



Research Article

Comparison between electrocoagulation and solvent extraction method in the process of the dechlorophyllation of alcoholic extracts from Moroccan medicinal plants *Petroselinum crispum*, *Thymus satureioides* and microalgae *Spirulina platensis*

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Abstract

The air-dried aerial leaves of *Petroselinum crispum*, *Thymus satureioides* and dried *Spirulina platensis* powder were separately extracted using a Soxhlet apparatus. The dark green solution obtained was subject to the dechlorophyllation by two techniques, the electrocoagulation and solvent extraction. The obtained absorbances, were particularly much lower for electrocoagulation process. This result showed that this technique can remove easily plant pigments in the extracts, without affecting the chemical composition on secondary metabolites.

Keywords Dechlorophyllation · Medicinal plants · Electrocoagulation · Solvent extraction · Chemical composition

1 Introduction

Petroselinum crispum which belongs to the family *Apiaceae*, is a species native to the central Mediterranean region, naturalized elsewhere in Europe and cultivated as an herb, a spice and a vegetable [1]. In Morocco, *P. crispum* is a medicinal plant known as the vernacular name “Baqdunis” and its extracts are known to be diuretic, smooth muscle relaxant and hepatoprotective in folk medicine [2]. The most important identified flavonoids compounds are quercetin, luteolin, kaemferol [3], and apigenin which were found to have antiinflammatory, antioxidant and anticancer activities [4]. The chemical composition of

the *P. crispum* oil was reported and a total of 34 components were identified in the literature in which myristicin and apiole were the major constituents. The presence of oxypeucedanin, a major furocoumarin in Parsley was also reported [5].

Thymus satureioides belong to *Lamiaceae* family. It's a North African species typical of arid habitats used in the Moroccan folk medicine in form of infuse and decoctions to treat whooping cough, bronchitis and rheumatism [6] and, generally also used, for its anti-inflammatory properties after topical or oral administration. *T. satureioides* is endemic species to Morocco and is a well-known aromatic

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plant producing flavonoids and an essential oil, rich in borneol, with high antimicrobial effect [7].

Spirulina, is an edible blue-green microalga (cyanobacterium) characterized by multicellular cylindrical trichomes in an open left-hand helix along the entire length [8]. This cyanobacterium has received much attention in culture and as a most promising and nutritious food source due to its high content of good quality protein as well as being rich in vitamins, minerals, and other components such as essential fatty acids and antioxidant pigments like carotenoids, chlorophyll and phycocyanin [9–12].

Spirulina is widely studied, not only for its nutritional value but also for its reported medicinal properties [13]. Several studies have shown that *Spirulina* and its extracts could prevent or inhibit cancer in humans and animals and have immuno-promoting effects [14, 15], antimicrobial activity [16, 17], antioxidant activity [18] and antihypertensive activity [19, 20].

Several studies have been engaged on the chemical characterization of oil and secondary metabolites from leaf or seeds extract of the above plants. However, during the extraction, the pigments, tannins and carbohydrates are usually presents and affected the quality of the crude extract. So they must be removed so as to obtain the secondary metabolites in a purified form. This problem is more pronounced with our plants, *P. crispum*, *T. satureioides* and *Spirulina platensis*, which always contains plant pigments, notably the chlorophylls. Conventional methods for removing these substances are solvent extraction (SE) and chromatography [21–23]. However, using solvents in both methods, became more difficult to remove and as a problematic volatile chemical waste in the extracts. In chromatography, expensive and usually unrecoverable adsorbents must be used, and even then, the efficiency of pigment removal is usually far from satisfactory.

The electrocoagulation (EC), an electrochemical technique in which aluminum and iron electrodes are dissolved, generates in situ coagulant agents, which will destabilize the colloidal particles. By EC a variety of unwanted dissolved particles and suspended matter, both organic and inorganic, can be effectively removed from an aqueous solution by electrolysis [24]. EC has been mainly used to treat various aqueous organic and inorganic wastes [25–27]. It was used also as a method in clarifying potable water [28, 29] dechlorophyllation of alcoholic plant extracts and the isolation alkaloids [30].

The effect of pH on electrocoagulation is also a very pronounced parameter which is taking account. Ghernaout et al. [31], while working on electrocoagulation of bentonite suspensions, they confirmed that pH has a great effect. Indeed, they found that at acid pH the neutralization of the superficial charges of the clays and at alkaline pH the enmeshment of the kaolin particles into a sweep floc. At neutral pH, the formed cations (Fe^{2+} and Fe^{3+}) neutralize colloidal particles and conduct to the hydroxides ($\text{Fe}(\text{OH})_2(\text{s})$ and $\text{Fe}(\text{OH})_3(\text{s})$) which adsorbate colloids and enhance sweep flocculation [31].

Reports concerning the use of electrocoagulation for dechlorophyllation for the Moroccan extracts for the plants studied have not been previously described before in the literature.

In continuation of our investigation of Moroccan aromatic flora, the object of this work is to study and to examine particularly the electrocoagulation as an alternative method of dechlorophyllation since it has received increased attention recently, mainly in the environmental field. To our knowledge, it is for the first time, we report here the dechlorophyllation of alcoholic plant extracts for three Moroccan medicinal plants by electrocoagulation and its influence on the chemical composition.

2 Materials and methods

2.1 Plant material

The leaves of *P. crispum* were collected in the North of Morocco in April 2016.

The *T. satureioides* were collected from Midelt region (430 km south east of Rabat) in June 2016.

The *S. platensis* were collected from Laayoune (650 km south of Rabat) and purchased from a local market in January 2016.

The plants materials were identified by Professor Leila EL GHAZI from the department of biology, Faculty of Sciences, University Hassan II of Casablanca, Morocco, according to the flora of Morocco [32]. A voucher specimen of the plants of air-dried leaves was deposited in chemistry and biological departments in the same faculty.

*Petroselinum crispum**Thymus satureioidis**Spirulina platensis*

2.2 Preparation of the alcoholic extract

50 g of each sample plant was extracted with 75% ethanol (200 mL) in a Soxhlet apparatus during 8 h. The resulting dark green solution was then subject to electrocoagulation for 2.5 h. The yields were calculated per weight of the dried plant material.

2.3 Dechlorophyllation by electrocoagulation

The conditions for electrocoagulation were as follows: two clean aluminium plates were used as electrodes, each of 3 × 15 cm dimensions. These were spaced 1.5 cm apart and dipped 7 cm into the magnetically-stirred solution containing 0.1% (w/v) NaCl as supporting electrolyte. The sodium chloride, used as supporting electrolyte, is necessary for the proper conduct of an electrocoagulation reaction, by preventing the formation of deposits on the surface of the anode and accelerating its dissolution and hence the kinetics of reaction.

Direct current (0.9 A, 16.9–31.6 V) from a power supply was then passed via the two electrodes through the solution, which was placed in a jacketed 250 mL beaker for occasional cooling during electrolysis. After 2.5 h of electrolysis, the mixture was filtered to afford a decolorized solution N°1. For this solution, the absorbance was measurement by a spectrophotometer (Model Unico—UV-2100) to determine the degree of decolourisation at 665–666 nm (corresponding to the presence of the green pigments) and at 408–410 nm (corresponding to the presence of the yellow pigments).

2.4 Dechlorophyllation by solvent extraction

Each plant material (50 g) was extracted with an organic solvent (chloroform) using a Soxhlet apparatus during 8 h. The green solution obtained was drying and 150 mL

of ethanol (75%) was then added. So as to obtain its dechlorophyllation, the mixture was refluxed for 2 h, and filtered using charcoal and the solution N°2 obtained was taken for absorbance measurement at 665–666 nm and at 408–410 nm.

2.5 Phytochemical analysis

The alcoholic crudes extracts of the plants were subjected to qualitative phytochemical screening for the identification of the various classes of active chemical constituents such as saponins, tannins, alkaloids, flavones aglycones, sterols and triterpenes using the method described in the literature [33–37].

2.5.1 Test for saponins

Five milliliter of alcoholic extract was vigorously shaken with 10 mL of distilled water for 2 min. The appearance of foam that persists for at least 15 min or the forming of an emulsion when olive oil was added confirmed the presence of saponins.

2.5.2 Test for tannins

One milliliter of alcoholic extract was mixed with 10 mL of distilled water and filtered. Ferric chloride (FeCl) reagent (3 drops) was added to the filtrate. A blue-black or green precipitate confirmed the presence of gallic tannins or catechol tannins, respectively.

2.5.3 Test for alkaloids

A small portion (0.2 mL) of the extract was stirred and placed in 1% aqueous hydrochloric acid (5 mL) on a steam bath. Then 1 mL of the filtrate was treated with Mayer's reagent (3 drops) while another portion was similarly treated with Dragedorff's reagent. Turbidity or precipitation with

these reagents was considered as evidence for the presence of alkaloids.

2.5.4 Identification of sterols and triterpenes

The alcoholic extract, 10 mL was placed in a small beaker and evaporated to dryness. The residue was dissolved in acetic anhydride (0.5 mL) and chloroform (0.5 mL). The solution was transferred into a dry test tube and concentrated sulphuric acid (2 mL) was added. Brownish red or violet rings at the zone of the contact with the supernatant and green or violet coloration denoted the presence of sterols and triterpenes.

2.5.5 Identification of flavone aglycones

A portion of the extract (2 mL) was heated, metallic magnesium and concentrated hydrochloric acid; (5 drops) were added. A red or orange coloration indicate the presence of flavones aglycones.

2.6 Total phenolic compounds

Total phenolic content was determined according to the Folin–Ciocalteu colorimetric method [38]. The sample (0.5 mL) and 2 mL of sodium carbonate solution (75 g/L) were added to 2.5 mL of 10%Folin–Ciocalteu reagent. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm. Gallic acid was used as standard for calibration curve. The results are expressed in mg gallic acid equivalents per gram extract (mg GAE/g extract). All determinations were performed in triplicate.

2.7 Flavonoids content

The content of this group of compounds were quantified as described by Ahn et al. [39]. To 0.5 mL of sample or standard, was added 0.5 mL of 2% AlCl_3 -ethanol solution. After 10 min at room temperature, the absorbance was measured at 420 nm. Quercetin was used as a standard for plotting the calibration curve, and the results are expressed in mg quercetin equivalents per gram extract (mg of QuE/g extract). Samples were analyzed in triplicate.

2.8 Polyphenol aglycones preparation

The polyphenol aglycones preparation was done according to Engida et al. [40]. The alcoholic extract was filtered through a Whatman N°2 filter paper; then the solvent was removed using a rotary evaporator at 60 °C. The dried extract was dissolved in a 88% aqueous methanol solution (40 mL), to which 0.5 g/L hydroquinone (HQ) and 6 N HCl (10 mL) were added, and the mixture heated at 80 °C for

1 h to hydrolyze glycosides to aglycons. HQ was incorporated as antioxidant to avoid flavonoid degradation. After cooling, the solution was made to a final volume of 100 mL by adding de-ionized water, and then filtered through a syringe filter for HPLC analysis. Full acid hydrolysis helps us to generate the free polyphenol aglycones contained in the alcoholic extract.

2.9 Polyphenol aglycones analysis

2.9.1 Apparatus and chromatographic conditions

The HPLC analyses were performed with an Agilent 1100 HPLC Series (Agilent, USA) equipped with a degasser G1322A, a quaternary gradient pump G1311A, an autosampler G1311A, a column oven G1316 A, a Zorbax SB-C18 reversed-phase analytical column 100 mm × 3,0 mm *i.d.*, 3,5 µm particle (Agilent, USA) operated at 48 °C.

The compounds were eluted with an isocratic elution of mobile phase water, acetonitrile, acetic acid (88%, 12%, 1%, respectively). The injection volume of all samples and the mixture of polyphenols standards was 5 µl. The flow rate was 1 mL/min. Polyphenols standards (10–30 mg/L) used were: apigenin, kaempferol, luteolin, quercetol, isoquercitrin, chlorogenic acid, gallic acid, catechin, vanillic acid, caffeic acid, syringic acid, rutin, ferulic acid, p-coumaric acid, sinapic acid and quercetin. Chromatograms were registered at 330 nm. The identification of phenolic compounds in the extract was accomplished by comparison of their retention times with those of pure standards. All the standard were procured from Sigma Chemical Company, USA.

The quantitative analysis of caffeic acid derivatives was made using the method described by AFNOR [41] and Lopes-Lutz et al. [42]. A set of seven standard dilutions of caffeic acid at 330 nm was used. A stock solution of 1 mg/mL in water, acetonitrile, acetic acid (88%, 12%, 1%, respectively) was prepared and stored at –20 °C. The

Table 1 Absorbance of plant extracts obtained after dechlorophyllation by electrocoagulation and by solvent extraction

Plant	Absorbance			
	Electrocoagulation (EC)		Solvent extraction (SE)	
	Solution N°1		Solution N°2	
	Green pigments	Yellow pigments	Green pigments	Yellow pigments
<i>P. crispum</i>	0.18	2.40	3.18	6.23
<i>T. satureioides</i>	0.34	1.15	2.70	4.70
<i>S. platensis</i>	0.22	1.33	1.69	3.92

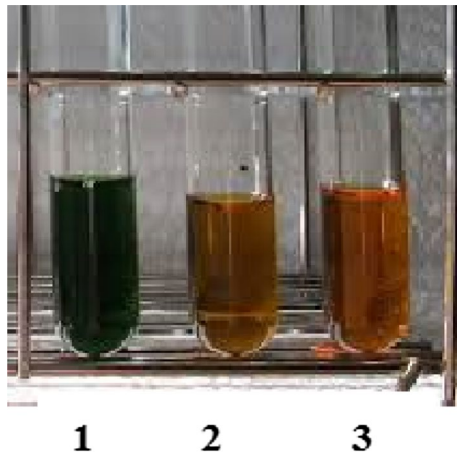


Fig. 1 Extracts color before dechlorophyllation (1), after dechlorophyllation by solvent extraction (2) and electrocoagulation (3)

calibration curves were linear over the range of 1–200 µg/mL with a correlation coefficient of ≥ 0.99 . For quantification, peak areas were correlated with concentrations in accordance with the calibration curves. Data are reported as means \pm standard deviations of triplicate independent analyses.

The HPLC method was validated in terms of linearity, limits of detection (LOD), limits of quantification (LOQ), recovery and intermediate precision. The calibration curves were linear with correlation coefficient ranging from 0.9909 to 0.9997. The values of LOD (0.22–0.93 mg/L), LOQ (0.65–2.70 mg/L), recovery ($\geq 97.2\%$) and precision in terms of retention time (%RSD ≤ 2.57) and peak area (%RSD ≤ 5.13) were satisfactory [43, 44].

3 Results and discussion

The alcoholic extract of *P. crispum* leaf gave a yield of 11.9% w/w and had a dark green colour. The *T. satureioides* extract gave a yield of 21.2% w/w and *S. platensis* extract produced a yield of 8.3% w/w.

Table 2 Phytochemical screening of the extracts of *P. crispum*, *T. satureioides* and *S. platensis*

Chemical constituents	<i>P. crispum</i>		<i>T. satureioides</i>		<i>S. platensis</i>	
	SE	EC	SE	EC	SE	EC
Saponins	–	–	++	+	+	++
Tannins	+++	–	++	–	++	–
Alkaloid	–	–	–	–	–	–
Sterols and triterpene	+	+	++	+	++	+
Flavone aglycones	++	++	++	+++	+	++

EC electrocoagulation and SE solvent extraction

– Negative result, + Positive result, ++ Present in high concentration and +++ Present in very high concentration

The absorbance at 665–666 nm corresponding to the green pigments and at 408–410 nm corresponding to the yellow pigments can be seen in Table 1 and Fig. 1. Examination of these results shows that all the absorbances were particularly high for solvent extraction. They were much lower for electrocoagulation. Indeed, using EC, the absorbance of green pigments was 0.18 for *P. crispum*, 0.34 for *T. satureioides* and 0.22 for *S. platensis*; the absorbances of yellow pigments were 2.40, 1.15 and 1.33 respectively for *P. crispum*, *T. satureioides* and *S. platensis*. It seems that dechlorophyllation by EC is more efficient than the conventional solvent fractionation method for all of the plants studied and the variations in absorbance are important between the two techniques. Our results are in accordance with the result previously reported in the literature by Kanlaya et al. [45] while working on the dechlorophyllation of alcoholic extracts from five plants (*Solanum laciniatum*, *Andrographis paniculata*, *Stevia rebaudiana*, *Centella asiatica*, *Cassia siamea*) by EC.

The phytochemical screening of *P. crispum*, *T. satureioides* and *S. platensis* extracts are given in Table 2. As can be seen, the results revealed the presence of saponins, sterols, triterpene, tannins and flavone aglycones. No alkaloids were found. In addition, using SE, the tannins were found in all extracts, but they were absent using EC. In our case, electrocoagulation seems to be an interesting method to remove the chlorophylls pigments and tannins which usually affect the quality of the extracts, than the conventional solvent fractionation method. The tannins, owing to their polyphenolic nature, are easily coagulated by the phenolate salt forming mechanism in addition to the adsorption mechanism [46]. In the context of electrocoagulation with electrodes of Al, in our case, if we consider tannins and chlorophylls pigments negatively charged colloids, they will tend to migrate towards the anode where there is oxidation of the aluminum (Reactions 1 and 2). They then increase the possibility of meeting with oxidized aluminum. This explains their elimination in the process of electrocoagulation [26].

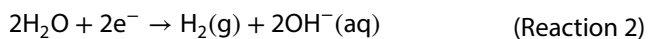
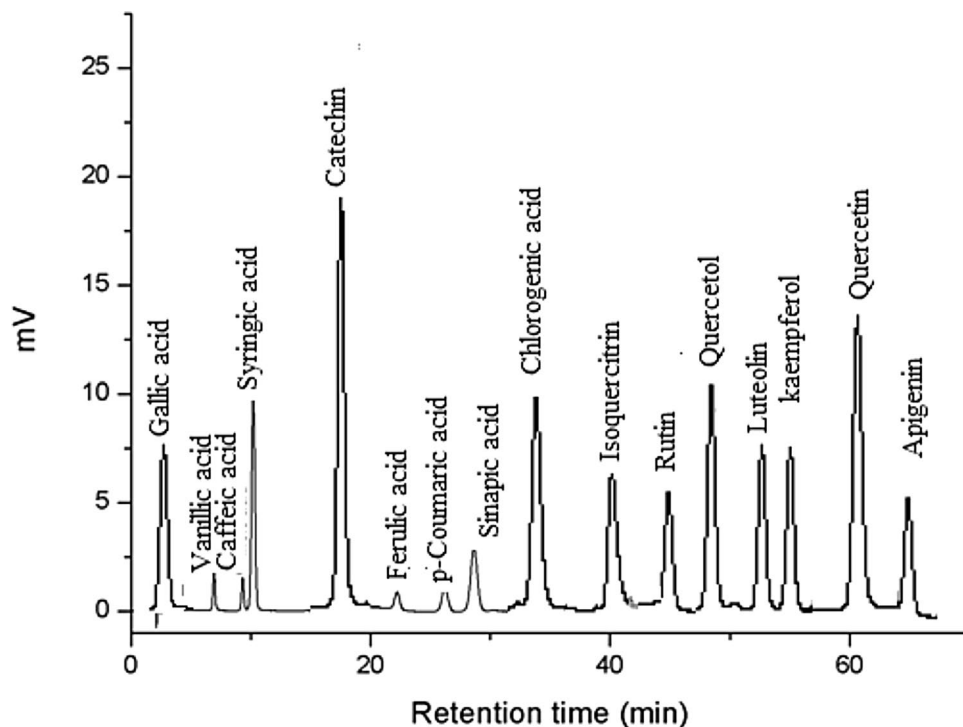


Table 3 Concentration of total phenols and flavonoid in extracts of *P. crispum*, *T. saturoioides* and *S. platensis*

	<i>P. crispum</i>		<i>T. saturoioides</i>		<i>S. platensis</i>	
	SE	EC	SE	EC	SE	EC
Total phenolics mg of GAE/g extract	36.5±0.4	39.5±0.4	69.5±0.4	70.2±0.4	29.5±0.4	28.4±0.4
Total flavonoid mg of QE/g extract	27.2±0.01	33.2±0.01	49.2±0.01	52.7±0.01	22.2±0.01	19.6±0.01

Results are given as mean ± standard deviation of three different experiments

GAE gallic acid equivalents, QE quercetin equivalents, EC electrocoagulation and SE solvent extraction

Fig. 2 HPLC Chromatogram of available polyphenol standards monitored at 330 nm and identified with retention time (min): gallic acid (2.80), vanillic acid (4.47), caffeic acid (9.45), syringic acid (11.15), catechin (19.7), ferulic acid (22.31), p-coumaric acid (26.51), sinapic acid (29.02), chlorogenic acid (35.12), isoquercitrin (40.14), rutin (46.25), quercetol (48.65), luteolin (53.17), kaempferol (55.39), quercetin (60.12) and apigenin (65.09)

3.1 Total phenolic and flavonoid composition

Results obtained in the present study shown in Table 3 revealed that using EC or SE method, the contents of total phenolic compounds and flavonoid for each plant are not affected. The *T. saturoioides* extracts have shown to contain a higher content of total phenolic (69.5–70.2 mg GAE/g) and flavonoid (49.2–52.7 mg QuE/g) compounds than the other extract. In addition for the cyanobacterium *S. platensis*, the amounts of total phenolic content found in our samples (19.6–28.5 mg GAE/g) are different of the Indian spirulina (17–43.2 mg GAE/g) [47].

To ensure that the chemical composition is not affected by the process of electrocoagulation, we also focused our study on the qualitative and quantitative analysis of polyphenol aglycones in the extracts. HPLC chromatogram of available polyphenol standards monitored at 330 nm is given in Fig. 2. The identified components and their percentages are given in Table 4.

The results shown that both EC and SE techniques, after acidic hydrolysis of the hydroalcoholic extract, the HPLC analysis revealed the presence of the same compounds in each plant and the qualitative and quantitative chemical composition is not affected by EC. For *P. crispum* five components were identified such as vanillic acid, luteolin, kaempferol, quercetin and apigenin. Some compounds have been previously reported from parsley

Table 4 Phenolic compounds (% of total) identified by HPLC, in the alcoholic extract of Moroccan medicinal plants after acidic hydrolysis

Peak N°	Compounds	Retention time (min)	Concentration (mg/100 g)					
			<i>P. crispum</i>		<i>T. satureioides</i>		<i>S. platensis</i>	
			SE	EC	SE	EC	SE	EC
1	Gallic acid	2.80	–	–	–	–	66.2	59.8
2	Vanillic acid	4.47	2.1	1.5	–	–	–	–
3	Caffeic acid	9.45	–	–	114.3	120.8	44.1	41.3
4	Syringic acid	11.15	–	–	–	–	–	–
5	Catechin	19.7	–	–	–	–	–	–
6	Ferulic acid	22.31	–	–	77.9	80.1	–	–
7	p-coumaric acid	26.51	–	–	63.4	59.8	11.5	9.8
8	Sinapic acid	29.02	–	–	–	–	–	–
9	Chlorogenic acid	35.12	–	–	42.1	39.7	–	–
10	Isoquercitrin	40.14	–	–	–	–	–	–
11	Rutin	46.25	–	–	–	–	12.3	14.1
12	Quercetol	48.65	–	–	–	–	–	–
13	Luteolin	53.17	47.2	48.6	35.1	33.8	–	–
14	Kaempferol	55.39	12.7	11.3	–	–	–	–
15	Quercetin	60.12	9.1	8.9	–	–	5.4	6.1
16	Apigenin	65.09	4.8	5.5	1.7	2.3	–	–

EC electrocoagulation and SE solvent extraction

– Not identified

extracts such as quercetin, luteolin, kaempferol [3] and apigenin [4].

For *T. satureioides*, six compounds were identified such as caffeic acid, ferulic acid, p-coumaric acid, chlorogenic acid, luteolin and apigenin. Those compounds have been previously reported in *T. satureioides* leaves by Ismaili et al. [48].

For *S. platensis* five components were identified such as gallic acid, caffeic acid, p-coumaric acid, rutin and quercetin. Machu et al. [47] reported the presence in the extract of *S. platensis* from India different phenolic compounds such as catechin hydrate, epicatechin, and pyrocatechol not present in our sample.

4 Conclusion

The electrocoagulation technique was found to be an interesting method for dechlorophyllation of our plant extracts than the conventional usage of organic solvents. It seems also not affecting the chemical composition. In Morocco, the result of this investigation will allow us to examine the possibility to promote this technique as another alternative environmentally friendly method to remove from the plants extracts the pigments, notably the chlorophylls.

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Compliance with ethical standards

Conflict of interest On behalf all coauthors, I confirm that we not have conflict of interest with any reviewers or any financial and personal relationships with other people or organizations that could inappropriately influence our work.

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