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Full Length Research Paper

Characterization of physicochemical parameters and the effect on the labeling of blood constituents with technetium-99m of a *Solanum melongena* commercial extract

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This investigation evaluated physicochemical parameters and the effect on the labeling of blood constituents with technetium-99m of a *Solanum melongena* aqueous extract. A commercial *S. melongena* was used to prepare an aqueous extract and visible absorbance spectrum, electric conductivity and refractive index were determined. Blood samples from *Wistar* rats were incubated with *S. melongena* extract or with 0.9% NaCl solution as control. After, stannous chloride as reducing agent and technetium-99m as sodium pertechnetate were added. Plasma and blood cells samples were separated. Samples of plasma and blood cells were also precipitated with trichloroacetic acid and soluble and insoluble fractions were separated. The radioactivity in each fraction was counted and the percentage of radioactivity incorporated was calculated. Data showed an absorbance peak at 500 nm and electric conductivity and refractive index were higher at the highest extract concentrations. *S. melongena* extract did not interfere on the labeling of blood constituents with technetium-99 m. The determination of these physicochemical parameters would contribute to characterize a *S. melongena* aqueous extract. Moreover, as the labeling of blood constituents with technetium-99m depends on the presence of reducing agent, the absence of effect of this extract in this radiolabeling process could be associated with its redox properties.

Key words: *Solanum melongena*, eggplant, labeling, technetium-99 m, physicochemical parameters.

INTRODUCTION

An important increase in the use of medicinal herbal products around the world has happened in the last years. In various countries, these products are classified and promoted as foods, not pharmaceuticals which facilitate their availability on the international market without requiring a medical prescription (González-Stuart, 2011). *Solanum melongena L.* (eggplant) is used as a natural product and belongs to the family Solonaceae. This plant family is further involved in our daily lives (Fukuoka et al., 2010). Eggplant is a native from India and is being an economically important vegetable crop in

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Asia and Africa (Van Eck and Snyder, 2006). On traditional medicine, eggplant has been used in weight-loss diets as it seems to increase the elimination of fat and fight the exceeding cholesterol (Gonçalves et al., 2006). Powerful antioxidants as flavonoids, vitamin A, ascorbic acid and phenolics compounds, such anthocyanins are in the eggplant (Nisha et al., 2009). 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) and the use of experimental models for the evaluation of possible biological effects of natural products have been utilized (Sulaiman et al., 2008; Chinou et al., 2007). In the development of the investigations with an extract of a natural product is important to determine some physicochemical parameters of this extract. The determination of the absorption spectrum (Frydman et al., 2008; Presta et al., 2007; Diniz et al., 2008), the electric conductivity (Frydman et al., 2008) and refractive index (Frydman et al., 2008) has been performed. Radionuclides have been used as a relevant tool in some of these models (Cicek et al., 2006; Das et al., 2002; Joseph et al., 2006; De et al., 2009; Diniz et al., 2008; Bustani et al., 2009). Among these radionuclides, the technetium-99m (^{99m}Tc) has been utilized (De et al., 2009; Diniz et al., 2008; Bustani et al., 2009).

Red blood cells labeled with ^{99m}Tc are radiopharmaceuticals and have been used in 'nuclear medicine' for measurement of blood volume (Vizzard et al., 2010), identification of hemangiomas (Borse et al., 2010), recognition of gastrointestinal bleeding (Currie et al., 2011) and other purposes (Saha, 2010). In this labeling process, a reducing agent (stannous chloride – $SnCl_2$) must be used. An experimental model based on the labeling of blood constituents with ^{99m}Tc has been used to assess some properties of various natural and synthetic products for different authors (De et al., 2009; Holanda et al., 2009; Bustani et al., 2009; Abreu et al., 2006).

Moreover, some authors have reported that some medicinal herbs are capable of altering the labeling of blood constituents with ^{99m}Tc (De et al., 2009; Benarroz et al., 2008; Presta et al., 2007). To our knowledge, following the searches in the PubMed (www.ncbi.nlm.nih.gov/sites/entrez), the absorption spectrum, the studies about the conductivity and the refractometry evaluation have not been carried out with a commercial *S. melongena* (SM) extract. The influence of this commercial on the labeling of blood constituents with ^{99m}Tc has not been also studied. The aims of this investigation were to determine some physicochemical parameters and the effect on the labeling of blood constituents with ^{99m}Tc of a *S. melongena* aqueous extract.

MATERIALS AND METHODS

Strategy in the PubMed

It was performed in PubMed (www.ncbi.nlm.nih.gov/sites/entrez) search using the key words "*S. melongena*" or "eggplant" and "absorbance spectrum", "electric conductivity", "refractive index" and using "*S. melongena*" or "eggplant" and "labeling".

Preparation of *Solanum melongena* extract

To prepare the extract, 450 mg of *S. melongena* dried extract from Herbarium (lot 822060, validity to May 2012) was added 10 ml of saline solution (0.9% NaCl) and agitated in vortex for 1 min. The preparation was centrifuged in a clinical centrifuge (2000 rpm, 15 min) and the supernatant obtained was considered to be 45 mg/ml.

Animals

All the experimental procedures have followed the Ethical Guidelines of the Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Brazil, with the protocol number CEA/213/2007. The animals were kept under environmental conditions (25±2°C, 12 h of light/dark cycle), water *ad libitum* and normal diet. Heparinized whole blood was withdrawn by cardiac puncture from adult male Wistar rats (3 to 4 months of age, 250 to 350 g of weight) under anesthesia by sodium thiopental, 60 mg/kg of weight.

Spectrophotometry of *Solanum melongena* extract

The absorbance spectrum (Analyser Comércio e Indústria Ltda, São Paulo, Brazil) was determined with the *S. melongena* extract (45 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 of the highest concentration of the extract at 500 nm was 1.47 ± 0.01. This value was considered as the marker of the reproducibility of the conditions used to prepare the extract.

Electric conductivity of *Solanum melongena* extract

The electric conductivity (mS/cm) of the *S. melongena* extracts at different concentrations (2.81, 5.62, 11.25, 22.5 and 45 mg/ml) was performed with a conductivimeter (Marte Balanças e Aparelhos de Precisão Ltda, São Paulo). Saline solution was used as the control. The value of electric conductivity (1.464 ± 0.004) of the extract at higher concentration was used as a second marker of the reproducibility of the conditions of the extract.

Refractive index of *Solanum melongena* extract

The refractive index (%BRIX) of *S. melongena* extracts at different concentrations (2.81, 5.62, 11.25, 22.5 and 45 mg/ml) was measured with a refractometer (Ningbo Utech International Co. Ltd., Ningbo, People's Republic of China) at room temperature. Saline solution was also used as the control. The value of the refractive index (1.40 ± 0.00) of the extract at higher concentration was used as a third marker of the reproducibility of the conditions of the

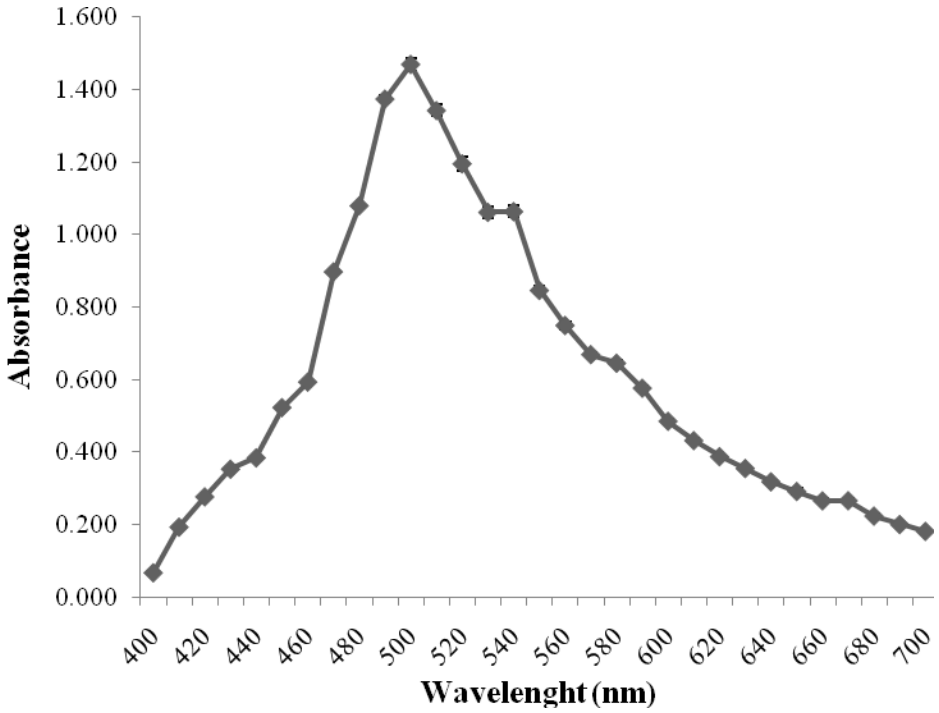

Figure 1. Absorbance spectrum of *Solanum melongena* extract (45 mg/ml). The absorbance spectrum was determined in the range of 400 to 700 nm at inervals of 10 nm. Saline solution (0.9% NaCl) was used as the blank.

extract.

***In vitro* radiolabeling of blood constituents**

Samples of heparinized blood were withdrawn by cardiac puncture. For the radiolabeling assay, these samples (500 µl) were incubated with SM extract (100 µl) at different concentrations (2.81, 5.62, 11.25, 22.5 and 45 mg/ml) for 1 h. After that, 500 µl of freshly prepared solution of stannous chloride (1.20 µg/ml) was added and incubated for 1 h. After this period of time, 100 µl of ^{99m}Tc (3.7 MBq) as sodium perthecnetate, recently milked from ^{99}Mo/^{99m}Tc generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo) was added and the incubation was continued for another 10 min. Then, blood samples were centrifuged (clinical centrifuge, 1500 rpm, 5 min) and plasma (P) and blood cells (BC) were separated. Aliquots of P and BC were also precipitated by trichloroacetic acid (5%) and insoluble (IF) ad soluble fractions (SF) were separated after centrifugation (1500 rpm, 5 min). Radioactivity (%ATI) in P, BC, IF-P, SF-P, IF-BC and SF-BC was determined in a well gamma counter (Gamma Counter, Gamma C-12, DPC Medlab, Los Angeles, CA, USA) and the percentage of radioactivity incorporated (%ATI) on each fraction was calculated as described previously (Bernardo-Filho et al., 1983).

Briefly, %ATI for each fraction was obtained by ratio between the radioactivity counting for a fraction and the sum of the radioactivity counting for this fraction and the complementary fraction multiplied by 100.

Statistical analysis

Data are reported as mean ± SD values of the absorbance, electric conductivity (mS/cm), refractive index (%BRIX), percentage of radioactivity (%ATI) from radiolabeling assay (n = 10 for each *S. melongena* concentration). The one-way analysis of variance - ANOVA test was performed to verify possible statistical differences ($p<0.05$) and 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 search that was done in the PubMed has revealed that no items were found with the key words "*S. melongena*" or "eggplant" and "absorbance spectrum", "electric conductivity", "refractive index" and 1 item using "*S. melongena*" or "eggplant" and "labeling". Figure 1 shows the absorbance spectrum of the *S. melongena* extract at higher concentration used (45 mg/ml) in the range of 400 to 700 nm. The data show an absorption peak of the extract (1.47 ± 0.01) at 500 nm. The electric conductivity of *S. melongena* extract at different concentrations is shown in Figure 2. The electric

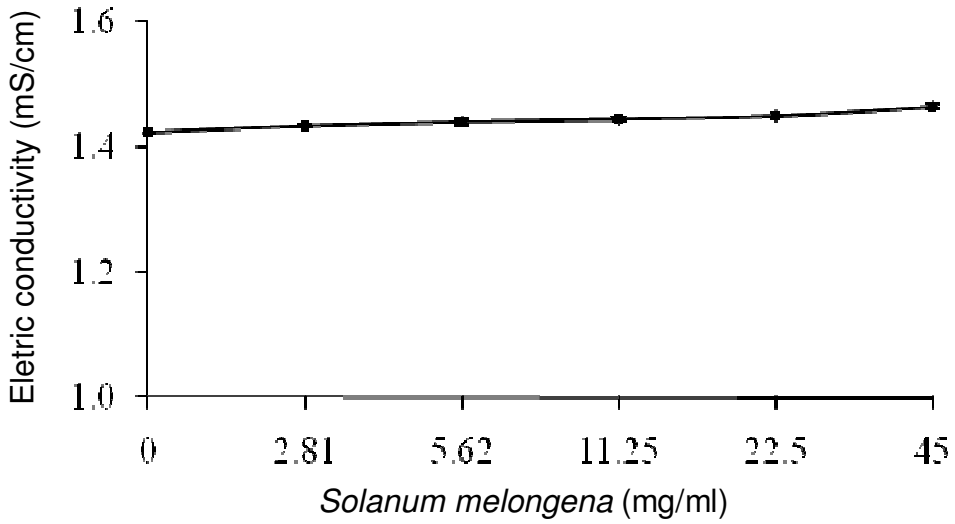

Figure 2. Electric conductivity of *S. melongena* extract at different concentrations. Dilutions were prepared in saline and the electric conductivity for each extract concentration was measured.

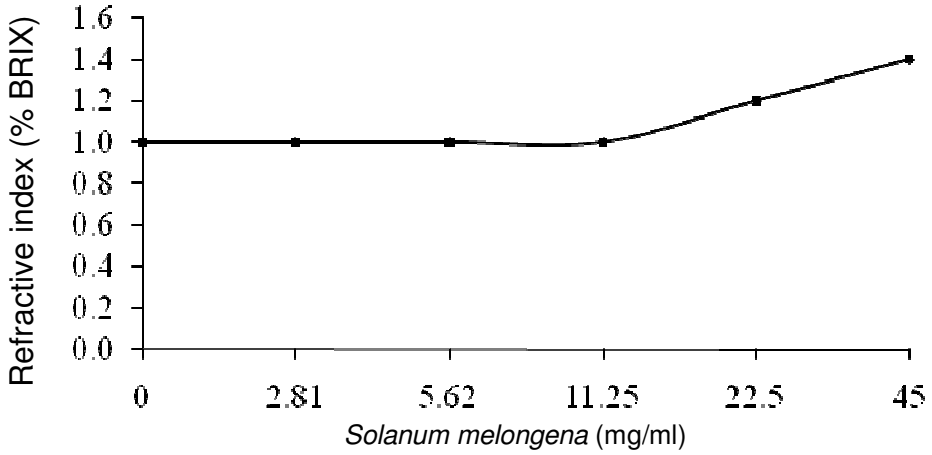

Figure 3. Refractive index of *S. melongena* extract at different concentrations. Dilutions were prepared in saline, and the refractive index for each concentration was measured.

conductivity medium (1.464 ± 0.004 mS/cm) was obtained at the higher extract concentration (45 mg/ml). Figure 3 shows the refraction index of *S. melongena* extract at different concentrations. The refractive index medium was (1.40 ± 0.00 %BRIX) at the higher concentration used (45 mg/ml). Table 1 presents the effects of *S. melongena* extract on the radioactivity distribution between cellular and plasma compartments. This data indicate no alteration (P>0.05) of ^{99m}Tc distribution on these compartments. Table 2 presents the effect of *S. melongena* on the fixation of ^{99m}Tc on insoluble and soluble fractions plasma proteins. As in Table 1, *S. melongena* was not capable of interfering significantly (P>0.05) on the fixation of the radioactivity on the insoluble and soluble fractions of plasma.

No significant (P>0.05) alteration on the fixation of radioactivity on proteins of blood cells from blood samples incubated with *S. melongena* (Table 3) was also

Table 1. Effect of *Solanum melongena* extract on the distribution of the radioactivity in plasma and cellular compartments.

	%ATI	
***Solanum melongena* (mg/ml)**	**P**	**BC**
0.9% NaCl	2.72±1.37	97.28±1.37
2.81	3.20±1.62	96.80±1.62
5.62	5.53±4.22	94.47±4.22
11.25	5.58±2.77	94.42±2.77
22.50	6.58±2.25	93.42±2.25
45.00	8.72±4.28	91.28±4.28

Blood samples from Wistar rats were incubated with *S. melongena* extract for 1 h and the labeling of blood constituents with ^{99m}Tc 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 *Solanum melongena* extract on the fixation of the radioactivity of the insoluble and soluble fractions of plasma.

	%ATI	
***Solanum melongena* (mg/ml)**	**IF-P**	**SF-P**
0.9% NaCl	72.03±4.68	27.97±4.68
2.81	78.30±8.31	21.70±8.31
5.62	79.11±9.92	20.89±9.92
11.25	73.03±8.01	22.97±8.01
22.50	78.65±6.46	21.35±6.46
45.00	77.05±6.89	22.95±6.89

Blood samples from Wistar rats were incubated with *S. melongena* extract for 1 h and labeling of blood constituents with ^{99m}Tc was carried out. Insoluble (IF) and soluble (SF) fractions from plasma (P) were isolated, radioactivity was counted and the percentage of incorporated radioactivity (%ATI) was calculated.

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

	%ATI	
***Solanum melongena* (mg/ml)**	**IF-BC**	**SF-BC**
0.9% NaCl	92.30±4.03	7.70±4.03
2.81	93.74±4.13	6.26±4.13
5.62	90.61±5.82	9.39±5.82
11.25	94.21±3.44	5.79±3.44
22.50	95.62±3.24	4.38±3.24
45.00	93.15±3.18	6.85±3.18

Blood samples from Wistar rats were incubated with *S. melongena* extract for 1 h and labeling of blood constituents with ^{99m}Tc 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.

found.

DISCUSSION

In the past two decades, there has been an exponential increase in the use of complementary and alternative medicine including herbal supplements use of herbal products in the United States as well as in other industrialized nations around the world (González-Stuart, 2011). *S. melongena* has been used in folk medicine as phytotherapic (Gonçalves et al., 2006). These facts have stimulated this investigation and a commercial *S. melongena* extract was used. No information about physicochemical parameters of *S. melongena* aqueous extract is available through PubMed. A publication (Capriles et al., 2002) with *S. melongena* and labeling was found in the PubMed however, this study was carried out within natural *S. melongena* that was purchased in a supermarket. The initial concern was to standardize the conditions of preparation of the extract used in the experiments. The analysis of the absorption spectrum shows that the *S. melongena* extract has a maximum absorbance at 500 nm (Figure 1). Different of Frydman et al. *(*2008) findings to extract of *Cordia salicifolia*; the electric conductivity of the *S. melongena* extract (Figure 2) is not dependent on concentration. In relation to refraction studies, we find higher refractive index at the highest concentration of *S. melongena* extract (Figure 3) according to Frydman et al. (2008) had described to extract of *C. salicifolia.* These physicochemical parameters could be used in other studies to characterize the preparation conditions of an aqueous *S. melongena* extract. It has been reported that several factors interfere in the labeling with ^{99m}Tc, among these are natural products or synthetic (Bernardo-Filho et al., 2005, Bustani et al., 2009; Presta et al., 2007).

Many studies demonstrate the effects of medicinal plants alter the *in vitro* labeling with ^{99m}Tc process *Cinnamomum zeylanicum* (Benarroz et al., 2008), *Ginko biloba*, (Moreno et al., 2004), *C. salicifolia*, (Frydman et al., 2008) and *Bacopa monnieri*, (De et al., 2009) are some examples of medicinal plants that were able to reduce the efficiency of *in vitro* radiolabeling. The data obtained with aqueous extract of *S. melongena* commercial indicates that there was no alteration on the labeling of the blood constituents with ^{99m}Tc. These results are consistent with Capriles et al. (2002) who found no change in the labeling of blood constituents with an extract obtained from fresh fruit of *S. melongena*, at the same concentrations of $SnCl_2$ used in both experiments. Moreover, this discussion is important to suggest that the commercial process to obtain a commercial *S. melongena* is not capable to alter properties of this product, at least considering the used experimental model. In conclusion our results suggest that some physicochemical parameters could be useful to characterize the *S. melongena* extract studied. Furthermore, with these findings are available scientific informations to aid other authors in studies with the *S. melongena*. In addition, probably the redox properties associated with the substances of the *S. melongena* extract could be responsible by the absence of effect of this extract on the labeling of blood constituents with ^{99m}Tc.

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