Comparative Evaluation of Essential Oils of *Helichrysum petiolare* Hilliard & B.L. Burtt Obtained from Solvent-Free Microwave and Hydrodistillation Extraction Methods

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Till date, no study has ever looked into the effect of extraction method on the chemical constituents of the essential oil of *Helichrysum petiolare*. This study therefore evaluated the effect of hydrodistillation and solvent free microwave extraction (SFME) methods on the chemical constituents of *H. petiolare* derived essential oils. The SFME derived essential oil was deep yellow and of higher yield than the pale yellow essential oil obtained through the hydrodistillation. There were substantial amounts of monoterpenes, monoterpene alcohols, sesquiterpenes, and sesquiterpene alcohols in both essential oils obtained. The SFME derived essential oil had 62 compounds as against the 52 derived through hydrodistillation. The SFME derived essential oil can therefore be suggested to be of better quality than of the hydrodistillation method. The compounds obtained in the essential oils have high pharmaceutical and cosmetic values, and as observed in this study, their quantity is dependent on method of extraction.

Keywords: Helichrysum petiolare, Hydrodistillation, Monoterpene, Sesquiterpene, Essential oil.

INTRODUCTION

South Africa harbours approximately 245 species of *Helichrysum*. Extracts of various *Helichrysum* species have been used to treat tropical infections, respiratory ailments, and as dressings in circumcision rites. Its administration by inhalation suggests that the volatile aromatic compounds may play a role in anti-infective therapies and several studies have indicated significant antimicrobial properties for *Helichrysum* oils [1].

Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain, as there is risk of unfavourable side effect. Furthermore, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety [2]. The compounds found in plants are of many kinds, but most are in four major biochemical classes: alkaloids, glycosides, polyphenols, and terpenes.

An essential oil is a concentrated hydrophobic liquid containing volatile (easily evaporated at normal temperatures) chemical compounds from plants. It is "essential" in the sense that it contains the essence of the plant's fragrance, *i.e.* the characteristic fragrance of the plant from which it is derived [3]. Unlike fatty oils, essential oils typically evaporate completely without leaving a stain or residue.

Essential oils are often used for aromatherapy, a form of alternative medicine in which healing effects are ascribed to aromatic compounds [4]. They can be extracted by different methods which include expression, solvent extraction, slow-folding process (sfumatura), absolute oil extraction, distillation (e.g. hydrodistillation, steam distillation and steam or water distillation), resin tapping, wax embedding, solvent free method and cold pressing [5,6]. However, most of these methods are time and energy consuming, and the essential oils eventually derived through them are either of small quantity or poor quality, there is therefore need for a faster, more energy sufficient method, which would yield higher quantity of essential oil with better quality.

It is therefore essential to compare the chemical components of essential oils extracted from *Helichrysum petiolare*

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using two methods, *i.e.* solvent-free microwave extraction and hydrodistillation methods.

EXPERIMENTAL

Sample collection: The whole plant of *Helichrysum petiolare* was collected from Hogsback situated in the Raymond Mhlaba Municipality of Eastern Cape Province, South Africa. The province falls within the latitudes 30°00′-34°15′S and longitudes 22°45′-30°15′E [7]. The plant collected was identified and authenticated by a qualified plant morphology expert and a specimen was submitted at the Giffen herbarium, University of Fort Hare, South Africa.

The fresh whole plant samples were subjected to essential oil extraction using solvent-free miocrowave extraction method and hydrodistillation method.

Solvent free microwave extraction: Solvent free microwave extraction was carried out with a Milestone DryDIST (2004) apparatus using the operational parameters described earlier [8]. The multimode reactor possesses a twin magnetron (2 800 W, 2450 MHz) with a maximum delivered power of 500 W in 5 W increments. Homogeneous microwave distribution throughout the plasma coated PTFE cavity is ensured by a rotating microwave diffuser with the temperature constantly monitored by an external IR sensor. Constant conditions of temperature and water were guaranteed by the reflux of condensed water, which was achieved by a circulating cooling system at 5 °C. *Helichrysum petiolare* plant (100 g) was placed into the reactor without addition of water or any solvent. The exhaustive extraction of the essential oil was obtained in 30 min.

Hydrodistillation: *Helichrysum petiolare* plant (100 g) was hydrodistilled for 3 h in an all-glass Clevenger apparatus in accordance with the description of the British Pharmacopoeia. Heat was supplied to the heating mantle (50 °C) and the essential oil was extracted exclusively with 4 L of water for 3 h. The essential oil was collected and analyzed immediately using GC-MS.

GC-MS analyses and identification of components: The chemical profiling of essential oil extracted through hydrodistillation and solvent free microwave extraction was performed using gas chromatography-mass spectroscopy (GC-MS) on an Agilent 6890 GC coupled to an Agilent 5975 MSD with a Zebron-5MS column (ZB-5MS 30 m \times 0.25 mm \times 0.25 μ m) (5%- phenylmethylpolysiloxane). GC grade helium was used as a carrier gas at a flow rate of 2 mL/min; splitless 1 µL injections were used (injector temp. = 280 °C; source temp. = 280 °C). Oven temp. was 70 °C, ramp 15 °C/min to 120 °C, ramp at 10 °C/min to 180 °C then ramp at 20 °C/min to 270 °C and hold for 3 min. Data was gathered with Chem station. Identification of the chemical components of the essential oils was accomplished by correlating their mass spectra and retention indices with those of the Wiley 275 library [2,8]. The spectrogram of each identified compound was determined using the integration of the peak areas.

RESULTS AND DISCUSSION

The essential oils obtained from *Helichrysum petiolare* plant through solvent free microwave extraction and hydrodis-

tillation were deep and pale yellow, respectively. Higher oil yield of 3.4 mL/100 g was obtained through solvent free microwave extraction compared to 2.6 mL/100 g obtained through hydrodistillation. The GC-MS spectra for the essential oil obtained by hydrodistillation and solvent free microwave extraction are shown in Figs. 1 and 2, respectively.

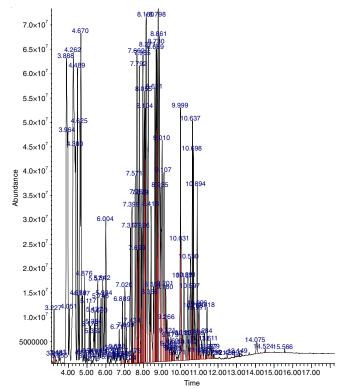


Fig. 1. GC-MS spectra data of *Helichrysum petiolare* essential oil using hydro distillation method

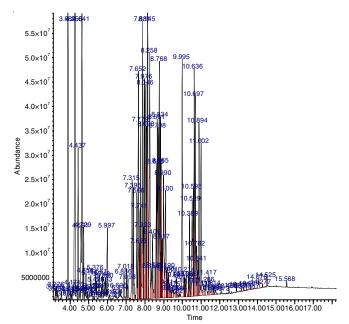


Fig. 2. GC-MS spectra data of *Helichrysum petiolare* essential oil using solvent-free microwave extraction

Tables 1 and 2 show the chemical compounds present in the essential oil extracted by each method, including their struc-

TABLE-1 KOVAT'S INDEX, RETENTION TIME, MOLECULAR AND STRUCTURAL FORMULA, AND MOLECULAR WEIGHT OF CHEMICAL COMPOUNDS HYDRODISTILLED FROM *Helichrysum petiolare*

S/N	Compounds	KI	RT	Area (%)	m.f.	Chemical component	Structure
1	(+)-α-Pinene	936.72	3.89	5.16	$C_{10}H_{16}$	Monoterpene	H Mun,
2	Camphene	944.83	4.05	0.62	$C_{10}H_{16}$	Monoterpene	
3	3-Carene	955.32	4.26	7.75	$C_{10}H_{16}$	Monoterpene	HILLING
4	Sabinene	961.19	4.38	1.43	$C_{10}H_{16}$	Monoterpene	
5	α-Thujene	966.62	4.49	2.55	$C_{10}H_{16}$	Monoterpene	
6	o-Diethylbenzene	973.38	4.63	1.66	$C_{10}H_{14}$	Monoterpene	
7	(+)-Sylvestrene	975.62	4.67	3.16	$C_{10}H_{16}$	Monoterpene	Millian
8	β-cis-Ocimene	979.45	4.75	0.37	$C_{10}H_{16}$	Monoterpene	
9	<i>m</i> -Propyltoluene	983.04	4.82	0.08	$C_{10}H_{14}$	Monoterpene	
10	γ-Terpinene	985.87	4.88	0.52	$C_{10}H_{16}$	Monoterpene	
11	Terpinolene	997.86	5.12	0.44	$C_{10}H_{16}$	Monoterpene	
12	Isodurene	1005.87	5.28	0.03	$C_{10}H_{14}$	Monoterpene	
13	Camphenilanol	1009.55	5.35	0.19	$C_{10}H_{18}O$	Monoterpene alcohol	ОН
14	α-Pyronene	1011.14	5.38	0.24	$C_{10}H_{16}$	Monoterpene	

15	α-Campholenal	1013.28	5.43	0.32	C ₁₀ H ₁₆ O	Monoterpene aldehyde	
16	trans-(-)-Pinocarveol	1020.45	5.57	0.69	$C_{10}H_{16}O$	Monoterpene alcohol	H HO
17	Pinocarvone	1029.15	5.75	0.57	$C_{10}H_{14}O$	Monoterpene ketone	
18	(-)-4-Terpineol	1033.93	5.84	0.51	$C_{10}H_{18}O$	Monoterpene alcohol	HO
19	α-Terpineol	1038.51	5.93	0.65	$C_{10}H_{18}O$	Monoterpene alcohol	, пант ОН
20	α-Thujenal	1041.99	6.04	1.25	$C_{10}H_{14}O$	Monoterpene aldehyde	OH OH
21	cis-Carveol	1047.86	6.12	0.10	C ₁₀ H ₁₆ O	Monoterpene alcohol	
22	D-Carvone	1057.96	6.33	0.05	$C_{10}H_{14}O$	Monoterpene ketone	У нин Н
23	Norpinane, 6,6-dimethyl-3-methylene-	1065.02	6.47	0.10	$C_{10}H_{16}$	Monoterpene	H
24	2,4-Decadienal	1081	6.79	0.07	$C_{10}H_{16}O$	Monoterpene aldehyde	
25	1,5,5-Trimethyl-6- methylene-cyclohexene	1088.96	6.95	0.06	$C_{10}H_{16}$	Monoterpene	
26	α-Cubebene	1077.11	7.10	0.23	$C_{15}H_{24}$	Sesquiterpene	
27	Copaene	1086.51	7.32	1.22	$C_{15}H_{24}$	Sesquiterpene	illina

28	α-Chamigrene	1091.55	7.43	0.19	C ₁₅ H ₂₄	Sesquiterpene	
29	Aromadendr-1-ene	1093.36	7.48	0.06	$C_{15}H_{24}$	Sesquiterpene	
30	α-Gurgujene	1097.46	7.57	1.37	$C_{15}H_{24}$	Sesquiterpene	
31	β-Сораепе	1102.97	7.70	0.56	$C_{15}H_{24}$	Sesquiterpene	
32	β-Gurjunene	1105.17	7.75	1.02	$C_{15}H_{24}$	Sesquiterpene	
33	Alloaromadendrene	1106.98	7.79	2.43	$C_{15}H_{24}$	Sesquiterpene	H _{IIII}
34	Humulene	1110.73	7.88	1.16	$C_{15}H_{24}$	Sesquiterpene	H H
35	Aromandendrene	1112.76	7.93	0.80	$C_{15}H_{24}$	Sesquiterpene	H _{Min}
36	Valencene	1120.43	8.10	1.97	$C_{15}H_{24}$	Sesquiterpene	
37	γ-Gurjunene	1122.84	8.16	4.44	$C_{15}H_{24}$	Sesquiterpene	
38	δ-Cadinene	1127.63	8.27	3.73	$C_{15}H_{24}$	Sesquiterpene	H

39	α-Amorphene	1131.72	8.37	0.62	C ₁₅ H ₂₄	Sesquiterpene	
40	Epiglobulol	1139.7	8.55	0.66	C ₁₅ H ₂₆ O	Sesquiterpene alcohol	HOIMIN H
41	(-)-Spathulenol	1145.65	8.69	2.90	C ₁₅ H ₂₄ O	Sesquiterpene alcohol	Hunder
42	Viridiflorol	1147.41	8.73	2.33	C ₁₅ H ₂₆ O	Sesquiterpene alcohol	но поменя
43	Ledol	1150.34	8.80	3.73	C ₁₅ H ₂₆ O	Sesquiterpene alcohol	но
44	Guaia-3,9-diene	1156.25	8.94	1.27	$C_{15}H_{24}$	Sesquiterpene	
45	Cyclosativene	1159.48	9.01	2.84	$C_{15}H_{24}$	Sesquiterpene	
46	β-Eudesmol	1163.66	9.11	2.30	C ₁₅ H ₂₆ O	Sesquiterpene alcohol	HO Julian
47	Cadalene	1167.72	9.20	0.58	$C_{15}H_{18}$	Sesquiterpene	
48	α-Bisabolol	1249.01	11.087	0.04	C ₁₅ H ₂₆ O	Sesquiterpene alcohol	OH
49	Geranyllinalool	1220.52	11.13	0.25	C ₂₀ H ₃₄ O	Diterpene alcohol	OH OH

50	Phytol	1227.81	11.28	0.30	$C_{20}H_{40}O$	Diterpene alcohol	он
51	trans-Geranylgeraniol	1246.62	11.68	0.07	$C_{20}H_{34}O$	Diterpene alcohol	он
52	(E)-Longipinane	1279.4	11.79	0.03	$C_{15}H_{26}$	Sesquiterpene	

TABLE-2 KOVAT'S INDEX, RETENTION TIME, MOLECULAR FORMULAR, STRUCTURAL FORMULA, AND MOLECULAR WEIGHT FOR THE SOLVENT-FREE MICROWAVE EXTRACTION (SFME)

S/N	Compounds	RI	RT	Area (%)	m.f.	Chemical component	Structure
1	β-Thujene	932.44	3.80	0.07	$C_{10}H_{16}$	Monoterpene	
2	α-Pinene	936.77	3.89	4.42	$C_{10}H_{16}$	Monoterpene	
3	Camphene	943.18	4.02	0.16	$C_{10}H_{16}$	Monoterpene	
4	Δ-car-3-ene	955.03	4.26	6.73	$C_{10}H_{16}$	Monoterpene	H
5	n-Decane	958.46	4.33	0.13	$C_{10}H_{22}$	Monoterpene	THE STATE OF THE S
6	α-Phellanderene	964.03	4.44	1.54	$C_{10}H_{16}$	Monoterpene	
7	4-Carene	968.46	4.52	0.14	$C_{10}H_{16}$	Monoterpene	
8	o-Cymene	964.03	4.59	0.74	$C_{10}H_{14}$	Monoterpene	
9	α-Ocimene	974.18	4.64	3.91	$C_{10}H_{16}$	Monoterpene	
10	cis-β-Ocimene	978.56	4.73	0.70	$C_{10}H_{16}$	Monoterpene	
11	<i>m</i> -Propyltoluene	982.09	4.80	0.14	$C_{10}H_{14}$	Monoterpene	
12	γ-Terpinene	984.88	4.86	0.33	$C_{10}H_{16}$	Monoterpene	

13	2-Phenylbutane	989.05	4.94	0.10	C ₁₀ H ₁₄	Monoterpene	\rightarrow
14	4-Ethyl- <i>m</i> -xylene	993.18	5.02	0.12	$C_{10}H_{14}$	Monoterpene	
15	2-Ethyl- <i>p</i> -xylene	1005.42	5.27	0.06	$C_{10}H_{14}$	Monoterpene	
16	Isodurene	1009.15	5.34	0.11	$C_{10}H_{14}$	Monoterpene	
17	Allo-Ocimene	1010.75	5.38	0.32	$C_{10}H_{16}$	Monoterpene	
18	α-Campholenal	1012.94	5.42	0.19	$C_{10}H_{16}O$	Monoterpene aldehyde	
19	L-trans-Pinocarveol	1019.95	5.56	0.44	C ₁₀ H ₁₆ O	Monoterpene alcohol	HO HO
20	Durene	1024.08	5.64	0.07	$C_{10}H_{14}$	Monoterpene	
21	(R)-Lavandulol	1025.82	5.68	0.14	$C_{10}H_{18}O$	Monoterpene alcohol	Inn., OH
22	Pinocarvone	1028.86	5.74	0.35	$C_{10}H_{14}O$	Monoterpene ketone	
23	Terpinen-4-ol	1033.48	5.83	0.21	$C_{10}H_{18}O$	Monoterpene alcohol	HO
24	α-Thujenal	1041.64	6.00	1.20	$C_{10}H_{14}O$	Monoterpene aldehyde	
25	cis-Carveol	1047.66	6.12	0.10	$C_{10}H_{16}O$	Monoterpene alcohol	ОН
26	D-(+)-Carvone	1058.16	6.33	0.05	C ₁₀ H ₁₄ O	Monoterpene ketone	

27	trans-Myrtanol	1061.89	6.40	0.12	C ₁₀ H ₁₈ O	Monoterpene alcohol	ОН
28	1.5.5-Trimethyl-6- methylene-cyclohexene	1085.87	6.89	0.29	$C_{10}H_{16}$	Monoterpene	
29	α-Cubebene	1073.62	7.02	0.30	$C_{15}H_{24}$	Sesquiterpene	
30	(-)-α-Copaene	1077.07	7.10	0.28	$C_{15}H_{24}$	Sesquiterpene	
31	β-Elemene	1089.87	7.40	1.27	$C_{15}H_{24}$	Sesquiterpene	
32	α-Gurjunene	1097.24	7.57	1.24	$C_{15}H_{24}$	Sesquiterpene	ÇH ₃
33	β-Caryophyllene	1100.95	7.65	2.86	$C_{15}H_{24}$	Sesquiterpene	H ₂ C H CH ₃
34	cis-β-Copaene	1102.72	7.69	0.54	$C_{15}H_{24}$	Sesquiterpene	H
35	β-Gurjunene	1104.78	7.74	0.94	$C_{15}H_{24}$	Sesquiterpene	HIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
36	Alloaromadendrene	1106.42	7.78	2.18	$C_{15}H_{24}$	Sesquiterpene	H _{Mm} , H
37	Humulene	1110.82	7.88	3.56	$C_{15}H_{24}$	Sesquiterpene	

38	Aromandendrene	1112.63	7.92	0.64	C ₁₅ H ₂₄	Sesquiterpene	H _{Mun}
39	Valencene	1114.91	7.98	2.84	$C_{15}H_{24}$	Sesquiterpene	
40	Germacrene D	1117.93	8.05	2.83	$C_{15}H_{24}$	Sesquiterpene	
41	α-Selinene	1119.96	8.09	2.05	$C_{15}H_{24}$	Sesquiterpene	· · · · · · · · · · · · · · · · · · ·
42	(+)-Ledene	1122.20	8.15	4.98	$C_{15}H_{24}$	Sesquiterpene	H
43	δ-Cadinene	1127.07	8.26	4.15	$C_{15}H_{24}$	Sesquiterpene	H
44	α-Cadinene	1131.29	8.36	0.51	$C_{15}H_{24}$	Sesquiterpene	
45	Epiglobulol	1139.18	8.54	0.39	C ₁₅ H ₂₆ O	Sesquiterpene alcohol	HOMMAN HO
46	Palustrol	1142.16	8.61	1.79	C ₁₅ H ₂₆ O	Sesquiterpene alcohol	НО
47	Ent-Spathulenol	1144.44	8.66	2.18	C ₁₅ H ₂₄ O	Sesquiterpene alcohol	OH H
48	Ledol	1146.47	8.71	2.08	C ₁₅ H ₂₆ O	Sesquiterpene alcohol	но

49	γ-Cadinene	1149.05	8.77	3.14	C ₁₅ H ₂₄	Sesquiterpene	H with H
50	Aromadendr-1-ene	1151.90	8.83	2.24	C ₁₅ H ₂₄	Sesquiterpene	
51	4-epi-Cubebol	1158.62	8.99	2.44	C ₁₅ H ₂₆ O	Sesquiterpene alcohol	IIIIIIIIII
52	(-)-Isoaromadendrene-(V)	1166.21	9.17	0.30	C ₁₅ H ₂₄	Sesquiterpene	
53	Cadalene	1167.24	9.19	0.46	$C_{15}H_{18}$	Sesquiterpene	
54	4.8a-Dimethyl-6-prop-1- en-2-yl-2,3,5,6,7,8- hexahydro-1 <i>H</i> - naphthalen-2-ol	1175.39	9.38	0.15	C ₁₅ H ₂₄ O	Sesquiterpene alcohol	HO
55	2-Isopropylidene-3- methylhexa-3,5-dienal	1217.06	9.52	0.10	C ₁₀ H ₁₄ O	Monoterpene aldehyde	
56	Cembrene	1188.57	10.46	0.14	$C_{20}H_{32}$	Diterpene	
57	Geranyl-α-terpinene	1195	10.60	1.11	$C_{20}H_{32}$	Diterpene	
58	Sclareol	1214.38	11.00	1.95	$C_{20}H_{36}O_2$	Diterpene alcohol	OH OH

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59	Phytol	1227.90	11.29	0.35	C ₂₀ H ₄₀ O	Diterpene alcohol	ОН
60	All-trans-farnesol	1271.72	11.61	0.19	C ₁₅ H ₂₆ O	Sesquiterpene alcohol	OH
61	All-trans-geranylgeraniol	1246.76	11.68	0.07	$C_{20}H_{34}O$	Diterpene alcohol	ОН
62	n-Eicosane	1265.33	12.07	0.04	$C_{20}H_{42}$	Diterpene	

tures, retention time and the Kovat's index. Fifty two chemical compounds were identified in the essential oil produced by hydrodistillation, while 62 compounds were identified in that produced by solvent free microwave extraction, this makes the latter a more preferable method of extraction than hydrodistillation in the quest to obtain high quality essential oil.

Among all the compounds of the hydrodistillation essential oil, E-longipinane, has the highest Kovat's index of 1279.4, while *n*-eicosane has the highest Kovat's index of 1265.33 among all the compounds of the solvent free microwave extraction essential oil. The two essential oils were characterized by monoterpenes, monoterpene alcohol, sesquiterpenes and sesquiterpenes alcohol, with trace amounts of monoterpene ketone, monoterpenes aldehyde, diterpene and diterpene alcohol.

Monoterpenes and their synthetic derivatives have been shown by studies to possess antifungal, antinociceptive, antispasmodic, antioxidant, antibacterial, antiarrhythmic, antitumor/ anticancer, antidiabetic, anti-inflammatory and antihistaminic activities [9-12]. Sesquiterpenes which were also found in abundance in the essential oil have also been reported to have antiinflammatory, antifungal, antibacterial, antidiabetic, antiulcer, anticancer and anaesthetic properties [13,14]. Diterpenes are also well known for diverse therapeutic properties which include antispasmodic, anti-inflammatory, antimicrobial properties [15]. The solvent free microwave extraction method (30 min) is six times faster than the hydrodistillation method (3 h), and the findings of this study show that the essential oil obtained through the solvent free microwave extraction method is higher in quantity and better in quality than that obtained through the hydrodistillation method. This finding supports the findings of Lucchesi et al. [16], who also arrived at similar conclusion, respectively, after carrying out extraction of essential oil from different herbs (basil, thyme, garden mint and *C. anisata* leaves) using the solvent free microwave extraction and hydrodistillation methods. The solvent free microwave extraction method is therefore more preferable in the extraction of essential oil of higher quantity and quality than the hydrodistillation method, as the method of extraction will determine the type, quality, and amount of constituent compounds of the essential oil.

Conclusion

Essential oils contain many therapeutic compounds which make them very useful in the treatment and prevention of diseases. The results obtained from this study also reflect the medicinal potential of *Helichrysum petiolare* and further justify its usage by local communities in the treatment of injuries and diseases. The essential oils obtained in this study possess compounds that have been proved by studies to have pharmaceutical, medicinal and cosmetic properties. The solvent free microwave extraction method therefore produced essential oil of higher quality and quantity than hydrodistillation method, and is therefore a more preferable method of extraction.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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