



# **Clean Technologies for Obtaining Biocomposites of Brazilian Ginseng *Pfaffia glomerata* (Spreng.) Pedersen: A Review**

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## **Authors' contributions**

*This work was carried out in collaboration between both authors. Author CBDO designed the study, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author OAS managed the analyses of the study and completed de investigation. Both authors read and approved the final manuscript.*

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## **ABSTRACT**

The Brazilian ginseng *Pfaffia glomerata* (Spreng.) Pedersen belongs to the *Amaranthaceae* family and has as its main component  $\beta$ -ecdysone, a phytoecdysteroid, found in the roots, stem, flowers and leaves of the plant. In the last years sustainability and the environment concern were decisive for the emerging supercritical fluid extraction and pressurized fluid extraction technologies to obtain biocomposites from the plant. These extraction technologies use solvents ( $\text{CO}_2$ , ethanol and water) and uses as controllable parameters pressure, flow, time and temperature. The combination of these factors generates atoxicity, no residue in the final extract and have a reduced energy cost and an excellent extraction yield. This work reviews the literature from 2007 to 2020 on the use of clean technology to obtain chemical biocomposites of interest in the areas of biology, agronomy, food and pharmaceuticals. It is was concluded that the supercritical fluid extraction and pressurized liquid extraction extracts were very efficient in obtaining  $\beta$ -ecdysone, since both presents low energy consumption, uses environmentally correct solvents which reduces harmful effects on the environment. Finally, to choose the best technology for extraction of other biocomposites depends on the chemical compound of interest.

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## 1. INTRODUCTION

A study in the main scientific journals about *Pfaffia glomerata* (Spreng.) Pedersen terminology was carried out at the Federal Institute of Paraná (IFPR), which covered the period from 2007 to 2020. A total of 250 results were found on the national periodical CAPES portal, 53 results in the scientific journal Elsevier and 49 results in the scientific journal Scielo. These results correspond to researches conducted in a large number of fields, mostly of them in the pharmaceutical area (26%) with direct implication on the health area, followed by agronomy (41%), biology (25%), and food (8%). In recent years, there was a growing demand for clean technologies using clean solvents and about (30%) of the researches are related to the supercritical extraction of *Pfaffia glomerata* biocomposites, which will be discussed later in the text. The objective of this work was to elaborate a literature review on the extraction methods, using clean technologies to obtain biocomposites of the *Pfaffia glomerata*.

The *Pfaffia glomerata* belongs to the *Amaranthaceae* family that presents about 170 genera and 2000 species [1], being found in Brazil about 20 genera, which are rich in nutrients and moisture. In Brazil, the main producing region is located between the states of Paraná, São Paulo and Mato Grosso do Sul. The main bioactive compound of interest is  $\beta$ -ecdysone, a natural ecdysteroid found mainly in the roots, but also being found in flowers, leaves and stem also contains the compound.

Concern for sustainability and the environment started a new way of obtaining secondary metabolites from plants, the so-called clean or "green" technology. This name is given due to the way the extracts are obtained, it is a technology that does not require the use of toxic solvents, leaves no residues in the final extract and works with a low energy consumption. Examples of this clean technology includes supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE). Such technologies employ CO<sub>2</sub>, ethanol, water or any other solvent considered clean along with the control of pressure, flow, time and temperature, thus optimizing the extraction process and improving the yields obtained.

Innumerable clean technologies have been used to obtain biocomposites present in *Pfaffia*

*glomerata*. Although the most explored part of the plant is still the root, the stem, flowers and leaves also show these compounds in varying amounts depending on the stage of maturation, plant nutrition and place of cultivation. Recent studies have shown that ginseng has great capacity for use in the food industry as surfactant, prebiotic and source of fructooligosaccharides (FOS). In the agronomic area studies have already been carried out on its use in phytoremediation of soil and water contaminated by heavy metals and the results obtained were excellent, in addition to its already established use in the pharmaceutical and medical industry, due to its antimicrobial, anti-inflammatory, antioxidant, antirheumatic, antitumor, antidiabetic and memory enhancement properties. In view of this, it is necessary to conduct studies with an approach to clean extraction processes in *Pfaffia glomerata* to obtain biocomposites, mainly  $\beta$ -ecdysone. The purpose of these studies is to identify the main clean technologies for obtaining chemical biocomposites present in Brazilian ginseng available in the literature.

## 2. PLANT SEASONALITY

*Pfaffia glomerata* is a plant well adapted to the Brazilian tropical climate, preferentially inhabiting riverside forests and rivers due to the high levels of nutrients and humidity. In addition, the development can take place in sandy soils or rich in organic matter and, in altitudes between 80 and 1000 m. The most commercially exploited part of the plant are the roots that contains the biocomposites in larger amounts while the aerial parts are generally discarded. Its therapeutic properties are attributed to the roots, although several scientific studies have shown that such components are also present in the aerial parts of the plant.

The genus *Pfaffia*, has 27 species distributed in Brazil, which are characterized as erect or semiprostrated herbs or sub-bushes that occur in savannas, rupestrian or clean fields, forest edges and riverbanks [2]. Dewick [3] mention that the roots of *Pfaffia* sp., known as Brazilian ginseng, have been used in folk medicine as tonic, aphrodisiac and antidiabetic [4].

A study carried out by [5] related to the geographical distribution of *Pfaffia glomerata*, *apud* [2] they classified the plant as having a

geographical distribution pattern of the "South American Wide" type. The species is very widespread in tropical and subtropical South America, reaching the southern border of the Rio da Prata [6]. In Brazil, it is found in the South (Paraná, Santa Catarina and Rio Grande do Sul), Southeast (São Paulo, Rio (Amazonas, Amapá, Pará, Rondônia and Roraima) and Northeast (Bahia, Maranhão, Paraíba and Pernambuco) [7].

Although the Flora List of Brazil does not report the occurrence of the species in the Federal District, there are two quotes from collections in this region deposited in the Embrapa Herbarium Genetic Resources and Biotechnology. This broad geographical distribution of *Pfaffia glomerata* results in populations with genetic variability and adaptability due to their presence in very different climatic and edaphic conditions [5] *apud* [8].

*Pfaffia glomerata* is a medicinal plant widely used as an adaptogenic herb. The species is a naturally found in Africa and in the Americas becoming the subject of pharmaceutical and commercial interest, mainly due to the accumulation of  $\beta$ -ecdysone in its roots. Brazil is considered the largest supplier of *Pfaffia glomerata* in the world. Due to the morphological similarity of its roots with those of *Panax ginseng* (Korean ginseng), the species has become known as Brazilian ginseng [9].

According to Corrêa-júnior [5], of the total *Pfaffia glomerata* extracted in Brazil, 12% is exported, the majority being sold as feed, which has a lower aliquot, a fact that overlook it is condition as a medicinal plant. About 30 tons of *Pfaffia glomerata* roots were monthly exported to Japan, supplied by extractivists from the Paraná River Bay area, estate of Paraná, and the municipality of Mogi das Cruzes, estate of São Paulo [10]. The international market price for  $\beta$ -ecdysone is approximately US\$ 85.00/g and it is estimated that in the last ten years, about 41 tons of ginseng roots have been exported to the European Union and Asia, fresh or dried, including cut, crushed and powder, corresponding to approximately US \$ 390,000 [11].

According to Corrêa-júnior [12] the ecological, agronomic and forestry aspects should be considered for the cultivation of *Pfaffia glomerata*. Besides, heevaluated that the species develops itself best at an average annual temperature of around 295 K, with maximum

averages of 306 K and a minimum of 290 K. On winter, loses leaves and totally paralyzes its growth and root production. The conditions imposed during plant cultivation also implicate in a direct influence on the biosynthesis and or the accumulation of metabolites [5].

About the harvest of the roots of *Pfaffia glomerata*, Corrêa-júnior [5] report that this can be done one year after planting at the end of winter. At this time the synthesized reserves have already been translocated to the roots [8], presenting higher productivity and higher levels of  $\beta$ -ecdysone [12].

### 3. MAIN CHEMICAL COMPOUNDS PRESENT IN *Pfaffia glomerata*

Have been found seven scientific articles in a search conducted in July of 2020 at the Portal Periódicos Capes, reporting as a focus the main chemical compounds presented in the *Pfaffia glomerata*. On the site research of the scientific journal Elsevier, Science Direct found twelve results for the major chemical compounds. Most of the researchers are Brazilian (about 95%), and some researches were done in partnership with foreign universities.

In most of the studies involving *Pfaffia glomerata* attempt elucidate the pharmacological effects of the extracts of this plant [13]. Research groups worldwide have demonstrated that the extracts have potential for the treatment of chronic inflammation [14]. On the therapeutic properties of *Pfaffia glomerata*, quotes antitumor effects, aphrodisiacs, antidiabetics and tonics in general [15], also acting as anti-inflammatory, immunostimulant, leukocytogenic, and internal cicatrizant.

Related to the gastrointestinal system of rats, they protect the gastric mucosa, reduce the number of lesions, increase the pH of the duodenum and reduce gastric secretions [16]. Antimicrobial activity against *Leishmania braziliensis* [14] and effects on melanogenesis [17].

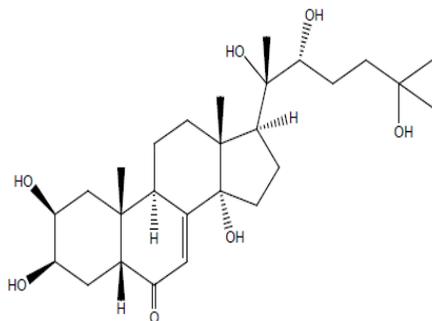
The therapeutic effects attributed to *Pfaffia glomerata* are due to the chemical compounds present in the plant. The main compounds found in the literature according to Silva [18] are: sitosterol, stigmasterol, allantoin, pfaffic acid and pfaffiaglicosides A, B, C, D, E and F [19], and the same authors detected an inhibitory effect of pfaffic acid and pfaffiaglycosides on the growth of B-16 melanoma tumor cells (model for studies of

metastatic tumors, with almost exclusive affinity for lung tissue). *Pfaffia glomerata* has been isolated from substances such as glomic acid, amphoteric acid and rubrosterone,  $\beta$ -ecdysone,  $\beta$ -D glucopyranosyloleate [20]. The levels of ecdysone in dried roots of *Pfaffia glomerata*, determined in several studies, varies between 0.64% and 0.76% [15] *apud* [21].

Scientific studies report the existence of several biocomposites in *Pfaffia glomerata*. The analyzes carried out with roots of different species accesses showed levels of  $\beta$ -ecdysone varying from 0.15 to 0.75% of the dry weight, showing the variability in relation to the accumulation of this metabolite by species [16,18,22]. The ecdysteroid  $\beta$ -ecdysone present in *Pfaffia glomerata* can be found in several plant organs, such as flowers, leaves, stems and roots [23,24], however, its commercial extraction comes mainly of the roots [25].

The main active constituents of the roots of *Pfaffia glomerata* according to [26] are saponins, which have already been identified by different authors in several studies. According to Shiobara [27] ecdysterone also called  $\beta$ -ecdysone (CAS n°8047-15-2) is characterized as the main saponin present in the roots of *Pfaffia glomerata*. More recently, Nakamura [17] identified 6 new constituents and 8 already known, of which  $\beta$ -ecdysone appeared in greater quantity.

Vardanega [28] mentions that  $\beta$ -ecdysone (Fig. 1), chemically defined as  $2\beta$ ,  $3\beta$ ,  $14\alpha$ ,  $20\beta$ ,  $22,25$ -hexahydroxy- $7$ -cholesten- $6$ -one is recognized as the main bioactive compound present in the roots of the Brazilian ginseng, responsible for the beneficial effects attributed to its use [14,16].



**Fig. 1. Structure of  $\beta$ -ecdysone**

To analyze the temporal and spatial distribution in *Pfaffia glomerata* (*Amaranthaceae*), Festucci –

buselli [24] evaluated that  $\beta$ -ecdysone was constantly detected in flowers, leaves, stems and roots, but its percentage varied throughout its development. The highest percentage of  $\beta$ -ecdysone was found in flowers (0.82%), roots (0.66%), leaves (0.60%) and stems (0.24%). While the stems had the lowest percentage of  $\beta$ -ecdysone (0.13% - 0.24%), followed by roots (0.42% - 0.66%) and flowers (0.47% - 0.82%). The leaves had the highest percentage variation of  $\beta$ -ecdysone (0.21% - 0.60%). The total amount of  $\beta$ -ecdysone in the leaves remained constant, showing a small variation, while the total amount of  $\beta$ -ecdysone in the roots increased over time, suggesting that  $\beta$ -ecdysone can be accumulated in the roots.

Another group of chemical compounds highly researched mainly in *Korean ginseng* are the ginsenosides. These chemical compounds are also investigated in *Pfaffia glomerata* in the form of saponins, as quoted by [29]. Until recently this group was poorly studied, but currently the theme has aroused much interest specially in the pharmaceutical area due to the satisfactory results reported in researches.

In a literature review on the application and molecular mechanisms of action of *Korean ginseng*, Kim [30] states that species rich in ginsenosides (triterpenesaponins) have the ability to inhibit the production of free radicals, stimulate the production of nitric oxide, improve blood circulation and muscle tone. According to Ryu [31], ginsenosides can be classified into two groups: intact ginsenosides and transformed ginsenosides, which are more bioavailable and bioactive than intact ginsenosides. Antitumor effects are usually attributed to transformed ginsenosides.

#### **4. CLEAN TECHNOLOGIES TO OBTAIN *Pfaffia glomerata* (SPRENG.) PEDERSEN BIOCOMPOSITES**

Plant-derived products are rich in a variety of secondary metabolites, such as terpenoids, alkaloids, and phenolic compounds [3]. Secondary metabolites are formed by biosynthetic reactions of plants and may function as attractive or for defense or signaling purposes [32]. The recovery of secondary metabolites by extraction is an essential process for the discovery of drugs that requires the recovery of chemical constituents from a complex matrix of plant material, necessitating the rupture of the cell walls of the plant [33]. There are numerous

methods to extract components of interest, usually starting as a mixture of active and inactive components that are fractionated to allow the identification of individual molecules [34].

Publications of the last two years, there has been an increase in contributions related to "green and supercritical solvents" (based on the *Scopus* survey, September 2017) aligning about 400 manuscripts, from which half corresponds to "green and supercritical solvents" [35].

Concerning the supercritical extraction of biocomposites from *Pfaffia glomerata*, 16 results were found on the *Science Direct* scientific research site belonging to the scientific journal *Elsevier* in a search conducted in July 2020. Khaw [34] evaluate that among the most ecological technologies (i.e., cleaner, using organic solvent amounts or reduced energy consumption through efficient dissolution or reduced processing times) for currently available herbs extraction are ultrasonic extraction, microwave assisted extraction, SFE, mechanical pressure and instantaneous relaxation control (DIC) [36].

Table 1 summarizes the greener technologies of extraction found. Of these, only SFE offers reduced processing energy inputs and an alternative solvent approach. In addition, recovery of the gaseous solvent in the depressurising of an SCF (i.e., reversion to a gas) state means that the phase separation is possible between a supercritical fluid (SCF) and the extracted molecules which are in solid or liquid state. Phase separation can facilitate the collection of pure CO<sub>2</sub> (gas) solvent so that it can be recirculated and stored, ready for reuse (lower CO<sub>2</sub> spent), thereby reducing energy consumption and increasing the overall sustainability of the extractions based in SCF [34].

According to Herrero [35], the increase in the production of academic researches involving green and supercritical solvents in the last two years (about 400 manuscripts), is not only due to the applications of green solvents in different high pressure, but also in new tools for modeling solvents. For instance, using molecular dynamic simulations (MD) [37] and also the Monte Carlo simulation was possible to investigate, among others, the phase equilibrium in the expanded CO<sub>2</sub> system and to predict its physicochemical properties as

an average standard for engineering processes [38].

Another interesting application that have been developed in recent years show the importance of solvent selection in the development of processes for the extraction of bioactive compounds from natural sources, it is possible to highlight those based on the theory of solubility parameters. Hansen's solubility parameters are a tool used to select the most suitable green solvents to selectively extract bioactive metabolites from natural sources [35]. Finally, the best solvents used in SFC to extract will depend on the physical chemical properties such Molecular weight, Critical temperature, Critical pressure and Density in critical point [34].

## 5. EMPIRICAL REVIEW

Several authors have reported the therapeutic and technological use of *Pfaffia glomerata*. Since 2007 there has been a significant increase in academic research, and in the year 2014 research has tripled compared to 2013 and previous years according to the scientific journal *Elsevier*. From 2014 onwards, there was a growing demand for clean technologies for extraction of biocomposites from roots, stem, flowers and leaves of *Pfaffia glomerata*, with SFE and PLE widely disseminated in academic, with potential use in the chemical, pharmaceutical and food industry.

*Pfaffia glomerata* has been shown to be an excellent source of biocomposites, although the most exploited part of the plant is still the root, flowers, leaves and stem, which have varying amounts of  $\beta$ -ecdysone. Several authors report in their research the benefits that the plant can offer, in both the pharmaceutical, food as the agronomic areas. The plant is well adapted to the Brazilian climate, preferentially inhabiting forests and riverbanks that have sandy soils rich in nutrients and humidity necessary for the good development of the plant. The Japanese pharmaceutical industry was a pioneer at *Pfaffia glomerata* biocomposite research in the 1970s, and researchers from all over the world have been publishing their experiences in a wide range of fields.

Vardanega [29] quote that in addition to the functional properties of Brazilian ginseng extracts reported in the literature, these can also be exploited for technological purposes due to their surfactant properties attributed to the presence of

saponins [39,40]. Recent studies have demonstrated that extracts of Brazilian ginseng were efficient for the stabilization of emulsions containing essential oils [41,42]. Stable emulsions containing 25% clove oil [43] and 3% annatto oil [42] were obtained using aqueous extract of Brazilian ginseng roots.

Also according to Vardanega [29], the surface properties of saponins have been known for many years, and as a result they are traditionally used as saponifying agents. Recently, new applications have been found such as the phytoremediation of contaminated soils [44], a hemolytic agent [45], antioxidant [46], and the use of a high molecular weight plastic film [47], absorption of lipids in the intestinal tract [48], solubilization of cholesterol in aqueous solutions [49], besides antimicrobial, antiviral and antitumor properties [50].

Through a chemical, pharmacological and toxicological evaluation of the extract of *Pfaffia glomerata*, [51] the results were obtained and concluded that the extract of the leaves of *Pfaffia glomerata* present significant antiulcerogenic, cytoprotective and antioxidant activity. This pharmacological activity may possibly be attributed to the presence of kaempferol and  $\beta$ -ecdysone. In another study, the antimicrobial activity of the crude extract of *Pfaffia glomerata* in microorganisms of the buccal cavity was evaluated, and the activity of *Enterococcus faecalis*, *Streptococcus salivarius*, *Streptococcus mutans*, *Candida albicans* and *Lactobacillus casei*. For the *Streptococcus anguinus*, *Streptococcus mitis* and *Streptococcus sobrinus* microorganisms there was activity, however, with no statistically significant difference between the three concentrations evaluated.

Evaluating the intestinal anti-inflammatory activity of *Pfaffia glomerata*, [52] observed that the methanolic extract of *Pfaffia glomerata* consists essentially of triterpenes and saponins, including  $\beta$ -ecdysone, which were concentrated in the butanolic fraction of *Pfaffia glomerata*, which in turn was evaluated in the process of induced intestinal inflammation, but were not able to prevent lesions formation.

In the search for new functions for the species, stands out the use of environmental services, due to its capacity to adapt to stressful environments, has been the focus of phytoremediation studies, a technique that uses plants as the soil and water decontamination

agent. *Pfaffia glomerata* seedlings grown at cadmium levels ranging from 20 to 80  $\mu$ M showed a certain degree of tolerance to this heavy metal [53]. However, because the species has a reasonable capacity to accumulate Mercury (Hg) in the roots, the use of *Pfaffia glomerata* as a phytostabilizer allows the Hg to be retained in the soil, avoiding contaminant expansion [54]. Therefore, the species has potential to be used in the extraction of environmental contaminants [5].

In order to evaluate the phytochemical and structural aspects of *Pfaffia glomerata* accessions with different nematodes [55], observed that the stimulus to the increase in  $\beta$ -ecdysone content may be related to the chemical response of the plant to nematode attack [56]. According to [55], the nematode-infected roots of *Pfaffia glomerata* presented levels of  $\beta$ -ecdysone compatible with the variation pattern (0.15% - 0.76%) observed by several authors in different entries and culture conditions [10,12,15,16, 57,58]. In commercial samples the  $\beta$ -ecdysone content fluctuates between 0.3 and 0.5% [57].

Another use of *Pfaffia glomerata* has been the studies as an ecological function using the flowering characteristic in practically every month of the year. Leite [58] studying arthropods associated with *Pfaffia glomerata* flowers recommended the species as a crop border, as a bait-plant, as well as to maintain parasitoids and predators that control the aphid populations. *Pfaffia glomerata* was also considered adequate to attract natural enemies, as it is an alternative source of nectar and pollen [5]. Therefore, the property of blossoming almost all year round, attracting pollinating insects and natural enemies of pests, can give the species an environmental function that is still unusual but of considerable use for agricultural practices guided by the agroecological principle [5].

Regarding the way of obtaining biocomposites, several authors have successfully exploited the use of clean technologies, such as extraction with SFE and PLE. The processes use controlled pressure, flow, time and temperature allied to the use of clean solvents, have been efficient in obtaining biocomposites from the *Pfaffia glomerata*.

It is important to differentiate these two clean extraction technologies that, although similar, have their peculiarities. SFE is a unitary contact operation based on equilibrium and physicochemical properties of supercritical fluids:

high solvation power, high diffusion coefficient, low surface tension and low viscosity [60]. For the extraction PLE, the application of pressure during the extraction process allows the use of temperatures above the boiling temperature of the solvents, without reaching the critical point [61]. These conditions improve the solubility of the solute in the solvent and the desorption kinetics from the solid matrix [62] *apud* [13].

Another point that draws attention to the clean technologies of extraction of biocomposites from *Pfaffia glomerata* is in relation to the sustainability. In the economic area one can mention the reduction of the energy cost in relation to a normal extraction system, where much energy and time is spent in the process. The SFE and PLE extractions use pressure, flow, time and controlled temperatures, aiming to obtain the maximum yield with the shortest possible time, economic and energy cost, optimizing the extraction process.

The solvents used are generally water, ethanol and CO<sub>2</sub> that can be reused at the end of the process, are non-toxic and do not leave residues in the final product, i.e., environmentally correct. The social dimension is directly affected by the beneficial effects resulting from the economic and environmental dimensions, as it improves the working environment and the quality of life of the people involved in the extraction process (local aspect), as well as contributing to the reduction of the effects of warming (global aspect).

Several researchers have been studying new technologies to obtain biocomposites aiming at the best extraction technique, that is, higher yield and lower energy cost. No consensus has yet been reached on the best technology to be applied, since each technique has its advantages and disadvantages. Since it is a physic-chemical process that has many variables, the choice of solvent, flow, time, pressure, and temperature are directly linked to the compound of interest, as well as the final yield of the extracted product.

In studies conducted with *Pfaffia glomerata* [63], the SFE study of Brazilian ginseng roots performed by [64] evaluated temperatures of 303 and 323K and pressures of 10, 20 and 30 MPa. The best extraction condition was at 323K and 20 MPa, where the kinetic study was conducted using ethanol as a cosolvent. In terms of extract yield (dry basis), this was higher when using 10% ethanol (0.52%). In the same study,

quantification of  $\beta$ -ecdysone was also performed, reaching 4.6%. But the results are still low. Santos and Albarelli [65] performed the PLE study of the *Pfaffia glomerata* roots, at 333K and 12 MPa, in which the focus of the work was the simulation of the process.

In their work, [63] aimed to study and optimize the extraction of  $\beta$ -ecdysone from roots *Pfaffia glomerata* using CO<sub>2</sub>, ethanol and the mixture ethanol: water (80:20) as solvents. It was possible to conclude that the increase in the amount of ethanol favored the extraction process, not only in terms of the amount of crude extract obtained, but also in terms of recovery of the compound of interest, in this case the ecdysteroid  $\beta$ -ecdysone. The results showed that ethanol has higher selectivity than CO<sub>2</sub> to extract this compound. Among the conditions studied, the maximal yield in terms of the compound of interest was the pressure of 20 MPa using the CO<sub>2</sub>: ethanol mixture (10:90, v/v) as extraction solvent. During the first hour of processing, 82% of the total yield and 89% of the total  $\beta$ -ecdysone content were obtained.

In the study of the kinetic behavior of the SFE process to obtain the Brazilian ginseng root extract using as solvent the CO<sub>2</sub> and ethanol as co-solvent, it was possible to conclude that the increase in the amount of ethanol favored the extraction process, not only in terms of the quantity of extract obtained, as well as in terms of recovering the compound of interest, in this case the ecdysteroid  $\beta$ -ecdysone. The results showed that ethanol has higher selectivity than CO<sub>2</sub> to extract this compound. Among the conditions studied, the maximal yield in terms of the compound of interest was the pressure of 20 MPa and 333K using the CO<sub>2</sub>: ethanol mixture (10:90, v/v) as extraction solvent. During the first hour of processing, 82% of the total yield and 89% of the total beta-ecdysone content were obtained [63].

According to the results obtained, the PLE condition selected for the extraction kinetics study was the one in which higher levels of  $\beta$ -ecdysone were obtained: 393K using pure ethanol solvent. As the pressure did not show significant influence, the lower value (8 MPa) was chosen to reduce the energy expenditure of the process. From the global extraction curve it was possible to verify that after approximately 26 minutes of processing (tCER) approximately 50% of extraction yield and 67.7% of  $\beta$ -ecdysone content were obtained. The total yield in terms of

**Table 1. Advantages and disadvantages of greener extraction methods**

<b>Methodology</b>	<b>Benefits</b>	<b>Disadvantages</b>	<b>Main fields of application</b>
DIC extraction (instant relaxation controlled by depressurized steam)	Avoids the loss and degradation of volatile and thermolabile compounds; short time contact; more effective process such as extraction with SFE; without solvents.	Essential oils was established to be very weak; High cost;	Food
Microwave assisted	Small extraction time, reduction of solvent and vegetable mass; extractions of primary and secondary metabolites	High cost of equipment; limited amount of sample; non-selective (large number of extracted compounds).	Natural products and food
Ultrasonic extraction	Successfully perform solvent-friendly; extractions of primary and secondary metabolites; Small extraction time; selective (large number of extracted compounds)	matrix heat	Natural products and food
Extraction with Supercritical Fluids (SFE)	Absence or limited solvent consumption to produce solvent-free extracts; suited for thermolabile biomolecules; composition of extracts with lower content of non-oxygenated compounds	Expensive equipment; small extraction capacity; many polar compounds are difficult to extract.	Food, chemical industry and pharmaceutical
Mechanical pressure	Solvent-Free; co-extraction of lipophilic and hydrophilic compounds; facilitating scale-up; low installation cost	inefficient for bioactive compounds	Food

mass of raw material obtained after 130 minutes of process was 7% (dry basis), being higher than in the SFE process. It was also possible to observe that in the studied condition a reactive extraction may be occurring, since the diffusional period was not reached [63].

Santos e Meireles [66] carried out a paper in which a new online process for the production of food-grade emulsions containing oily extracts, i.e. oil-in-water (O/W) emulsions, was presented in one step. This process was called SFE Emulsions. With this process, emulsions containing extracts of supercritical fluids can be obtained directly from plant materials. The aim in the design of this process is to propose a new fast way to obtain emulsions of supercritical fluid extracts.

Currently, the conventional emulsion formulation method is a two-step procedure, i.e. first extraction of supercritical fluid to obtain an extract; and second, the emulsion formulation using another device. Another variation of the process was successfully tested and validated, giving rise to a new process: EPFE (Emulsions of Extractions of Pressurized Liquids). Both processes exploit the supercritical miscibility of CO<sub>2</sub> essential oils, in addition, the EPFE process exploits the emulsion properties of pressurized aqueous plant extracts rich in saponin. The viability of this latter process was demonstrated using roots of *Pfaffia glomerata* as source of extract rich in saponin, water as extraction solvent and clove essential oil, extracted directly with supercritical CO<sub>2</sub>, as a dispersed model phase. In addition, examples of pressurized fluid coupled processes applied to add value to bioactive food compounds developed over the last five years [66].

The supercritical CO<sub>2</sub> was selective to the saponins with greater capacity to reduce the surface tension of aqueous solutions, when used with ethanol as cosolvents and, in general, the process of sequential extraction in fixed bed using the "green" solvents presented as a good alternative to the use of organic solvents to extract and fractionate saponins present in different vegetable sources [39].

The use of a PLE technique is an attractive second alternative [40], because it allows rapid extraction and reduced solvent consumption. PLE allows rapid extraction (less than 30 min) of analytes in closed and inert environments under high pressures (not exceeding 20 MPa) and

temperatures (299-473K). Thus, extraction of solvents that are inefficient in extraction at low temperatures can be much more efficient at the elevated temperatures used in PLE.

According to Vardanega [40], PLE usually uses safe solvents (GRAS), such as ethanol and water [67]. However, the use of aqueous surfactant solutions as alternative solvent systems in PLE has been reported for the extraction of ginsenosides from ginseng roots (*Panax quinquefolium*). When compared to the use of pure water or methanol, the presence of a common nonionic surfactant (Triton X-100) in water at a concentration above its critical concentration of micelles was shown to increase the amount of extracted ginsenosides. The advantages of using aqueous nonionic surfactant solutions have also been demonstrated in comparing the performances between ultrasonic assisted extraction and PLE. These advantages may be provided by the effect of improving the solubility of micelles by Triton X-100.

According to Vardanega [68] recently prebiotic compounds were found in roots of Brazilian ginseng, such as FOS. Prebiotic compounds have been extensively studied to be added to the human diet to promote well-being and other health benefits. Based on these properties, extraction in subcritical water, an environmentally friendly process, was performed to obtain the bioactive compounds of Brazilian ginseng roots (BGR) and aerial parts (BGA) for the use of the whole plant.

For the BGR, the effects of the temperature (353-453K) and the static extraction time (5-15 min) on the extraction yield,  $\beta$ -ecdysone and FOS content in the extracts were evaluated; while for BGA, the effects of temperature (353-453K), pressure (2-12 MPa) and static extraction time (5-10 min) were evaluated on extraction yield and  $\beta$ -ecdysone content in extracts, both with complete statistical techniques of factor analysis and analysis of variance (ANOVA). The extracts of BGR showed a  $\beta$ -ecdysone content of up to 0.7 g 100 g<sup>-1</sup> extract and a FOS content of up to 8.8 g 100 g<sup>-1</sup> extract, which means that BGR can be considered a major source of these bioactive compounds. Meanwhile, BGA extracts had a  $\beta$ -ecdysone content of 0.3 g 100 g<sup>-1</sup> extract [68].

The following is a summary Table 2 with the main research on extraction using clean technologies in *Pfaffia glomerata* roots.

**Table 2. Researchs on extraction of biocomposites using clean technologies in *Pfaffia glomerata* roots**

Used part of <i>P. glomerata</i>			Target chemical composite	Analytical methodology for the identification of chemicals compounds	Pression (MPa)	Temperature (K)	Methodology used for extraction	Solvent	Reference
Roots	Steam	Leaf							
X	X	X	$\beta$ -ecdysone	HPLC	-	299	Soxhlet.	Methanol	[24]
X	-	-	Saponins and $\beta$ -ecdysone	HPLC	10, 20 and 30	303 and 323	SFE	CO <sub>2</sub> , ethanol and water	[64]
X	-	-	Saponins and $\beta$ -ecdysone	HPLC	12	333	PLE	Simulation of the extraction process	[65]
X	-	-	Ginsenosides	HPLC	24	318	UASFE <sup>1</sup> and UAPLE <sup>2</sup>	CO <sub>2</sub> , Ethanol and water	[28]
X	-	-	Saponins	HPLC	30	323	SFE	CO <sub>2</sub> , Ethanol and water	[69]
X	-	-	$\beta$ -ecdysone	HPLC	20	333	SFE	CO <sub>2</sub> , ethanol(10:90)	[63]
X	-	-	$\beta$ -ecdysone	HPLC	8	393	PLE	Ethanol:water (80:20) and ethanol	[70]
X	X	X	$\beta$ -ecdysone and Fructooligosaccharides(FOS)	HPLC	2-12	353-453	SWE	Water	[68]
X	-	-	$\beta$ -ecdysone and saponins	HPLC	0.1, 5 and 10	333	HPLC pump <sup>3</sup> .	Ethanol and water	[28]

<sup>1</sup>Ultrasound-assisted SFE; <sup>2</sup>Ultrasound-assisted PLE; <sup>3</sup>The intensified extraction (IE) was performed in two stages, sequentially. Ethanol was used as extraction solvent in the first step and water was used in the second

**Table 3. Main researches about a used part of *Pfaffia glomerata*, biocomposites, yield and economic use**

Used part of <i>P. glomerata</i>	Biocomposite and Yield	Economic uses	Reference
Roots	Better results: Global extraction yield – 5.8%	Pharmaceutical	[64]
Roots, Steam, Leaf	Simulation : Method I –Global extraction yield: 5.2%; $\beta$ -ecdysone: 5.6%, Method II - Global extraction yield: 62.9%; $\beta$ -ecdysone: 0.52%	Food	[68]
Roots	Saponins – 55%, $\beta$ -ecdysone – 5.6%, Fructooligosaccharides – 7.9%	Food	[29]
Roots	Global extraction yield – 40.5%	Pharmaceutical	[65]
Roots		Food	[42]
Roots	Global extraction yield:82%; $\beta$ -ecdysone: 89%	Pharmaceutical	[13]
Roots	Saponins – 6.9%	Food and Pharmaceutical	[69]
Roots	Better results: Global extraction yield – 2.12%; Antioxidant Activity extraction – 42%	Pharmaceutical and Foods	[43]
Roots	Better Results: Global extraction yield – 50%; $\beta$ -ecdysone – 5%	Pharmaceutical and Foods	[70]

Table 3 shows the main researches on the yield of biocomposites extracted from different parts of *Pfaffia glomerata* with different clean technologies (Ultrasound-assisted SFE, Ultrasound-assisted PLE, The intensified extraction (IE) was performed in two stages, sequentially etc). The yield results are quite different from each other, since the extraction methods are different. There are other variables that can interfere in the yields like the type of solvent (polar or non-polar), time, flow, part and origin of the plant. Finally, it was found that none of these researches cited the flower as a subject of study of yield.

## 6. CONCLUSION

The review demonstrated that *Pfaffia glomerata* (Spreng.) Pedersen has numerous applications in the pharmaceutical industry, as well as an enormous potential in the food area as a technological agent, surfactant and in the agronomic area as phytoremediate of soil and water contaminated with heavy metals. In addition the plant has an enormous potential to obtain biocomposites, mainly  $\beta$ -ecdysone by clean technologies. However, there are almost no studies on obtaining ginsenosides. Finally, clean technologies for obtaining biocomposites from *Pfaffia glomerata* such as SFE and PLE have proven to be very efficient, since both act with low energy consumption and the solvents used are environmentally correct, reducing the harmful effects on the environment. The choice of the best technology to be applied will depend on the compound of interest, that is, there is a need for further studies in the field. In the case of obtaining  $\beta$ -ecdysone, the two technologies were satisfactory presenting excellent extraction yield.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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