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ORIGINAL PAPER

Essential oil composition and antioxidant activity of *Stachys officinalis* subsp. *algeriensis* (Lamiaceae) from a wild population in Tunisia

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Abstract

In continuation of our research focus on the characterization of the volatile components of the spontaneous Tunisian flora, the aim of the present work was to carry out the chemical analysis of the essential oil of wood betony, *Stachys officinalis* subsp. *algeriensis* (de Noë) Franco (Lamiaceae). The volatiles were extracted by hydro-distillation and determined by gas chromatography adopting a flame ionization detector and gas chromatography coupled to mass spectrometry technique. 35 constituents were identified representing 97.7% of the total essential oil. Wood betony oil was found to be rich in sesquiterpene hydrocarbons (41.1%) and by oxygenated sesquiterpenes (31.7%). The main metabolites were δ-cadinene (13.7%), followed by cubebol (12.4%), *epi*-cubebol (7.4%), and α-calacorene (6.9%). The present study—the first—on the antioxidant activity of essential oils obtained from this species collected in Tunisia, showed that the oils exhibit moderate antioxidant activity and reduce 2,2′-diphenyl-1-picrylhydrazyl (radical DPPH$^{\cdot}$) with an effective concentration (IC_{50}) value of 3.9 mg/mL.

Keywords *Stachys officinalis* subsp. *algeriensis* (de Noë) Franco · Lamiaceae · Gas chromatography · Sesquiterpenes · Antioxidant activity

Introduction

For thousands of years, medicinal and aromatic plants containing potentially bioactive natural products have been widely used to treat diseases and to maintain the human health. Essential oils produced by the plants—often responsible for this biological activity—are typically volatile complex mixtures of mono and sesquiterpenes, phenylpropanoids and their oxygenated derivatives. Many of them have a significant usage in traditional medicine, food flavoring and pharmaceutical industries [1]. About 300 plant-derived essential oils are commercially used for food preservation, pharmacological purposes and in aromatherapy [2], due to their capacities to provide secondary metabolites useful for protection against bacteria, viruses, fungi, and pests. Medicinal plants belonging to the Lamiaceae (Labiatae) family are known for their aromatic smell; they are composed of a large number of widely used culinary herbs including rosemary, basil and marjoram which are characterized by a high content of essential oils [3]. Throughout the centuries, *Stachys* L. species were used in traditional medicine for numerous purposes, including the treatment of inflammatory diseases, ulcers, sclerosis of the spleen, genital tumors, infected wounds, cough, diarrhea, fevers, sore throat, liver disorders, heart weakness, internal bleeding, headaches, migraine and neuralgia [4–9]. *Stachys* species are also appreciated as alimentary plants. Their edible tubers are likely to contain higher concentrations of nutritionally important components such as carbohydrates and proteins

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[10]. Many of them are useful to improve the taste of jelly or yogurt and as seasonings and flavorings [11]. This is the case with *Stachys palustris*, an edible medicinal plant distributed in humid habitat in Europe, its great potential as functional food have been described. For example, its young shoots were cooked and used as asparagus substitute, while its tuber, consumed raw or cooked is used to flavor bread [12]. *Stachys* herbal extracts are reported to possess various medicinal benefits, including anti-inflammatory, antitoxic, antimicrobial, antioxidant effects and also for their effectiveness as natural preservatives which is widely explored in food chemistry [9]. *Stachys officinalis* subsp. *algeriensis* (de Noë) Franco [13], commonly known as wood betony, is one of the very important aromatic and medicinal plants within the Lamiaceae family. Widespread throughout the world, common in many areas in Europe and North Africa, this perennial herb contains relatively high amounts of tannins, bitter substances, a small amount of essential oils and other active substances [14]. In traditional phytotherapy, the aerial parts of wood betony are commonly used for the treatment of a wide range of disorders, especially as a nervine and tonic for strengthening the nervous system, and as an external application to wounds [15–17] Other properties have been linked to wood betony as an astringent, antidiarrheal, diuretic, antiseptic, febrifuge, cholagogue and vulnerary [18]. In Romania it is recommended to alleviate and heal pulmonary catarrh, asthma, and diarrhea (oral use), gout, gallstones and kidney stones, as well as for the treatment of cuts and varicose ulcer (in external use) [19, 20]. Three European herbals during sixteenth century mentioned that a broth made from the boiled roots of *Betonica officinalis* was used to ease polyarthritis, gout, hip pains and lameness of the limbs [21]. Tea made with the aerial parts of *B. officinalis* collected in Kosovo, have a reputation of being a tranquilizing agent with sedative, antispasmodic, and diuretic properties [22]. Phytochemically, previous investigations of wood betony extracts led to the isolation and identification of iridoids, flavonoids, phenolic acids such as caffeic, chlorogenic, neochlorogenic, 3,5-dicaffeoylquinic and *p*-coumaric acids, choline, betaine, and stachydrine [19]. Iridoids represented good chemotaxonomic markers of *Stachys* genus. Harpagide, ajugoside, aucubin, acetyl harpagide and harpagoside have been identified as the main monoterpenoids present in *B. officinalis* [23]. It has been made evident from a number of studies, *Stachys* essential oils consist mainly of sesquiterpenes and oxygenated sesquiterpenes [24, 25]. Samples from different wild populations of wood betony collected in Croatia, in Montenegro, in Serbia, and in Kosovo have been studied [22], where a natural variation of the oil constituents was detected and rationalized by the differences in environmental conditions. The antimicrobial and antifungal activities showed by *Stachys* genus render them particularly useful as a natural conservative and by consequences as an important genus in the food industry [7].To our knowledge, there are no published data on the traditional use of *Stachys officinalis* subsp. *algeriensis* (de Noë) Franco growing spontaneously in Tunisia or in Northern Africa [26] and until now, the chemical composition of its essential oil have never been described. This investigation is therefore the first report on the detailed composition characterization and antioxidant effects of wild wood betony collected in Kroumiria region (North-West of Tunisia).

Materials and methods

Plant material and isolation of essential oil

Aerial parts of Tunisian wood betony were collected by the end of the blooming stage and during the fruiting period (September–October 2013) (Fig. 1) from "Melloula" region. The plant grows spontaneously in mixed pine-oak forests (Tabarka, Kroumiria) with coordinates 36°56′40.41″N; 08°42′27.85″E, altitude between (157 and 161) m a.s.l. Plant specimens were identified by one of the authors (REM), botanist in the Laboratory of Botany and Plant Ecology, Faculty of Sciences of Bizerta, Jarzouna, Bizerta-Tunisia, where voucher specimens [LAM/St-off-alg (B-off-alg); 00119/2013] have been deposited (a duplicate is deposited in Herb. Univ. Monastir). The material, previously air-dried at ambient temperature (about 200 g) was subjected to hydrodistillation for 3 h in a Clevenger type apparatus. To separate the volatile oil from the condensate water phase, an extraction with *n*-hexane was made. The organic volatile fraction was collected by decantation, dried over anhydrous sodium sulphated, filtered. After solvent evaporation, the essential oil (yielding 0.09%) was stored in brown glass bottle tightly closed, at 4 °C until analysis.

Gas chromatography

The GC analysis of the oil was carried out on Varian-Agilent Technologies 450 gas chromatograph equipped with FID, using stationary phase CP Sil-8-CB (30 m × 0.25 mm, 0.25 µm film thickness) column under the experimental conditions reported [27–30]. Nitrogen was a carrier gas at 1.0 mL/min flow rate. Temperature programming was 60–220 °C at 3 °C/min, for injector and detector temperatures were 230 and 250 °C, respectively. The injection volume was 1.0 µL of 1% solution diluted in *n*-hexane; split ratio was 1:50.

Gas chromatography–mass spectrometry

The GC–MS analysis of the oil was carried out on Thermo Scientific Trace Ultra GC interfaced with a Thermo

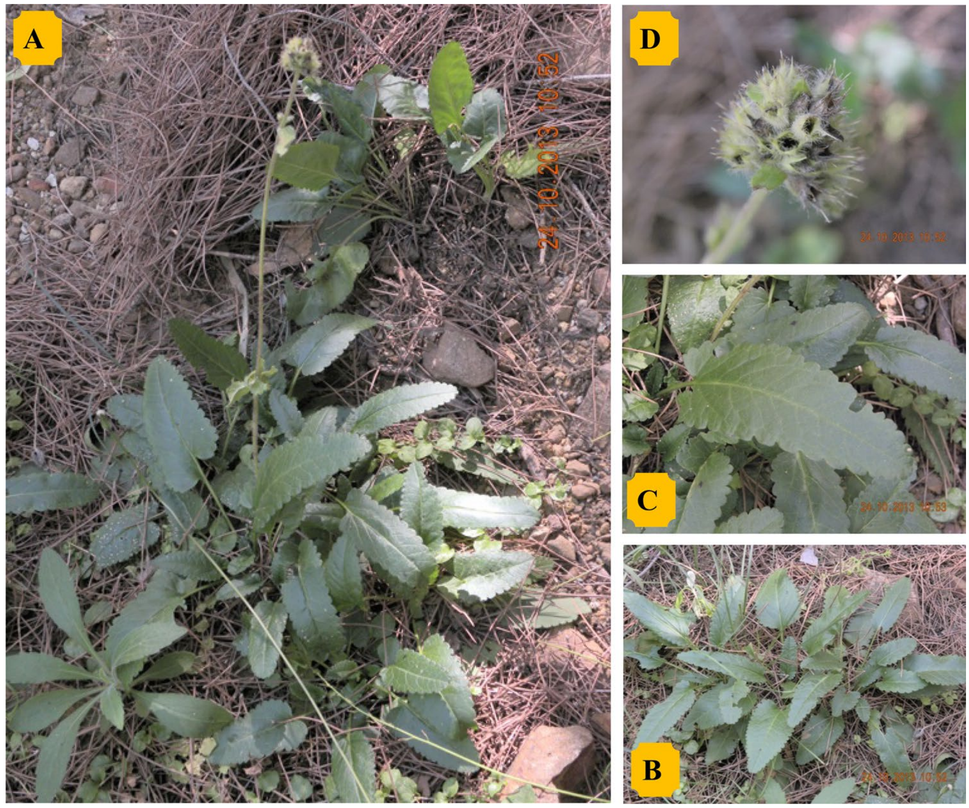

Fig. 1 The Tunisian subendemic wood betony *Stachys officinalis* subsp. *algeriensis* (de Noë) Franco. **a** Plant in its natural habitat. **b** Many basal long petiolated leaves in rosette. **c** Obcordate leaf, crenulated in border, hairless and smooth in the adaxial face. **d** Thick inflorescence on the top of an erecting single flowering spike, (Photos of Ridha EL MOKNI)

Scientific ITQ 1100 Mass Spectrometer fitted with TG-5 (30 m × 0.25 mm, 0.25 µm film thicknesses) column. The oven temperature was programmed from 60 to 220 °C at 3 °C/min using helium as a carrier gas at 1.0 mL/min. The injector temperature was 230 °C, injection volume 1.0 µL of 1% solution prepared in *n*-hexane; split ratio 1:50. MS ionization energy was taken at 70 eV. Mass range was set from 40–450 Da. All the experimental parameters were applied from those reported earlier [28, 30].

Identification of the components

Identification of constituents were done on the basis of Retention Index (RI, determined with reference to homologous series of *n*-alkanes (C_8–C_{25}), under identical experimental condition), MS library search (NIST 08 MS Library (Version 2.0 f) and WILEY MS 9th Edition), and by comparison with MS literature data [31], and co-injection of authentic standards from Sigma–Aldrich, India (≥ 98% purity). The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor.

Antioxidant activity

DPPH$^{\bullet}$ radical-scavenging assay: 2,2′-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$) free radical assay was carried out to measure the free radical-scavenging activity as reported previously [32]. A volume of 1.0 mL of each ethanol solution from Tunisian wood betony prepared at different concentrations was mixed with an equal volume of ethanolic solution of DPPH$^{\cdot}$ (0.1 mM). Corresponding blank samples were prepared and quercetin (62–1000 µg/mL) was used as reference standard. The disappearance of the DPPH$^{\cdot}$ was measured after 30 min of incubation at room temperature. The antioxidant effect was expressed in radical-scavenging activity (RSA) calculated according to the formula of Yen and Duh [33].

$$\%\,\mathrm{RSA} = \left[(\mathrm{As\ control} - \mathrm{As\ sample})/\mathrm{As\ control}\right] \times 100,$$

where As control is absorbance of the control sample ($t = 0$ h).

As sample is the absorbance of a tested sample at the end of the reaction ($t = 1$ h).

The essential oil concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting percentage of radical-scavenging activity (% RSA) against the Tunisian wood betony essential oil concentration.

Results

Chemical composition

The hydro-distillation of the dried aerial parts of Tunisian subendemic wood betony yielded a pale-yellow colored oil (0.09%). Table 1 summarizes the volatile metabolites characterized in their elution order using a non-polar stationary phase capillary column (TG-5 Column) and identified according to the relative retention indexes and to their mass spectra (Fig. 2). 35 compounds were identified, representing 97.7% of the total essential oil. Sesquiterpene hydrocarbons

Table 1 Chemical composition of the essential oil of the Tunisian subendemic wood betony, *Stachys officinalis* subsp. *algeriensis* (de Noë) Franco

No.	Compound	RT	$RI_{Calculated}$	$RI_{Literature}$	%	Identification
1	*α*-Pinene	5.64	918	939	4.2	RI, MS, CI
2	*β*-Pinene	6.69	949	980	0.5	RI, MS
3	Myrcene	7.11	961	991	3.3	RI, MS, CI
4	*o*-Cymene	7.99	987	1022	0.7	RI, MS
5	Limonene	8.27	995	1031	5.7	RI, MS, CI
6	*γ*-Terpinene	9.24	1024	1062	4.8	RI, MS, CI
7	*m*-Cymene	10.09	1049	1082	1.1	RI, MS
8	6-Camphenol	11.25	1083	1109	0.3	RI, MS
9	*trans*-Limonene oxide	12.18	1110	1139	0.2	RI, MS
10	Methyl salicylate	13.75	1156	1190	t	RI, MS
11	Thymol	18.13	1285	1290	1.5	RI, MS, CI
12	Carvacrol	18.47	1295	1298	t	RI, MS, CI
13	*α*-Cubebene	21.18	1375	1351	0.5	RI, MS
14	*α*-Copaene	22.22	1405	1376	0.9	RI, MS
15	*β*-Cubebene	22.71	1419	1390	1.5	RI, MS
16	*β*-Caryophyllene	23.83	1453	1418	3.1	RI, MS, CI
17	*β*-Gurjunene	24.23	1464	1432	1.6	RI, MS
18	*α*-*trans*-Bergamotene	24.64	1476	1436	0.5	RI, MS
19	*α*-Humulene	24.98	1486	1454	4.1	RI, MS
20	*cis*-Muurola-4(14),5-diene	25.12	1491	1460	t	RI, MS
21	*γ*-Muurolene	25.95	1515	1477	0.3	RI, MS
22	*γ*-Curcumene	26.26	1524	1480	2.3	RI, MS
23	Germacrene D	26.52	1532	1480	3.9	RI, MS
24	*epi*-Cubebol	26.71	1537	1493	7.4	RI, MS
25	Cubebol	27.48	1560	1514	12.4	RI, MS
26	*cis*-Calamenene	27.59	1563	1521	0.8	RI, MS
27	*δ*-Cadinene	27.86	1571	1524	13.7	RI, MS
28	Cadina-1,4-diene	28.15	1580	1532	1.0	RI, MS
29	*α*-Calacorene	28.25	1583	1542	6.9	RI, MS
30	Caryophyllene oxide	29.71	1626	1581	2.9	RI, MS, CI
31	1-*epi*-Cubenol	31.55	1680	1627	0.8	RI, MS
32	Cubenol	32.11	1696	1642	3.8	RI, MS
33	Apiol	32.26	1701	1680	2.6	RI, MS
34	*β*-Bisabolol	33.15	1727	1671	2.3	RI, MS
35	Khusinol acetate	38.82	1894	1816	2.1	RI, MS
Monoterpene hydrocarbons				20.3%		
Oxygenated monoterpenes				0.5%		
Sesquiterpene hydrocarbons				41.1%		
Oxygenated sesquiterpenes				31.7%		
Phenyl derivatives				4.1%		
Total identified				97.7%		

RI Retention Index relative to C_8–C_{25} *n*-alkanes on TG-5 column, *MS* NIST and Wiley library and the literature, *t* trace (< 0.1%), *CI* co-injection of authentic standards (C98% purity)

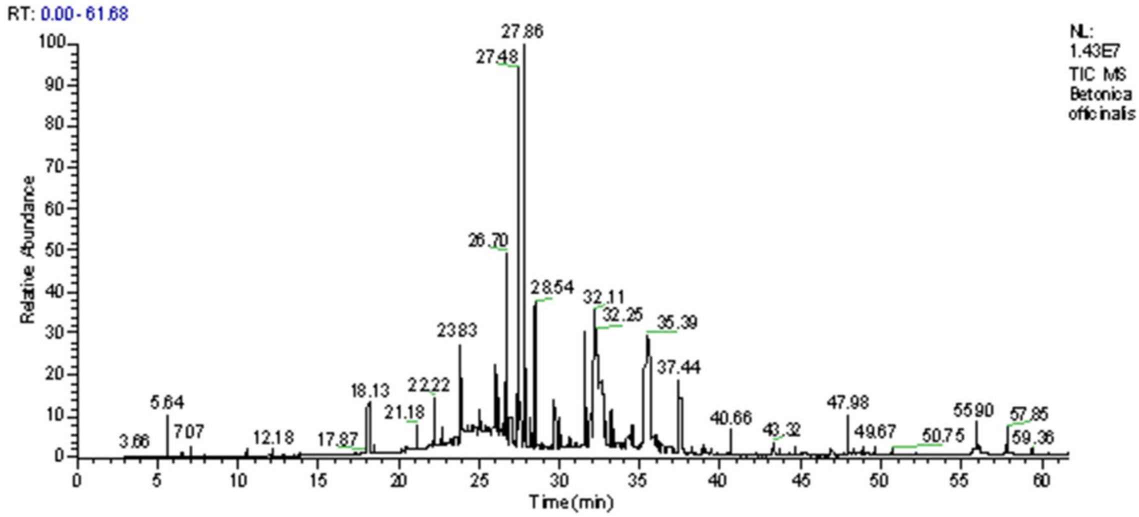

Fig. 2 GC-TIC chromatogram of the essential oil of the Tunisian subendemic wood betony *Stachys officinalis* subsp. *algeriensis* (de Noë) Franco

(41.1%) constituted the most dominant group, followed by the oxygenated sesquiterpenes and by, respectively, the monoterpene hydrocarbons which account for 31.7 and 20.3% of the total constituents, while phenolic derivatives and oxygenated monoterpenes are scarcely represented in this essential oil (4.1 and 0.5%, respectively). The main components (dominant chemotypes) identified in the Tunisian wood betony were δ-cadinene (13.7%), followed by cubebol (12.4%), *epi*-cubebol (7.4%) than α-calacorene (6.9%). Thus, the chemical profile of ours ample is globally consistent with those reported for other members of *Stachys* genus, for which sesquiterpenoids are of common occurrence, rather, low amounts of phenylpropanoids were detected [23, 34, 35].

DPPH$^{\cdot}$ radical-scavenging activity

This is the first report on the antioxidant activity of a Tunisian subendemic wood betony essential oil, from North Africa. The free radical-scavenging ability of the tested sample of the essential oil was determined using the DPPH$^{\bullet}$ assay and was compared to that of quercetin. Table 2 shows the reducing power of the essential oil and the control as a function of their concentration. As expected, the scavenging effects increased as the concentration of the tested sample increased. The present investigation depicts that, as anticipated, the essential oils has a lower antioxidant activity compared with quercetin. The IC_{50} values were 3.9 and 0.069 mg/mL for, essential oil and for quercetin, respectively. This might be explained by the low level of hydrogen-donating constituents in the volatile oil, especially phenolic derivatives (4.1%).

Table 2 Inhibition % and IC_{50} concentrations of DPPH-scavenging capacity of the Tunisian subendemic wood betony (*Stachys officinalis* subsp. *algeriensis* (de Noë) Franco) essential oil

Bioactive samples	C (µg/mL)	Inhibition percentage	IC_{50} (mg/mL)
Tunisian wood betony essential oil	125	1.4 ± 0.20	3.90
	250	14.2 ± 0.5	
	500	17.57 ± 0.73	
	1000	23.14 ± 0.60	
	2000	38.85 ± 1.30	
	4000	53.85 ± 1.50	
	8000	66.28 ± 0.80	
Quercetin control	62	46.17 ± 0.05	0.069
	125	89.57 ± 0.16	
	250	90 ± 0.72	
	500	92.57 ± 0.17	
	1000	96.42 ± 0.45	

IC_{50} the concentration of the sample required to inhibit 50% of the radical DPPH$^{\cdot}$

Discussion

The results of this study showed for the first time the essential oil composition of a sample of wood betony from Northwest Tunisia, North Africa, and highlights the considerable qualitative and quantitative variation between the major organic volatiles of the Tunisian subendemic wood betony essential oil and other populations from several European countries [9, 20, 22]. It is apparent from the data that the analyzed sample possessed a high level of sesquiterpene hydrocarbons (41.1%) and belongs to the δ-cadinene chemotype (13.7%). While germacrene D, being the main constituent of the essential oils identified in Croatian [36] and

Serbian [37] wood betony, is quantified in a few amount in our sample and also in that from Italy [17]. The main constituents of the leaf essential oil analyzed in Kosovo were α-pinene, 1-octen-3-ol, β-bourbonene, (*E*)-caryophyllene and germacrene D [22]. The occurence of sesquiterpenes in the *Stachys* genus is evident according to a wide number of previous studies [23, 34]. However, the predominance of germacrene D depends on the studied species, on different plant tissues and locations [17, 38]. Furthermore, previously published data [9] on the antioxidant effects of six *Stachys* species collected from Lebanon, Greece and Italy (European origin), showed that these oils, which have IC_{50} values between 0.482 and 1.26 mg/mL had better antioxidant activity than the Tunisian wood betony essential oil (IC_{50} value of 3.9 mg/mL). Significant changes, in the chemical composition and antioxidant effects between the Tunisian subendemic wood betony essential oil and other *Stachys* samples from different localities in other areas of the world, might arise from environmental (climatic, seasonal, geographical) and genetic differences. This might confirm well this membership to an ecotype (strictly North African in the beginning) different from other ecotypes of the northern coast of the Mediterranean region and could argue in favor of a distinction between at least two major ecotypes; the first, would be the North African (subsp. *algeriensis*) whereas, the second comprises the European subspecies (subsp. *serotina*; subsp. *velebitica*; subsp. *haussknechtii*, etc.). Previous studies indicated that predominant compounds in the extracted oils exhibit a broad spectrum of biological activities (anxiolytic, anti-inflammatory and anti-nociceptive) [23]. The remarkably high content of sesquiterpene hydrocarbons, especially δ-cadinene, could be of interest for further phytochemical studies and biological investigations, taking into account that δ-cadinene was shown to inhibit the growth of *Streptococcus mutans* (one of the most important cariogenic bacteria) and *Propionibacterium acnes* (one of the bacteria responsible for acne). It has also shown antiproliferative effects against different cell lines [39, 40]. Thus, a correlation between the chemical composition of the tested essential oil and its biological activity should be assessed for a relationship to the δ-cadinene and cubebol present in high percentages in the wood betony growing spontaneously in Tunisia.

Conclusion

The essential oils of the native North-African wood betony collected in North-West of Tunisia, *Stachys officinalis* subsp. *algeriensis* (de Noë) Franco, were investigated by capillary GC/FID and GC/MS for the first time. 35 organic volatiles were identified by comparison of their retention indexes and mass spectra with those of the literature. The first study on the antioxidant activity of the essential oils, rich in sesquiterpene hydrocarbons and dominated by the biologically active terpenes δ-cadinene and cubebol, showed a moderate free radical-scavenging ability. This antioxidant activity might be due to the synergistic interactions between different volatiles and is dependent on the nature and the concentration of the antioxidants. Thus, further extensive chromatographic studies on volatile extracts of this subendemic taxon (occurring largely almost in its natural area, Algeria–Tunisia–Morocco–Iberian Peninsula), would be useful for the identification of new antioxidants and various potentially bioactive products.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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