Influence of an aqueous extract of *Coriandrum sativum* leaves on the labeling of blood constituents with technetium-99m and determination of some of its physical parameters

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*Coriandrum sativum* (coriander) extract (leaves) (CE) is used to treat diabetes, dyspepsia, loss of appetite, seizures, insomnia, and anxiety. In the investigations about the effects of a plant extract, it is important to determine some of its physicochemical parameters. Red blood cells labeled with technetium-99m (\(^{99m}\)Tc) is used in nuclear medicine as well as in experimental models in basic research. The aim of this work was to determine some physicochemical parameters of an aqueous extract of CE and its effect on the labeling of blood constituents with \(^{99m}\)Tc. Determinations of the absorption spectrum (AS), electric conductivity (EC), and pH have been performed using different concentrations of CE. Results showed that CE had a maximum absorbance at 480 nm. EC was inversely correlated with the concentration. We found the highest value of pH at the lower concentration of the extract (0.05 mg/ml). There was no significant (\(P>0.05\)) alteration on the labeling of the blood constituents with \(^{99m}\)Tc. In conclusion, physicochemical parameters could be useful to characterize the CE studied. Probably, the redox properties associated with the substances of this extract could be responsible by the absence of effect on the labeling of blood constituents with \(^{99m}\)Tc.

Key words: *Coriandrum sativum*, coriander, labeling, technetium-99m, physicochemical parameters, blood.

INTRODUCTION

Natural products are used by the population *in natura* or in the form of pharmaceutical preparation, and their use has grown worldwide (Firenzuoli et al., 2005).

*Coriandrum sativum* (coriander) belongs to the Apiaceae family. It is used as a condiment as regards to its intense aroma. It is native to Southern Europe and the Middle East. Coriander (leaves) is used by the Egyptians as a medicinal plant for its digestive and soothing properties. In folk medicine, *C. sativum* (leaves) is utilized
as herbal medicine to treat diabetes (Deepa and Anuradha, 2011), but it is also considered to treat dyspepsia, loss of appetite, seizures, insomnia, and anxiety (Breevort, 1996; Reyes et al., 2010). Experimental studies using different methodologies demonstrated that C. sativum (leaves) contains antioxidants molecules, such as flavonoids glycosides (isoquercitrin, quercitrin, and rutin), caffeic and chlorogenic acids, and tannins. Other molecules are found like linanool (monoterpene alcohol), linoleic acid, sugars, and proteins (Reyes et al., 2010; Budavari et al., 1999; Leung and Foster, 1996).

As the use of medicinal herbs has increased in the world, it is important to be aware of the possibilities of health complications (González-Stuart, 2011). In the regard, the use of experimental models for the evaluation of possible undesirable effects of these natural products is required (Chinou et al., 2007; Sulaiman et al., 2008). This implies a better knowledge of the physicochemical parameters of the extract, such as the absorption spectrum (Presta et al., 2007; Diniz et al., 2008; Frydman et al., 2008; Carmo et al., 2011), the electric conductivity (Frydman et al., 2008; Carmo et al., 2011), and pH (Ameh et al., 2010).

A relevant tool to be used in some experimental models is the radionuclide (Diniz et al., 2008; Cicek et al., 2006; Das et al., 2002; Joseph et al., 2006; De et al., 2009; Bustani et al., 2009). Among these radionuclides, technetium-99m (99mTc) has been utilized (Diniz et al., 2008; De et al., 2009; Bustani et al., 2009).

Red blood cells labeled with 99mTc are used in nuclear medicine (Saha, 2010) as well as in experimental models in basic research (Frydman et al., 2008; De et al., 2009; Bustani et al., 2009; Souza et al., 2011). The labeling process involving 99mTc requires the presence of a reducing agent and the most used for this purpose is the stannous chloride (SnCl2), present in several kits used in nuclear medicine (Saha, 2010). An experimental model based on the labeling of blood constituents with 99mTc has been used to evaluate the impact of various natural and synthetic products (De et al., 2009; Bustani et al., 2009; Holanda et al., 2009; Abreu et al., 2006). Since some medicinal herbs are capable of altering the labeling of blood constituents (Presta et al., 2007; De et al., 2009; Benarroch et al., 2008).

Databases are also used to verify the scientific interest in a subject (Santos-Filho et al., 2011; Maiworm et al., 2011; Santos-Filho et al., 2012), as PubMed (www.ncbi.nlm.nih.gov/sites/entrez) and Scientific Electronic Library Online (Scielo) (www.scielo.org). The number of papers in PubMed and Scielo using C. sativum is very small. This fact encourages the intensification of scientific studies on this natural product.

The aim of this investigation was to determine some physicochemical parameters of a C. sativum aqueous extract and its effect on the labeling of blood constituents with 99mTc.

MATERIALS AND METHODS

Strategy in the PubMed and Scielo

A search (March 8, 2012) using the key words “Coriandrum sativum” or “Coriander” was performed in PubMed (www.ncbi.nlm.nih.gov/sites/entrez) and Scielo (www.scielo.org).

Preparation of C. sativum extract

A commercial preparation (lot 0028, validity June, 2011) of C. sativum was purchased from Distribuidora de Cereais Crowne Ltd, (Rio de Janeiro, Brazil). All the experiments were performed during the validity of the product. The extract of this product was prepared using 0.08 g of dried leaves in 10 ml of saline solution (0.9% NaCl). This preparation was centrifuged for 15 min at 1500 rpm (clinical centrifuge) and the extract obtained was considered to be 8 mg/ml.

Animals

Anticoagulated (sodium heparin) blood was withdrawn from male Wistar rats weighing between 250 and 300 g, aging from 3 to 4 months. The animals were kept under care at average temperature of 25°C, relative humidity around 55% and light/dark cycle of 12 h and were fed with standard diet and water ad libitum. All experiments were conducted following the standards of the Comitê de Ética Para o Uso de Animais Experimentais (CEUA), Instituto de Biologia Roberto Alcantara Gomes. This project was approved with the registration number CEUA/042/2011. The animals were handled according to the standards recommended by the Colégio Brasileiro de Experimentação Animal-COBEA, Princípios Éticos na Experimentação Animal (1991).

Spectrophotometry of C. sativum extract

The absorbance spectrum (Analyser Comércio e Indústria Ltda., São Paulo, Brazil) was determined with the coriander extract (0.8 mg/ml) prepared as described earlier in the range of 400 to 700 nm. Saline solution was used as the blank. The absorbance was measured at each interval of 10 nm. The value of the absorbance was maximum (0.108 ± 0.003) at 480 nm with a C. sativum (coriander) extract of 8 mg/ml. This value was considered as the marker of the reproducibility of the conditions used to prepare the extract.

Electric conductivity of C. sativum extract

The electric conductivity (mS/cm) of the coriander extract was performed with a conductivimeter (Marte Balanças e Aparelhos de Precisão Ltda. São Paulo, Brazil). Saline solution was used as the control. The value of electric conductivity (0.478 ± 0.05) (extract 8 mg/ml) was also used as a second marker of the reproducibility of the conditions used in the preparation of the extract.

pH of C. sativum extract

The pH of the coriander extracts was measured with a pH meter (PHTEK, pH/MV/temp, pH5-3B, Labiocenter produtos para
Figure 1. Absorbance spectrum of *C. sativum* extracts (0.8 mg/ml). The absorbance spectrum was determined in the range of 400 to 700 nm at intervals of 10 nm. Saline solution (0.9% NaCl) was used as the blank.

**In vitro radiolabeling of blood constituents**

Aliquots of 0.5 ml of anticoagulated blood obtained by cardiac puncture from male Wistar rats (3 to 4 months old, 250 to 350 g, n = 10) were mixed *in vitro* for 1 h at room temperature with the extract in different concentrations (0.8, 0.4, 0.2, and 0.1 mg/ml). Blood aliquots were also incubated under the same conditions with NaCl 0.9% as control. After the incubation period, 0.5 ml of SnCl₂ (Sigma, USA, 1.2 µg/ml) was added and incubated for 1 h, whereupon 0.1 ml (3.7 MBq) of sodium pertechnetate (Na⁹⁹ᵐTcO₄⁻) recently eluted from ⁹⁹ᵐMo/⁹⁹ᵐTc generator (Instituto de Pesquisas Energeticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo) was added and incubation continued for another 10 min. After the incubation time, samples were centrifuged (1500 rpm, 5 min) and plasma (P) and blood cells (BC) were separated.

Other aliquots of 0.02 ml of P and BC were precipitated in 1.0 ml of trichloroacetic acid (TCA, 5%), and were centrifuged again, separating the soluble fractions (SF) and insoluble (IF) of P and BC. The radioactivity in each fraction was counted in a gamma counter (Gamma C-12, DPC Medlab, Los Angeles, CA, USA) and the percentage of radioactivity (%ATI) from radiolabeling assay (n = 10 for each *C. sativum* concentration).

To evaluate the possible interference of the coriander extract in blood radiolabeled, one-way analysis of variance (ANOVA) test was performed to verify possible statistical differences (P<0.05), followed by Dunnet post-test. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, CA, USA).

**RESULTS**

The web search (March 8, 2012) using PubMed revealed 133 items with the keyword “*Coriandrum sativum*” and 238 with “Coriander”, whereas search within Scielo revealed 32 and 46 publications, respectively.

Figure 1 shows the absorbance spectrum of the *C. sativum* extract at the highest concentration used (8 mg/ml) in the range of 400 to 700 nm. The data show an absorption peak of the extract (0.108 ± 0.011) at 480 nm. It was used as a marker of the reproducibility of the extract preparation.

The electric conductivity of *C. sativum* extract at different concentrations is as shown in Figure 2. At the
Figure 2. Electric conductivity of *C. sativum* extract at different concentrations. Dilutions were prepared in saline, and the electric conductivity for each extract concentration was measured.

Highest extract concentration (8 mg/ml), the mean value of the electric conductivity was 0.478 ± 0.046 mS/cm. It was used as a second marker of the reproducibility of the preparation of the extract.

Figure 3. pH index of *C. sativum* extract at different concentrations. Dilutions were prepared in saline, and the refractive index for each concentration was measured.

Figure 3 shows the pH index of *C. sativum* extract at different concentrations. The refractive index medium was 6.56 ± 0.03 at the highest concentration used (8 mg/ml). It was used as a third marker of the...
reproducibility of the preparation of the extract.

Table 1 presents the effects of *C. sativum* extract on the radioactivity distribution between cellular and plasma compartments. These data indicate no alteration (P>0.05) of 99mTc on both blood and plasma compartments.

Table 2 presents the effect of *C. sativum* on the fixation of 99mTc in insoluble and soluble fractions of blood specimens. Blood samples from Wistar rats were incubated with *C. sativum* extract for 1 h and labeling of blood constituents with 99mTc was carried out. Plasma (P) and blood cells (BC) were isolated, radioactivity was counted and the percentage of incorporated radioactivity (%ATI) was calculated.

**Table 1.** Effect of *C. sativum* extract on the distribution of the radioactivity in blood compartments.

<table>
<thead>
<tr>
<th><em>C. sativum</em> (mg/ml)</th>
<th>P (%)</th>
<th>BC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>2.66 ± 1.41</td>
<td>97.34 ± 1.41</td>
</tr>
<tr>
<td>1</td>
<td>2.93 ± 1.82</td>
<td>97.07 ± 1.82</td>
</tr>
<tr>
<td>2</td>
<td>3.40 ± 2.84</td>
<td>96.60 ± 2.84</td>
</tr>
<tr>
<td>4</td>
<td>3.15 ± 2.72</td>
<td>96.85 ± 2.72</td>
</tr>
<tr>
<td>8</td>
<td>2.90 ± 1.68</td>
<td>97.10 ± 1.68</td>
</tr>
</tbody>
</table>

Blood samples from Wistar rats were incubated with *C. sativum* extract for 1 h and labeling of blood constituents with 99mTc was carried out. Plasma (P) and blood cells (BC) were isolated, radioactivity was counted and the percentage of incorporated radioactivity (%ATI) was calculated.

**Table 2.** Effect of *C. sativum* extract on the fixation of the radioactivity of the insoluble and soluble fractions of plasma.

<table>
<thead>
<tr>
<th><em>C. sativum</em> (mg/ml)</th>
<th>IF-P (%)</th>
<th>SF-P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>72.53 ± 4.38</td>
<td>27.47 ± 4.38</td>
</tr>
<tr>
<td>1</td>
<td>73.38 ± 5.65</td>
<td>26.62 ± 5.65</td>
</tr>
<tr>
<td>2</td>
<td>74.48 ± 4.33</td>
<td>25.56 ± 4.33</td>
</tr>
<tr>
<td>4</td>
<td>66.35 ± 7.47</td>
<td>33.65 ± 7.47</td>
</tr>
<tr>
<td>8</td>
<td>74.18 ± 6.40</td>
<td>25.82 ± 6.40</td>
</tr>
</tbody>
</table>

Blood samples from Wistar rats were incubated with *C. sativum* extract for 1 h and labeling of blood constituents with 99mTc was carried out. Insoluble (IF) and soluble (SF) fractions from blood cells (P) were isolated, radioactivity was counted and the percentage of incorporated radioactivity (%ATI) was calculated.

No significant (P>0.05) alteration on the fixation of radioactivity on blood cells proteins obtained from BC fraction isolated from whole blood incubated with *C. sativum* (Table 3) was also found.

**Table 3.** Effect of *C. sativum* extract on the fixation of the radioactivity of the insoluble and soluble fractions of blood cells.

<table>
<thead>
<tr>
<th><em>C. sativum</em> (mg/ml)</th>
<th>IF-BC (%)</th>
<th>SF-BC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>91.17 ± 5.24</td>
<td>8.83 ± 5.24</td>
</tr>
<tr>
<td>1</td>
<td>93.76 ± 5.49</td>
<td>6.24 ± 5.49</td>
</tr>
<tr>
<td>2</td>
<td>90.08 ± 7.19</td>
<td>9.92 ± 7.19</td>
</tr>
<tr>
<td>4</td>
<td>92.91 ± 4.42</td>
<td>7.09 ± 4.42</td>
</tr>
<tr>
<td>8</td>
<td>91.46 ± 4.79</td>
<td>8.54 ± 4.79</td>
</tr>
</tbody>
</table>

Blood samples from Wistar rats were incubated with *C. sativum* extract for 1 h and labeling of blood constituents with 99mTc was carried out. Insoluble (IF) and soluble (SF) fractions from blood cells (BC) were isolated, radioactivity was counted and the percentage of incorporated radioactivity (%ATI) was calculated.

**DISCUSSION**

Natural products have been used by humans as a food, as well as herbal medicines. The use of these products is growing worldwide, studies on their biological effects are relevant to help understand the action in the form of extracts or isolated compounds of these products. Likewise, these investigations may allow a safer use of these products (Rotblatt and Ziment, 2002).

There is no exact description about folk preparation and intake of coriander extracts, which vary by region or country where coriander is consumed (Deepa and Anuradha, 2011; Breevort, 1996; De Smet, 2002; Reyes et al., 2010). Moreover, in comparison with other natural products, the number of publication about coriander is still limited. These facts have stimulated this investigation. Furthermore, physical properties such as absorbance spectrum, electric conductivity and pH of coriander extracts are not available through PubMed and Scielo. These physical parameters could be important and useful for the characterization and the preparation conditions of an aqueous coriander extract.

The initial concern was to standardize the conditions of preparation of the aqueous extract used in the experiments. The analysis of the absorption spectrum shows that the *C. sativum* extract has a maximum absorbance at 480 nm (Figure 1). Similar findings are reported by Frydman et al. (2008) for *Cordia salicifolia* extract. The electric conductivity of the *C. sativum* extract (Figure 2) is inversely dependent on the concentration. As for pH studies, we found the highest value at the lowest concentration of *C. sativum* extract (Figure 3), whereas this trend is inverse in results described by Frydman et al. (2008) with an extract of *C. salicifolia*.

It has been reported that several factors interfere in labeling with 99mTc, among them are natural or synthetic products (Presta et al., 2007; Bustani et al., 2009; Bernardo-Filho et al., 2005). Many studies demonstrate that medicinal plants alter the *in vitro* labeling with 99mTc, as *Cinnamomum zeylanicum* (Benarroz et al., 2008), *Ginkgo biloba* (Moreno et al., 2004), *C. salicifolia* (Filho et al., 2005). These physical parameters could be important and useful for the characterization and the preparation conditions of an aqueous coriander extract.
(Frydman et al., 2008), and Bacopa monnieri (De et al., 2009).

The data obtained with the aqueous extract of a commercial preparation of C. sativum indicate that there was no significant alteration in the distribution of the $^{99m}$Tc in plasma and blood cells compartments. The fixation of the $^{99m}$Tc on blood cells proteins is also not altered. The antioxidant properties of some substances present in the coriander extract described by some authors (Reyes et al., 2010; Budavari et al., 1999; Leung and Foster, 1996) could be implied in the absence of alteration in the blood radioactivity distribution. In addition, although alterations in the measurements of the electric conductivity (Figure 2) and pH (Figure 3) were found, these findings do not seem to interfere in the radiolabeled of the blood constituents.

In conclusion, our results suggest that some physicochemical parameters could be useful to characterize the C. sativum extract studied. Probably the antioxidant properties associated with the substances of C. sativum extract could be responsible by the absence of effect of this extract on the labeling of blood constituents with $^{99m}$Tc.

ACKNOWLEDGEMENTS

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