# INFUSIONS AND DECOCTIONS OF MIXED HERBS USED IN FOLK MEDICINE: SYNERGISM IN ANTIOXIDANT POTENTIAL

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**Running Head:** Synergism in antioxidant potential of mixed herbs from folk medicine

**ABSTRACT**

Infusions (herbal teas) and decoctions are frequently used to administer oral doses of herbs. Although some herbs are used as single ingredients, they are often prepared as mixtures, as reported by numerous ethnobotanical surveys. The present work was carried out to identify the different types of interaction (synergistic, additive and antagonistic effects) which may be found in the antioxidant activity of preparations from mixtures of the popular herbs *Aloysia citrodora* (lemon verbena), *Foeniculum vulgare* (fennel) and *Mentha spicata* (spearmint). Herbs were prepared using traditional methods, and the effects after different periods of storage, up to 120 days, were also evaluated. Antioxidant activity was evaluated using DPPH radical scavenging activity, reducing power, and inhibition of lipid peroxidation by the β-carotene-linoleate system and the TBARS assay. Known antioxidant compounds such as total phenolics, flavonoids, ascorbic acid and reducing sugars were also determined. Spearmint was found to be present in the herb mixtures with the greatest antioxidant activity and these also had the highest flavonoid content. The most potent antioxidant activity was found in combinations of different herbs, suggesting synergistic effects.

*Keywords*: Herbal mixtures; Infusions/Decoctions; Synergistic effects; Antioxidant activity.

## INTRODUCTION

Many research groups are examining the chemical nature and activity of natural antioxidants in fruits, vegetables, grains, herbs and other foods. Most antioxidants isolated from higher plants are polyphenols, which show biological activity as antibacterial, anti-carcinogenic, anti-inflammatory, antiviral, anti-allergic, estrogenic, and immune-stimulating effects. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelating potential (Larson, 1988; Atoui *et al.*, 2005).

Tea (specifically refers to infusions prepared from the leaves of *Camellia sinensis*) and herbal teas (known also as tisanes, that are infusions made from roots, flowers, leaves, seeds or twigs of other plants) contribute to the major source of phenolic compounds in our diet (Shahidi, 2000; Shikanga *et al.*, 2010). Herbal teas are the most popular non-alcoholic beverages in the world, because of the multitude of associated health benefits.

Spontaneous wild flora (e.g. fennel), as well as cultivated exotic species (e.g. lemon-verbena and spearmint), introduced in homegardens long time ago, are usually picked and kept in the best conditions for use throughout the year. Their popularity is due to their fragrance, flavor and medicinal properties, mostly those concerning the digestive and respiratory systems. Particularly, lemon-verbena (*Aloysia citrodora* Palau; *port.* limonete, erva-luísa, doce-lima, lucialima) infusions are used for its stomachic, sedative, febrifuge and antispasmodic effects (Camejo *et al*., 2003; Cunha *et al*., 2007; Carvalho, 2010). Infusions and decoctions of fennel (*Foeniculum vulgare* Mill.; *port.* funcho, fiolho, fionho, erva-doce) are prepared for the respiratory, gastrointestinal and genitourinary systems (Camejo *et al.*, 2003; Novais *et al.*, 2004; Cunha *et al*., 2007; Carvalho, 2010). Spearmint (*Mentha spicata* L.; *port.* hortelã-pimenta) tea or infusion is

considered a digestive beverage and has traditionally been used in the treatment of headaches and respiratory and digestive disorders (Cunha, 2007; Carvalho, 2010).

In a previous report we described the effects of preparation methods (infusion and decoction) and storage period in the antioxidant potential of herbal water oral dosages of the mentioned herbs (Guimarães *et al.*, 2010). Nevertheless, these species are widely combined in mixtures in order to enhance their pharmacological effects as reported in several ethnobotanical surveys (Frazão-Moreira *et al.*, 2009; Carvalho, 2010). Most herbal practitioners and skilful healers have learned from their ancestors that herbal mixtures can be useful to increase the medicinal properties of individual species, to reduce some kind of toxicity and to improve the taste of some oral forms. The folk use of mixtures is based on an empirical concept of similarity of the therapeutic effects (e.g fennel and spearmint are both individually used as a digestive), as well as on the assumption of the sum of the benefits (Carvalho, 2010).

The generally accepted medical opinion that recommends the use of concentrated granules of a single herb (Cheng *et al.*, 2005) should be reconsidered. Additional therapeutic effects to those derived from a single herb might be gained by using herbal mixtures (Kuijun *et al.*, 2009).

Total antioxidant capacity in natural matrixes is attributed to three different types of interaction: synergistic (Hsu *et al.*, 2005; Queirós *et al.*, 2009), antagonistic (Wang *et al.*, 2000, Pinelo *et al.*, 2004), and additive (Philpott *et al.*, 2004; Heo *et al.*, 2007) effects. Frequently, the antioxidant activity is due to a combination of phytochemicals, resulting in additive and/or synergistic effects. This explains why no single antioxidant can replace the combination of natural phytochemicals in foods and achieve their health benefits (Liu, 2004).

The present work aims to evaluate the different types of interaction in the antioxidant activity of infusions and decoctions from mixtures of herbs (lemon-verbena, fennel and spearmint) used in folk medicine.

## MATERIALS AND METHODS

**Standards and reagents.** All the solvents were of analytical grade purity; methanol was supplied by Lab-Scan (Lisbon, Portugal). The standard used in the antioxidant activity assays, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), L-ascorbic acid, gallic acid and (+)-catechin were purchased from Sigma (St. Louis, MO, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

**Plant material and samples.** Aerial parts of the three species were gathered in June 2009, in Bragança, Trás-os-Montes, north-eastern Portugal. The selected sites and gathering practices took into account local consumers criteria and the optimal growth stage preferences for preparing herbal beverages, such as infusion and decoction. Thus, fennel flowering shoots (stems, leaves and flower buds) were collected in half shade sites at the edges of woods. Shoots (stems and leaves) from lemon-verbena and spearmint were gathered in two homegardens with informants' agreement. Morphological key characters from the Flora Iberica were used for plant identification. Voucher specimens are kept in the Herbarium at the Escola Superior Agraria de Bragança.

The three species were used fresh (immediately after being collected), and shade-dried (after being stored in a dark, dry and room temperature place, for 30, 60 and 120 days), simulating informants' usual conditions. Using fresh and dried materials, three types of mixtures were prepared according to traditional practices as documented in ethnobotanical surveys carried out in the Portuguese North-eastern region (Frazão-Moreira *et al.*, 2009; Carvalho, 2010): lemon-verbena + fennel, lemon-verbena + spearmint and fennel + spearmint.

**Preparation of the samples.** According to informants' procedures (Carvalho, 2010), preparing half a litter of an infusion or decoction requires a handful of fresh plant material. Therefore, a half of a handful of each fresh sample was weighted (to calculate the corresponding dry weight) and then mixed, as recommended by informants, to prepare the oral forms. Herbal dosage forms were prepared by decoction and infusion using samples with different storage times. For time zero, fresh samples were used (7.5 g of lemon-verbena; 9 g of fennel; 5 g of spearmint). In the subsequent times of storage (30, 60 and 120 days) dry weight corresponding to the mentioned fresh weights were used (3 g of lemon-verbena; 3 g of fennel; 1 g of spearmint aerial parts). The codes used to identify each sample are shown in **Table 1**.

*Decoctions.* The sample was added to 500 mL of distilled water, and heated (heating plate, VELP scientific) until boiling. The mixture was left stand at boiling temperature for 5 min and at room temperature for 5 minutes more, and then filtered under reduced pressure. The obtained decoction was frozen, lyophilized (Ly-8-FM-ULE, Snijders, Holland) and redissolved in water at a concentration of 2.5 mg/mL.

*Infusions.* The sample was added to 500 mL of boiling distilled water and left to stand at room temperature for 5 minutes, and then filtered under reduced pressure. The obtained

infusion was frozen, lyophilized and redissolved in water at a concentration of 2.5 mg/mL.

**Evaluation of antioxidant activity**

The antioxidant activity was evaluated by DPPH radical-scavenging activity, reducing power, inhibition of β-carotene bleaching in the presence of linoleic acid radicals and inhibition of lipid peroxidation using TBARS in brain homogenates, according to procedures previously described by the authors (Guimarães et al., 2010). The extract concentrations providing 50% of antioxidant activity or 0.5 of absorbance ($EC_{50}$) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene bleaching and TBARS assays) or absorbance at 690 nm (reducing power assay) against extract concentrations. Trolox was used as standard.

**Evaluation of antioxidants**

Total phenolics, flavonoids, ascorbic acid and reducing sugars were estimated following spectrophotometer assays described by the authors (Guimarães et al., 2010).

Total phenolics and flavonoids were calculated using gallic acid ($9.4 \times 10^{-3}$ - $1.5 \times 10^{-1}$ mg/mL) and (+)-catechin ($4.5 \times 10^{-3}$ - $2.9 \times 10^{-1}$ mg/mL) to obtain the standard curves. The results were expressed as mg of gallic acid equivalents (GAE) and mg of (+)-chatequin equivalents (CE), respectively for phenolics and flavonoids, per g of decoction/infusion.

Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid ($6.0 \times 10^{-3}$ -$1.0 \times 10^{-1}$ mg/mL), and the results were expressed as mg of ascorbic acid per g of decoction/infusion.

Reducing sugars were estimated using glucose to calculate the standard curve (0.2-1.5

mg/mL); the results were expressed as mg of reducing sugars per g of decoction/infusion.

### Statistical analysis

All the assays were carried out in triplicate in three different samples of each single herb. The results were expressed as mean values and standard deviation (SD), and were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$ (SPSS v. 16.0 program).

## RESULTS AND DISCUSSION

Lemon-verbena, fennel and spearmint are some of the species widely used as single ingredients or as mixtures for herbal remedies. Traditional healers and local herbal practitioners believe that mixtures have particular attributes which are not encountered when using single plants. Besides, some herbal mixtures are used to improve taste which, in a few cases, it is a problem for some consumers. They empirically consider that mixtures increase the therapeutic efficacy of the beverages, but until recently there has been little evidence to demonstrate that this is the case (Carvalho, 2010).

The antioxidant properties of decoctions and infusions prepared mixing two herbs with storage periods up to 4 months were evaluated by four different assays: DPPH radical scavenging capacity, reducing power and inhibition of lipid peroxidation using β-carotene-linoleate model system and TBARS assay in brain homogenates (**Table 2**). Antioxidant compounds present in decoctions/infusions, including phenolics, flavonoids, ascorbic acid and reducing sugars, were also determined (**Table 3**).

The presence of spearmint in the mixtures seemed to increase their antioxidant properties (significantly lower $EC_{50}$ values; $p<0.05$), which is in agreement to previous

results reporting this herb as having the highest antioxidant activity (Guimarães *et al.*, 2010). In fact, the samples F+S $I_{30}$ and $D_{60}$, L+S $I_0$ and $D_{60}$, F+S $I_0$, and F+S $I_{30}$ and L+S $D_{60}$, revealed the highest DPPH scavenging activity, reducing power, β-carotene bleaching inhibition and TBARS formation inhibition, respectively (**Table 2**). Some of the mentioned mixtures (F+S $I_{30}$ and F+S $I_0$) revealed high levels of phenolics. Furthermore, the mixtures with spearmint showed the highest concentration of flavonoids (**Table 3**) that are the main phenolic compounds in plants.

Otherwise, decoctions and infusions obtained from lemon-verbena and fennel (L+F) showed the lowest DPPH scavenging activity, reducing power and TBARS formation inhibition (**Table 2**). Fennel and lemon-verbena used individually in decoctions or infusions were also reported as having the lowest antioxidant potential (Guimarães *et al.*, 2010).

The method of infusion gave better or similar (no significantly statistical differences, $p<0.05$) antioxidant activity than the corresponding decoction, with the following exceptions: L+S $I_{30}$ in DPPH assay, L+S $I_{30}$, L+S $I_{60}$, F+S $I_{60}$ in reducing power assay, L+F $I_0$, L+S $I_{120}$ in β-carotene assay, and L+S $I_{60}$ in TBARS assay.

It was not possible to find a tendency of antioxidant properties evolution along the storage period. After 4 months of storage the herbs kept the antioxidant potential (low $EC_{50}$ values).

Phenolics and flavonoids were the main antioxidant compounds found in all the herbal beverages. The samples L+F $I_0$ (438.08 mg GAE/g), L+S $I_{120}$ (83.71 mg CE/g), L+F $I_{120}$ (11.00 mg/g) and L+S $I_{120}$ (0.84 mg/g) revealed the highest concentrations in phenolics, flavonoids, ascorbic acid and reducing sugars, respectively. It was not possible to find significantly negative linear correlations between the individual antioxidants analysed

and the $EC_{50}$ values of antioxidant activity. Therefore, other antioxidants than the herein quantified are contributing to the antioxidant potential of the herbal beverages.

The types of interactions (synergistic, additive or antagonistic effects) observed in the antioxidant activity of the herbal mixtures are given in **Table 4**. For DPPH radical scavenging activity assay, it was observed a synergism (increase of antioxidant capacity) in more than 80% of the samples. An antagonistic effect was obtained only in L+S $D_0$, L+S $I_{30}$ and L+F $D_{60}/I_{60}$. Considering the reducing power assay, the synergism was the main effect, being observed in 58% of the samples. All the mixtures obtained with herbs with a storage period of 30 days, the decoction L+F and the infusions L+F and L+S prepared with herbs with a storage period of 60 days presented antagonism; an additive effect was observed in L+S $D_{120}$. Only in the β-carotene bleaching assay, the main effect was antagonism (50% of the samples), followed by synergism (42%) and additive effects (8%). Once more, the synergist effect predominated in the TBARS inhibition assay, being observed in all the samples, unless in L+S $I_{60}$ that showed antagonism (**Table 4**).

Overall, L+F $D_0/I_0$, F+S $D_0/I_0$, F+S $I_{60}$, L+F $I_{120}$ and F+S $I_{120}$ revealed synergist effects in all the antioxidant activity assays. Two of these mixtures also presented the highest phenolic (L+F $I_0$; 438.08 mg GAE/g; **Table 3**) and ascorbic acid (L+F $I_{120}$; 11.00 mg/g; **Table 3**) contents. **Figure 1** shows the synergisms observed in L+F $I_{120}$ mixture.

Infusions of L+S prepared from herbs with 30 and 60 days of storage revealed antagonistic effects in all the antioxidant activity assays, unless in the TBARS inhibition assay and DPPH scavenging activity assay, respectively. **Figure 2** shows the antagonisms observed in L+S $I_{60}$ mixture.

## CONCLUSION

The results have shown that these preparations are in fact more effective as mixtures and have confirmed and validated the empirical uses of local healers and consumers. Spearmint was present in the mixtures with highest antioxidant properties and with highest flavonoids contents. Generally, the method of infusion gave better or similar antioxidant activity than the corresponding decoction. After 4 months of storage the herbs kept the antioxidant potential (low $EC_{50}$ values). Synergism was the main effect observed in the present study. Therefore, the generally accepted medical opinion that recommends the use of concentrated granules of a single herb should be reconsidered. Additional therapeutic effects to those derived from a single herb might be gained by using herbal mixtures.

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**Table 1.** Identification of the samples.

| Time of storage | Mixed herbs | Herbal tea | Code |
|---|---|---|---|
| 0 days | Lemon-verbena + Fennel | Decoction | L+F $D_0$ |
| | | Infusion | L+F $I_0$ |
| | Lemon-verbena + Spearmint | Decoction | L+S $D_0$ |
| | | Infusion | L+S $I_0$ |
| | Fennel + Spearmint | Decoction | F+S $D_0$ |
| | | Infusion | F+S $I_0$ |
| 30 days | Lemon-verbena + Fennel | Decoction | L+F $D_{30}$ |
| | | Infusion | L+F $I_{30}$ |
| | Lemon-verbena + Spearmint | Decoction | L+S $D_{30}$ |
| | | Infusion | L+S $I_{30}$ |
| | Fennel + Spearmint | Decoction | F+S $D_{30}$ |
| | | Infusion | F+S $I_{30}$ |
| 60 days | Lemon-verbena + Fennel | Decoction | L+F $D_{60}$ |
| | | Infusion | L+F $I_{60}$ |
| | Lemon-verbena + Spearmint | Decoction | L+S $D_{60}$ |
| | | Infusion | L+S $I_{60}$ |
| | Fennel + Spearmint | Decoction | F+S $D_{60}$ |
| | | Infusion | F+S $I_{60}$ |
| 120 days | Lemon-verbena + Fennel | Decoction | L+F $D_{120}$ |
| | | Infusion | L+F $I_{120}$ |
| | Lemon-verbena + Spearmint | Decoction | L+S $D_{120}$ |
| | | Infusion | L+S $I_{120}$ |
| | Fennel + Spearmint | Decoction | F+S $D_{120}$ |
| | | Infusion | F+S $I_{120}$ |

**Table 2.** Antioxidant activity ($EC_{50}$ values; mg/mL) of decoctions/infusions obtained from mixed herbs after different times of storage. The results are expressed as mean ± SD (n=9). In each column different letters mean significant differences ($p<0.05$).

| Samples | DPPH scavenging activity | Reducing power | β-Carotene bleaching inhibition | TBARS inhibition |
|---|---|---|---|---|
| L+F $D_0$ | 0.29 ± 0.00 cb | 0.15 ± 0.00 j | 0.17 ± 0.05 f | 0.08 ± 0.01 fde |
| L+F $I_0$ | 0.28 ± 0.00 cb | 0.16 ± 0.00 j | 0.29 ± 0.01 ef | 0.09 ± 0.01 cde |
| L+S $D_0$ | 0.28 ± 0.14 cb | 0.11 ± 0.00 k | 0.29 ± 0.04 ef | 0.06 ± 0.00 jigh |
| L+S $I_0$ | 0.14 ± 0.01 jhi | 0.08 ± 0.00 n | 0.31 ± 0.01 ef | 0.06 ± 0.00 jfigh |
| F+S $D_0$ | 0.24 ± 0.04 cfed | 0.09 ± 0.00 lnm | 0.33 ± 0.14 ef | 0.07 ± 0.01 jfigh |
| F+S $I_0$ | 0.19 ± 0.00 gfh | 0.09 ± 0.00 lnm | 0.15 ± 0.04 f | 0.06 ± 0.00 jfigh |
| L+F $D_{30}$ | 0.23 ± 0.01 gfed | 0.85 ± 0.01 a | 0.49 ± 0.03 de | 0.12 ± 0.00 b |
| L+F $I_{30}$ | 0.23 ± 0.02 cfed | 0.66 ± 0.04 b | 0.43 ± 0.01 def | 0.07 ± 0.00 figh |
| L+S $D_{30}$ | 0.18 ± 0.00 ghi | 0.28 ± 0.01 e | 1.26 ± 0.41 a | 0.05 ± 0.00 jih |
| L+S $I_{30}$ | 0.22 ± 0.00 gfed | 0.42 ± 0.00 d | 1.33 ± 0.18 a | 0.05 ± 0.00 ji |
| F+S $D_{30}$ | 0.14 ± 0.00 jhi | 0.45 ± 0.02 c | 0.95 ± 0.01 bc | 0.06 ± 0.00 jigh |
| F+S $I_{30}$ | 0.10 ± 0.02 j | 0.20 ± 0.01 hg | 0.48 ± 0.01 de | 0.05 ± 0.00 j |
| L+F $D_{60}$ | 0.38 ± 0.01 a | 0.29 ± 0.06 e | 0.23 ± 0.03ef | 0.17 ± 0.01 a |
| L+F $I_{60}$ | 0.27 ± 0.02 cbd | 0.29 ± 0.01 e | 0.26 ± 0.05 ef | 0.11 ± 0.01 cb |
| L+S $D_{60}$ | 0.13 ± 0.00 ji | 0.08 ± 0.01 n | 1.16 ± 0.48 ba | 0.05 ± 0.01 jigh |
| L+S $I_{60}$ | 0.14 ± 0.00 jhi | 0.10 ± 0.01 lkm | 0.82 ± 0.29 c | 0.09 ± 0.00 cd |
| F+S $D_{60}$ | 0.12 ± 0.01 j | 0.09 ± 0.00 nm | 0.72 ± 0.40 dc | 0.07 ± 0.01 fge |
| F+S $I_{60}$ | 0.13 ± 0.00 ji | 0.10 ± 0.00 lk | 0.32 ± 0.08 ef | 0.06 ± 0.00 jigh |
| L+F $D_{120}$ | 0.30 ± 0.01 b | 0.29 ± 0.01 e | 0.39 ± 0.05 ef | 0.10 ± 0.00 c |
| L+F $I_{120}$ | 0.24 ± 0.00 ced | 0.25 ± 0.00 f | 0.15 ± 0.02 f | 0.11 ± 0.01 cb |
| L+S $D_{120}$ | 0.24 ± 0.01 cfed | 0.18 ± 0.00 hi | 0.20 ± 0.04 ef | 0.08 ± 0.01 fde |
| L+S $I_{120}$ | 0.18 ± 0.01ghi | 0.15 ± 0.00 j | 0.44 ± 0.03 def | 0.07 ± 0.00 fgh |
| F+S $D_{120}$ | 0.21 ± 0.01 gfe | 0.21 ± 0.01 g | 0.23 ± 0.05 ef | 0.10 ± 0.05 c |
| F+S $I_{120}$ | 0.20 ± 0.00 gfe | 0.18 ± 0.00 i | 0.22 ± 0.07 ef | 0.06 ± 0.00 jfigh |

**Table 3.** Antioxidant compounds present in decoctions/infusions obtained from mixed herbs after different times of storage. The results are expressed as mean ± SD (n=9). In each column different letters mean significant differences ($p<0.05$).

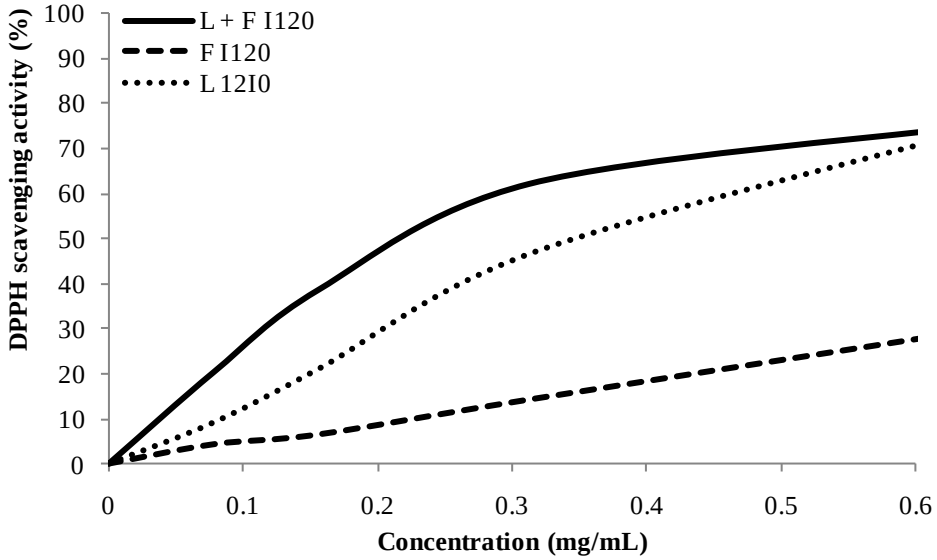
| Samples | Phenolics (mg GAE/g) | Flavonoids (mg CE/g) | Ascorbic acid (mg/g) | Reducing sugars (mg/g) |
|---|---|---|---|---|
| L+F $D_0$ | 389.73 ± 4.00 b | 25.02 ± 1.67 gf | 8.44 ± 0.04 fe | 0.25 ± 0.04 k |
| L+F $I_0$ | 438.08 ± 0.19 a | 26.05 ± 1.13 f | 6.33 ± 0.08 jk | 0.19 ± 0.01 l |
| L+S $D_0$ | 368.35 ± 2.07 c | 24.76 ± 0.21 gf | 8.22 ± 0.20 fe | 0.45 ± 0.01 gf |
| L+S $I_0$ | 236.04 ± 1.09 ji | 36.92 ± 0.52 ed | 6.74 ± 0.18 ji | 0.40 ± 0.01 ih |
| F+S $D_0$ | 242.47 ± 2.56 i | 34.87 ± 0.78 ed | 5.83 ± 0.21 l | 0.37 ± 0.02 ij |
| F+S $I_0$ | 337.75 ± 1.21 d | 26.59 ± 0.14 f | 5.03 ± 0.12 m | 0.41 ± 0.01 gh |
| L+F $D_{30}$ | 228.46 ± 9.21 j | 12.85 ± 1.72 jlik | 7.46 ± 0.23 g | 0.26 ± 0.01 k |
| L+F $I_{30}$ | 236.26 ± 10.86 ji | 13.35 ± 2.00 jlik | 7.41 ± 0.15 hg | 0.36 ± 0.01 j |
| L+S $D_{30}$ | 365.34 ± 0.73 c | 18.65 ± 0.21 gih | 6.80 ± 0.21 i | 0.52 ± 0.03 e |
| L+S $I_{30}$ | 292.41 ± 3.95 f | 14.80 ± 0.25 jik | 8.03 ± 0.29 f | 0.48 ± 0.05 f |
| F+S $D_{30}$ | 342.93 ± 22.40 d | 17.06 ± 1.86 jih | 6.24 ± 0.19 lk | 0.57 ± 0.06 d |
| F+S $I_{30}$ | 399.27 ± 6.60 b | 23.20 ± 0.12 gfh | 6.76 ± 0.05 ji | 0.18 ± 0.02 l |
| L+F $D_{60}$ | 116.58 ± 1.71 m | 2.71 ± 0.27 m | 10.17 ± 0.05 dc | 0.36 ± 0.00 ij |
| L+F $I_{60}$ | 157.32 ± 2.09 m | 7.44 ± 027 lm | 8.33 ± 0.51 fe | 0.38 ± 0.00 ij |
| L+S $D_{60}$ | 273.93 ± 2.98 g | 7.55 ± 0.89 lm | 8.68 ± 0.12 e | 0.76 ± 0.01 b |
| L+S $I_{60}$ | 293.31 ± 9.34 f | 10.05 ± 0.16 lk | 7.31 ± 0.26 hg | 0.20 ± 0.01 l |
| F+S $D_{60}$ | 317.21 ± 2.11 e | 10.31 ± 0.24 lk | 8.51 ± 0.38 e | 0.26 ± 0.02 k |
| F+S $I_{60}$ | 272.14 ± 0.61g | 11.90 ± 0.67 jlk | 6.97 ± 0.09 hi | 0.71 ± 0.01 c |
| L+F $D_{120}$ | 163.48 ± 6.57 m | 42.46 ± 0.59 d | 10.49 ± 0.63 bc | 0.59 ± 0.00 d |
| L+F $I_{120}$ | 191.22 ± 4.15 l | 59.92 ± 16.17 c | 11.00 ± 0.43 a | 0.58 ± 0.00 d |
| L+S $D_{120}$ | 259.02 ± 2.55 h | 82.61 ± 1.54 a | 9.72 ± 0.16 d | 0.70 ± 0.00 c |
| L+S $I_{120}$ | 280.46 ± 0.96 g | 83.71 ± 5.82 a | 9.77 ± 0.16 d | 0.84 ± 0.00 a |
| F+S $D_{120}$ | 213.78 ± 4.69 k | 74.96 ± 3.43 b | 10.75 ± 0.14 ba | 0.73 ± 0.01 cb |
| F+S $I_{120}$ | 240.83 ± 2.75 i | 57.72 ± 2.24 c | 9.81 ± 0.05 d | 0.74 ± 0.00 cb |

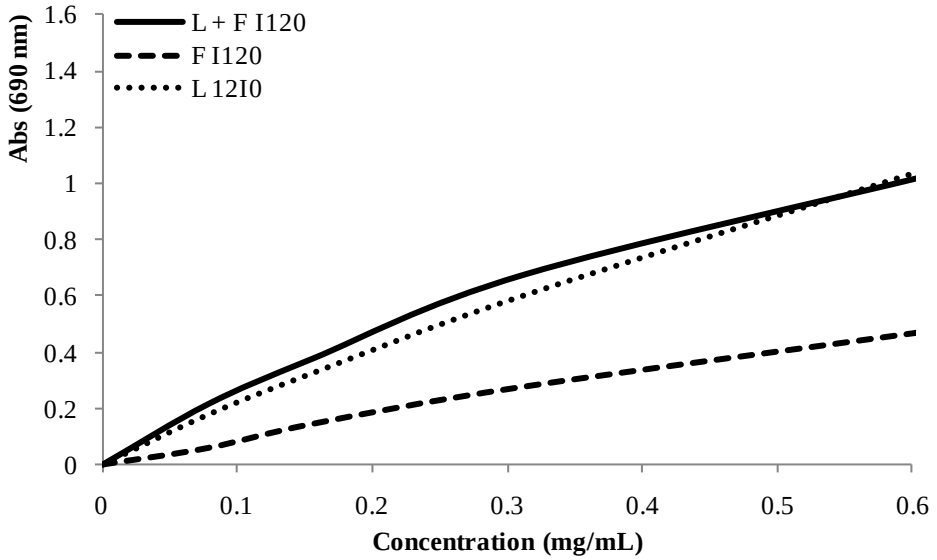
**Table 4.** Theoretical[a] *versus* experimental values of antioxidant activity $EC_{50}$ (mg/mL) of decoctions/infusions obtained from mixed herbs after different times of storage.

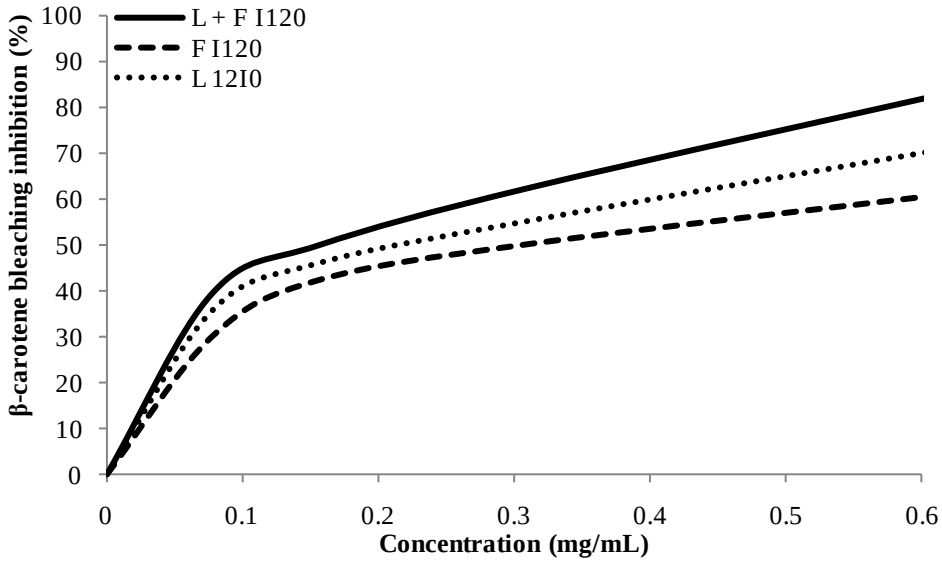
| Samples | DPPH Scavenging activity | | | Reducing power | | | β-Carotene bleaching inhibition | | | TBARS inhibition | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Theoretical | Experimental | Effect | Theoretical | Experimental | Effect | Theoretical | Experimental | Effect | Theoretical | Experimental | Effect |
| L+F $D_0$ | 0.39 | 0.29 | S | 0.28 | 0.15 | S | 0.72 | 0.17 | S | 0.66 | 0.08 | S |
| L+F $I_0$ | 0.34 | 0.28 | S | 0.18 | 0.16 | S | 0.39 | 0.29 | S | 0.43 | 0.09 | S |
| L+S $D_0$ | 0.20 | 0.28 | AN | 0.12 | 0.11 | S | 0.37 | 0.29 | S | 0.44 | 0.06 | S |
| L+S $I_0$ | 0.18 | 0.14 | S | 0.10 | 0.08 | S | 0.14 | 0.31 | AN | 0.20 | 0.06 | S |
| F+S $D_0$ | 0.34 | 0.24 | S | 0.22 | 0.09 | S | 0.54 | 0.33 | S | 0.50 | 0.07 | S |
| F+S $I_0$ | 0.28 | 0.19 | S | 0.12 | 0.09 | S | 0.30 | 0.15 | S | 0.37 | 0.06 | S |
| L+F $D_{30}$ | 0.36 | 0.23 | S | 0.21 | 0.85 | AN | 0.47 | 0.49 | A | 0.37 | 0.12 | S |
| L+F $I_{30}$ | 0.26 | 0.23 | S | 0.17 | 0.66 | AN | 0.32 | 0.43 | AN | 0.23 | 0.07 | S |
| L+S $D_{30}$ | 0.23 | 0.18 | S | 0.12 | 0.28 | AN | 0.28 | 1.26 | AN | 0.19 | 0.05 | S |
| L+S $I_{30}$ | 0.17 | 0.22 | AN | 0.08 | 0.42 | AN | 0.12 | 1.33 | AN | 0.14 | 0.05 | S |
| F+S $D_{30}$ | 0.22 | 0.14 | S | 0.13 | 0.45 | AN | 0.29 | 0.95 | AN | 0.29 | 0.06 | S |
| F+S $I_{30}$ | 0.18 | 0.10 | S | 0.13 | 0.20 | AN | 0.25 | 0.48 | AN | 0.15 | 0.05 | S |
| L+F $D_{60}$ | 0.21 | 0.38 | AN | 0.17 | 0.29 | AN | 0.75 | 0.23 | S | 0.34 | 0.17 | S |
| L+F $I_{60}$ | 0.21 | 0.27 | AN | 0.17 | 0.29 | AN | 0.61 | 0.26 | S | 0.26 | 0.11 | S |
| L+S $D_{60}$ | 0.16 | 0.13 | S | 0.09 | 0.08 | S | 0.47 | 1.16 | AN | 0.10 | 0.05 | S |
| L+S $I_{60}$ | 0.15 | 0.14 | S | 0.09 | 0.10 | AN | 0.33 | 0.82 | AN | 0.07 | 0.09 | AN |
| F+S $D_{60}$ | 0.15 | 0.12 | S | 0.13 | 0.09 | S | 0.58 | 0.72 | AN | 0.30 | 0.07 | S |
| F+S $I_{60}$ | 0.14 | 0.13 | S | 0.13 | 0.10 | S | 0.46 | 0.32 | S | 0.24 | 0.06 | S |
| L+F $D_{120}$ | 1.15 | 0.30 | S | 0.61 | 0.29 | S | 0.28 | 0.39 | AN | 0.26 | 0.10 | S |
| L+F $I_{120}$ | 0.75 | 0.24 | S | 0.46 | 0.25 | S | 0.23 | 0.15 | S | 0.29 | 0.11 | S |
| L+S $D_{120}$ | 0.28 | 0.24 | S | 0.19 | 0.18 | A | 0.08 | 0.20 | AN | 0.11 | 0.08 | S |
| L+S $I_{120}$ | 0.26 | 0.18 | S | 0.17 | 0.15 | S | 0.16 | 0.44 | AN | 0.16 | 0.07 | S |
| F+S $D_{120}$ | 1.03 | 0.21 | S | 0.49 | 0.21 | S | 0.24 | 0.23 | A | 0.24 | 0.10 | S |
| F+S $I_{120}$ | 0.65 | 0.20 | S | 0.38 | 0.18 | S | 0.24 | 0.22 | S | 0.22 | 0.06 | S |

[a]The theoretical values were calculated considering additive contributions of the individual herbs (Guimarães et al. 2010).

A - Additive effect: theoretical and experimental values reveal differences lower than 5%. S - Synergistic effect: experimental values are more than 5% lower for $EC_{50}$ when compared with theoretical values. AN – Antagonistic effect: experimental values are more than 5% higher for $EC_{50}$ when compared with theoretical values.


DPPH scavenging activity (%)
L + F I120
F I120
L 12I0
100
90
80
70
60
50
40
30
20
10
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration (mg/mL)


Abs (690 nm)
L + F I120
F I120
L 12I0
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration (mg/mL)


β-carotene bleaching inhibition (%)
L + F I120
F I120
L 12I0
100
90
80
70
60
50
40
30
20
10
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration (mg/mL)

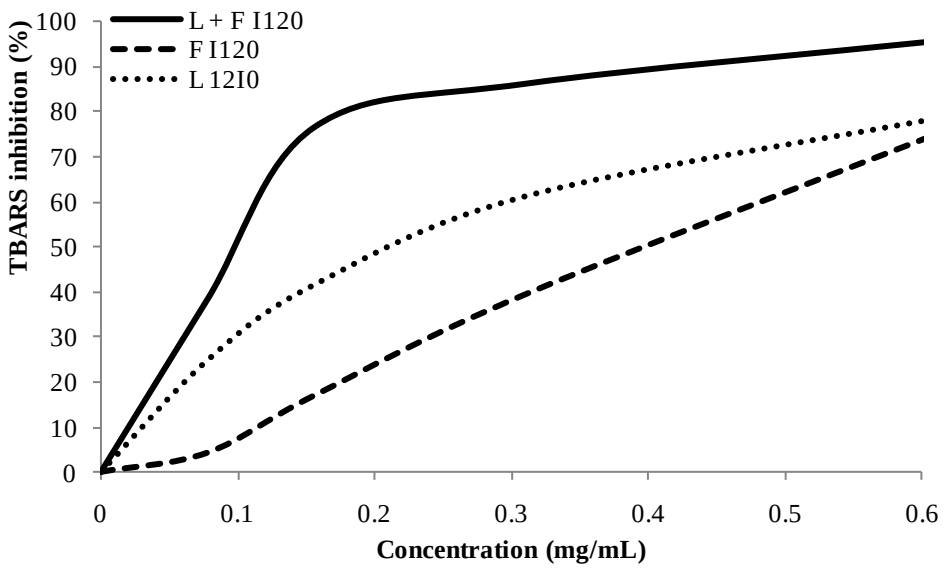
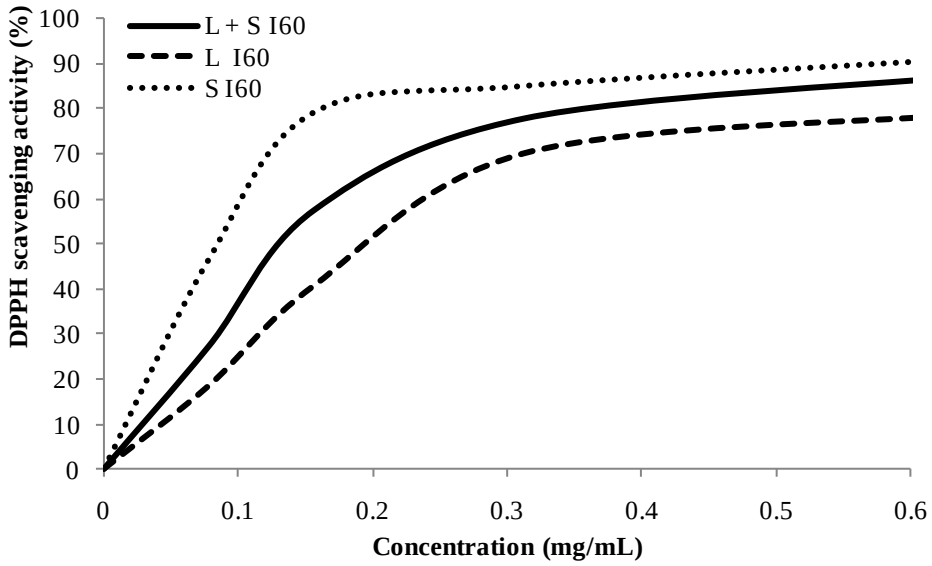
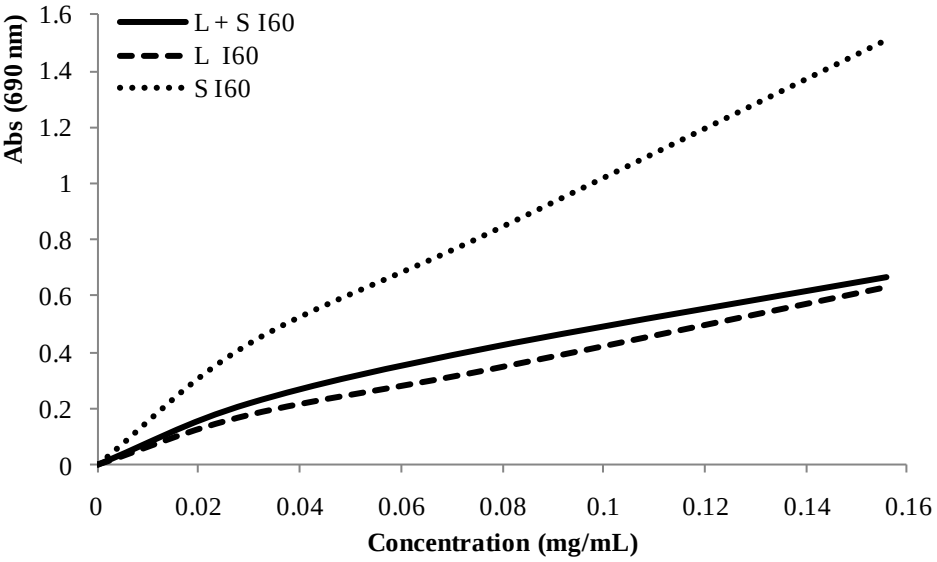



**Figure 1.** Synergistic effect in DPPH scavenging activity, reducing power, β-carotene bleaching inhibition and TBARS inhibition: the example of L+F $I_{120}$ mixture.


DPPH scavenging activity (%)
100
90
80
70
60
50
40
30
20
10
0
L + S I60
L I60
S I60
0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration (mg/mL)


Abs (690 nm)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
L + S I60
L I60
S I60
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
Concentration (mg/mL)

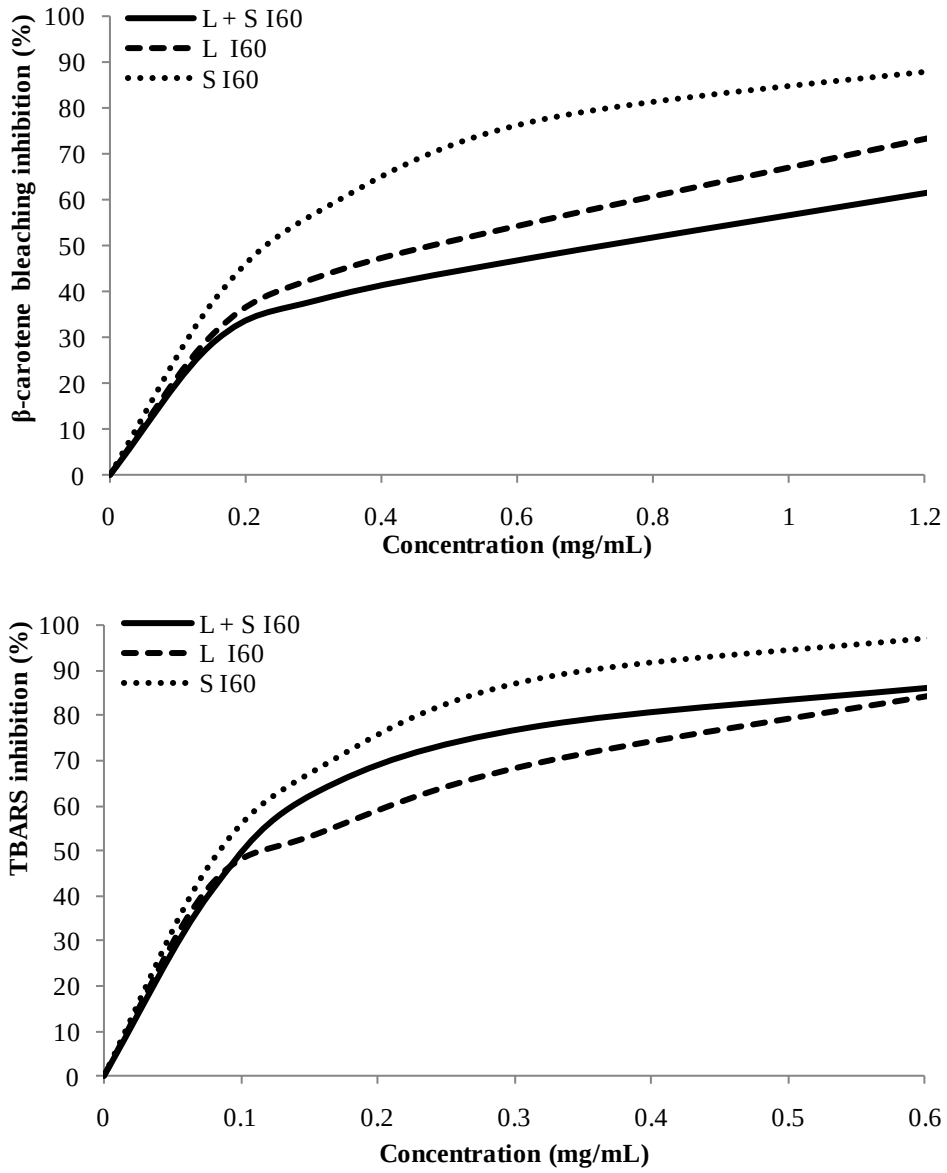



**Figure 2.** Antagonist effect in reducing power, β-carotene bleaching inhibition and TBARS inhibition: the example of L+S $I_{60}$ mixture.