



# Oxyprenylated secondary metabolites: a survey of their innovative extraction methodologies

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**Abstract** Oxyprenylated secondary metabolites of plant, fungal, and microbial origin have emerged as biologically active natural compounds with a great potential for the next future. While originally obtained from the respective sources merely by conventional techniques of the phytochemical practice like maceration, percolation, and Soxhlet extractions, during the last five years novel and alternative experimental protocols to get such chemicals in higher yields and purity degree and/or in form of enriched phytopreparations have been developed. Such new processes include ultrasounds and microwaves assisted extractions, use of auxiliary agents like deep eutectic solvents and QuEChERS, employment of novel extractive solvents like supercritical CO<sub>2</sub> and subcritical butane, accelerated solvent extractions, and finally extractions in the heterogeneous phase using solid supports. All these new methodologies proved to be very powerful and efficient in terms of yields and selectivity in concentrating classes of secondary metabolites from the respective matrices, comparing favorably to the already and widespread “classic” extraction techniques. All the developed processes can be framed in a green chemical context stating the

employment of nontoxic, non-pollutant, environmentally friend, recyclable, easy to handle and to store solvents and reagents. The aim of this review article is to make a detailed survey of these alternative extraction processes for oxyprenylated secondary metabolites providing not only the most relevant examples of the recent literature, but also concrete suggestions and addresses to accomplish future research activities in the same field.

**Keywords** Auraptene · Extraction · Imperatorin · Oxyprenylated secondary metabolites · Umbelliprenin

## Abbreviations

ASE	Accelerated solvent extraction
CD	Cyclodextrin
DES	Deep eutectic solvents
DLLME	Dispersive liquid–liquid microextraction
MW	Microwave
QuEChERS	Quick, easy, cheap, effective, rugged, and safe
r.t.	Room temperature
SX	Soxhlet
TTO	Tea tree oil
UHPLC-	Ultra high performance liquid chromatography/photodiode array
PDA	chromatography/photodiode array
US	Ultrasound

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## Introduction

Isolation of a pure target substance from a plant matrix for pharmacological evaluation purposes still remains a challenging and a very problematic experimental step. It is notorious how this represents a significant barrier in natural product development. Oxyprenylated secondary metabolites from plants, fungi, protozoa, and bacteria have been subject of intensive studies during the last two decades. This class of rare natural products are in turn classified on the basis of their central core linked to one or more differently functionalized 3,3-dimethylallyl, geranyl, or farnesyl side chains via ethereal bonds and thus comprise phenylpropanoids, polyketides, xanthenes, and alkaloids (Fiorito et al. 2018). Investigations on the pharmacological properties of such phytochemicals pointed out their great potential as chemopreventive and therapeutic agents of acute and chronic syndromes affecting humans like cancer, inflammation, stroke, neurological disorders, dysmetabolisms, and several others. A plethora of review articles to this concern were reported in the recent literature (Tayarani-Najaran et al. 2021; Preziuso et al. 2020; Fiorito et al. 2020a, b; Bibak et al. 2019; Fiorito et al. 2019a, b, c; Fiorito et al. 2018). The most part of studies aimed at investigating and depicting the pharmacological properties of oxyprenylated secondary metabolites have been accomplished with samples of chemical synthetic origin and/or after exhaustive extractions of huge masses of vegetable material. In this latter case the active principles have been obtained by the respective matrices employing common and “classic” phytochemical techniques like maceration, percolation, and use of Soxhlet apparatuses. Indeed, maceration was the first and practically only extractive techniques applied before other processes emerged as valid and in some instances better alternatives. The main drawback of “classic” methods relies in the low yields by which metabolites of interest can be extracted, thus implying that a huge quantity of plant material must be treated to obtain the quantities necessary to perform significant and predictable pharmacological in vitro and in vivo assays. Indeed, the low abundance in nature is a common feature of the most part of oxyprenylated phenylpropanoids and polyketides so far characterized (Fiorito et al. 2019a). With the scope of increasing extractive yields and purity degree, during the last five

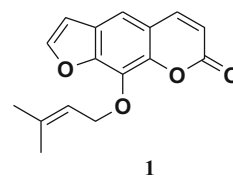
years novel, alternative, and more powerful methodologies have been developed. Such new techniques comprise UAs and MWs assisted extractions, use of auxiliary agents like DES and QuEChERS, of supercritical CO<sub>2</sub> and subcritical butane as extractive solvents, ASE, and finally adsorption on lamellar solids and similar sorbents. All these were capable to provide the desired phytochemicals in increased yields and, more interestingly, were shown to selectively concentrate classes of oxyprenylated secondary metabolites from the respective matrices. Thus, these novel methods compare favorably respect to the above mentioned “classic” extraction techniques. Furthermore, all the set up processes can be regarded as green chemical ones as they employ nontoxic, non-pollutant, environmentally friend, recyclable, easy to handle and to store solvents and reagents. By this review article we wish to provide a detailed survey of the in so far reported alternative extraction processes for oxyprenylated secondary metabolites mentioning not only the most relevant examples of the recent literature to this concern, but also concrete suggestions and addresses to accomplish future research activities in the same field.

## Ultrasounds assisted extractions

The first successful application of USs assisted extraction of oxyprenylated phenylpropanoids dates back to 2012 when Waksmundzka-Hajnos and coworkers isolated imperatorin (**1**) (Fig. 1) from the fruits of *Peucedanum verticille* (L.) Koch ex DC. (Apiaceae).

These authors noticed a great difference between the process carried out at 60 and 20 °C for the same time and using petroleum ether as the solvent in terms of extractive yields, 6.68 and 2.05 mg/g, respectively. The application of USs was also by far more efficient than the extraction promoted by MWs and pressurized 80% MeOH (Waksmundzka-Hajnos et al. 2012).

**Fig. 1** Structure of imperatorin (**1**)



In 2014 Genovese and coworkers successfully applied a combination of USs and maceration for the qualitative and quantitative analysis of boropinic acid (2) and 4'-geranyloxyferulic acid (3), that were for the first time detected as additional components of the phytochemical pool of pulps and exocarp of grapefruits, *Citrus paradisi* Macfad (Rutaceae) (Genovese et al. 2014). At the moment, this is the only example in the literature of the contemporary application of a "classic" technique such as maceration with an innovative one such as the USs. Such a combination allowed to get the desired phytochemicals in higher yields respect to the application of individual processes and to highlight how the content of both boropinic acid (2) and 4'-geranyloxyferulic acid (3) largely depend on the geographical origin of the vegetable material used for the extraction steps.

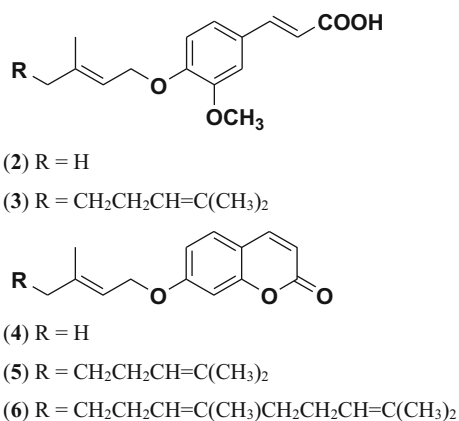
In 2016 Taddeo and coworkers compared the efficiency of the US-based process with respect to maceration employing different solvents. In particular the attention of these Authors focused on four oxyprenylated secondary metabolites, namely boropinic acid (2), 4'-geranyloxyferulic acid (3), 7-isopentenylcoumarin (4), and auraptene (5) (Fig. 2) and employing raw propolis as the matrix of natural origin (Taddeo et al. 2016).

In the contest of this study, five mixtures were used as the extracting solvents: absolute EtOH, EtOH/H<sub>2</sub>O 3:7, olive oil, a 1.5% w/w aqueous solution of  $\beta$ -CD, a biphasic 1:1 combination of a 1.5% w/w aqueous solution of  $\beta$ -CD and olive oil. These proved to extract compounds (2–5) selectively and with different

efficiency. In particular, when USs were applied (10 min, r.t.), boropinic acid (2) and 7-isopentenylcoumarin (4) (181.3  $\mu$ g/g) were extracted in the highest yield (213.1  $\mu$ g/g of dry propolis) when the hydroalcoholic solvent was employed, while the same occurred for 4'-geranyloxyferulic acid (3) (107.1  $\mu$ g/g) and auraptene (5) (74.3  $\mu$ g/g) with absolute EtOH. Despite being fairly efficient, the comparison between USs-based extraction and maceration (96 h) proved to be slightly superior in terms of extractive yields, especially when considering oxyprenylated coumarins for which values recorded with the application of this "classic" technique were about 50% higher.

One year later the same research group adopted a slight modification of the above mentioned USs-based extraction method investigating the chemical composition for 4'-geranyloxyferulic acid (3), 7-isopentenylcoumarin (4), auraptene (5), and umbelliprenin (6) (Fig. 2) in three plants, namely dill (*Anethum graveolens* L.), anise (*Pimpinella anisum* L.), and wild celery (*Angelica archangelica* L.), all belonging to the Apiaceae family (Taddeo et al. 2017). The same solvents as previously outlined were used with the replacement of olive oil and the biphasic 1:1 combination of a 1.5% w/w aqueous solution of  $\beta$ -CD and olive oil with a 1:1 mixture of a 1.5% w/w aqueous solution of  $\beta$ -CD and EtOH. Also in this case a comparison with maceration in terms of extractive yields was accomplished. The pattern of results obtained by Taddeo and coworkers using the vegetable matrices derived from these three plants was quite similar to that of the study carried out in 2016: maceration proved to be in all cases a better extraction methods that USs-based one with the only exception of umbelliprenin (6) from seeds of *P. anisum* (10.22  $\mu$ g/g of dry extract in the case of extraction with the 1.5% w/w aqueous solution of  $\beta$ -CD and 9.41  $\mu$ g/g with absolute EtOH, threefold and 2.7-fold the best value recorded after maceration with the 1:1 mixture of a 1.5% w/w aqueous solution of  $\beta$ -CD and EtOH, respectively), and auraptene (5) from the roots of *A. archangelica* (127.29  $\mu$ g/g in the case of extraction with absolute EtOH with respect to 119.62  $\mu$ g/g after maceration with the same solvent).

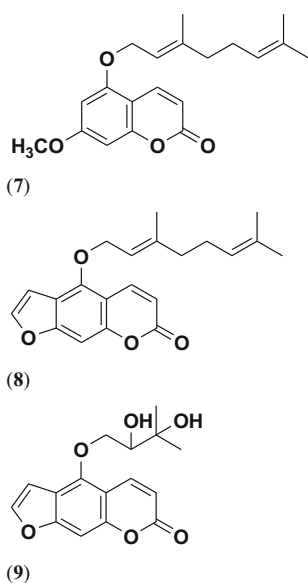
Also for the extract of aerial parts and root extracts of the edible herb *Amaranthus retroflexus* L. (Amaranthaceae), for only one phytochemical (e.g. umbelliprenin and *n*-hexane as the solvent) out of six, ultrasonication (10 min) was shown to be more



**Fig. 2** Structure of boropinic acid (2), 4'-geranyloxyferulic acid (3), 7-isopentenylcoumarin (4), auraptene (5), and umbelliprenin (6)

effective than 96 h maceration (32.15  $\mu\text{g/g}$ ). On the contrary, when applied to the extraction with absolute EtOH of seeds of *Ferulago campestris* (Besser) Grecescu, USs were shown to be the best methodology providing umbelliprenin (**6**) in a concentration value of 221.35  $\mu\text{g/g}$  of dry extract, around 0.8-fold higher than “classic” maceration in the same solvent (Taddeo et al. 2019).

Also for the most recent literature example of USs promoted extraction with absolute EtOH of oxyprenylated coumarins, this process proved to be the best one when used for the qualitative and quantitative analysis of auraptene (**5**) and umbelliprenin (**6**) as novel components of the phytochemical pool of seeds of *Punica granatum* L. (Lythraceae). In fact, these two secondary metabolites have been obtained in 1.99  $\mu\text{g/g}$  of dry extract and 6.53  $\mu\text{g/g}$  respectively. It is noteworthy that, even after the application of an experimental time of just 1 min, the USs-assisted maceration performed significantly better in terms of yields (around 18-fold) than “classic” maceration in the same solvent carried out over a period of 96 h (Fiorito et al. 2019b). Finally, USs assisted extraction proved to be efficient also in the case of other differently substituted biologically active coumarins like 5-geranyloxy-7-methoxycoumarin (**7**), bergamottin (**8**), and oxypeucedanin hydrate (**9**) (Fig. 3), that were recently obtained from fruit extracts of two



**Fig. 3** Structure of 5-geranyloxy-7-methoxycoumarin (**7**), bergamottin (**8**), and oxypeucedanin hydrate (**9**)

*Citrus* species, namely *C. latifolia* (Yu Tanaka) Tanaka and *C. aurantifolia* (Christm.) Swingle in the concentrations range 352–392  $\mu\text{g/g}$  of fresh weight, 302–349  $\mu\text{g/g}$ , and 256–357  $\mu\text{g/g}$ , respectively. Compound (**9**) has been also extracted from *C. sinensis* (L.) Osbeck, *C. limonia* Pehr Osbeck, and *C. reticulata* Blanco (Ramírez-Pelayo et al. 2019).

The different pattern of results obtained so far with the use of USs as the extraction technique can be explained by the great complexity of the vegetable matrices listed above from a chemical point of view. In some cases, this may lead to sequestering some categories of secondary metabolites, also making it necessary to apply longer extraction times. In the frame of UAs assisted extractions this latter statement means a massive increase of temperatures, thus leading to an extensive chemical degradation of the oxyprenylated portion of the phytochemical matrix. Nevertheless, results outlined for the two last examples, namely *F. campestris* and *P. granatum* are more than encouraging to try to better and sharply set up USs-based processes in order to obtain higher yields and selectivity for selected categories of oxyprenylated plant metabolites.

### Microwaves assisted extractions

The application of MWs assisted extraction for oxyprenylated secondary metabolites is limited to very few examples reported in the recent literature. This partial lack of know-how may be due to the fact that operational temperatures that can be reached using MWs apparatuses, even after short periods of time (Bagade and Patil 2021), render this method of low applicability for such phytochemicals, that may be chemically thermolabile in certain instances. To date only three examples have been reported in the literature, and all in 2019 and all by the same research group.

In the first study, Taddeo and coworkers compared the efficiency of some extraction techniques, including MWs-based one for umbelliprenin (**6**) from seeds extracts of *A. graveolens*, *P. anisum*, and *F. campestris* (Taddeo et al. 2019). Only when a 7:3 EtOH/H<sub>2</sub>O mixture was used as the solvent, MWs assisted extraction compared favorably to maceration and USs-based one providing compound (**6**) in the following concentrations: 11.33  $\mu\text{g/g}$  of dry extract for *A.*

*graveolens*, 7.15 µg/g for *P. anisum*, and finally 9.14 µg/g for *F. campestris*. Notably, in the course of this study, it was observed how MWs was not practically applicable when a 1.5% aqueous solution of β-CD was employed as the extractive solvent. This may be due to a partial degradation of this auxiliary agent by high operational temperatures eventually reached during the course of the MWs extractions, thus largely reducing the extraction capacities by β-CD.

A similar pattern of results have been obtained in the case of the qualitative and quantitative analysis of the same umbelliprenin (**6**) and auraptene (**5**) from seeds extract of *P. granatum*, for which the application of MWs-based technique provided concentration values higher than those obtained with maceration, but fivefold less than the USs process (Fiorito et al. 2019b).

The most recent example reported in the literature by Fiorito and coworkers represents the most successful one for the effective extraction of compounds (**2–6**) from three well known edible plants, namely spinach (*Spinacia oleracea* L., Amaranthaceae, leaves), goji (*Lycium barbarum* L., Solanaceae, berries), and quinoa (*Chenopodium quinoa* Willd., Amaranthaceae, seeds) (Fiorito et al. 2019c). Three mixtures were tested as the extractive solvents, namely absolute EtOH, EtOH/H<sub>2</sub>O 7:3, and EtOH/H<sub>2</sub>O 3:7, with the first one, as in several other cases, providing the best yields. Recorded values were 4.36 µg/g of dry extract for boropinic acid (**2**), detected only in spinach extracts, in the range 2.01–21.49 µg/g for 4'-geranyloxyferulic acid (**3**), in the range 2.33–8.47 µg/g for 7-isopentenylcoumarin (**4**), in the range 7.91–28.09 µg/g for auraptene (**5**), and finally in the range 8.33–49.22 µg/g for umbelliprenin (**6**). The presence of these last four samples was revealed only in spinach and quinoa extracts. Despite its limited applications in the phytochemical practice applied to the extraction and isolation of oxyprenylated phenylpropanoids from plant matrices, the results and outcomes of this latter study are extremely encouraging to, as in the previous case, further explore the general usefulness, applicability, and limitations of MWs promoted processes. In particular there is a concrete need of correlation (also in terms of mathematical model, like surface response methodology) between MWs potency employed, operational times, and the putative chemical degradation of the

metabolites herein under investigation. Indeed, no studies to this concern have been reported in the literature, thus such a topic could represent an attractive address to accomplish future investigations.

### Deep eutectic solvents promoted extractions

The use of DESs for plant extraction purposes is a novel and challenging field of research. Improved yields, selectivity, versatility, and ease of recycling and storage are the main advantages of this novel technique (Ivanović et al. 2020). For what concerns oxyprenylated phenylpropanoids, only one article to this frame has been reported in the literature.

In 2018 Ferrone and coworkers investigated the application of a DLLME using DESs as the extracting solvent for the qualitative and quantitative determination of boropinic acid (**2**), 4'-geranyloxyferulic acid (**3**), 7-isopentenylcoumarin (**4**), and auraptene (**5**) in olive, soy, peanuts, corn, and sunflower oils (Ferrone et al. 2018). To this aim, four DESs, namely glycolic acid/trimethylglycine (Gly/TMG), phenylacetic acid/trimethylglycine (PhAA/TMG), 2-furoic acid/trimethylglycine (Fur / TMG), and S-(+)-mandelic acid/trimethylglycine (Man/TMG), were employed. The selection of the listed DESs as was based mainly on their electrostatic, hydrophobic, and π-π interaction capacities with the target analytes. The extraction procedure consisted first in a dilution of each oil sample with *n*-hexane followed by the addition of the solution of each DES in isopropanol and extraction at r.t. for 10 min followed by the separation of the two phases obtained and subsequent UHPLC-PDA analysis. The presence of an aromatic ring as a key structural feature of DESs, by enhancing the possibility of hydrophobic and van der Waals interactions with the 3,3-dimethylallyloxy and geranyloxy side chains as well as with aromatic portion of cinnamate or coumarin cores, largely provided higher extraction yields for the desired secondary metabolites. With the only exception of Fur/TMG, a parallelism between decreasing polarity order of the DESs (Man/TMG > Fur/TMG > PhAA/TMG) and their increased extractive capacities towards geranyl and dimethylallyl ethers of umbelliferone was recorded. PhAA/TMG was thus selected as the DES with the best extractive capacities, also on the basis the assessed enrichment factors on solutions of the pure

chemical standards under investigation. Recorded values were the following: 3.48–17.54  $\mu\text{g/mL}$  for boropinic acid (2), 2.35–12.08  $\mu\text{g/mL}$  for 4'-geranyloxyferulic acid (3), 1.20–12.92  $\mu\text{g/mL}$  for 7-isopennyloxy coumarin (4), and 0.06–14.82  $\mu\text{g/mL}$  for auroptene (5).

Results and outcomes of this recent study surely deserve to be further investigated. Data reported above shed light on the great potential of DESs as extractive agents of differently functionalized oxyprenylated secondary metabolites, being up to now among the best methodologies in terms of extractive yields. Stating the numerous vegetable matrices reported so far in the literature to contain such phytochemicals and the ease of synthesis of a plethora of differently structurally substituted DESs (and consequently ease of modulation of chemico-physical properties), it is easy to imagine how numerous studies can be quickly and comprehensively carried out in the near future.

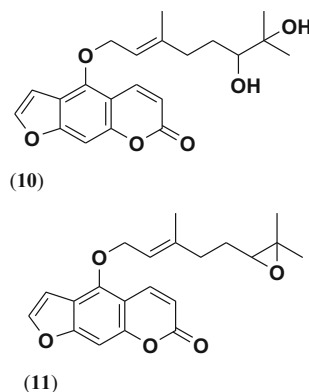
### QuEChERS promoted extractions

As for DESs, also the use of QuEChERS for the extraction of oxyprenylated secondary metabolites has found until now a very limited application. Only two reports have been published in the literature and both focused on only *O*-prenylfuranocoumarins.

In 2016 Lee and coworkers set up a new method for the qualitative and quantitative analysis of photoactive furanocoumarins from grapefruits (whole fruits) and successfully submitted to treatment with QuEChERS this vegetable matrix, being able to extract and quantify three oxyprenylated furanocoumarins, namely bergamottin (8), 6,7 dihydrobergamottin (10), and epoxybergamottin (11) (Fig. 4) (Lee et al. 2016).

The successful application of this technique allowed also these Authors to determine compounds (9–11) also in the plasma and urine of healthy volunteers before and after ingestion of grapefruit or grapefruit juice.

In a similar way Cook and coworkers in 2017 determined a wider panel of oxyprenylated furanocoumarins from some plants belonging to the Apaiaceae family (Cook et al. 2017). Also in this case the experimental step based on the use of QuEChERS was crucial for the effective quantification of the secondary metabolites of interest. In both studies,



**Fig. 4** Structure of 6,7-dihydrobergamottin (10) and epoxybergamottin (11)

however, no speculations on the role of QuEChERS have been done. In particular the effect of key parameters like the quantity added to accomplish the extraction process, the experimental time and temperature, and similar ones were not taken into the proper consideration. Consequently, there are still many gaps to fill in this particular field and there are therefore concrete addresses for future investigations aimed at much better define the role played by QuEChERS in the extraction of oxyprenylated secondary metabolites, other than prenyloxyfuranocoumarins, from plant matrices.

### Supercritical CO<sub>2</sub> promoted extractions

The only paper about the use of supercritical CO<sub>2</sub> for the extraction of oxyprenylated secondary metabolites have been reported in 2020 by Woźniak and coworkers. These Authors used such extractive solvents to investigate the qualitative and quantitative profile of a panel of furanocoumarins, comprised the oxyprenylated ones imperatorin (1), bergamottin (8), and 6,7 dihydrobergamottin (10) from *A. archangelica*, *C. paradisi*, *Cnidium monnieri* (L.) Cusson (Apiaceae), and *Psoralea corylifolia* L. (Fabaceae) (Woźniak et al. 2020). Several chemico-physical parameters were applied to select the best experimental conditions providing the desired secondary metabolites in the highest yield. In particular, Woźniak and coworkers examined nine combinations of temperature (40, 60, and 80 °C) and pressure (10, 20, and 40 MPa) and finally assessed that furocoumarins, regardless of the

plant matrix of origin, exhibited the highest solubility in supercritical carbon dioxide at a temperature of 80 °C and pressure of 40 MPa. It is noteworthy to highlight how the presence of prenyloxy and geranyloxy groups as structural features of the investigated furanocoumarins increased their solubility by 35% and 90%, respectively. The recorded data for prenylated compounds were in good agreement with theoretical calculations done by the Chrastil equation, that allowed to calculate the solubility of an analyte with given temperature and density of a supercritical fluid in a simple binary system. These authors applied also the broken plus intact cell model to predict the kinetics of extraction of furanocoumarins from the respective vegetable extract and also in this case there was a very good agreement between theoretical and practical data. The one reported by Woźniak and coworkers is a pioneering investigation in which a very detailed study of chemo-physical parameters and the rate influencing the overall extraction process for oxyprenylated furanocoumarins, thus determining the fate of extractive yields. In particular, the proper application of mathematical models allowed to predict the overall solubility of selected phytochemicals in individual solvents, thus providing a strong rationale for the concrete development of an experimental protocol adaptable to each plant matrix. The content of this investigation can be absolutely claim as a model to follow also for other categories of oxyprenylated secondary metabolites.

### Subcritical butane promoted extractions

The use of subcritical butane as a clean and green solvent for the extraction of vegetable material is emerging as a valid, alternative, and powerful technique in the phytochemical practice (Liang et al. 2019). Such a methodology have been successfully recently applied also to oxyprenylated secondary metabolites.

In 2020 Genovese and coworkers investigated the performance of the subcritical butane extraction of a panel of compounds (2–6) from grapefruit peels under a counter-current mode using a handmade apparatus coupled to UHPLC analysis and compared the recorded data with those obtained as a result of the US- and MW-assisted macerations (both carried out for 10 min) with absolute ethanol (EtOH) (Genovese

et al. 2020). Applying the same chemo-physical parameters used for the extraction of several other categories of secondary metabolite, but longer times (up to 24 h) these authors observed how for three out of five oxyprenylated phytochemicals under investigation better yields were recorded for the subcritical butane based process respect to both the US- and MW-ones. Quantification data were the following: 0.234 mg/g of dry extract for 7-isopentenylcoumarin (4) (+ 28.2% respect to US and + 38.9% respect to MW), 1.035 mg/g for auraptene (5) (+ 37.3 and + 48.3%), and finally 0.211 mg/g for boropinic acid (2) (+ 10.9 and + 15.6%). In no cases umbelliprenin (6) and 4'-geranyloxyferulic acid (3) were detected.

This technology was applied by the same research group soon after to investigate the presence and quantify umbelliprenin (6) in extracts from *Artemisia vulgaris* L. (Fam. Asteraceae) (Fiorito et al. 2020a, b). Also in this case, under the same experimental conditions, subcritical butane proved to largely be the most efficient extractive solvents and umbelliprenin was detected with a concentration of 0.180 mg/g of dry extract. Such a value was higher than those recorded after the application of “classic” maceration, and US- and MW-promoted extractions (+ 77.2, + 66.1, and + 67.8% respectively). It is also noteworthy how the use of pentane as the extracting solvent in a SX apparatus provided umbelliprenin in a concentration of only 0.04 mg/g.

Although preliminary, results from both studies showed that butane-based processes have been shown to be safe from an operational point of view, to employ non-polluting chemicals, and to provide good to excellent extractive yields. Moreover, butane has the great advantage to be used as subcritical fluid, requiring low critical temperature and pressure, in a continuous counter-current process ending with a vacuum removal at low temperatures. All these properties allow also to prevent to a large extent the chemical degradation of thermally labile plant secondary metabolites like most of oxyprenylated phenylpropanoids are. Furthermore, the subcritical butane under counter-current mode was largely a more efficient methodology than “classic” overnight maceration and similar ultrasounds- and microwaves-assisted routes as well as SX extractions.

## Accelerated solvent extractions

ASE is a method of extraction first introduced by Richter and coworkers in 1995 that uses high temperature and pressure resulting in taking less time and requiring less solvent, and in most instances providing better analyte recovery (Richter et al. 1996). The elevated temperature is necessary to increase extraction efficiency of desired analytes, while the elevated pressure is used to keep the solvent in a liquid state as the temperature increased above its boiling point. For what concerns oxyprenylated phenylpropanoids, this technique have been applied only for the extraction of imperatorin (**1**). In two reports appeared in the literature.

In 2004 Waksmundzka-Hajnos and coworkers described the application of ASE to obtain compound (**1**) from the fruits of *Archangelica officinalis* L. (Apiaceae) (Waksmundzka-Hajnos et al. 2004a) and *Pastinaca sativa* L. (Apiaceae) (Waksmundzka-Hajnos et al. 2004b). In the case of *A. officinalis* extraction with petroleum ether, followed by methanol (100 °C, 60 bar) yielded 19.08 mg/g of this furanocoumarin while SX and US-based processes (extraction with petroleum ether, followed by methanol), gave significantly lower yields (– 31.2 and – 26.6% respectively). In the case of *P. sativa*, the application of ASE provided a value (15.12 mg/g) only slightly better than that recorded applying US-promoted extraction (+ 4.5%).

ASE was also applied for the extraction of imperatorin from the fruits of *Heracleum leskowitzii* L. (Apiaceae). Different temperature conditions (80, 90, 100, and 110 °C) and solvent (MeOH, Et<sub>2</sub>O, and CH<sub>2</sub>Cl<sub>2</sub>) were investigated. Recorded data showed that CH<sub>2</sub>Cl<sub>2</sub> performed slightly better than MeOH and Et<sub>2</sub>O. A temperature-dependent increase in the extraction yield of imperatorin was also observed passing from 80 to 110 °C. (17.87 mg/100 g, 18.06 mg/100 g, 18.22 mg/100 g, and 19.07 mg/ 100 g at 80 °C, 90 °C, 100 °C, and 110 °C respectively). In this context it is noteworthy to underline how the application of the SX method provided very poor yield due to the thermal degradation of furanocoumarins (Skalicka-Wozniak and Głowniak 2012).

Although limited to the extraction of only one secondary metabolite, also ASE appears to be a powerful methodology with a great potential applicable in the next future to a wide range of oxyprenylated

secondary phytochemicals. In this context it has to be pointed out how ASE, although requiring high temperatures, it preserves these natural compounds from heat-promoted chemical degradation.

## Extractions in the heterogeneous phase

The use of solid sorbents of different chemical origin and structure is a practice widely employed for the extraction and/or analysis of plant extracts (Raks et al. 2018). The first example reported in the literature about the extraction in the heterogeneous phase of oxyprenylated secondary metabolites have been reported in 2018 by Scotti and coworkers. These Authors carried out the qualitative and quantitative analysis of compounds (**2–6**) in the essential oil of *Melaleuca alternifolia* (Maiden and Betche) Cheel (Myrtaceae), the well-known TTO, after treatment of the same with Al<sub>2</sub>O<sub>3</sub> (Brockmann activity II) followed by desorption with MeOH and CH<sub>2</sub>Cl<sub>2</sub> (Scotti et al. 2018). Only 7-isopentenylcoumarin (**4**), boropinic acid (**2**), and 4'-geranyloxyferulic acid (**3**) were detected providing the following concentration values: 44.03 µg/g TTO, 1.01 µg/g and 130.51 µg/g respectively. Under the same experimental conditions SiO<sub>2</sub> gel (pre-treated with Et<sub>3</sub>N) provided as well good extractive yield but less than about 30%.

The second example has been reported very recently by Fiorito and coworkers. These Authors established a convenient and powerful process to get auraptene (**5**)-enriched blends from *Citrus limon* L. Osbeck (Rutaceae) peels powder with enhanced antioxidant properties respect to the crude phyto-preparation. The process these authors set up consisted in the selective adsorption of compound (**5**) by bentonite followed by desorption and evaporation of solvents to get the final product. In this case the sorbent allowed to concentrate in the solid phase auraptene and most of the antioxidant active principles present in the lemon peel powder crude extract, thus rendering it a blend with interesting and promising functional food and nutraceutical properties (Fiorito et al. 2021).

Results from this latter investigation seem to suggest that some solid sorbent may exhibit selectivity in extracting individual molecules or group of natural products. Thus, also for oxyprenylated phenylpropanoids, the extraction in the heterogeneous phase



**Table 1** Survey of the main data outlined in the present review article

Extraction method	Phytochemicals	Source	Ref.	
Ultrasounds	Imperatorin (1)	<i>P. verticille</i> (L.) Koch ex DC. (Apiaceae)	Waksmundzka-Hajnos et al. (2012)	
	Boropinic acid (2)	<i>C. paradisi</i> Macfad (Rutaceae) Propolis	Genovese et al. (2014) Taddeo et al. (2016)	
	4'-Geranyloxyferulic acid (3)	<i>C. paradisi</i> Macfad (Rutaceae) Propolis	Genovese et al. (2014) Taddeo et al. (2016)	
	7-Isopentenylxycoumarin (4)	Propolis <i>A. graveolens</i> L. (Apiaceae) <i>P. anisum</i> L. (Apiaceae) <i>A. archangelica</i> L. (Apiaceae)	Taddeo et al. (2016) Taddeo et al. (2017)	
	Auraptene (5)	Propolis <i>A. graveolens</i> L. (Apiaceae) <i>P. anisum</i> L. (Apiaceae) <i>A. archangelica</i> L. (Apiaceae) <i>P. granatum</i> L. (Lythraceae)	Taddeo et al. (2016) Fiorito et al. (2019b)	
	Umbelliprenin (6)	<i>A. graveolens</i> L. (Apiaceae) <i>P. anisum</i> L. (Apiaceae) <i>A. archangelica</i> L. (Apiaceae) <i>A. retroflexus</i> L. (Amaranthaceae) <i>P. granatum</i> L. (Lythraceae)	Taddeo et al. (2017) Taddeo et al. (2019) Fiorito et al. (2019b)	
	5-Geranyloxy-7-methoxycoumarin (7)	<i>Citrus</i> spp. (Rutaceae)	Ramírez-Pelayo et al. (2019)	
	Bergamottin (8)	<i>Citrus</i> spp. (Rutaceae)	Ramírez-Pelayo et al. (2019)	
	Oxypeucedanin hydrate (9)	<i>Citrus</i> spp. (Rutaceae)	Ramírez-Pelayo et al. (2019)	
	Microwaves	Boropinic acid (2)	<i>S. oleracea</i> L, Amaranthaceae <i>L. barbarum</i> L., Solanaceae <i>C. quinoa</i> Willd, Amaranthaceae	Fiorito et al. (2019c)
		4'-Geranyloxyferulic acid (3)	<i>S. oleracea</i> L, Amaranthaceae <i>L. barbarum</i> L., Solanaceae <i>C. quinoa</i> Willd, Amaranthaceae	Fiorito et al. (2019c)
		7-Isopentenylxycoumarin (4)	<i>S. oleracea</i> L, Amaranthaceae <i>L. barbarum</i> L., Solanaceae <i>C. quinoa</i> Willd, Amaranthaceae	Fiorito et al. (2019c)
		Auraptene (5)	<i>P. granatum</i> L. (Lythraceae) <i>S. oleracea</i> L, Amaranthaceae <i>L. barbarum</i> L., Solanaceae <i>C. quinoa</i> Willd, Amaranthaceae	Fiorito et al. (2019b) Fiorito et al. (2019c)
Umbelliprenin (6)		<i>A. graveolens</i> L. (Apiaceae) <i>P. anisum</i> L. (Apiaceae) <i>A. archangelica</i> L. (Apiaceae) <i>P. granatum</i> L. (Lythraceae) <i>S. oleracea</i> L, Amaranthaceae <i>L. barbarum</i> L., Solanaceae <i>C. quinoa</i> Willd, Amaranthaceae	Taddeo et al. (2017) Fiorito et al. (2019b) Fiorito et al. (2019c)	

**Table 1** continued

Extraction method	Phytochemicals	Source	Ref.	
Deep eutectic solvents	7-Isopentenylcoumarin (4)	Olive oil Soy oil Peanuts oil Corn oil Sunflower oil	Ferrone et al. (2018)	
	Auraptene (5)	Olive oil Soy oil Peanuts oil Corn oil Sunflower oil	Ferrone et al. (2018)	
QuEChERS	Bergamottin (8)	<i>C. paradisi</i>	Lee et al. (2016)	
	6,7 Dihydrobergamottin (10)	<i>C. paradisi</i>	Lee et al. (2016)	
	Epoxybergamottin (11)	<i>C. paradisi</i>	Lee et al. (2016)	
	Oxyprenylated furanocoumarins	Apiaceae species	Cook et al. (2017)	
Supercritical CO <sub>2</sub>	Imperatorin (1)	<i>A. archangelica</i> <i>C. paradisi</i> <i>C. monnieri</i> (L.) Cusson (Apiaceae) <i>P. corylifolia</i> L. (Fabaceae)	Woźniak et al. (2020)	
		Bergamottin (8)	<i>A. archangelica</i> <i>C. paradisi</i> <i>C. monnieri</i> (L.) Cusson (Apiaceae) <i>P. corylifolia</i> L. (Fabaceae)	Woźniak et al. (2020)
		6,7 Dihydrobergamottin (10)	<i>A. archangelica</i> <i>C. paradisi</i> <i>C. monnieri</i> (L.) Cusson (Apiaceae) <i>P. corylifolia</i> L. (Fabaceae)	Woźniak et al. (2020)
Subcritical butane	Boropinic acid (2)	<i>C. paradisi</i>	Genovese et al. (2020)	
	4'-Geranyloxyferulic acid (3)	<i>C. paradisi</i>	Genovese et al. (2020)	
	7-Isopentenylcoumarin (4)	<i>C. paradisi</i>	Genovese et al. (2020)	
	Auraptene (5)	<i>C. paradisi</i>	Genovese et al. (2020)	
	Umbelliprenin (6)	<i>C. paradisi</i>	Genovese et al. (2020)	
Accelerated solvent extraction	Imperatorin (1)	<i>A. officinalis</i> L. (Apiaceae)	Waksmundzka-Hajnos et al. (2004a)	
		<i>P. sativa</i> L. (Apiaceae)	Waksmundzka-Hajnos et al. (2004b)	
		<i>Heracleum leskowi</i> L. (Apiaceae)	Skalicka-Wozniak and Głowniak (2012)	
Extractions in the heterogeneous phase	Boropinic acid (2)	<i>M. alternifolia</i> (Maiden and Betche) Cheel (Myrtaceae)	Scotti et al. (2018)	
	4'-Geranyloxyferulic acid (3)		Scotti et al. (2018)	
	7-Isopentenylcoumarin (4)		Scotti et al. (2018)	
	Auraptene (5)	<i>M. alternifolia</i> (Maiden and Betche) Cheel (Myrtaceae) <i>C. limon</i> L. Osbeck (Rutaceae)	Scotti et al. (2018) Fiorito et al. (2021)	
	Umbelliprenin (6)		Scotti et al. (2018)	

with differently chemically functionalized solid sorbents is a very promising field of research.

## Conclusions

The aim of this review comprehensive review article was to gather literature data pointing out the application of non-conventional techniques for the extraction of biologically active naturally occurring oxyprenylated phenylpropanoids.

A survey of the data outlined in the present manuscript is given in Table 1, in which we listed all the extraction methods and the phytochemicals that were obtained in the best yield and selectivity after its application.

The search for more effective and powerful processes to this context is a field of more and more growing interest, as such a group of plant active principles is emerging as a class of natural products claimed to exert valuable pharmacological effects with a great potential for future chemopreventive and therapeutic applications. The application of the title techniques is moving nowadays through the direction of obtaining increased extractive yields from the respective vegetable sources and more recently to have more selectivity, as it was put in evidence to a certain extent by the use of solid sorbents like lamellar solids. Thus, the future research activities should be addressed towards two main ways: first, the application of already reported methodologies, like those described herein, to other subclasses of oxyprenylated secondary metabolites (e.g. *O*-prenylpolyketides, *O*-prenylalkaloids, *O*-prenylxanthones, and *O*-prenylflavonoids) deriving also from hitherto unexplored plant matrices in order to test the overall performance and efficiency of individual processes, second the establishment of novel methods tailored on the specific structure of oxyprenylated derivatives. A very good example to this aim could be setting up a molecularly imprinted polymer-based methodologies. Such a technique is a relatively recent one (Kamaruzaman et al. 2021) and this technique is relatively new and has the undoubted advantage of being able to extract specific chemical structures with a high degree of selectivity, since the stationary phase is designed on the basis of the latter.

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