

**Phytopharmacological preparations as predictors of plants bioactivity:  
a particular approach to *Echinacea purpurea* (L.) Moench antioxidant  
properties**

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## **Abstract**

Numerous evidences have confirmed the multitude of health benefits of plant products and its derived formulations. *Echinacea purpurea* (L.) Moench is a good example widely used due to its therapeutic properties. In the present study, the antioxidant properties of *E. purpurea* hydroethanolic and aqueous extracts obtained from dry or fresh raw material were evaluated and compared with dietary supplements based on the same plant (tablets and syrup), in order to conclude about the most active phytopharmacological preparation or formulation. The chemical composition of the different samples was also assessed. Overall, the hydroethanolic extract of fresh plant revealed the highest activity, directly related with its higher contents in phenolic ( $229.22 \pm 4.38$  mg GAE/mL), flavonoids ( $124.83 \pm 7.47$  mg GAE/mL), organic acids ( $8.89 \pm 0.10$  g/100 g) and tocopherols ( $4.55 \pm 0.02$  mg/100 g). Tablets followed by syrup revealed the worst effect, positively correlated with the lowest abundance in bioactive molecules. The weak *in vitro* antioxidant potential of commercial phytopharmacological formulations could be related with their chemical composition, including the addition of excipients. Further studies are necessary to deepen knowledge on this area, namely focusing *in vivo* experiments, in order to establish upcoming guidelines to improve the quality and bioavailability of phytopharmacological formulations.

*Keywords:* Medicinal plants; purple cone flower; aqueous/hydroethanolic extracts; antioxidant activity

## Introduction

Ethnopharmacological preparations have been secularly used for their efficient and specific health improving effects. The whole plant, but mainly specific plant parts comprise the most commonly raw materials used to develop phytopharmacological formulations [1–3]. Bioactive molecules represent the pivotal focus of attention, and their abundance in natural matrices clearly determines their preference. In fact, the bioactive potential of botanical preparations is directly dependent to the richness in the pool of phytochemicals, from which some of them are biologically active, while other ones need to be metabolized and/or extracted to exert beneficial effects [2,4,5].

*Echinacea purpurea* (L.) Moench, commonly known as purple coneflower, is a member of the *Echinacea* genus (Asteraceae), a group of wild and perennial plants, native from the North of America [6,7]. *E. purpurea* possess a secular story of use, being even known by primitive societies as “anti-infectious” agent, due to a great potential to treat viral and bacterial infections, varying from a simple acne and ulcers to mild septicemias [6–9]. Interestingly, over the years, its immunomodulatory properties have been increasingly reported both through *in vitro* as also *in vivo* studies, which confirm its traditional use. However, other biological effects have been described, also derived from their modulatory potential, such as antifungal, antiviral, antibacterial, anti-inflammatory, antioxidant and even antitumor properties [6,10–12]. Not least interesting to highlight is the fact that, despite to the wide variety of bioactive constituents that have been isolated, it was not already possible to identify and to relate the most important phytochemical with a specific biological potential, due to their complex chemical structures; so, synergic reactions have been stated as the basis of these promissory bioactivities [13].

Stimulation of the natural killer (NK) cells activity is considered one of the most representative immunomodulatory potentialities of *E. purpurea*; in fact, these cells play a crucial role in the defensive status of body, through phagocytosis, free radicals generation, intermediaries of the inflammatory process and in the secretion of a wide variety of biochemically different substances, among them enzymes, anti and pro-inflammatory cytokines, such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), nitric oxide (NO) and reactive oxygen species (ROS), which increase the ability of the body to impair the tumor growth and to eliminate a wide variety of bacteria and fungi [14–17]. Therefore, through modulation pathways, *E. purpurea* acts in a multitude of biological processes. For example, Matsiopa et al. observed that, in carbon tetrachloride intoxicated mouse, Echinacea tincture greatly improved catalase and glutathione transferase enzymes activity [18]. On the other hand, Dogan et al. in a colite-induced model, described a mucosal protective effect, mainly conferred by the Echinacea antioxidative constituents, caffeic acid and echinacoside [19].

Considered one of the most controversial and current hot topic, premature aging and oxidative stress-related diseases among active population have demanded an increasing attention by the medical community [20–22]. Indeed, not only environmental and alimentary patterns acts as main triggering factors, but also free radicals overproduction by organic metabolism plays an important role [23–26]. In spite of the human body possess proper detoxifying mechanisms, the loss of the organic homeostasis leads to several changes in metabolic pathways which consequently improves the oxidative stress, mainly characterized by lipid, protein and DNA modifications, and then the likelihood of diseases/disorders occurrence [27,19]. *E. purpurea* has been showing promissory antioxidant properties, most of them directly related with its richness in phenolic compounds, including flavonoids and phenolic acids [6,19,28]. Furthermore,

and despite availability of commercial products containing *E. purpurea*, their antioxidant properties should be determined and compared with the raw material and derived extracts, which was the main purpose of this pioneer study.

## **Materials and methods**

### *Samples and samples preparation*

*From raw material.* The raw material of *Echinacea purpurea* (L.) Moench (aerial parts and inflorescences) was provided by “Cantinho das Aromáticas”, an organic and certified farm from Vila Nova de Gaia, Portugal (<http://cantinhodasaromaticas.blogspot.pt/>), and was used as (i) fresh material, which was then lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA); (ii) and dried material using heated and forced atmosphere, and packaged for bulk marketing. Both samples were reduced to powder and submitted to different procedures to obtain the following extracts: infusions (mixing 1 g with 200 mL of boiling distilled water for 5 min, and filtering under reduced pressure); decoctions (mixing 1 g with 200 mL of distilled water and boiling for 5 min, plus more 5 min at room temperature and filtering under reduced pressure); and hydroethanolic extracts (stirring 4 g with 30 mL of ethanol: water (80:20, v/v) for 1 h, filtering, re-extracting in the same conditions and removing the ethanol in a rotary evaporator (Büchi R-210, Flawil, Switzerland).

The water was removed from all the extracts by lyophilisation and the following stock solutions were prepared: aqueous extracts: 5 mg/mL and hydroethanolic extract (20 mg/mL), from which several dilutions were performed in order to evaluate the antioxidant activity.

*From dietary supplements.* Two dietary supplements were used, namely tablets (Echinaforce Kids® tablets, A. Vogel) and syrup (Syrup ECHINACIN®, Madaus), both based on extracts prepared from dried aerial parts of *E. purpurea*. These supplements were selected considering the results from a preliminary inquiry conducted in pharmacies of Bragança, Portugal (data not shown).

The tablets (300 mg) contained concentrated hydroalcoholic extract of *E. purpurea* (6.2 mg) and excipients (sorbitol, vegetal magnesium stearate and natural orange flavour). Each tablet was pulverized, dissolved in 10 mL of distilled water and then filtered under reduced pressure, in order to obtain a stock solution of 0.62 mg/mL.

Syrup (100 mL of solution) contained dried and pressed extract of *E. purpurea* (2.34 g) and excipients (potassium sorbate, anhydrous citric acid, xanthane, flavours and purified water). Therefore, a syrup concentration of 23.4 mg/mL was used as stock solution.

Successive dilutions were prepared from the initial stock solutions of both dietary supplements in order to evaluate the antioxidant activity.

#### *Standards and reagents*

The solvents n-hexane 95%, acetronile 99% and ethyl acetate 99.98% and HPLC grade were purchased to Fisher Scientific (Loures, Portugal). Ethanol and analytical grade were acquired in Fisher Chemical (Lisbon, Portugal). The standards of sugars and derivatives, organic acids, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid and (+)-catechin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tocopherols standards and tocol (50 mg/mL) were provided from Matreya (Pleasant Gap, Pensilvânia, EUA), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Alfa Aesar (Ward Hill, MA, EUA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

### *Evaluation of the antioxidant activity*

*Antioxidant activity assays.* The antioxidant activity of all extracts was evaluated through four different *in vitro* assays.

Scavenging effects on DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals (RSA) was evaluated using an ELX800 microplate Reader (Bio-Tek Instruments, Inc; Winooski, VT, USA), and calculated as a percentage of DPPH discolouration through the formula:  $[(A_{\text{DPPH}} - A_{\text{S}}) / A_{\text{DPPH}}] \times 100$ , where  $A_{\text{S}}$  is the absorbance of the solution containing the sample at 515 nm, and  $A_{\text{DPPH}}$  is the absorbance of the DPPH solution [29].

Reducing power (measured by ferricyanide Prussian blue assay) (RP) was evaluated by the capacity to convert  $\text{Fe}^{3+}$  into  $\text{Fe}^{2+}$ , measuring the absorbance at 690 nm in the microplate Reader mentioned above. Sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1 % w/v, 0.5 mL) were added at the different concentration solutions (0.5 mL). Then, the mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10 % w/v, 0.5 mL) was added. The final mixture (0.8 mL) was put in a 48-wells, and also deionised water (0.8 mL) and ferric chloride (0.1 % w/v, 0.16 mL) was joined. Finally, the absorbance was measured at 690 nm [29].

$\beta$ -Carotene bleaching inhibition (CBI) was evaluated through the  $\beta$ -carotene/linoleate assay; the zero time absorbance was measured at 470 nm in a spectrophotometer (AnalytikJena, Jena, Germany). The neutralization of linoleate free radicals avoids  $\beta$ -carotene bleaching, which is measured by the formula:  $(\beta\text{-carotene absorbance after 2h of assay} / \text{initial absorbance}) \times 100$  [29].

Lipid peroxidation inhibition (LPI) was evaluated in porcine brain cell homogenates by the decreasing in thiobarbituric acid reactive substances (TBARS); the colour intensity of the malondialdehyde-thiobarbituric acid (MDA-TBA) adduct was measured by its

absorbance at 532 nm; the inhibition ratio (%) was calculated using the following formula:  $[(A - B)/A] \times 100\%$ , where A and B were the absorbance of the control and the sample solution, respectively.

The results were expressed in  $EC_{50}$  values, i.e. sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay [29]. Trolox was used as positive control.

*Determination of total antioxidants.* Total phenolics were estimated according with [Wolfe et al. \(2003\)](#), with some modifications. Folin-Ciocalteu (2.5 mL, previously diluted in water 1:10 v/v) was added to an aliquot (0.5 mL) of each sample preparation, and sodium carbonate (75 g/L, 2 mL). The mixture was centrifuged during 15 s and let stand for 30 min at 40°C, in order to allow the coloration development. The corresponding absorbance was measured at 765 nm (Analytikjena, Jena, Germany). Gallic acid was used to calculate the standard curve (0.05-0.8 mM:  $y = 1.683x + 0.044$ ;  $R^2 = 0.999$ ), and the results were expressed in mg of gallic acid equivalents (GAE) by mL of extract solution.

Total flavonoids were determined according [Jia et al. \(1999\)](#), with some modifications. An aliquot (0.5 mL) of each sample solution was mixed with deionized water (2 mL), and then with  $NaNO_2$  solution (5 %, 0,15 mL). After 6 min, an  $AlCl_3$  solution (10 %, 0,15 mL) was added and let to rest for 6 min more. Afterwards, a NaOH solution (4%, 2 mL) and distilled water were added to perform a final volume of 5 mL. Finally, the obtained mixture was allowed to stand during 15 min, and the pink color intensity was measured at 510 nm. (+)-Catechin was used to determine the standard curve (0.0156-1.0 mM;  $y = 0.98766x - 0.0008$ ;  $R^2 = 0.999$ ), and the results were expressed in mg of (+)-catechin equivalents by mL of extract solution.



### *Determination of the chemical composition*

*Free sugars and derivatives.* Free sugars and derivatives were determined by High Performance Liquid Chromatography (HPLC), coupled to a detector of refraction index (HPLC-RI), as described by [Barros et al. \(2010\)](#). Each sample (1 g) was enriched with melezitose as an internal standard (IS, 5 mg/mL) and further extracted (except the syrup, which was directly analyzed after dilution) with ethanol 80%, at 80 °C, during 30 min. The resulted suspension was centrifuged (refrigerated centrifuge Centorium K240R-2003) at 15000g, during 10 min. The supernatant was concentrated and vestiges of lipids were removed by washing three times with ethyl ether (10 mL). After concentration, solid residues were dissolved in water to a final volume of 5 mL. Sugars were determined by using an HPLC system (HPLC, Knauer, Smartline system, Berlin, Germany) at 35°C, coupled with a RI detector (Knauer Smartline 2300) and a column 100-5 NH<sub>2</sub> Eurospher (4.6 × 250 mm, 5 µm, Knauer). The mobile phase was acetronile/deionized water, 70:30 (v/v), with a caudal of 1 mL/min. Sugars identification was done by comparison of the relative retention times of the sample peaks with standards, and its quantification by using the internal pattern method.

*Organic acids.* Organic acids were determined by following the previous procedure described by [Pereira et al. \(2012\)](#). The samples (2 g) were extracted (except the syrup, which was directly analyzed after dilution), being shacked with methaphosphoric acid (25 mL), at 25 °C and 150 rpm, during 45 min and, then filtered first through Whatman n°4 paper, and next through filters of nylon (0.2 µm). The analysis was performed in an ultra fast liquid chromatography (UFLC, Shimadzu 20A) system, with a column of reverse phase C18 SphereClone (4.6 × 250 mm, 5 µm, Phenomenex, Torrance, CA,

USA), operating at 35 °C. The elution was carried out with sulfuric acid 3.6 mM by using a caudal of 0.8 mL/min and the detection was carried out with a photodiode array (PDA) detector, in which 215 nm and 245 nm (only for ascorbic acid) were the preferential wavelengths. Finally, organic acids were quantified by comparison the respective areas of registered peaks with the calibration curves obtained from the commercial standards.

*Tocopherols.* Tocopherols were determined by using an optimized procedure described by Barros et al. (2010). Previously to the extraction procedure, BHT in hexane (10 mg/mL; 100 µL) and the IS in hexane (tocol: 50 µg/mL; 400 µL) solutions were added to the samples (500 mg), and then homogenized (except the syrup, which was directly analyzed after dilution) with methanol (4 mL) during 1 min (vortex). Afterwards, hexane (4 mL) was added, homogenized once again during 1 min (vortex), mixed with a concentrated NaCl aqueous solution (2 mL), homogenized (1 min) and centrifuged (5 min, 4000g). The supernatant was carefully transferred to a vial, and each sample was re-extracted twice again with hexane. The combined extracts were dried in a nitrogen stream, re-dissolved in 2 mL of hexane, dehydrated with anhydrous sodium sulfate, filtered through a LC disposable filter (0.22 µm), transferred to a vial injection and analyzed by HPLC. The HPLC equipment (Knauer) was coupled to a detector of fluorescence (FP-2020, Jasco, Easton, MD, USA). All the data were analyzed by using Clarity 2.4 (DataApex) software. The chromatographic separation was achieved by using a normal phase column of Polyamide II (4.6 × 250 mm, 5 µm, YMC Waters, Lisboa, Portugal), at 30°C. The mobile phase used was a mixture of hexane and ethyl acetate (70:30, v/v) with a caudal of 1 mL/min. The quantification was based in the

fluorescence signal, by using an internal standard method and by comparison with the standards.

### **Statistical analysis**

Three samples were used for each preparation and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analyzed using a Student's *t*-test, in order to determine the significant difference among different samples, with  $\alpha = 0.05$ . This treatment was carried out using SPSS v. 22.0 program (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA, IBM Corp.).

### **Results and discussion**

#### *Antioxidant activity of phytopharmacological preparations based on E. purpurea*

There are several methods that can be used to assess the *in vitro* antioxidant activity of natural matrices, from which several constrains and advantages are commonly associated [35–37]. In the present work, the free radical scavenging activity (RSA), reducing power (RP),  $\beta$ -carotene bleaching inhibition (CBI) and lipid peroxidation inhibition (LPI) assays were used.

**Table 1** shows the results of the antioxidant activity of hydroethanolic and aqueous extracts obtained from dry or fresh *E. purpurea*, as also of dietary supplements based on the same plant (tablets and syrup). A global comparison was performed in order to find the most active phytopharmacological preparation or formulation.

No significant statistical differences were found between the RSA of all the extracts prepared from fresh material, the hydroalcoholic extract of dried plant and the commercial tablets, which showed the highest capacity. The lowest RSA was observed

for the syrup. In relation to the RP ability, the hydroalcoholic extract prepared from the fresh plant and tablets presented the most pronounced effects, while the syrup was once again the least effective. Concerning to the CBI, the hydroalcoholic extract of the fresh plant continued to be the most effective, while the aqueous extract prepared by infusion of the dried material presented the lowest activity. Lastly, the hydroalcoholic extract of the fresh plant revealed to be the most active in LPI, while syrup provided the lowest potential. In addition, and once lipid peroxidation is responsible for progressive and significant damages on the membrane potential of cells, the study of natural matrices with protective effects is of the utmost importance. For example, neuronal cells are highly affected by free radicals and reactive oxygen species (ROS), being neurodegenerative disorders one of the most frequent worldwide aging-related diseases [20,38–40]. In fact, brain cells are highly susceptible to oxidative damages, once carries an intense and continuous supplying of oxygen; furthermore, it has no effective detoxifying systems and antioxidant defenses, apart from its richness in vulnerable substances (i.e., polyunsaturated fatty acids, catecholamines, ions from the transition metals such as iron, etc.) [38,41–43]. In this case, through TBARS assay, it was observed a high inhibition of lipid peroxidation; so, purple coneflower extracts and related commercial preparations revealed to be promissory antioxidant agents.

The highest antioxidant potential was achieved by the hydroalcoholic extract of the fresh plant (in all the assays), which is directly related with its highest content in phenolics and flavonoids. Similar evidences were observed regarding the lowest concentration of phenolics and flavonoids found in the syrup, which provided the lowest antioxidant activity (**Table 1**). It should be also highlighted that the aqueous extract prepared by infusion of the dried plant also presented a weak effect, directly correlated with its scarce content in flavonoids and phenolics. It is important to highlight that

phenolic compounds have been increasingly related with the antioxidant potential of natural matrices; in fact, they have important chemical structures with reducing abilities, which confer significant effects at a level of free radicals neutralization [28,42,44,45]. They also act both in the initiation steps and in the propagation of the oxidative pathways, by neutralizing damaging effects, once forms stable intermediate compounds (conferred by its phenolic rings and hydroxyl radicals) [46–49].

[Henning et al. \(2011\)](#) by evaluating the antioxidant potential of several herbs and spices, observed that not only different extraction solvents as also phytochemical forms (dry, fresh and blended paste) cause direct interferences on their phenolic contents and consequently affects the final bioactivity of the studied matrices. In the same line, [Pinela et al. \(2012\)](#) by determining the antioxidant potential, phenolic and ascorbic acid contents in different samples of *Tuberaria lignosa* (Sweet) Samp., achieved significant differences that varied according with the plant origin (wild vs. commercial), preparation method (infusion vs. decoction) and even storage procedures (freeze vs. shade-dried). The authors observed a higher antioxidant potential, phenolics and flavonoids contents in the wild samples; overall, freeze-dried preparation presented the most promissory antioxidant effects; however, while freeze-dried decoction preparation presented the highest abundance in flavonoids ( $19.48 \pm 0.50$  mg/g), shade-dried infusion was more rich in phenolics ( $250.14 \pm 0.57$  mg/g) [51].

In the present study, the hydroalcoholic extract of the fresh aerial parts of *E. purpurea* evidenced the most promissory antioxidant effects, while syrup exhibit the weakest potential, which is in accordance with previous studies. For example, several authors demonstrated that the dried extracts of purple coneflower and its derived syrups were not efficient to reduce symptoms, duration and time of colds [52–54]. On the other hand, [Almeida et al. \(2011\)](#) by assessing the antioxidant potential of several dietary

supplements, among them pills, capsules and infusions, achieved significant differences in the final bioactive potential. In spite of capsules presented the highest antioxidant potential, evaluated by DPPH scavenging activity, reducing power and TBARS inhibition assays, when combined with other preparations (namely infusions and resveratrol) a pronounced improvement on their final potential (nearly to 92% of cases) was observed [55]. Therefore, the combination of several plant preparations appears to improve and confer additional health benefits. For example, [Pereira et al. \(2014\)](#) studied the effects of the type of plant-based formulations and compositional mixtures on the antioxidant and cytotoxic activities of dietary supplements. The authors observed the existence of synergistic reactions when plant-derived formulations were used in combination; furthermore, and apart from that the syrup presented the highest antioxidant potential, followed by infusion preparation and then pills, it was the infusion preparation that provided the most pronounced toxic effects on hepatocellular carcinoma cell lines (HepG2) [56]. It means that probably other interfering factors, such as excipients, can exert significant modulatory effects and, therefore, while in the infusion preparation the bioactive molecules are free and able to exert their biological activity, they can be linked with other chemical constituents in phytopharmacological preparations (such as syrup and pills).

#### *Chemical composition of phytopharmacological preparations based on *E. purpurea**

**Table 2** shows the obtained results of the chemical composition of *E. purpurea* dried and fresh raw material, respectively; being identified and then quantified four different sugars and tocopherols, and six organic acids. In general, fresh plant (which was lyophilized immediately after recollection) presented the highest abundance in chemical constituents: sugars > tocopherols > organic acids. By analyzing the free sugars

contents, fructose, glucose and arabinose were detected in dried and fresh material, and, for the last one, sucrose was also detected. While glucose was the most abundant in the dried aerial parts of *E. purpurea* ( $1.15 \pm 0.02$  g/100 g), sucrose ( $4.39 \pm 0.17$  g/100 g) predominated in the fresh plant. In relation to the tocopherols contents, the fresh plant presented all the tocopherol isoforms ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol), being  $\alpha$ -tocopherol ( $3.84 \pm 0.04$  mg/100 g) the most representative, while in the dried plant  $\delta$ -tocopherol was absent. Lastly for organic acids, six were detected in dried and fresh material, being succinic, citric, quinic and malic acids the most abundant; citric acid was the most abundant in the fresh plant ( $5.19 \pm 0.18$  g/100 g), while succinic acid was the prevalent in the dried material ( $2.15 \pm 0.07$  g/100 g).

A particular attention should be given to tocopherols, once they are important natural antioxidants in foods. Their action as free radicals scavengers and hydrogen donators, confer a pronounced protection to the organisms against several disorders, among them degenerative and cardiovascular diseases [24]. So, tocopherols present a pivotal importance at intracellular level, and its absence increase the membrane fragility and consequent vulnerability to the free radicals attack. Among them,  $\alpha$ -tocopherol is the isoform with the highest biological potential [27], which explains and even confirms the obtained promissory antioxidant potential of the fresh preparation obtained from *E. purpurea*. Other authors have already confirmed the interesting tocopherols abundance in *Echinacea* seeds. For example, [Oomah et al. \(2006\)](#) by evaluating the physicochemical characteristics of three different *Echinacea* species, observed that not only  $\alpha$ -tocopherol was the most abundant tocopherol isoform in *E. purpurea* seeds, but also its content varied according to the *Echinacea* species, harvesting time and plant source/origin. However, and to the authors' knowledge, there are no studies evaluating

the respective sugars, tocopherols and organic acid contents in aerial parts of *E. purpurea*.

**Table 3** shows the obtained results for the chemical composition of two phytopharmacological formulations (i.e. tablets and syrup) based on *E. purpurea*. In spite of syrup presented the highest abundance in sugars, being xylitol ( $15.25 \pm 0.55$  g/100 mL) the most prominent (which is in agreement with the indication of the package label), in the tablets only sorbitol ( $0.34 \pm 0.01$  g/tablet) and vestigial amounts of sucrose were detected. Among the following organic acids identified, namely citric, oxalic, malic, xiquimic and succinic acids, citric acid was the most abundant in tablets ( $1.41 \pm 0.21$  g/tablet), while in syrup it was succinic acid ( $0.93 \pm 0.01$  g/100 mL). Regarding tocopherols, only  $\alpha$ -tocopherol was quantified in tablets ( $0.14 \pm 0.01$   $\mu$ g/tablet), while in the syrup no tocopherol isoforms were identified.

The pronounced differences observed between commercial and traditional phytopharmacological preparations appears to be directly related with the type of excipients used. For example, by comparing the sugars contents with those presents in dried and fresh plant, xylitol and sorbitol were absent; it means that both of them derived from external addition (i.e. excipients), respectively in the syrup and tablets based on *E. purpurea*. However, it is interesting to highlight that, at the same time, it was possible to confirm the labeled nutritional information. There are several reports evidencing the modulatory effects of preservatives on the products bioavailability [58,59]. In fact, not all the bioactive molecules are easily solubilized, chemically stable or organoleptically acceptable; therefore, it is crucial to proceed with their homogeneity in order to improve the tolerability by consumers. However, it is also important to ensure that no blocking effects and interferences will occur with their use, much more than focusing the organoleptic acceptance of plant-derived supplements.



## **Conclusion**

Considering the magnitude of oxidative stress-related disorders, the use of efficient alternatives, namely based on natural matrices have shown to constitute an upcoming and promissory approach to improve the health and wellbeing of individuals.

In the present experiment, and despite the existence of several reports that evaluated the bioactive potential of *E. purpurea*, it was very interesting to observe that depending on different procedures used to process raw materials (fresh material or dried under heated and forced atmosphere; infusion, decoction, others), noticeable variations in the antioxidant potential were obtained. So, it is possible to conclude that not only the extraction solvent but also the extraction conditions and the formulation act as conditioning factors to the final bioactivity. Otherwise, the type of raw plant material (fresh or dried) used, also plays a crucial role. In this case, the hydroalcoholic extract of the fresh plant proved to be the most antioxidant, while the syrup, prepared with dried plants, presented the lowest activity. All of these achievements appear to be directly correlated with the contents in phenolics and flavonoids. However, further studies are necessary in order to assess the *in vivo* potential and related modes of action, including the establishment of therapeutic and prophylactic doses.

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**Table 1.** Antioxidant potential of different phytopharmacological formulations based on *Echinacea purpurea* (mean  $\pm$  SD).

EC <sub>50</sub> values (mg/mL)	Dried plant			Fresh plant			Supplements	
	Hydroethanolic	Aqueous (I)	Aqueous (D)	Hydroethanolic	Aqueous (I)	Aqueous (D)	Tablets	Syrup
DPPH scavenging activity (RSA)	0.76 $\pm$ 0.02 <sup>d</sup>	2.41 $\pm$ 0.22 <sup>b</sup>	1.43 $\pm$ 0.03 <sup>c</sup>	0.28 $\pm$ 0.22 <sup>d</sup>	0.47 $\pm$ 0.05 <sup>d</sup>	0.35 $\pm$ 0.07 <sup>d</sup>	0.28 $\pm$ 0.01 <sup>d</sup>	26.38 $\pm$ 1.2 <sup>a</sup>
Reducing power (RP)	0.55 $\pm$ 0.04 <sup>d</sup>	1.82 $\pm$ 0.05 <sup>b</sup>	0.96 $\pm$ 0.01 <sup>c</sup>	0.18 $\pm$ 0.01 <sup>g</sup>	0.40 $\pm$ 0.01 <sup>e</sup>	0.29 $\pm$ 0.01 <sup>f</sup>	0.17 $\pm$ 0.02 <sup>g</sup>	2.20 $\pm$ 0.02 <sup>a</sup>
$\beta$ -carotene bleaching inhibition (CBI)	5.51 $\pm$ 0.63 <sup>b</sup>	7.05 $\pm$ 0.36 <sup>a</sup>	4.10 $\pm$ 0.10 <sup>c</sup>	0.36 $\pm$ 0.03 <sup>f</sup>	1.95 $\pm$ 0.18 <sup>de</sup>	2.21 $\pm$ 0.10 <sup>d</sup>	*	1.79 $\pm$ 0.03 <sup>e</sup>
TBARS inhibition (LPI)	0.84 $\pm$ 0.01 <sup>c</sup>	1.64 $\pm$ 0.01 <sup>b</sup>	0.72 $\pm$ 0.02 <sup>d</sup>	0.04 $\pm$ 0.01 <sup>g</sup>	0.45 $\pm$ 0.02 <sup>e</sup>	0.30 $\pm$ 0.02 <sup>f</sup>	0.07 $\pm$ 0.01 <sup>h</sup>	2.27 $\pm$ 0.06 <sup>a</sup>
Phenolics (mg GAE/mL)	49.26 $\pm$ 1.34 <sup>e</sup>	13.79 $\pm$ 0.56 <sup>g</sup>	24.96 $\pm$ 0.46 <sup>f</sup>	229.22 $\pm$ 4.38 <sup>a</sup>	55.02 $\pm$ 0.16 <sup>d</sup>	73.32 $\pm$ 2.33 <sup>c</sup>	154.61 $\pm$ 2.74 <sup>b</sup>	9.67 $\pm$ 0.07 <sup>h</sup>
Flavonoids (mg CE/mL)	22.82 $\pm$ 2.06 <sup>e</sup>	5.65 $\pm$ 0.30 <sup>f</sup>	7.49 $\pm$ 1.30 <sup>f</sup>	124.83 $\pm$ 7.47 <sup>a</sup>	29.09 $\pm$ 1.76 <sup>d</sup>	52.33 $\pm$ 2.45 <sup>c</sup>	73.76 $\pm$ 1.13 <sup>b</sup>	3.29 $\pm$ 0.06 <sup>f</sup>

(I)- infusion; (D)- decoction; GAE- gallic acid equivalents; CE- (+)-catechin equivalents. \*- Pro-oxidant effect. EC<sub>50</sub> values correspond to the sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay. In each row, different letters means significant differences between extracts ( $p < 0,05$ ).

**Table 2.** Chemical composition of different raw materials derived from *Echinacea purpurea* (mean  $\pm$  SD).

	Dried plant	Fresh plant*	t-Students test p-value
<b>Sugars</b>	(g/100 g)	(g/100 g)	
Arabinose	0.32 $\pm$ 0.01	1.02 $\pm$ 0.03	<0.001
Fructose	0.07 $\pm$ 0.02	3.45 $\pm$ 0.16	<0.001
Glucose	1.15 $\pm$ 0.02	4.08 $\pm$ 0.23	<0.001
Sucrose	n.d.	4.39 $\pm$ 0.17	-
Sum	1.54 $\pm$ 0.01	12.94 $\pm$ 0.59	<0.001
<b>Organic acids</b>	(g/100 g)	(g/100 g)	
Oxalic acid	0.32 $\pm$ 0.04	0.36 $\pm$ 0.02	0.072
Quinic acid	1.98 $\pm$ 0.12	1.10 $\pm$ 0.04	0.001
Malic acid	1.09 $\pm$ 0.05	1.04 $\pm$ 0.10	0.291
Shikimic acid	0.02 $\pm$ 0.0001	0.01 $\pm$ 0.001	0.003
Citric acid	1.23 $\pm$ 0.01	5.19 $\pm$ 0.18	<0.001
Succinic acid	2.15 $\pm$ 0.07	1.19 $\pm$ 0.04	<0.001
Sum	6.79 $\pm$ 0.04	8.89 $\pm$ 0.10	<0.001
<b>Tocopherols</b>	(mg/100 g)	(mg/100 g)	
$\alpha$ -Tocopherol	0.14 $\pm$ 0.04	3.84 $\pm$ 0.04	<0.001
$\beta$ -Tocopherol	0.15 $\pm$ 0.01	0.49 $\pm$ 0.01	<0.001
$\gamma$ -Tocopherol	0.060 $\pm$ 0.001	0.07 $\pm$ 0.01	0.158
$\delta$ -Tocopherol	n.d.	0.15 $\pm$ 0.01	-
Sum	0.35 $\pm$ 0.04	4.55 $\pm$ 0.02	<0.001

\* and lyophilized material. n.d., not detected.



**Table 3.** Chemical composition of different phytopharmacological formulations based on *Echinacea purpurea* (mean  $\pm$  SD).

	Syrup	Tablets
<b>Sugars</b>	(g/100 mL)	(g/tab)
Xylitol	15.25 $\pm$ 0.55	nd
Fructose	0.53 $\pm$ 0.01 <sup>b</sup>	nd
Glucose	0.25 $\pm$ 0.02 <sup>c</sup>	nd
Sucrose	nd	tr
Sorbitol	nd	0.34 $\pm$ 0.01
Sum	16.03 $\pm$ 0.57 <sup>a</sup>	0.34 $\pm$ 0.01
<b>Organic acids</b>	(g/100 mL)	(mg/tab)
Oxalic acid	0.25 $\pm$ 0.01 <sup>b</sup>	0.17 $\pm$ 0.02
Quinic acid	nd	tr
Malic acid	0.22 $\pm$ 0.02 <sup>b</sup>	0.51 $\pm$ 0.04
Shikimic acid	tr	0.01 $\pm$ 0.00
Citric acid	0.41 $\pm$ 0.01 <sup>c</sup>	1.41 $\pm$ 0.21
Succinic acid	0.93 $\pm$ 0.01 <sup>c</sup>	0.65 $\pm$ 0.03
Fumaric acid	tr	tr
Sum	1.81 $\pm$ 0.01 <sup>c</sup>	2.75 $\pm$ 0.20
<b>Tocopherols</b>	(mg/100 mL)	( $\mu$ g/tab)
$\alpha$ -Tocopherol	nd	0.14 $\pm$ 0.01
Sum	nd	0.14 $\pm$ 0.01

Tab, tablet; n.d., not detected; tr.- traces.