the tongue and the palate (2.46 mL of product)	Dillon's Whipping Cream = 4.0; Gerber Applesauce Stage 1 = 9.0

and with S-compounds representing ~ 8.0%. Finally, in the *Diplotaxis*-based products, the ITC family had the highest content (~ 89.0%); however, the second-highest content was that of aldehydes (~ 9.0%), which did not play a predominant role in the previous samples. The third chemical family was organic acids (~ 2.3%); only these three families were present in the cooked preparations.

Allyl isothiocyanate was the main volatile compound, representing 77.5% of the total identified compounds in all samples analyzed. Within the ITC family, this compound represented ~ 95.0% of the total content, was present in all samples, and was the predominant one in all samples except M1. The general order for the content of ally isothiocyanate was *Diplotaxis erucoides* > *Diplotaxis*-based products > wasabi > mustard. Another important compound was acetic acid, which was present in 7 out of the 13 samples under analysis. It was the second most abundant volatile compound in M3 and M4 (11 and 15%, respectively), and in the case of sample M1, acetic acid (~ 30%) predominated even above allyl isothiocyanate (Table 3). Usually, mustard recipes contain vinegar (acetic acid) and its content depends on the brands and markets [25]. The case of eugenol should be highlighted as well, because it predominated in two of the mustard samples (M1 and M3), reaching a content as high as 22% in the M1 sample. Eugenol is the predominant volatile compound of clove, and this spice is quite usual as a flavoring of mustards [26].

The degradation of glucosinolates leads to the presence of intermediate isothiocyanates, which in turn produce compounds such as carbon oxide sulfide and carbon disulfide [27, 28]. In fresh samples of *Diplotaxis erucoides*, COS was not found, as in the Diplotaxis-based dishes. However, appreciable amounts of these compounds (COS and CS_2) appeared in all commercial samples.

Regarding the sample complexity (those having a high number of volatile compounds), mustards had compounds from all families, especially the M1 sample which had up to 21 compounds; in contrast, only 4 compounds (hexanal, allyl isothiocyanate, *trans*-2-hexenal, hexanoic acid) were found in the two *Diplotaxis*-based products (DeC and DeM). A similar trend and composition have been previously reported in *Diplotaxis tenuifolia* [22, 24].

Organic acids and sugars

Six organic acids were identified (Table 4), with phytic and citric acids being the predominant ones. This acid profile is typical of the Brassicaceae family [29, 30]. Citric acid

Table 3 Volatile compounds (% of total area of identified compounds) of *Diplotaxis erucoides* plant, commercial mustard and wasabi samples, and *Diplotaxis*-based products

Code	Compound	RT (min)	KI	ANOVA‡	*Diplotaxis erucoides* (%)			Mustards (%)					Wasabis (%)			Dishes (%)	
					Stems	Leaves	Pods	M1	M2	M3	M4	M5	W1	W2	W3	DeC	DeM
V1	Carbon oxide sulfide	1.933	496	***	ND c¥	ND c	ND c	ND c	26.70 a	ND c	0.07 b	ND c	ND c	0.04 b	0.05 b	ND c	ND c
V2	Ethanol	2.269	517	*	ND e	ND e	ND e	0.78 a	ND e	0.41 b	0.18 c	0.16 c	ND e	0.02 d	0.02 d	ND e	ND e
V3	Dimethyl sulfide	2.429	527	*	2.93 a	1.47 b	1.79 b	ND d	ND d	ND d	ND d	ND d	0.06 c	0.07 c	0.02 c	ND d	ND d
V4	Carbon disulfide	2.585	537	**	ND e	ND e	ND e	0.98 d	ND e	3.33 c	9.47 b	19.44 a	8.84 b	11.63 b	3.05 c	ND e	ND e
V5	Ethyl acetate	3.094	568	*	ND c	ND c	ND c	5.17 a	ND c	0.51 b	0.19 b	0.42 b	ND c	ND c	ND c	ND c	ND c
V6	Acetic acid	3.212	576	***	ND d	ND d	ND d	30.16 a	ND d	11.02 b	15.04 b	16.00 b	0.08 c	0.15 c	0.10 c	ND d	ND d
V7	Methylacrylonitrile	3.919	619	*	ND d	ND d	ND d	ND d	1.03 b	2.34 a	1.69 ab	2.19 a	ND d	0.05 c	0.03 c	ND d	ND d
V8	Hexanal	6.502	756	*	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	2.92 a
V9	Allyl isothiocyanate	8.248	828	***	83.63 c	95.07 a	96.34 a	26.51 f	67.11 d	70.09 d	69.10 d	58.33 e	86.5 b	83.85 c	94.15 a	90.02 b	87.27 b
V10	*trans*-2-Hexenal	8.921	820	*	0.22 b	0.80 b	0.26 b	ND c	ND c	ND c	ND c	ND c	ND c	ND c	ND c	8.00 a	9.41 a
V11	Isobutyl isothiocyanate	9.539	874	*	ND e	ND e	ND e	ND e	0.33 b	0.62 a	0.16 c	0.31 b	0.27 b	0.33 b	0.03 d	ND e	ND e
V12	3-Butenyl isothiocyanate	11.327	935	*	0.29 e	0.29 e	0.39 e	0.98 d	2.41 b	4.26 a	1.58 c	1.71 c	0.17 e	0.17 e	0.04 e	ND f	ND f
V13	1.8-Cineole	12.099	960	*	0.10 b	ND c	ND c	1.13 a	ND c	0.9 a	ND c	ND c	ND c	ND c	ND c	ND c	ND c
V14	*α*-Phellandrene	13.366	1001	*	ND b	ND b	ND b	0.77 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V15	Hexanoic acid	14.350	1032	*	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	1.61 a	ND b
V16	Limonene	14.394	1033	*	ND b	ND b	ND b	0.51 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V17	Phellandrene	14.548	1038	*	ND b	ND b	ND b	0.55 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V18	*γ*-Terpinene	15.694	1074	*	ND b	ND b	ND b	0.33 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V19	Nonanal	16.033	1085	*	ND b	0.12 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V20	Camphor	16.939	1114	*	ND b	ND b	ND b	ND b	ND b	0.21 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V21	Phenylacetaldehyde	17.710	1139	*	0.59 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V22	Estragole	17.760	1141	*	ND c	ND c	ND c	1.24 a	0.13 b	ND c	ND c	ND c	ND c	ND c	ND c	ND c	ND c
V23	*α*-Terpineol	17.976	1147	*	ND b	ND b	ND b	0.37 a	ND b	0.20 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V24	Linalool	18.915	1178	*	ND b	ND b	ND b	0.36 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V25	Decanal	19.112	1184	**	0.22 ab	0.18 b	0.19 b	0.43 a	ND c	ND c	ND c	ND c	ND c	ND c	ND c	ND c	ND c
V26	Benzenepropanenitrile	20.766	1239	*	ND d	ND d	ND d	ND d	ND d	ND d	0.13 ab	ND d	0.09 b	0.24 a	0.03 c	ND d	ND d
V27	Benzylacetone	20.777	1239	*	ND b	ND b	ND b	ND b	0.31 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V28	2-Pyrrolidinethione	22.437	1295	*	ND d	ND d	ND d	ND d	0.77 c	1.89 a	1.36 b	0.62 c	ND d	ND d	ND d	ND d	ND d
V29	Triacetin	22.751	1305	*	ND b	ND b	ND b	ND b	ND b	ND b	ND b	0.13 a	ND b	ND b	ND b	ND b	ND b
V30	Ethyl octanoate	22.859	1309	*	ND b	ND b	ND b	0.20 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V31	Terpinen-4-ol	23.061	1316	*	ND b	ND b	ND b	4.34 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V32	Eugenol	23.313	1325	***	ND c	ND c	ND c	22.17 a	ND c	1.94 b	ND c	ND c	ND c	ND c	ND c	ND c	ND c
V33	Ar-Curcumene	25.770	1412	*	ND b	ND b	ND b	ND b	0.23 a	0.24 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V34	*β*-Cyclocitral	26.250	1430	*	ND b	0.12 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
V35	Phenethyl isothiocyanate	26.967	1457	***	11.04 a	1.55 d	0.66 e	ND f	0.63 e	1.49 d	0.61 e	0.34 e	3.64 b	3.14 b	2.14 c	ND f	ND f
V36	*trans*-Anethole	28.443	1516	**	ND b	ND b	ND b	1.99 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b

Table 3 (continued)

Code	Compound	RT (min)	KI	ANOVA‡	*Diplotaxis erucoides*			Mustards (%)					Wasabis (%)			Dishes (%)	
					Stems	Leaves	Pods	M1	M2	M3	M4	M5	W1	W2	W3	DeC	DeM
V37	*trans*-Cinnamaldehyde	29.452	1568	*	ND b	ND b	ND b	0.52 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b

‡ *, **, and ***, significant at $p<0.05$, 0.01 and 0.001, respectively

¥Values (mean of three replications) followed by the same letter, within the same row, were not significantly different ($p<0.05$). Tukey's least significant difference test

was the only compound found in all samples and its content ranging between 0.018 and 11.32 g kg^{-1} (W2 and *Diplotaxis*-stems, respectively). Typically, phytic acid is present in *Brassicaceae* family [31]; however, it was not present in neither *Diplotaxis erucoides* plant nor consequently in the *Diplotaxis*-based products (DeC and DeM). Phytic acid reached very high contents in both mustard and wasabi samples, ranging between 318 and 2085 g kg^{-1} (M3 and W3, respectively). The other four organic acids (fumaric, malic, oxalic and tartaric) were only found in *Diplotaxis erucoides* at low contents or even traces.

Additionally, six sugars were identified among all samples (Table 4), with fructose, glucose and sucrose being the predominant ones, and the first two compounds being present in all samples. Fructose was the most abundant sugar in wasabi, while both glucose and sucrose played an important role in both mustards and wasabis. Besides, the maltose content was relatively high in two mustard samples (M1 and M2). Finally, *Diplotaxis* and *Diplotaxis*-based products had very low sugar content with their profile being controlled by glucose. In other Brassicaceae plants, such as *Eruca sativa*, the sugar contents were higher than those of *Diplotaxis erucoides*, but never reached those of the commercial mustards and wasabis [32].

Sensory evaluation

The panel described the sensory profile of four samples under analysis: (1) *Diplotaxis*-based cream (DeC), (2) *Diplotaxis*-based mayonnaise (DeM), (3) mustard M3, and (iv) wasabi W2; the last two samples were previously selected by the panel as those having the closest flavor to that of *Diplotaxis erucoides* and were fully described.

Diplotaxis-ID odor and aroma are the perception (with the product outside or inside the mouth, respectively) of aromatics commonly associated with or identified as freshly cut *Diplotaxis erucoides*; these aromatics may be described as green, pungent, sharp, sulfury. The samples W2 and DeM showed the highest intensity of the *Diplotaxis*-ID attributes, followed by M3 and DeC (Fig. 2). It is worth mentioning that the intensity of this key attributes can be increased by decreasing the temperature (as close as possible to room temperature) at what the cream and mayonnaise were prepared (40 °C) because the myrosinase activity decreases as the temperature increases [33]. Both *Diplotaxis*-based products had the high intensity of the herbaceous-green attribute; which can be linked to the high contents of *trans*-2-hexenal, which main sensory descriptor is green/herbaceous [34]. The high sweetness of the DeC was probably due to the whipped cream used in its formulation. Mustards and wasabis had high saltiness and sourness due to the addition of NaCl and citric/acetic acids [35, 36]. Finally, DeM had the highest bitterness

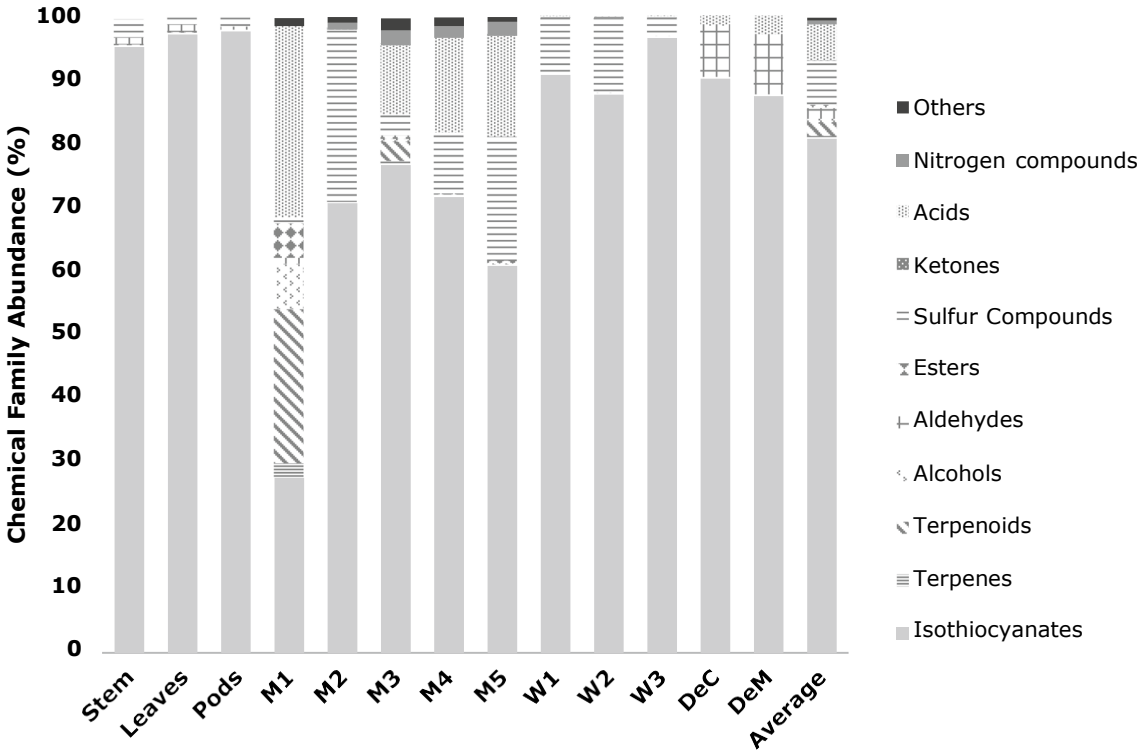

Fig. 1 Chemical families (% of total area of identified compounds) of the volatile compounds found in *Diplotaxis erucoides* plant, commercial mustard and wasabi samples, and *Diplotaxis*-based products

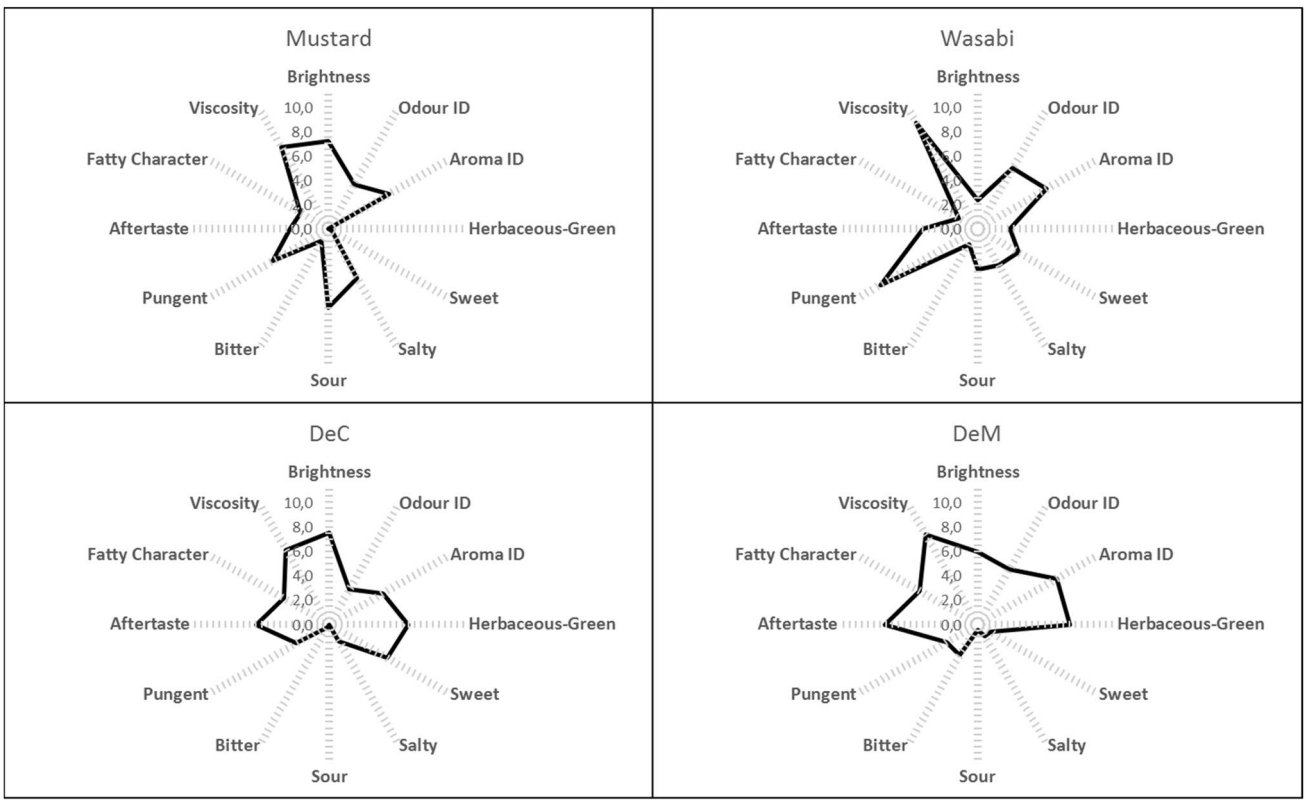

Fig. 2 Sensory profiles (appearance, flavor and texture) of mustard and wasabi samples and *Diplotaxis*-based products (cream: DeC and mayonnaise: DeM)

Table 4 Organic acids and sugars (g kg^{-1}) found in *Diplotaxis erucoides* plant (stems, leaves, and pods), commercial mustard and wasabi samples, and *Diplotaxis*-based products

			Diplotaxis erucoides (g kg^{-1})[a]			Mustards (g kg^{-1})[a]					Wasabis (g kg^{-1})[a]			Dishes (g kg^{-1})[a]	
Code	Compound	ANOVA[‡]	Stem	Leaves	Pods	M1	M2	M3	M4	M5	W1	W2	W3	DeC	DeM
A1	Citric	**	0.018 d[¥]	0.038 d	0.020 d	9.77 ab	8.24 b	6.22 c	7.92 b	7.44 bc	7.66 bc	11.32 a	7.41 bc	6.20 c	5.91 c
A2	Fumaric	NS	Trace	Trace	Trace	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A3	Malic	NS	Trace	0.023	Trace	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A4	Phytic	NS	ND	ND	ND	1501	431	2085	825	915	751	710	318	ND	ND
A5	Oxalic	NS	Trace	Trace	Trace	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A6	Tartaric	*	0.003 a	0.028 a	0.026 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
S1	Arabinose	*	0.042 a	0.028 a	0.027 a	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b	ND b
S2	Fructose	**	Trace c	Trace c	Trace c	Trace c	18.66 b	Trace c	Trace c	Trace c	262 a	303 a	205 a	Trace c	Trace c
S3	Galactose	*	0.050 a	Trace b	0.038 a	ND c	ND c	ND c	ND c	ND c	ND c	ND c	ND c	ND c	ND c
S4	Glucose	***	0.100 f	Trace g	0.102 f	Trace g	50.80 c	82.04 b	102 a	94.35 a	40.92 d	27.14 e	35.32 d	0.262 f	0.230 f
S5	Maltose	*	ND d	ND d	ND d	23.31 b	54.49 a	ND d	ND d	ND d	23.12 b	19.13 b	5.27 c	ND d	ND d
S6	Sucrose	***	0.010 g	0.002 g	0.015 g	66.83 d	101 b	3.99 e	7.47 e	ND h	82.74 c	137 a	93.16 b	0.070 f	0.043 f

[‡]*NS* not significant at $p < 0.05$

*, **, and ***, significant at $p < 0.05$, 0.01 and 0.001, respectively

[¥]Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different ($p < 0.05$). Tukey's least significant difference test

[a]*M* mustard; *W* wasabi; *DeC Diplotaxis erucoides* cream; *DeM Diplotaxis erucoides* mayonnaise; *Trace* below the limit of quantification; *ND* not detected

due to its relatively high hexanal content, among other factors [34, 37]. One of the most important attributes in this type of products is pungency. In this case, W2 had the highest intensity, followed by M3 and with the *Diplotaxis*-based products having the lowest intensity. The pungency in fresh *Diplotaxis erucoides* plant is high, but myrosinase was inactivated during the sauces preparation and they were not as pungent as expected [33]. It has been recently demonstrated that cooking can significantly affect the glucosinolate concentrations [38, 39]. However, both *Diplotaxis*-based products showed long aftertaste, especially DeM, and it is linked to the high intensity of key attributes such as *Diplotaxis*-ID odor/aroma and herbaceous-green notes.

The relationship between the chemical analyses and the sensory attributes is quite interesting and clear. Saltiness and sourness were high in the samples of commercial mustard and wasabi, while they were very low (< 2.0) in the *Diplotaxis*-products; these results agreed well with the higher NaCl and of organic acids in the samples W2 and M3. The high intensity of the sweetness in the *Diplotaxis*-cream is due to the whipped cream used in its preparation, while the sweet taste of the W3 is due to its high content of sugars. Finally, the long aftertaste of the *Diplotaxis* products is linked with their high content of *trans*-2-hexenal.

Conclusions

Two healthy (low in sugars) and local alternatives to commercial mustard and wasabi have been developed using a wild edible plant, *Diplotaxis erucoides*, growing as a weed in the Mediterranean regions of Spain. Allyl isothiocyanate was the main volatile compound in mustards and wasabis, and this is why they were used as control samples. Citric acid, sucrose, glucose and fructose were added as preservation agents and sweeteners to commercial mustards and wasabis; however, these compounds are not needed in the *Diplotaxis*-based products, making them more natural and safer. The sensory profile of the *Diplotaxis*-based products was characterized by high intensity of *Diplotaxis*-ID odor/aroma, herbaceous flavor, and long aftertaste; however, some pungency was missing and can be improved by preparing the cream and mayonnaise at a temperature close to room temperature to avoid the inactivation of the myrosinase enzyme, which is the responsibility of the production of the allyl isothiocyanate, the main compound behind the pungency of Brassicaceae family. Considering all the above, it can be concluded that *Diplotaxis erucoides* is a sustainable alternative to produce spicy/pungent sauces.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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