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Compressed fluids and phytochemical profiling tools to obtain and characterize antiviral and anti-inflammatory compounds from natural sources[☆]

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ABSTRACT

Many natural compounds, found mainly in plants, are associated with the treatment of various diseases. The search for natural therapeutic agents includes compounds with antiviral and anti-inflammatory activities. Among the many steps involved in bioprospection, extraction is the first and most critical step for obtaining bioactive compounds. One of the main advantages of using compressed fluids extraction is the high quality of the final product obtained due to the use of green solvents, while the selectivity towards target compounds can be tuned by adjusting the process parameters, especially pressure, temperature and solvent characteristics. In this review, a discussion is provided on the power of compressed fluids, such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) and subcritical water extraction (SWE) to obtain antiviral and anti-inflammatory compounds from natural sources. In addition, an adequate knowledge about the identity and quantity of the compounds present in the extract is essential to correlate biological activity with chemical composition. Phytochemical profiling tools used for identification and quantification of these bioactive natural compound are also discussed. It can be anticipated that after the current SARS-CoV-2 pandemic, the search of new natural compounds with antiviral and anti-inflammatory activity will be a hot research topic, so, this review provides an overview on the technologies currently used that could help this research.

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1. Introduction

In nature, there is a wide variety of plant-derived compounds with different biological activities, including antiviral and anti-inflammatory. Bioprospection can be defined as “*the methodical search for novel pharmaceutical (and other) products from living beings*”. The search for natural compounds against viral infections is a very hot topic due to the large number of related diseases and the

ease of viral spread [1–3]. This problem is even more evident now due to the SARS-CoV-2 pandemic. On the other hand, the search for natural anti-inflammatory compounds is also an important issue, since inflammation is suggested to play a crucial role in many diseases, including viral infections [4,5]; in fact, the infection mediated by SARS-CoV-2 has strongly been associated with several inflammatory processes.

Polyphenols, terpenes, fatty acids and polysaccharides are some types of natural compounds that have been tested as antiviral and anti-inflammatory agents. These compounds are usually present in spices, flowers and leaves of many plants and herbal species used in traditional medicine in many countries. In addition, interesting compounds can be obtained from food, agricultural by-products and marine sources (e.g., seaweeds and microalgae) [6]. The evaluation of natural compounds as therapeutic agents is not intended to replace synthetic drugs, but to alleviate their use, attenuate the toxic side-effects and to help to develop new candidates of future

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pharmaceuticals [1,7,8]. However, extraction, identification and *in vitro* or *in vivo* confirmation of their activity are challenges that need to be overcome for the development and use of these natural bioactive compounds.

The first question raised is the technology needed to obtain these compounds, standardize the product and make the process scalable. In this sense, it is important to consider the actual need for using sustainable techniques and solvents, able to comply with the Green Chemistry and Green Engineering principles [9]. Among the different techniques that can be employed, those based on compressed fluids fulfill these requirements and have already been used to extract target bioactive compounds from different biomasses [6,10]. Techniques such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) and subcritical water extraction (SWE), stand out from conventional techniques due to the use of non-toxic solvents (or minimizing its amount), short extraction times and highly tunable selectivity, improving the quality of extracts and valuing applications as therapeutic agents.

Once the natural extract is obtained using the mentioned green extraction processes and its bioactivity is confirmed using *in vitro* or *in vivo* assays, an in depth study of its chemical composition is mandatory, frequently facing the challenge of identifying and elucidating the structure of compounds many times new and unknown. For this reason, the analytical identification and quantification of compounds are essential tasks that complement the extraction step. Chromatographic techniques coupled to (tandem) high resolution mass spectrometry are widely employed due to its capacity to identify the structure, composition and concentration of compounds in a very fast and sensitive way [11]. Interestingly, once the phytochemical profile of the extracts is obtained, the analytical tool selected can assist in improving the extraction focusing on the target bioactive compound(s).

In this review, we discuss the applicability and highlight the power of compressed fluids as an alternative to classical extraction methods to obtain natural compounds with viral and inflammatory properties. Besides, the main analytical techniques used for their adequate phytochemical profiling are also discussed (see Fig. 1).

2. Natural compounds with antiviral and anti-inflammatory activity

2.1. Antiviral activity

Plants have been studied as natural sources of antiviral compounds for treating different viruses, such as herpes, influenza and hepatitis, among others [3]. Recently, Zhang et al. [12] through an *in silico* study, reported 13 natural compounds found in 26 Chinese herbs commonly used to treat viral respiratory infections, as potentially active to treat Covid-19; these compounds have been confirmed to directly inhibit important proteins in SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome) and, considering the genetic similarities between SARS and MERS coronavirus and the new SARS-CoV-2, it is expected that they could be also effective against the new coronavirus. This type of studies opens a horizon for technologies capable of selectively extracting these compounds, such as compressed fluids techniques. It is expected that, in addition to the domestic use of medicinal herbs, obtaining the compounds through a standardized process will facilitate the development of vaccines, adjuvants and/or drugs. In this section we present the studies on the antiviral activity of compounds obtained by compressed fluids found in literature. An overview of the natural matrices used as sources of compounds, the class of compounds obtained, the extraction technique and conditions applied, as well as the analytical technique used for the phytochemical characterization is provided in Table 1. All these studies employed *in vitro* approaches to confirm the antiviral bioactivity. The antiviral biological mechanisms of these compounds are out of the scope of this work.

Herpes simplex virus (HSV) affects adults and children, there is no cure or vaccine and, currently, there is an urgent need to develop new treatments due to the appearance of strains resistant to acyclovir, the drug most frequently used to reduce the infection time and pain produced by HSV [2]. According to data in Table 1, terpenes extracted by compressed fluids are the class of compounds most studied as antivirals. Monoterpenes as camphor, borneol and 1,8-cineole found in the supercritical extracts of sage (*Salvia officinalis*), and carvacrol and thymol from oregano

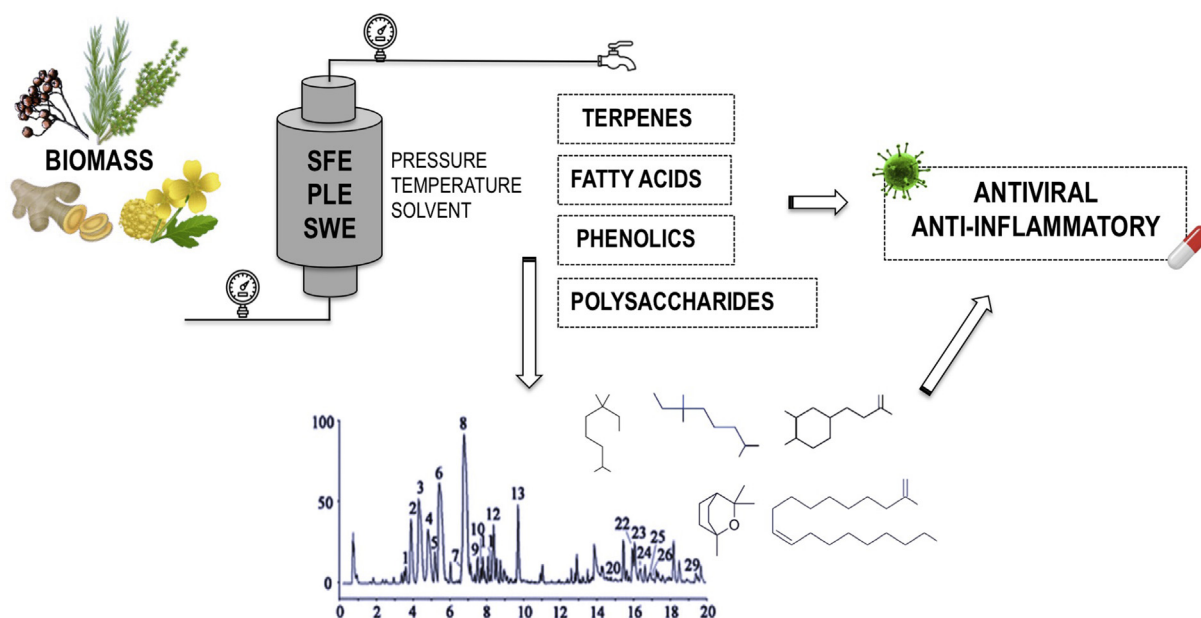


Fig. 1. Schematic representation of the steps involved in obtaining antiviral and anti-inflammatory compounds.

Table 1
Antiviral activity of natural compounds obtained by compressed fluids (all of them assayed *in vitro*).

Natural source	Technique	Conditions	Compounds	Analytical characterization	Virus	Remarks	Ref.
<i>Melia azedarach</i> (fruits)	SFE	30 MPa, 50°C; 1.65 g/min	Fatty acids (linoleic, palmitic and myristic)	GC-MS (ESI +/-)-TOF-MS	Herpes simplex and Bovine Diarrhea Virus	Inhibition of virus replication > 94%	[15]
	SFE	30 MPa, 50°C 30% EtOH	Phenolics (caffeic acid and malic acid)				
<i>Oregano (Origanum vulgare)</i>	SFE	30 MPa, 40°C, 50 g/min	Terpenes (carvacrol and thymol)	GC-MS	Herpes simplex	Significant inhibition of virus replication. IC ₅₀ = 1.88 µg/mL IC ₅₀ = 5.33 µg/mL	[13]
<i>Sage (Salvia officinalis)</i>	SFE		Terpenes (borneol, camphor, and 1,8-cineole)	GC-MS			
Thyme species	SFE	30 MPa, 40°C, 50 g/min	Terpenes (thymol, carvacrol and borneol)	GC-MS	Herpes simplex	IC ₅₀ = 1.74–2.25 µg/mL	[14]
Tangerine pericarp (Guangchenpi)	SFE	25 MPa, 35°C for 2 h, 20L/h	Flavonoids (tangeretin and nobiletin)	HPLC-PDA	Respiratory syncytial virus (RSV)	Potent activity comparable to the positive control, ribavirin	[20]
Heather leaves (<i>Calluna vulgaris</i>)	SFE	30 MPa, 50°C for 3 h, 60 g/min, 15% EtOH	Terpenes (oleanolic and ursolic acid)	HPLC-UV	Hepatitis C	Superior activity for higher contents of oleanolic and ursolic acids in the extracts	[49]
<i>Cinnamomi ramulus</i> (herb)	SFE	Not informed	Flavonoid (cinnamaldehyde)	GC-MS	Herpes simplex Respiratory syncytial virus (RSV)	SFE extract was more efficient than water and ethanol extracts. IC ₅₀ = 56.55 e 40.37 µg/mL	[16]
<i>Centipeda minima</i> (whole herb)	SFE	20 MPa at 40°C for 2 h, 20 L/h	Terpene (pseudoguaianolide)	HPLC ESI-HRMS, UV, IR, and ¹ H, ¹³ C NMR	Influenza A (H1N1)	Better activity than the positive control ribavirin.	[22]
<i>Chlorella vulgaris</i> (seaweeds)	PLE	10 MPa, 150°C for 20 min, EtOH	Terpene (phytol)	GC-MS	Herpes simplex	Cells pretreated with 75 µg/mL before adding the virus inhibited 70% of the virus infection. IC ₅₀ = 80.23 µg/mL	[17]
<i>Haematococcus pluvialis</i> (microalgae)	PLE	10 MPa, 100°C for 20 min, EtOH	Fatty acids	GC-MS	Herpes simplex	IC ₅₀ = 99.59 µg/mL	[19]
<i>Dunaliella salina</i> (microalgae)	PLE	10 MPa, 160°C for 15 min, EtOH	Terpenes (naptalene, β-ionone, neophytadiene)	GC-MS	Herpes simplex	IC ₅₀ = 152.73 µg/mL	[19]
<i>Himanthalia elongate</i> (seaweeds)	PLE	10 MPa, 100°C for 20 min, EtOH	Fucosterol and palmitic acid	GC-MS	Herpes simplex	Effective only when added before the virus, simultaneously with the virus or immediately after viral infection. IC ₅₀ = 83.24 µg/mL IC ₅₀ = 59.07 µg/mL	[18]
<i>Himanthalia elongate</i> (seaweeds)	SWE	10 MPa, 100°C for 20 min	Polysaccharide (fucoindans)	GC-MS	Herpes simplex		[18]
<i>Chlorella vulgaris</i> (seaweeds)	SWE	10 MPa, 150°C for 20 min, water	Polysaccharide (β-glucan)	GC-MS	Herpes simplex	Cells pretreated with 75 µg/mL before adding the virus inhibited 90% of the virus infection. IC ₅₀ = 33.93 µg/mL IC ₅₀ = 98.61 µg/mL	[17]
<i>Haematococcus pluvialis</i> (microalgae)	SWE	10 MPa, 100°C for 20 min	Polysaccharide (mannan)	GC-MS	Herpes simplex		[19]
<i>Dunaliella salina</i> (microalgae)	SWE	10 MPa, 160°C for 15 min	Polysaccharide (β-glucan)	GC-MS		IC ₅₀ = 85.34 µg/mL	[19]
<i>Brassica juncea</i> (brown mustard)	SWE	SWE – 110°C for 10 min	Brassinosteroids	–	Influenza A (H1N1)	50.35% antiviral activity at maximum nontoxic concentration (0.5 mg/mL)	[21]
<i>Nizamuddiniana zanardinii</i> (Brown alga)	SWE	SWE -150°C for 10 min	Polysaccharide (fucoindans)	GC-MS	Herpes simplex	Activity similar to the positive control, acyclovir. IC ₅₀ = 0.607 µg/mL	[46]

IC₅₀: concentration to reduce the viral activity in 50%; CTD₁₀: non-toxic concentration resulting in cell survival of 90%.

(*Origanum vulgare*) and thyme (*Thymus vulgaris*) extracts were pointed out as responsible for the antiviral activity against HSV [13,14]. This virus has also demonstrated to be sensitive to fatty acids, mainly linoleic acid extracted from *Melia azedarach* fruits [15]; and cinnamaldehyde, a flavonoid extracted from *Cinnamomi ramulus* [16].

Polysaccharides are another class of natural compounds, which have been highlighted for their effective antiviral activity against various viruses. In Table 1, we can see that algae and microalgae are the most common sources of antiviral polysaccharides [17–19], and HSV has been the target virus in these studies. However, a common

conclusion in all these studies was that the antiviral activity was higher when the host cells were pretreated with polysaccharides before the virus infection. In this sense, including sub- and super-critical extracts in normal diets may allow to boost the immune response. Therefore, the effects of future viral infections may be lower, reducing the spread of the outbreak by considering these polysaccharides pretreatment.

One of the main diseases triggered by a virus is acute respiratory infection, especially influenza viruses. Langeder et al. [1] reported that flavonoids represent the most important class of compounds studied for treating this type of viruses. Few studies have been

found in literature dealing with the antiviral activity of compounds obtained by compressed fluids in acute respiratory infections. The supercritical extract rich in flavones, tangeretin and nobiletin, extracted from the tangerine pericarp [20], as well as, cinnamaldehyde from *Cinnamomi ramulus* [16], showed antiviral activity against the respiratory syncytial virus, an infection that mainly affects babies and children. In addition, antiviral activity against influenza A (H1N1) was attributed to the presence of brassinosteroids found in brown mustard [21], and brevilin A, a natural sesquiterpene lactone extracted from the herb *Centipeda minima*, which interestingly exhibited better activity than positive control ribavirin [22], giving a clear idea about the great possibilities of these antiviral natural compounds.

2.2. Anti-inflammatory activity

Terpenes, fatty acids and phenolics represent the classes of compounds extracted by compressed fluids already tested for anti-inflammatory disorders. In general, the anti-inflammatory activity of these compounds is related to the inhibition of pro-inflammatory pathways associated to many diseases. The overview of anti-inflammatory compounds extracted by SFE is provided in Table 2. The compounds extracted by PLE and SWE with anti-inflammatory activity are presented in Table 3. The data provide information on the natural source and the class of compounds obtained responsible of the anti-inflammatory activity. The anti-inflammatory biological mechanisms of these compounds are out of the scope of this work.

Terpenes are the compounds most frequently studied in extracts from compressed fluids with anti-inflammatory activity. These compounds are widely distributed in many plants, mainly in aerial parts (leaves and stems) and underground parts (roots). The anti-inflammatory potential of terpenes were described by Hortelano et al. [23], revealing that these compounds can be promising natural therapeutic agents. As can be seen in Table 2, terpenes from turmeric species (*Curcuma* sp.) were strongly associated with anti-inflammatory activity, both *in vitro* and *in vivo* studies. *In vitro*, terpenes were assayed as inhibitors of lipoxygenase and proteinase [24,25], key enzymes of inflammatory pathways. *In vivo*, the therapeutic effect of turmeric compounds was demonstrated against periodontitis [26] and paw edema in rats [25], which was related to the synergistic action of curcuminoids, phenolic acids and volatiles present in the extract.

Camphor, borneol, phytol and eucalyptol from the edible flower *Chrysanthemum indicum* also showed potential anti-inflammatory action against acute lung injury in mice [27], a clinical disease with excessive inflammatory response and high mortality rates, and against pulmonary inflammation caused by the anti-tumor drug used to treat rats with hepatoma tumor [8]. Additionally, the effectiveness of *C. indicum* compounds against age-related brain and liver injury was demonstrated by the synergistic action of terpenes, flavonoids and phenolic acids in response to the production of inflammatory mediators [28].

Anti-inflammatory activities against gastrointestinal disorders were also reported for fatty acids extracted from *Bidens pilosa* [29] and phytosterols of *Physalis angulate* [30], which attenuated the inflammation symptoms when used as preventive treatment. Other compounds such as *Z*-ligustilide (phtalide) and 6-gingerol (polyphenol) extracted from a mixture of ginseng and ginger (*Angelica sinensis* and *Zingiber officinal*) also relieved intestinal inflammation in rat models [31]. Finally, the *in vitro* gastrointestinal release of γ -oryzanol, a terpene obtained from rice bran, exhibited a potent anti-inflammatory activity, which helped to prevent colorectal cancer [32].

Among the phenolic compounds, those belonging to the groups of catechol derivatives, coumarins, and flavonoids were considered the best anti-inflammatory compounds, based on their structure, molecular weight, polarity, among other physicochemical properties [7]. Although phenolic compounds have already been presented as an alternative to the treatment of inflammation [33], few studies have assayed the anti-inflammatory potential of phenolic compounds extracted by compressed fluids as shown in Tables 2 and 3. Flavonoids (catechin, quercetin and resveratrol) and the sesquiterpene (caryophyllene oxide) obtained from *Leptocarpha rivularis* stalks, exhibited an inhibitory effect against to inflammatory disorders caused by diabetes mellitus type 2 [34]. Chlorogenic acid (phenolic acid) and luteolin-7-O-glucoside (flavonoid), extracted from flowers of *Lonicera japonica* [35], together with phenolics, lutein and β -carotene (carotenoids) extracted from spinach, were associated with suppression of inflammatory effects in macrophage cells [36]. The high content of resveratrol (stilbene) and phenolic compounds (phenolic acids and flavonoids) of grape stems attenuated the inflammatory activity in an atherosclerotic environment model [37]. However, oxyresveratrol present in *Morus alba* branches showed greater activity than resveratrol in inhibiting inflammation in a leukocyte migration model [38]. Isorhamnetin and kaempferol from seabuckthorn (*Hippophae rhamnoides*) leaves showed effective activity against pro-inflammatory cytokines produced in response to Tetanus and Diphtheria toxoids, infectious diseases caused by bacteria [39]. The authors suggested that these compounds could be used as a potential vaccine adjuvant.

Collectively, these findings corroborate the antiviral and anti-inflammatory potential of bioactive compounds obtained from different natural sources extracted using compressed fluids' techniques. In the following section, we will discuss the applicability of compressed fluids in the extraction of the above mentioned compounds.

3. Green extraction techniques for obtaining antiviral and anti-inflammatory compounds

Green extraction is a term derived from Green Chemistry, whose 12 principles were suggested by Anastas and Warner [9]. In summary, the principles set out the ways in which chemical products and processes can be more sustainable. In this context, Chemat et al. [40] define the green extraction of natural products as based on processes with low energy consumption, which allow the use of alternative solvents or non-toxic solvents, renewable raw material, and yield safe and high-quality extracts/products without contaminants. The compressed fluids techniques can satisfy many of the green extraction principles, depending on the solvents and conditions used for extracting the natural compounds.

Compressed fluids extraction techniques operate at medium to high pressures. Temperature and pressure are the main variables of these processes, which can change the physicochemical properties of the solvent. Depending on the technique (SFE, PLE, or SWE), the solvent can be in sub- or supercritical conditions. SFE mainly uses CO₂ as extracting solvent (supercritical CO₂, SC-CO₂), because of its mild supercritical conditions (31.2°C and 7.38 MPa) that allows extracting at low temperatures. Other benefits associated to the use of CO₂ are its low cost, non-flammability and GRAS (Generally Recognized As Safe) status. Moreover, after extraction CO₂ is released as gas (which can be recycled) and therefore, no solvent residues are found in the extract or in the unextracted matrix that can be further used or processed. Co-solvents can be used to assist in the extraction of compounds with low affinity for CO₂ (compounds of medium and high polarity). If the amount of co-solvent is high enough to form two phases or one phase above the bubble point curve, but below the

Table 2
Natural compounds with anti-inflammatory activity obtained by SFE.

Natural source	Extraction Conditions	Compounds	Analytical characterization	Type of study	Remarks	Ref.
<i>Aloysia gratissima</i> (leaves)	20 MPa, 60°C for 120 min, 2 g/min	Terpenes (guaiol, pinocampnone, caryophyllene oxide and spathulenol)	GC-MS	<i>In vivo</i>	Significant reduction of mice paw edema up to 3 h after injury induction (10 mg/kg)	[50]
<i>Bidens pilosa</i> (aerial parts)	30 MPa, 40°C, 5 L/min	Fatty acids (palmitic, oleic, linoleic, linolenic)	GC-MS	<i>In vivo</i>	Effective in preventing or treating intestinal inflammation in rats (25–100 mg/kg)	[29]
Carvi seeds (<i>Carum carvi</i>)	20 MPa, 40°C, 14 mL/min for 180 min	Fatty acids, sterols (β -Sitosterol) and polyphenols	GC-FID GC-MS	<i>In vitro</i>	Strong anti-inflammatory activity compared to conventional extraction. IC ₅₀ = 24. μ g/mL	[59]
<i>Arctium lappa</i> (leaves)	9 MPa and 40°C	Terpenes (limonene, carvone)	GC-FID GC-MS	<i>In vivo</i>	Gastroprotective effects in rats (300 mg/kg)	[58]
	20 MPa, 60°C for 40 min	Terpenes (lupeol acetate, phytol and α -amyrin)	GC-MS	<i>In vitro</i>	Co-solvent improved the extraction yield, as well as the inhibitory activity. IC ₅₀ = 74.69 μ g/mL	[51]
Turmeric (<i>Curcuma</i> sp.)	40 MPa, 45°C, 2 mL/min, 20% isopropanol for 60 min	Terpenes (Isborneol, curdione, vellaral, procurcumiadiol, germacrone)	LC-Q-TOF-MS, ESI(+) GC-Q-TOF-MS, EI	<i>In vitro</i>	Co-solvent increased lipoxygenase inhibition in 62.5–86.55%	[24]
	35 MPa, 65°C, 2 mL/min for 150 min, 30% EtOH	Terpenes (curcuminoids) and phenolics	LC-Q-TOF-MS, ESI(+)	<i>In vivo</i>	The specie with higher amount of curcuminoids showed lower activity due to limited bioavailability; 1 g/kg reduced 70% of the inflammation in rats	[25]
	40 MPa, 50°C, 3.2 L/min	Terpene (xanthorrhizol)	–	<i>In vivo</i>	Attenuation of periodontal inflammation in rats (30–100 mg/kg/day)	[26]
Neem leaves (<i>Azadirachta indica</i>)	Not informed (Nisarga Ltd., Satara, Maharashtra, India)	Terpenes	–	<i>In vitro</i>	Reduced inflammation associated with colon cancer	[70]
				<i>In vivo</i>	Decreased the oral cancer cell proliferation in rats (200 mg/kg)	[71]
<i>Angelica sinensis</i> and <i>Zingiber officinal</i> (roots) (7:4 w/w)	8 MPa, 55°C, 25 L/h,	Phthalide (ligustilide) Phenolic (6-gingerol)	GC-MS HPLC-UV	<i>In vivo</i>	Ameliorated colitis symptoms by decreasing oxidative stress and suppressing inflammatory mediators in rats (60 mg/kg)	[31]
Ginger (<i>Zingiber officinale</i>)	22.7 MPa, 50°C for 3 h	Not identified	–	<i>In vivo</i>	Rat paw edema decreased by 24.66% (25 mg/kg)	[72]
Pepper (fruits)	20 MPa, 50°C, for 1 h	Amide, terpenes, olefins and ester	HPLC-PDA, UV GC-TQ-MS	<i>In vitro</i>	Anti-inflammatory and antitumor effects were better than the reflux and ultrasonic ethanol extracts. IC ₅₀ = 4.19 μ g/mL	[67]
	10–30 MPa, 40°C, 2 L/min for 60 min	Alkaloid (Piperine)	HPLC-UV	<i>In vitro</i>	Activities comparable with ascorbic acid and diclofenac sodium (200 μ g g/mL)	[73]
White pepper, long pepper, cinnamon, saffron and myrrh mixture	30 MPa, 50°C for 2 h	Terpenes (β -selinene, aromadendrene, β -elemene, cis-piperitol)	GC-MS	<i>In vivo</i>	Inhibition of peripheral inflammatory pain in rats (62.5–1000 mg/kg)	[45]
<i>Valeriana glechomifolia</i> (aerial and subterraneous parts)	9 MPa, 40°C, 6.67 $\times 10^{-4}$ kg/s.	Terpenes (valtrate, acevaltrate)	HPLC ¹ H and ¹³ C NMR	<i>In vivo</i>	The activity was comparable or better than the positive control in the treatment of inflammation induced in rats (1–30 mg/kg)	[74]
<i>Achillea millefolium</i> (leaves)	Fractionation of ultrasound-assisted extract – 15 MPa, 40°C	Terpenes (camphor, artemisia ketone and borneol)	HPLC-QTOF-MS, ESI (–) GC-MS	<i>In vitro</i>	The higher the concentration of terpenes, the greater the anti-inflammatory activity.	[44]
<i>Leptocarpha rivularis</i> (stalks)	40 MPa, 60°C, 3.7–4.6 L/min	Terpene (caryophyllene oxide) Phenolics (quercetin, kaempferol and resveratrol)	HPLC-DAD GC-FID	<i>In vitro</i>	The highest activity was obtained with the presence of co-solvent. IC ₅₀ = 2.7 mg/mL α -glucosidase; IC ₅₀ = 15.1 mg/mL α -amylase	[34]

(continued on next page)

Table 2 (continued)

Natural source	Extraction Conditions	Compounds	Analytical characterization	Type of study	Remarks	Ref.
<i>Acanthus ilicifolius</i> Linn (leaves)	Not informed	Alkaloid (2-benzoxazolinone)	HPTLC-UV	<i>In vivo</i>	Oral safety and anti-inflammatory activity were assayed (>2000 mg/kg)	[66]
Leaves and stems of cipó-puçá	40 MPa, 40°C, and 10% EtOH, 4.52 g/min	Terpenes, phenolic compounds, flavonoid	HPTLC-UV	<i>In vivo</i>	Anti-inflammatory response in the central nervous system of rats	[65]
Rice bran	45 MPa, 60°C	Terpenes (γ -oryzanol, tocotrienol and tocopherol)	HPLC-UV	<i>In vitro</i>	Potent activity comparable to the positive control in the treatment of inflammatory bowel diseases. IC ₅₀ = 15–28 μ g/mL	[32]
<i>Apium graveolens</i> (seeds)	Separex®, Champigneulles, France	Terpene (Senkyunolide)	UPLC-UV	<i>In vivo</i>	Reduced dandruff formation and soothes the human scalp	[60]
<i>Ligusticum chuanxiong</i>	35 MPa, 60°C, 20 L/h for 3 h	Terpene (ligustilide and senkyunolide)	GC-MS	<i>In vivo</i>	Mitigated liver and kidney injury in D-galactose-treated mice	[52]
<i>Physalis angulata</i> (aerial parts)	30 MPa, 40°C, 5 L/min for 150 min	Phytosterols	–	<i>In vivo</i>	Modulation of pathways and mediators of the intestinal inflammatory response in rats (25–100 mg/kg)	[30]
<i>Pinus densiflora</i> (needle)	30 MPa, 60°C, for 2 h. CO ₂ and ethanol flow rate of 140 and 10 mL/min	Not identified	–	<i>In vitro</i>	Inhibitory effect on the expression of pro-inflammatory mediators	[75]
<i>Perilla frutescens</i> (leaves)	40 MPa, 50°C, 60 mL/min for 3 h	Terpene (isoegomaketone-IK)	HPLC-UV	<i>In vitro</i>	SFE exhibited approximately 10-fold higher IK content and much stronger anti-inflammatory activity compared with ethanol extract	[61]
Brown Seaweed (<i>Undaria pinnatifida</i>)	20 MPa, 45°C, 250 mL/min for 30 min	Fatty acid (palmitic acid)	GC-MS	<i>In vivo</i>	Active against mouse ear inflammation. IC ₅₀ = 87 μ g/ear	[57]
<i>Lonicera japonica</i> (flower buds)	15, 25 and 35 MPa at 45°C for 2 h	Polyphenols (chlorogenic acid and luteolin-7-O-glucoside)	UPLC-ESI-MS/MS GC-MS	<i>In vitro</i>	SFE extracts showed anti-inflammatory activity superior to water and ethanol. IC ₅₀ = 100 μ g/mL	[35]
<i>Litsea japonica</i> (fruit)	30 MPa, 60°C, 60 mL/min for 180 min	Flavonoids and lactones	HPLC-UV	<i>In vitro</i>	Modulation of expression of pro-inflammatory mediators	[62]
<i>Ishige okamurae</i> (alga)	40 MPa, 40°C for 2 h	Fatty acids	GC-FID	<i>In vitro</i>	Anti-inflammatory response in macrophage cells	[76]
Ginger (<i>Zingiber officinale</i>)	30 MPa, 30°C 1.42 \times 10 ⁻⁴ kg/s	Terpenes	GC-MS	<i>In vitro</i>	Inhibition of pro-inflammatory cytokine production	[53]
Rosemary (<i>Rosmarinus officinalis</i>)	25 MPa, 40°C, 1.13 \times 10 ⁻⁴ kg/s	Terpenes	GC-M	<i>In vitro</i>	Inhibition of pro-inflammatory cytokine production	[53]
<i>Thunbergia laurifolia</i> (leaves)	Not identified	Not identified	–	<i>In vivo</i>	Acceleration of burn wound healing in rats treated with gel with 10% extract	[77]
Copaiba leaves (<i>Copaifera</i> sp.)	20 MPa, 60°C, 8.33 \times 10 ⁻⁵ kg/s for 2 h	Not identified	–	<i>In vivo</i>	Neuroprotective effects in stroke induced in rat brains (50 mg/kg)	[78]
<i>Ledum palustre</i> (aerial parts)	9 MPa and 40°C	Terpenes (palustrol, ledol, ascaridole)	GC-MS	<i>In vivo</i>	Inhibition of the hind paw edema in rats (50–80%)	[56]
Marjoram (<i>Origanum majorana</i>)	30 MPa, 40°C, 60 g/min	Terpenes (sabinene hydrate and terpineol)	GC-MS	<i>In vitro</i>	Inhibition of pro-inflammatory cytokine secretion and gene expression (10 μ g/mL)	[54]
Sweet basil (<i>Ocimum basilicum</i>)	30 MPa, 40°C, 60 g/min	Terpenes (Linalool and eugenol)	GC-MS	<i>In vitro</i>	Inhibition of pro-inflammatory cytokine secretion and gene expression (10 μ g/mL)	[55]
Sage (<i>Salvia officinalis</i>)	30 MPa, 40°C, 50 g/min	Terpenes (camphor, borneol, 1,8 cineole)	GC-MS	<i>In vitro</i>	Suppression of pro-inflammatory cytokine production (30 μ g/mL)	[55]
Seabuckthorn leaves (<i>Hippophae rhamnoides</i>)	35 MPa, 60°C, 25% EtOH	Flavonoids (myricetin, isorhamnetin)	HPLC-UV HPTLC	<i>In vivo</i>	Reduction of inflammation in response to tetanus and diphtheria toxoids in rats (100 μ g/rat)	[39]

Table 2 (continued)

Natural source	Extraction Conditions	Compounds	Analytical characterization	Type of study	Remarks	Ref.
	20 MPa, 50°C, 25% EtOH	Flavonoid (isorhamnetin)	HPLC-UV	<i>In vivo</i>	Attenuation of endotoxin-induced sepsis in rats (27 µg/kg)	[63]
	20 MPa, 50°C, 25% EtOH	Flavonoid (isorhamnetin)	HPLC-UV	<i>In vivo</i>	Significant reduction in the induced-arthritis inflammation (270 µg/kg)	[64]
<i>Morus alba</i> (Branches)	Not identified	Phenolic (oxyresveratrol)	GC-MS	<i>In vitro</i>	Inhibition of pro-inflammatory pathways	[38]
<i>Chrysanthemum indicum</i> (flowers)	25 MPa, 45°C, 20 L/h for 4 h	Fatty acids (glyceryl 2-linoleate, glycidyl palmitate, ethyl linoleate and ethyl oleate); Terpenes (camphor, (isoborneol, eucalyptol caryophyllene oxide);	GC-MS HPLC-UV	<i>In vivo</i>	Mitigation of liver and brain injury in rats (100–300 mg/kg)	[28]
	Institute of New Drug Research & Development Guangzhou University of Chinese Medicine	Terpenes (camphor, borneol, eucalyptol, thymol, curcumene)	GC-MS HPLC-UV	<i>In vivo</i>	Attenuation of inflammation pathways associated with tumor activities	[8]
				<i>In vivo</i>	Attenuation of inflammation induced by acute lung injury in mice (120 mg/kg)	[27]
	25 MPa, 45°C, 20 L/h for 4 h	Terpenes (eucalyptol, bornyl acetate, caryophyllen, caryophyllene oxide); Phenolics (thymol, bisabolol oxide); Alkenes (curcumene, verbenol)	GC-MS HPLC-UV	<i>In vivo</i>	Attenuation of edema induced in rats (40 mg/kg)	[68]
	25 MPa, 45°C for 3h	Not identified	–	<i>In vivo</i>	The extract was mixed in a 3:3:1 (w/w) ratio with patchouli oil and turmeric oil. Significant suppression of edema in rats (170 mg/kg)	[79]
Spinach leaves	35 MPa, 40°C, 60 g/min for 6 h	Carotenoids (lutein and β-carotene)	HPLC-UV	<i>In vitro</i>	SFE extract showed higher activity than PLE extract	[36]

EtOH: ethanol; ESI: Electrospray ionization source; EI: Electronic impact ionization source, HPTLC: High-performance thin-layer chromatography.

Table 3
Anti-inflammatory activity of natural compounds obtained by PLE and SWE.

Natural source	Conditions	Compounds	Analytical characterization	Type of study	Remarks	Ref.
Grape stems	PLE - 70% EtOH, 120°C for 10 min	Phenolics (quercetin, catechin, epicatechin, gallic acid, resveratrol)	HPLC-UV	<i>In vitro</i>	Stems showed better activity related to the presence of resveratrol	[37]
Grape seeds	PLE - 75% EtOH, 20°C for 11 min					
Spinach leaves	PLE - 10 MPa, 80°C, 50:50 EtOH:water for 10 min	Phenolic compounds	–	<i>In vitro</i>	PLE extract showed lower activity than SFE extract	[36]
<i>Aureobasidium pullulans</i> (fungus)	SWE - Commercial process Itochu Sugar (Aichi, Japan)	Polysaccharide (β-glucan)	HPLC-RID	<i>In vitro</i>	Low molecular weights fractions increased inhibitory activity	[80]
<i>Citrus unshiu</i> (peel)	SWE - 165°C for 15 min under a pressure below 3.2 MPa	Flavonoid-hesperidin	HPLC-UV	<i>In vitro</i>	SWE extract showed higher anti-inflammatory response compared to hot water and ethanol extractions (10 mg/mL)	[48]

critical composition, the extracting solvent is called gas-expanded liquid (GXL) and mainly uses carbon dioxide to expand the liquid [41]. In fact, by changing only the amount of CO₂ in the solvent, the same system can highly modify its physicochemical properties (such as dielectric constant, transport properties, hydrogen-bonding ability, miscibility and acidity), behaving as switchable solvents at medium-high pressure, as shown in Fig. 2. For further reading on this topic, see Herrero et al. [41].

SFE has some advantages compared to conventional techniques. One of the most interesting is related to the tunability of the solvent. Above the critical conditions of temperature and pressure, the change of the binomial T and P can generate a “new solvent” with different values of solubility, diffusivity and viscosity, modifying its extraction power [42]. PLE (also called Accelerated Solvent Extraction, ASE®, or Pressurized Solvent Extraction) and SWE operate under subcritical conditions, at temperatures above the

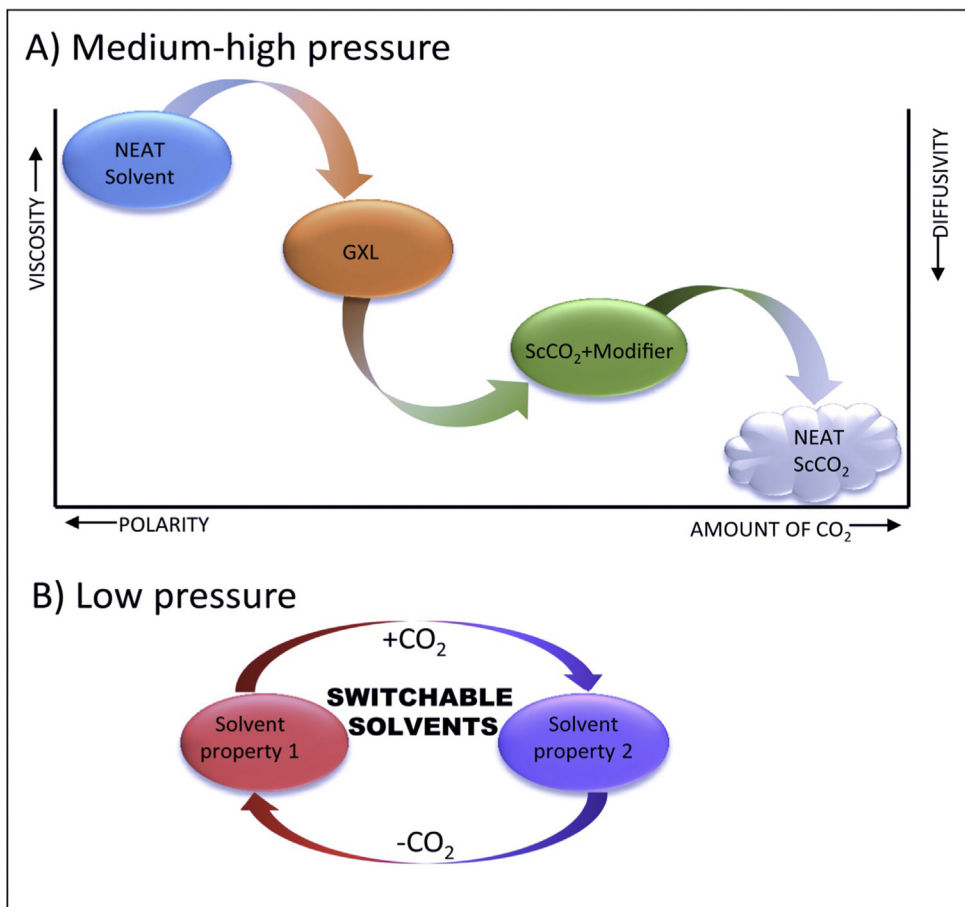


Fig. 2. Systems using carbon dioxide to modify the chemical and/or physical properties of the solvents. A) Carbon dioxide-expanded liquids (CXLs) and B) Switchable Solvents. Reprint from Ref. [41], with permission of Elsevier.

atmospheric boiling point of the solvent, usually ethanol and/or water, and below its critical temperature (and applying a pressure high enough to maintain the solvent in liquid state). In general, non-polar compounds, such as terpenes, fatty acids and sterols are extracted by SFE due to the solvent characteristics, as can be seen in Table 2, and also described in Fig. 3; while polar or semi-polar compounds are preferentially extracted by GXL, PLE and SWE (Table 3). PLE and SWE processes, generally use higher

temperatures, unlikely SFE and GXL. The binomial T and P allows the solvent to remain in a liquid state while varying its physico-chemical characteristics (lower viscosity, higher transport properties) yielding a more powerful extraction. High temperatures combined with high pressures may modify the dielectric constant of the solvent and, therefore, the selectivity of the extracted compounds, in addition to promoting the accelerated effect of mass transfer [43]. The principles and instrumentation of compressed fluid extraction techniques have been very well described in previous reviews, and more information on specific characteristics of extraction conditions can be found in the following references [6,43].

Regarding the applicability of compressed fluids for extraction of antiviral and anti-inflammatory compounds, the major inputs come from SFE, being terpenes the main target family. According to Table 1, the pressures and temperatures used to extract antiviral compounds varied between 20 and 30 MPa and 40–50°C, respectively. Table 2 shows that depending on the matrix, anti-inflammatory terpenes were recovered at SFE conditions ranging from 9 to 40 MPa and temperatures from 30 to 65°C, with different CO₂ flow rate and use or absence of co-solvent. In general terms, monoterpenes are more soluble at lower densities (40–50°C, 8–9 MPa), while hydrocarbonated and oxygenated terpenes are more soluble at higher densities (40–50°C, 10–20 MPa). For the same natural matrix, caraway (*Carum carvi*) seeds, monoterpenes were extracted at 9 MPa and 40°C, while fatty acids and sterol were extracted at 20 MPa and 40°C. That is, by increasing the pressure, compounds with a higher molecular weight and polar functional

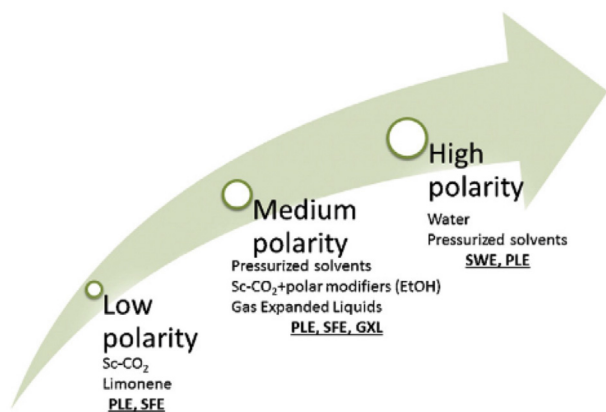


Fig. 3. Green solvents and environmentally friendly technologies used to extract high added-value products from natural sources. Reprint from Ref. [69], with permission of Elsevier.

groups were obtained due to the change in CO₂ density. This same effect was also observed in the extraction of isorhamnetin from seabuckthorn (*Hippophae rhamnoides*) leaves [39]. Increasing the pressure from 30 to 35 MPa resulted in a 23% increase in yield, which can be correlated with the change in the mass transfer and the solvation power of the solvent associated with pressure. The antisolvent power of SC-CO₂ has been also exploited for fractionating an ethanolic extract obtained from the leaves of yarrow (*Achillea millefolium*) [44]. In this work, through a supercritical antisolvent fractionation (SAF), two fractions were obtained containing terpenes (less soluble in the mixture CO₂:EtOH) and flavonoids. Thus, the authors attributed the differences in the anti-inflammatory activity to the class of compounds found in each fraction, being more active, in this case, the terpenes-rich fraction obtained at pressures of 15 MPa.

In general, the non-polar compounds were very effective as antiviral agents when compared to polar compounds, based on their IC₅₀ values. Bitencourt et al. [15], applied a sequential extraction (50°C and 30 MPa) of four steps using CO₂, CO₂:ethanol (70:30 m/m), pure ethanol and ethanol:water (30:70 v/v) to *Melia azedarach* fruit sample. Results showed that only the compounds recovered in the first two steps (fatty acids) were effective against herpes (HSV). Also, SC-CO₂ extracts presented 90% inhibition against Bovine Diarrhea Virus, an effect associated to its content in fatty acids and terpenes. The other fractions were rich in phenolic compounds with no antiviral activity. Zhou et al. [16] tested compounds obtained by SFE, hydrodistillation (HD) and reflux extraction (RE) against HSV and observed more efficient results with SFE extracts due to differences in the chemical profiles. Santoyo et al. [18] evaluated water, hexane and ethanol as solvent for obtaining antiviral compounds by PLE. The ethanolic extract presented more efficient inhibition than hexane and water extracts against HSV. The major compounds were fucosterol and palmitic acid, whose presence was higher in the most active extracts. These examples demonstrated the interest of SFE technology for the obtainment of natural bioactive compounds compared, for instance, to conventional stem distillation and solvent extraction. In this sense, the high temperatures employed for steam distillation can lead to the conversion of terpenes or thermolabile phenolics to less active molecules, reducing the quality of the final extract. Moreover, solvent extraction can contaminate the essential oils [45]. Even if SWE uses also high temperatures, the combination with short extraction times and the absence of oxygen, can selectively concentrate antiviral compounds from natural sources, such as polysaccharides [18,19,46]. In this case, the temperature is more important than pressure to promote the depolymerization of the polysaccharides and favor their solubility due to the reduction of their molecular weight. The range of temperatures for recovery of polysaccharides vary between 120 and 250°C depending on the type and source of biomass [47], while pressure is kept around 10 MPa when dealing with SWE.

Terpenes, fatty acids, phenolic compounds, sterols, flavonoids and carotenoids were the main classes of compounds reported as anti-inflammatory agents obtained by compressed fluids techniques. Again, terpenes, sterols and fatty acids were mainly obtained using supercritical CO₂. The use of ethanol as cosolvent (30% ethanol/70% CO₂) significantly increased the yield of curcuminoids, which are lipophilic phenolic compounds [25]. In that work, authors tested methanol, ethanol, acetone and isopropanol + ethanol as modifiers, and suggested some synergistic effects of ethanol under optimum conditions (35 MPa/65°C with 30% ethanol), such as the increase in the solvent polarity and density by the mixture CO₂:ethanol, improving the solubility of curcuminoids; the swelling of the matrix by ethanol, which improves the mass transfer rate; and the reduction in the viscosity of the solvent [25].

Moreover, these authors optimized the conditions for Enzyme-Assisted Extraction (EAE) coupled to SFE, which resulted in a higher amount of curcuminoids. The combination of enzymes and supercritical fluids resulted in a highly anti-inflammatory extract, whose power was increased by the use of alum as adjuvant.

Compounds like carotenoids, phenolics and flavonoids were extracted from different natural sources using PLE and SWE. For instance, a mixture of water and ethanol (50:50) at 80°C was efficient to recover phenolic compounds from spinach leaves with good anti-inflammatory activity. However, low amounts of carotenoids were found in this extract compared to SFE, presenting both extracts an important anti-inflammatory activity [36]. Phenolic compounds extracted by SWE were also active anti-inflammatory agents. Min et al. [48] evaluated anti-inflammatory activity of citrus peel compounds extracted at 165°C for 15 min and compared with hot water and ethanol extraction. SWE extracts had higher total phenolic and flavonoid contents and better anti-inflammatory activity than those from conventional techniques, showing that biological active compounds can be recovered at high temperatures conditions and short times.

In summary, the extraction conditions of target compounds depend on a series of factors, such as: 1) the knowledge of the biomass/natural source composition; 2) the chemical characteristics of the compound(s); 3) the affinity between the solvent and the compound(s); 4) the solvent physicochemical properties, linked to pressure and temperature changes; 5) the need for biomass pretreatments; among others. It is important to note that it is possible to select some extraction conditions based on the general characteristics of the compound, such as polarity and thermostability, but extraction conditions should be optimized for each natural source and each target compound. The variety of natural sources also evidences the need of more studies that clarify mechanisms of antiviral and anti-inflammatory activities of the target compounds.

4. Phytochemical profiling of natural antiviral and anti-inflammatory compounds

A broad variety of natural compounds, including terpenes/terpenoids, fatty acids, flavonoids, polysaccharides (fucoidans, β -glucan) and some alkaloids (piperine, 2-benzoxazolinone) with demonstrated antiviral and anti-inflammatory activity have been identified in SFE, PLE and SWE extracts from a large variety of natural sources. Considering this chemical diversity, the use of different analytical platforms is frequently required for their characterization, providing complementary analytical information, and increasing the number and reliability of identified phytochemical compounds. Thus, both liquid chromatography (LC) and gas chromatography (GC) platforms hyphenated to different types of detectors, including LC-ultraviolet (UV), LC-mass spectrometry (MS), LC-high resolution (HR)MS, GC-flame ionization detector (FID), GC-MS and GC-HRMS, are commonly used for phytochemical profiling of antiviral and anti-inflammatory compounds, as summarized in Tables 1–3.

Analytical methods based on GC-MS are the most widely reported in literature for the profiling of antiviral agents in compressed fluid extracts. The non-polar nature of SFE extracts makes them particularly suitable for GC analysis. Antiviral agents such as terpenes and terpenols (e.g., δ -cadinene, calamenene, cubenol, 1-epi-cubenol, α -pinene, germacrene, β -caryophyllene, thymol, carvacrol, borneol) are GC amenable compounds that can be analyzed by GC-MS without any derivatization step. Fatty acids such as linoleic, palmitic and myristic acid, obtained in antiviral SFE fractions have been detected by GC-MS as ester derivatives [19] and by direct infusion in the ESI source of a TOF-MS system [15].

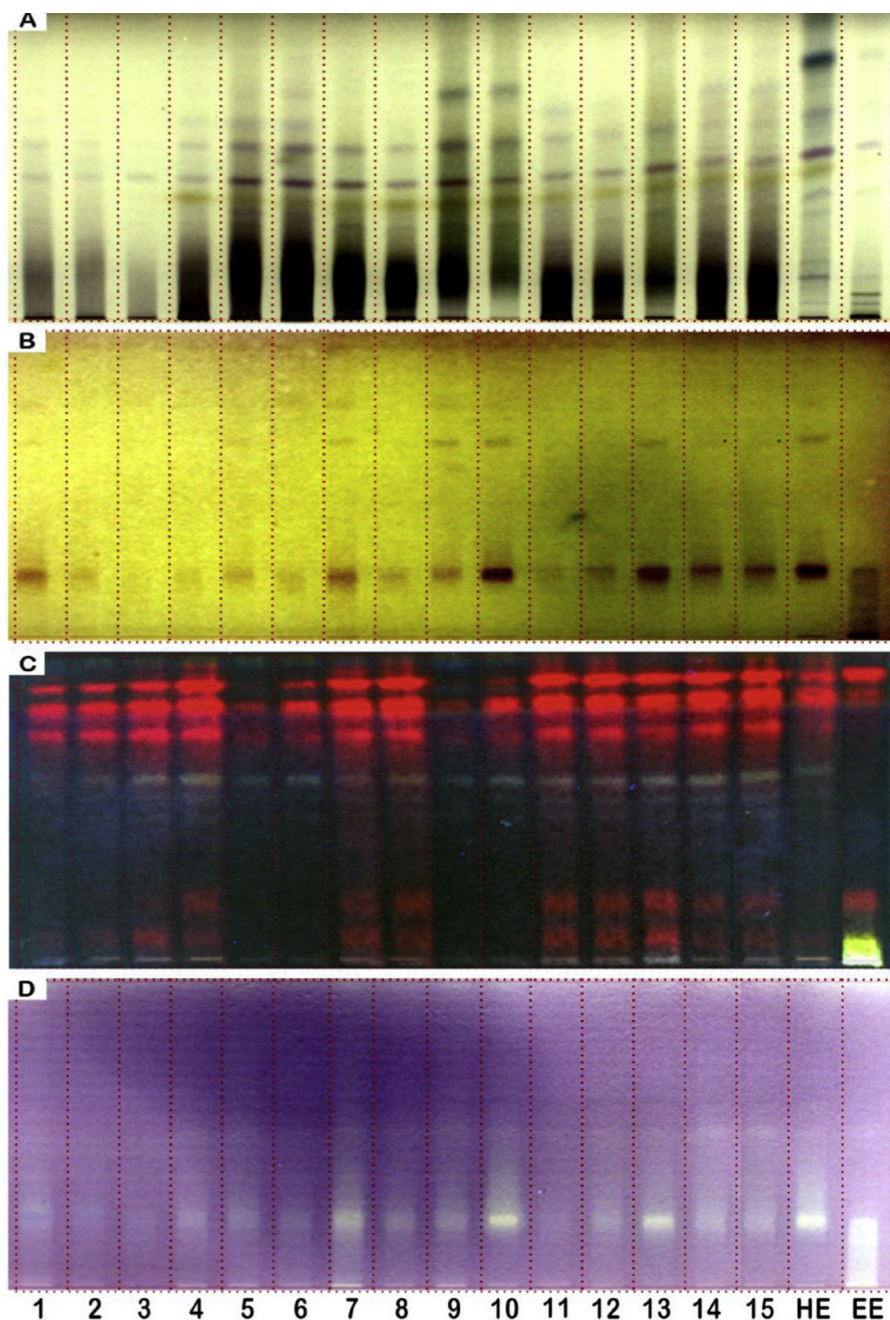


Fig. 4. HPTLC of *C. sicyoides* extracts (assays 1–15) supercritical extracts; (HE) hexane extract; (EE) ethanolic extract; derivatized plates with: (A) VS; (B) FeCl₃; (C) NP/PEG; (D) DPPH; mobile phase: dichloromethane, methanol and formic acid (95:4:1). Reprinted from Ref. [65], with permission from Elsevier.

Antiviral polysaccharides, such as β -glucan and fucoidans, have been determined by GC-MS in SWE extracts after hydrolysis with TFA and subsequent derivatization (e.g., trimethylsilylation) [18,19,46]. In most of these works, separation is normally performed using non-polar HP-5, DB-5 or ZB-5 (5 %-phenyl- 95 %-dimethylpolysiloxane) GC capillary columns, operating with a single quadrupole MS analyzer in full scan acquisition mode, and electronic impact (EI) as the standard ionization mode. Due to the highly reproducible and standardized mass fragmentation pattern obtained by EI, fixing the ionization energy at 70 eV, provides spectral patterns that can be easily and systematically searched in well-established MS databases (e.g. NIST MS database, Fiehn Lib, Wiley, etc.). Additionally, to identify compounds more precisely,

their linear retention indices (RIs) are frequently calculated [11]. For these reasons, GC-MS represents an affordable and powerful tool, considered as the first choice for identification and structure elucidation of unknown volatile and semi-volatile organic compounds in natural extracts.

Alternatively, for the characterization of more polar antiviral terpenoids (oleanolic and ursolic acid) [49], or flavonoids (tangeretin and nobiletin) [49], LC-based approaches were primarily used, in combination with UV detection. Confident structural elucidation of a small group of antiviral sesquiterpene lactones (pseudoguaianolides) were achieved combining HPLC-UV analysis with electrospray ionization (ESI)-HRMS, infrared (IR), and ¹H, ¹³C nuclear magnetic resonance (NMR) spectral information [22].

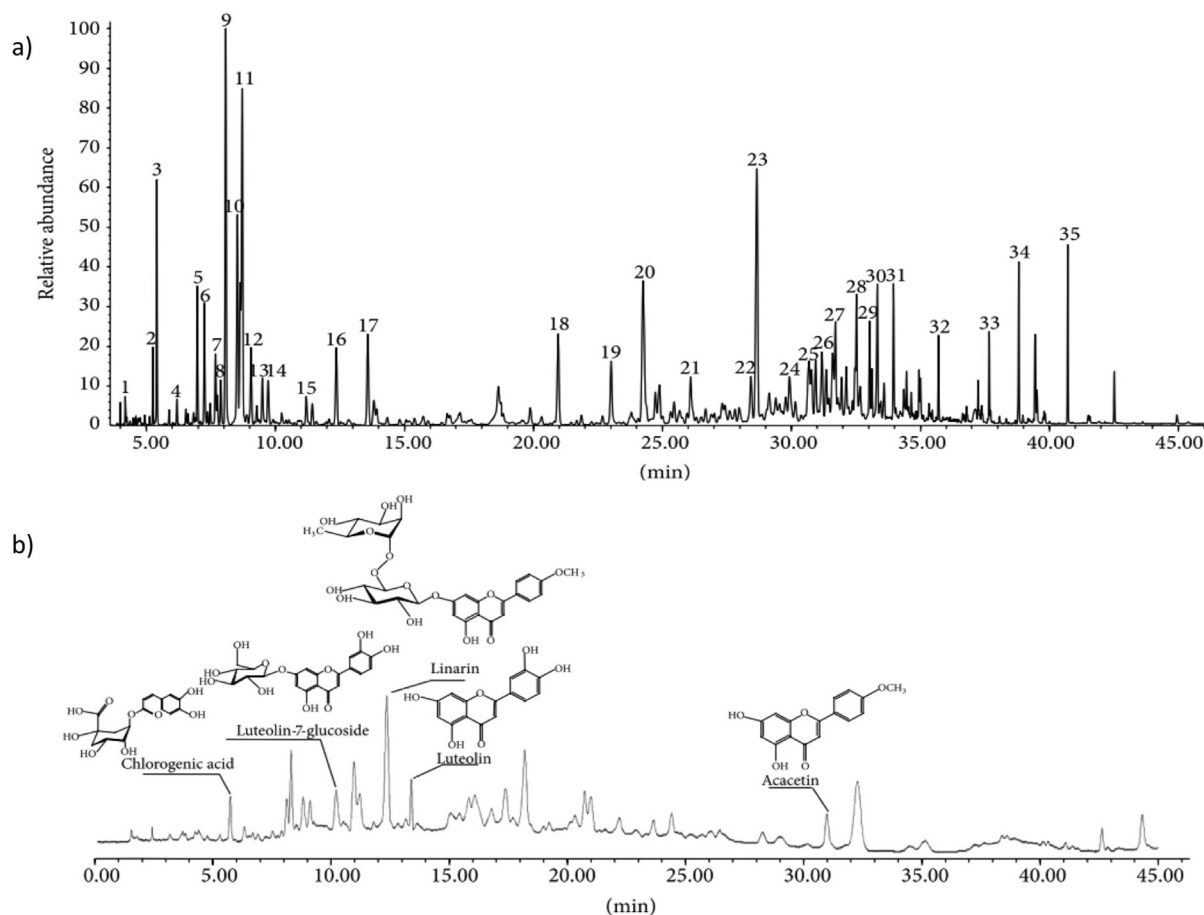


Fig. 5. GC-MS and HPLC chromatograms of *C. indicum* SFE extract (CiSFE). (a) GC-MS chromatograms of the *n*-hexane layers of CiSFE. (b) HPLC chromatograms of the 75% methanol layers of CiSFE. Reprinted from Ref. [68], with permission from Elsevier.

Terpenes are also major compounds in SFE with anti-inflammatory properties. *In-vitro* and/or *in-vivo* activity of tested extracts has been attributed to structures like guaioil, pinocampone, caryophyllene oxide, spathulenol, isoborneol, curdione, velleral, procurcumadiol, germacrone, curcuminoids, β -selinene, aromadendrene, β -elemene, or *cis*-piperitol, among others. GC-MS methods are also the traditional choice for the profiling analysis of these terpenoids [38,45,50–56], as well as for fatty acids (e.g. palmitic, oleic, linoleic, linolenic, EPA and mead acids) [29,57], that contribute to the anti-inflammatory composition of essential oils and other natural extracts obtained by SFE.

GC-FID is a low-cost analytical alternative, which has been reported for the analysis of anti-inflammatory fatty acids, sterols and terpenes in SFE extracts [58,59]. However, the lack of unambiguous identification is an important drawback of FID-based methods. In those works, even when GC-FID was proposed as determination analytical method, the identification of the analytes was later confirmed using GC-MS.

Besides GC-based platforms, HPLC methods are also widely reported in literature for the profiling analysis of natural extracts obtained using compressed fluids with anti-inflammatory properties. In particular, methods based on reversed-phase LC with C8 and C18 columns have been reported for polar terpene derivatives (caryophyllene oxide, γ -oryzanol, tocotrienol and tocopherol, senkyunolide, isoegomaketone-1K), phenolic compounds (quercetin, kaempferol and resveratrol, myricetin, isorhamnetin) and alkaloids (piperine). In this HPLC-based methods, UV detection is the most

popular choice [32,34,60–64]. However, the need of standards is here mandatory for an adequate identification of compounds by HPLC-UV.

High-performance Thin Layer Chromatography (HPTLC) methods can also be useful strategies for simultaneous phytochemical screening and bioactivity testing, by developing the plates with appropriate solvent and subsequent derivatization with different reagents. The phytochemical screening of anti-inflammatory *C. sicyoides* extracts obtained by SFE was performed by HPTLC-UV (Fig. 4), applying specific derivatization reagents such as vanillin–sulfuric acid for the terpenes, steroids, and fatty acids; ferric chloride for phenolic compounds; and diphenylboryloxethylamine (NP) and polyethylene glycol 4000 (PEG) for flavonoids identification [65]. Another HPTLC methodology was employed in parallel for authentication and quantification of anti-inflammatory alkaloid 2-benzoxazolinone content in a standardized SFE extract of *Acanthus ilicifolius* [66].

Complementary structural information and wider coverage of phytochemicals with different physicochemical properties can be obtained combining GC-MS and HPLC-UV or better HPLC-MS/MS approaches. In this line, several authors have reported characterization results of anti-inflammatory SFE extracts, integrating both LC and GC hyphenated platforms. Thus, Guo et al. [67] compared the anti-inflammatory activity of three different *Piper longum* extracts, with similar major constituents according to their HPLC-UV chromatograms, whereas comprehensive profiling analysis by GC-MS of the most active extract obtained by SFE allowed the

identification of forty-six phytochemicals. Similarly, qualitative profiling of a *Angelica sinensis* and *Zingiber officinale* Roscoe's SFE extract (AZ-SFE) was based on GC-MS analysis, whereas the contents of major compounds ligustilide and 6-gingerol in AZ-SFE were determined by HPLC-UV using an Inertsil ODS-C18 column [31]. Following a hyphenated MS/MS-based approach, Hsu et al. [35] determined the amount of the bioactive polyphenols chlorogenic acid and luteolin-7-O-glucoside in *Lonicera japonica* SFE extracts by HPLC-MS/MS in ESI(+) mode. The chemical composition profiles were comparatively evaluated by GC-MS analysis using a high-polarity DB-WAX capillary column. Moreover, the anti-inflammatory properties and chemical composition of SFE extracts of *Chrysanthemum indicum* have been comprehensively tackled by several authors. In these works, bioactive polyphenols were identified and quantified by HPLC-DAD, whereas terpenes profiling was carried out by GC-MS analysis [8,27,28,68]. A total of 35 compounds were identified by GC-MS, and 5 phenolics (chlorogenic acid, luteolin-7-glucoside, linarin, luteolin and acacetin) were reconfirmed and quantitatively determined by HPLC-UV [68], as illustrated in Fig. 5.

In recent works, the implementation of hyphenated HRMS-based techniques, via liquid chromatography (namely, LC-QTOF-MS) and gas chromatography (namely, GC-QTOF-MS), have significantly boosted the untargeted profiling of anti-inflammatory compounds. Thus, the *Curcuma* oleoresins SFE extracts were subjected to LC-QTOF-MS analysis. Operating in positive ESI(+) mode, high contents of curcuminoids with anti-inflammatory capacity were tentatively identified [25]. Alternatively, accurate mass from HPLC-QTOF-MS analysis in negative ESI(-) mode allow Villalva et al. [44] to confidently assign the identity of phenolic compounds selectively precipitated in the SAF process to improve the anti-inflammatory activity of the separator fractions of *Achillea millefolium* L. extract. These HRMS-based approaches significantly increase the speed of analysis, improving selectivity, resolution and efficiency, and providing improved structural determination capabilities compared to other methods (UV, IR and NMR). Furthermore, hybrid QTOF-MS instruments have been largely adopted as powerful tools for untargeted analysis of complex plant extracts, due to their capability of providing accurate mass data, and structural information from HR-MS/MS fragmentation [11].

Both GC and LC-hyphenated QTOF-MS platforms are highly complementary tools, providing MS and MS/MS data for compounds with different physicochemical properties, increasing the coverage in phytochemical profiling. In this regard, Vijayan et al. [24], reported HRMS data from both LC- and GC-QTOF-MS/MS platforms, improving the understanding on the phytochemical composition of *Curcuma* oleoresins SFE extracts with anti-inflammatory and anti-bacterial activity. LC-(ESI+)-QTOF-MS analysis showed methane type of monoterpenes and guaiane type of sesquiterpenes, whereas the GC-QTOF-MS results revealed mainly sesquiterpenes like curdione and monoterpene molecule like isocamphol [24].

5. Conclusions and future perspectives

New and well characterized biological activities have been attributed to several natural compounds that can exhibit complementary therapeutic effects against different diseases. Compressed fluids are powerful green extraction techniques with demonstrated capacity to obtain bioactive compounds with antiviral and anti-inflammatory activity. Despite this, the number of compounds extracted by compressed fluids tested as antiviral so far is comparatively small compared to the number of compounds tested as anti-inflammatory. The ability of compressed fluids to extract

targeted compounds can be used to provide compounds with required antiviral characteristics. Moreover, conjunction of compressed fluids technologies and phytochemical profiling analytical methodologies based on high-resolving power LC and GC platforms, hyphenated to complementary detectors (HP(T)LC-UV, HPLC-MS/MS, LC-HRMS, GC-MS and GC-HRMS) or based on stand-alone detection systems (FT-IR, ESI-HRMS, ¹H- or ¹³C-NMR), have been successfully implemented for targeted and untargeted profiling of antiviral and anti-inflammatory compounds in complex natural extracts. In particular, HRMS-hyphenated techniques provide useful structural information for discovery and exploration of new chemical constituents from natural sources. Despite their use is still limited in the profiling analysis of antiviral and anti-inflammatory compressed fluids extracts, the ever growing implementation of HRMS-based techniques in combination with UV, IR and NMR spectral data can improve phytoconstituents identification from natural sources, which will increase the success rate in the discovery of new therapeutic agents with antiviral and anti-inflammatory properties.

The search of natural compounds against viral diseases and associated inflammatory responses has been going on for years. However, after the current SARS-COV-2 pandemic, we anticipate that the search of new natural compounds with antiviral and anti-inflammatory activity against COVID-19 will be a very hot research topic, so, the integrated approach reported in this review can play an essential role on the development of new drugs that assist in the treatment or prevention of this disease in the future.

Acknowledgments

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