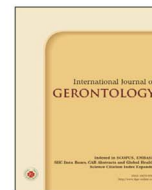




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## Original Article

# Adjunctive Therapeutic Effects of Cinnamomum Camphora Forest Environment on Elderly Patients with Hypertension

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## SUMMARY

**Background:** Many studies have demonstrated the beneficial effects of the mixed forest environment on human health. However, few studied the effect of the single tree species forest on human health. This study was explored the effect of *C. camphora* forest environment on elderly patients with hypertension (HTN).

**Methods:** 31 elderly patients with essential HTN were randomly divided into two groups. Blood pressure (BP), pulse oxygen saturation (SpO<sub>2</sub>%), heart rate (HR), heart rate variability (HRV) and levels of plasma high-sensitivity C-reactive protein (hs-CRP), as well as profile of mood states (POMS) test, were measured. Environment factors of two experimental sites were monitored. Categorical variables and continuous data were analyzed by Chi-square and t-test, respectively.

**Results:** After forest bathing, subjects in the forest group showed significantly lower levels of diastolic blood pressure (DBP), low frequency (LF), the ratio of low frequency and high frequency (LF/HF) and hs-CRP, and greatly higher levels of SpO<sub>2</sub>% and high frequency (HF) than that of in control group. Furthermore, negative mood subscale scores of POMS were significantly lower following forest bathing, while the positive score was much higher. However, the main components of volatile organic compounds (VOCs) in the two experimental sites were obviously different.

**Conclusion:** *C. Camphora* environment significantly decreased the DBP and inflammatory level, balanced the autonomic activity and improved the mood state of participates, which might be associated with the biological effectiveness of terpenes, implying that *C. Camphora* environment might be an adjunctive therapy for HTN patients.

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## 1. Introduction

With the world's cities becoming increasingly congested and polluted, China's government has advocated the construction of healthy city. Public green space is the important content of the healthy city project. In general, public green space includes parks and reserves, sporting fields, riparian areas like stream and river banks, greenways and trails, community gardens, street trees, and nature conservation areas, as well as less conventional spaces such as green walls, green alley ways, and cemeteries.<sup>1</sup> Those urban green spaces provided a wide range of ecosystem services that could help combat many urban ills and improve life for city dwellers — especially their health. In the past decades, researchers have showed that psychological well-being, mental health and the prevention of obesity were linked to urban parks and green space.<sup>2–4</sup> Early in 1982, one forest agency of Japan first proposed incorporating forest bathing trips into a good lifestyle.<sup>5</sup> After development of more than 30 years, forest bathing had well development in Japan. As one type of ecosystem services, forest bathing (also named forest therapy or natural therapy) is one of the important ways to interact with out-

door environments. There are abundant resources in China; development forest bath might be the most economic way of forestry economic transition in China. Most researches have demonstrated that forest bathing could alleviate the stress and anxiety,<sup>4,6–10</sup> boost the immune function<sup>11–13</sup> and increase the expression of anti-cancer proteins.<sup>11</sup> In addition, our previous works have reported that forest bathing have positive influence on young healthy student, and that it might be used as an adjunctive therapy on elderly patients with hypertension (HTN), chronic obstructive pulmonary disease (COPD) and chronic heart failure (CHF).<sup>14–20</sup>

Although many researchers have focused on the forest bathing, most of them were studied the mixed forest environment, the forest of single tree species remained attracted less attention. Cinnamomum camphora (*C. camphora*), the “civic tree” of Hangzhou, is an evergreen broad-leaved tree belonging to the family Lauraceae, which is widely distributed in south China. Due to its beautiful shape, *C. camphora* was usually used as a virescent tree in urban gardens and streets. As one of the important economic trees in China, *C. camphora* has long been prescribed in traditional medicines for the treatment of inflammation-related diseases such as rheumatism, sprains, bronchitis, asthma, indigestion and muscle pains.<sup>21</sup> Based on its anti-inflammatory functions, more value of *C. camphora* to human health remained to be developed. Therefore, in current

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study, we performed a forest bathing at an urban park of single tree species (*C. camphora* forest environment) to determine the influence of tree-species-specific forest bathing, *C. camphora* forest environment, on elderly patients with HTN.

## 2. Patients and methods

### 2.1. Subjects and study design

This study was performed at Jingshan town, which located at the Northwest of Hangzhou city, Zhejiang province. A *C. camphora* forest farm was used as the experimental site, which was covered a square of 86,658 m<sup>2</sup> and included 230 various camphor tree species. For comparison, a typical suburban site located in Jingshan town was used as the control.

From 19 October 2017 to 21 October 2017, 31 patients with HTN from Hangzhou city were participated the study. They were randomly divided into two groups consisting of 11 in control group and 20 in forest group according to the ratio of 1:2. The inclusion and exclusion criteria were as follow: (1) patients with diagnosed essential hypertension; (2) aged from 60 to 75 years; (3) blood pressure (BP), with or without medical control, less than 180/110 mmHg; (4) class I–II cardiac function according to the criteria of American New York Heart Association; (5) capable of taking care of themselves in daily life. The exclusion criteria are: (1) catching cold or suffering other acute diseases two weeks prior to the trial or during the trial process; (2) chronic history, including cancer, serious liver, kidney, brain, heart, lung diseases, etc.; (3) acute myocardial infarction or cerebrovascular accident within six months; and (4) experienced a severe trauma or a major surgery.

The experimental program were described as previous study.<sup>14</sup> In briefly, two groups were activated according to this procedure: On the first day, all participates were blooded without eating in the morning at Zhejiang hospital. After breakfast, one group was sent to the forest site, the other to the suburban site. In the afternoon, after taking lunch and noon break in their resting room, the subjects were arranged to sit quietly for 2 h at 3:00 pm–5:00 pm in the each experimental sites. On the second day, the subjects were arranged to sit quietly as the first day on the morning and afternoon. On the morning of the third day, subjects were blooded without eating. After breakfast, both two groups were taken to Hangzhou city. Two hotels nearing the two indicated experimental sites offered similar accommodation conditions. The distance between the forest site and the corresponding hotel was similar to that of the control. During the study period, the physical activities were controlled, and smoking and alcoholic or caffeinated beverages were not allowed.

All procedures of our study were in line with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the ethics committee of Zhejiang Hospital. Signed informed consent was obtained from every subject.

### 2.2. BP, heart rate (HR), oxygen saturation (SpO<sub>2</sub>) and heart rate variability (HRV) measurement

Systolic, diastolic blood pressure (SBP, DBP) and HR were obtained every day before and after the experiment from the right arm using a portable digital sphygmomanometer (HEM-7000-E, Omron, Kyoto, Japan). The fingertip pulse oximeter (YUWELL YX301) was used to measure pulse SpO<sub>2</sub> at the initial and the end of the experiment. HRV, including low frequency (LF), high frequency (HF) and the ratio of low frequency and high frequency (LF/HF), were

assessed by SA-3000P (Medicore Inc., Seoul, Korea) at the initial and the end of the experiment.

### 2.3. Bio-indicators determination

Plasma level of high-sensitivity C-reactive protein (hs-CRP) was determined by an automatic biochemistry analyzer (Hitachi Model 7600 Series Automatic Analyzer, Japan).

### 2.4. Profile of mood states (POMS) evaluation

The standard version of the POMS questionnaire was used to measure mood states.<sup>22</sup>

### 2.5. Air quality assessment

The air quality in the two sites was monitored, simultaneously. The level of negative oxygen ions was detected by an air ion counter (KEC-900 Type, Shenzhen Yuan Hengtong Technology Co., Ltd., Shenzhen, China). The concentration of particulate matter less than 2.5 μm in diameter (PM<sub>2.5</sub>) was measured by a portable dust monitor (Dustmate Type, Beijing Liyang Tech Technology Co. Ltd., Beijing, China).

### 2.6. Volatile organic compounds (VOCs) sampling and analysis

A ZC-Q-type air sampler, made by the Zhejiang Hengda instrumentation, CO, LTD., was used to collect volatile compounds. These leaves flourish in a sunny position and non-injured were used to analysis the volatile organic compounds (VOCs). The bag volume capacity is 0.1 m<sup>3</sup>, the headspace gases generated in the bag were sampled 3 min after inflation and held for 30 min at a rate of 0.1 m<sup>3</sup>.min<sup>-1</sup>.

TDS-GC-MS was used for the analysis of the volatile components. A TDS3 instrument was used for thermal desorption (Gerstel, Germany), with the following parameters: He carrier gas at a constant pressure of 20 kPa, inlet temperature of 250 °C, desorption temperature of 250 °C held for 10 min, cold trap temperature of -100 °C held for 3 min and injector temperature of 260 °C produced by rapid heating. An agilent model 7890A-5975C GC-MS instrument (Agilent Technologies, Santa Clara, CA, USA) with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was employed to analysis the VOCs with the following parameters: oven temperature held at 40 °C for 3 min, then ramped to 112 °C at a rate of 6 °C min<sup>-1</sup>, held for 3 min, increased to 250 °C at a rate of 6 °C min<sup>-1</sup>, increased to 270 °C at a rate of 10 °C min<sup>-1</sup> and held for 5 min. The interface and ion source temperatures were maintained at 280 and 230 °C, the quadrupole temperature was 150 °C and the MS detector was operated in the full-scan electron ionization (EI) mode with electronic impact ionization (ionization voltage 70 eV), scanning the mass range from 29 to 400 m/z.

Data were analyzed using the X calibur software system (version 1.2). The identities of volatile components were confirmed by comparing their mass spectra with those obtained from the National Institute for Standards and Technology (NIST, 2008). The quantitative analysis of volatile components was performed based on peak areas in the total ion chromatographs (TIC) by the area normalization method.

### 2.7. Data analysis

Categorical variables were compared by Chi-square analysis.

Independent samples t-test or paired samples t-test was used to compare continuous data. All statistical analyses were completed using the SPSS 19.0 software (SPSS China, Shanghai, China),  $p < 0.05$  was considered statistically significant.

### 3. Results

#### 3.1. Clinical characteristics of the participants

The clinical characteristics of the participants were shown in supplementary file (Table 1). No significant differences in the baseline characteristics of the participants, including gender, age, body mass index (BMI), SBP, DBP, HR, SpO<sub>2</sub>%, HRV, hs-CRP and POMS score, were observed between the two groups.

#### 3.2. Effects of *C. camphora* forest on BP and SpO<sub>2</sub>%

After experiment, the level of DBP was significantly lower in the forest group than in control group ( $67.95 \pm 2.69$  vs.  $71.64 \pm 4.20$ ,  $p < 0.05$ ; Figure 1). In contrast, the value of SpO<sub>2</sub>% was significantly higher in forest group than in control group ( $97.55 \pm 0.82$  vs.  $98.10 \pm 0.64$ ,  $p < 0.05$ ; Figure 1). There was no obvious alteration was observed for HR and SBP.

#### 3.3. Influence of forest bathing on HRV

As an important tool for studying autonomic control of the heart and autonomic dysfunction, HRV has been widely measured in heart diseases such as cardiac infarction, heart failure, arrhythmia and syncope.<sup>23</sup> As shown in Figure 2, *C. camphora* forest bathing significantly decreased the levels of LF ( $35.40 \pm 13.98$  vs.  $50.88 \pm 14.32$ ,  $p < 0.05$ ) and LF/HF ( $0.68 \pm 0.52$  vs.  $1.36 \pm 1.29$ ,  $p < 0.05$ ), when compared with the control group. Instead, HF level was significantly higher in forest group than that in the control group ( $60.54 \pm 14.26$  vs.  $48.37 \pm 14.42$ ,  $p < 0.05$ ).

**Table 1**  
Baseline level of the indicators of subjects before experiment (Mean  $\pm$  SD).

	Baseline of control group (n = 11)	Baseline of forest group (n = 20)	p-value
Age (year)	73.91 $\pm$ 6.640	73.50 $\pm$ 5.889	0.861
Gender (male/female)	12/8	7/4	0.106 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	23.564 $\pm$ 2.176	23.759 $\pm$ 1.768	0.788
SBP (mmHg)	137.64 $\pm$ 5.887	139.25 $\pm$ 12.594	0.693
DBP (mmHg)	72.45 $\pm$ 4.275	73.75 $\pm$ 7.691	0.611
HR (bpm)	70.00 $\pm$ 5.514	74.40 $\pm$ 7.769	0.108
SpO <sub>2</sub> (%)	97.09 $\pm$ 1.700	97.85 $\pm$ 1.089	0.202
LF (m/s <sup>2</sup> )	39.786 $\pm$ 21.766	40.681 $\pm$ 15.888	0.896
HF (m/s <sup>2</sup> )	59.760 $\pm$ 22.164	59.319 $\pm$ 15.888	0.949
LF/HF	0.918 $\pm$ 0.781	0.887 $\pm$ 0.903	0.922 <sup>b</sup>
hs-CRP (mg/L)	3.064 $\pm$ 1.777	3.135 $\pm$ 2.968	0.943 <sup>b</sup>
<b>POMS</b>			
Tension-anxiety (T)	14.874 $\pm$ 1.794	14.686 $\pm$ 1.489	0.757
Depression-dejection (D)	29.091 $\pm$ 2.343	29.105 $\pm$ 1.747	0.985
Anger-hostility (A)	23.18 $\pm$ 1.328	24.00 $\pm$ 1.170	0.086
Vigor-activity (V)	23.82 $\pm$ 0.982	23.65 $\pm$ 1.089	0.674
Fatigue-inertia (F)	15.45 $\pm$ 1.214	14.55 $\pm$ 1.234	0.059
Confusion-bewilderment (C)	16.00 $\pm$ 0.775	15.60 $\pm$ 1.095	0.294

<sup>a</sup> The chi-squared test was used; <sup>b</sup> Mann-Whitney U test was used; others were analyzed by using the independent-samples t test.

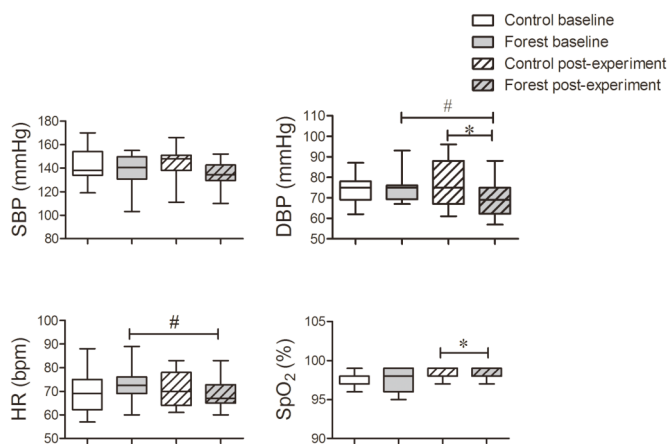
Abbreviation: BMI, body mass index; SDP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; hs-CRP, high-sensitive-reactive protein.

#### 3.4. Inflammatory cytokines level

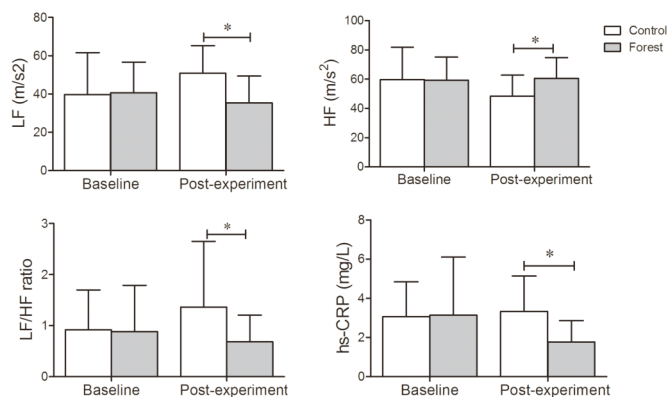
As one of the key inflammatory factors, hs-CRP, was analyzed in present study. At the end of the experiment, subjects in the forest group were showed a significantly lower level of hs-CRP than that of the control group ( $1.77 \pm 1.09$  vs.  $3.34 \pm 1.80$ ,  $p < 0.05$ , Figure 2). This result suggested that *C. camphora* forest bathing could inhibit the level of inflammatory response in elderly patients with HTN.

#### 3.5. Mood state of participates

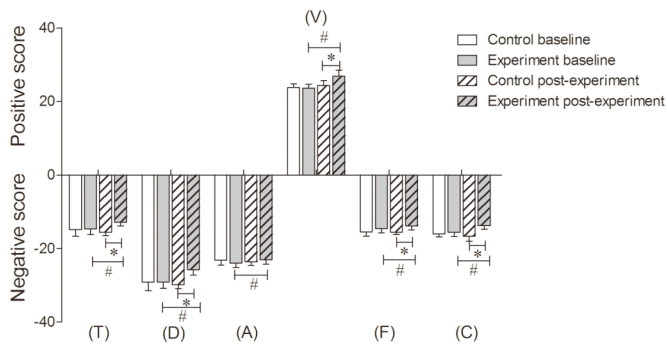
From the POMS subscale score, as is shown in Figure 3, after forest bathing, much lower scores of tension-anxiety (T), depression (D), confusion (C) and fatigue (F) were found in the forest group compared with the control group (T,  $12.90 \pm 0.97$  vs.  $15.55 \pm 0.93$ ,  $p < 0.05$ ; D,  $25.72 \pm 1.45$  vs.  $29.82 \pm 1.08$ ,  $p < 0.05$ ; C,  $13.75 \pm 0.97$  vs.  $16.64 \pm 1.36$ ,  $p < 0.05$ ; F,  $13.80 \pm 1.11$  vs.  $15.55 \pm 0.69$ ,  $p < 0.05$ ) or itself baseline scores (T,  $12.90 \pm 0.97$  vs.  $14.69 \pm 1.49$ ,  $p < 0.05$ ; D,  $25.72 \pm 1.45$  vs.  $29.11 \pm 1.75$ ,  $p < 0.05$ ; C,  $13.75 \pm 0.97$  vs.  $15.60 \pm 1.10$ ,  $p < 0.05$ ; F,  $13.80 \pm 1.11$  vs.  $15.55 \pm 1.23$ ,  $p < 0.05$ ), while it has opposite trend in the score of vigor-activity (V,  $26.90 \pm 1.59$  vs.  $24.36 \pm 1.29$ ,  $p < 0.05$ ).



**Figure 1.** Effects of *C. camphora* forest bathing on BP-related indicators. N = 11 (in control), 20 (in forest). SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SpO<sub>2</sub>%, pulse oxygen saturation. \*  $p < 0.05$  analyzed by independent sample t-test; #  $p < 0.05$  analyzed by paired sample t-test.



**Figure 2.** Influences of *C. camphora* forest bathing on HRV and inflammatory cytokines. N = 11 (in control), 20 (in forest). LF, low frequency; HF, high frequency; LF/HF, ratio of low frequency/high frequency; hs-CRP, high-sensitive C reactive protein. \*  $p < 0.05$  analyzed by independent sample t-test.



**Figure 3.** *C. camphora* forest bathing improved the mood state of subjects. N = 11 (in control), 20 (in forest). T, tension-anxiety; D, depression; A, anger-hostility; F, fatigue; V, vigor-activity; C, confusion. \*  $p < 0.05$  analyzed by independent sample t-test; #  $p < 0.05$  analyzed by paired sample t-test.

### 3.6. Environment factors of two experimental sites

We monitored the air quality and VOCs of the experimental and control sites in the present study (Table 2). Results showed that except the air humidity, the other factors including temperature, negative ions, PM2.5 (particulate matter  $\leq 2.5 \mu\text{m}$ ), PM10 (particulate matter  $\leq 10 \mu\text{m}$ ) and comfort index of two sites were similar. The air humidity of experiment group was significantly higher than the control group.

In addition, the components of forest aerosols of *C. camphora* were analyzed in this study (Table 2). From the results, components of the VOCs of two experimental sites were obviously different. The VOCs of the control sites were mainly consisted by aromatics ( $48.99 \pm 9.05$ ) and esters ( $35.74 \pm 10.52$ ), which were significantly higher than experimental sites (aromatics:  $29.75 \pm 11.27$ ; esters,  $14.79 \pm 6.89$ ). However, in the experimental sites, the proportion of terpenes ( $29.26 \pm 6.84$ ) and aldehydes ( $13.68 \pm 3.30$ ) in VOCs were markedly higher than control site (terpenes:  $2.66 \pm 3.34$ ; aldehydes: 0).

## 4. Discussion

Nowadays, artificial stimulations that filled in urban environments have been considered as a negative factor on human health, more attention has thus been paid on the natural environment.<sup>24</sup> Healthy city is the desirable of people all over the world. As one of the environmental friendly street trees, *C. camphora* is widely distributed in south China, which provided a rare natural green park environment for people who lived in city. In this natural green park environment, a large number of volatile and non-volatile substances called phytoncides (wood essential oil) were emitted from plants, which has proved to beneficial for human bodies. However, different environments have various microenvironments which may have discrepancy influence on different subjects. Therefore, we speculated that special forest environment, for example *C. camphora* forest environment, might have different influenced on HTN patients.

In this study, we showed that *C. camphora* forest bathing decreased the level of DBP, increased the level of  $\text{SpO}_2\%$ . In the HRV analysis, values of LF and LF/HF ratio were significantly lower in forest group than that in control group, while HF value was statistical higher than controls. These results suggested that subjects in a forest environment had more enhanced parasympathetic and lower sympathetic nervous activity than those in a suburban environment. Similar HRV response were often seen in yoga therapy.<sup>25,26</sup> In addition, *C. camphora* forest environment significantly reduced the hs-CRP level and improved the mood state of participates in comparison to the control group.

**Table 2**

The air quality and constituent of VOCs in two experimental sites.

	Control site	Experimental site
Temperature ( $^{\circ}\text{C}$ )	$19.87 \pm 1.57$	$17.86 \pm 1.30$
Humidity (%)	$71.65 \pm 5.20$	$83.48 \pm 2.39^*$
Negative ions ( $\text{m}^{-3}$ )	$304.68 \pm 150.42$	$492.99 \pm 97.50$
PM2.5 ( $\text{mg}/\text{m}^3$ )	$0.04 \pm 0.02$	$0.03 \pm 0.01$
PM10 ( $\text{mg}/\text{m}^3$ )	$0.05 \pm 0.01$	$0.04 \pm 0.01$
Comfort index	$72.33 \pm 3.51$	$69.00 \pm 3.00$
Terpenes (%)	$2.66 \pm 3.34$	$29.26 \pm 6.84^*$
Hydrocarbons (%)	0	0
Aromatics (%)	$48.99 \pm 9.05$	$29.75 \pm 11.27^*$
Aldehydes (%)	0	$13.68 \pm 3.30^*$
Ketones (%)	$2.44 \pm 2.31$	0
Alcohols (%)	0	0
Esters (%)	$35.74 \pm 10.52$	$14.79 \pm 6.89^*$
Organic acids (%)	0	$6.65 \pm 8.35$
Others (%)	$10.17 \pm 2.92$	$5.86 \pm 6.18$

PM2.5, particulate matter  $< 2.5 \mu\text{m}$  in aerodynamic diameter; PM10, particulate matter  $< 10 \mu\text{m}$  in aerodynamic diameter; \*  $p < 0.05$ , independent-samples t-test were used.

In the present study, there were no obviously different in the air quality of the two experimental sites, including temperature, negative ions, PM2.5, PM10 and comfort index, but except for the air humidity (in Table 2). The air humidity of experiment group was significantly higher than the control group. However, the components of VOCs in the two experimental sites were notably different. The major components of the *C. camphora* forest atmosphere were terpenes, aromatics, esters and aldehydes, which account over 80 percent. It is worth mentioning that terpenes, produced by various plants, including *C. camphora* forest, are associated with not only anti-inflammation but also anti-bacterial effect, and that terpenes are the main ingredients of the VOCs of *C. camphora*, accounting 29.26% for *C. camphora* forest atmosphere (detected by our team), 50.83% for flowers, 70.75% for leaves and 78.22% for branches.<sup>27–30</sup> Previous studies have demonstrated the beneficial effects of various terpenes on immune and neuronal health.<sup>21,31–35</sup> It was reported that terpenes exerted anti-inflammatory effects by inhibiting various pro-inflammatory pathways, including mitogen-activated protein kinases (MAPKs), nuclear factor kappa B (NF- $\kappa$ B), c-Jun N-terminal kinase (JNK) in many diseases.<sup>31–34</sup> Porres-Martínez et al. reported that monoterpenes, such as  $\alpha$ -pinene and 1, 8-cineole exerted neuro-protective effects by regulating gene expression.<sup>35</sup> They protected PC12 cells against oxidative stress through reactive oxygen species (ROS) scavenging and induced the nuclear Nrf2 factor though enhancing the expression of antioxidant enzymes.<sup>35</sup> In addition, terpenes also have multiple physiology properties, such as anti-tumor and antifungal, etc.<sup>21,36</sup> Therefore, *C. camphora* forest bathing decreasing DBP, LF, and LF/HF ratio, increasing the  $\text{SpO}_2\%$ , inhibiting the hs-CRP and improving the POMS might be associated with the biological effectiveness of terpenes.

In conclusion, although the sample size was small, it is worth noting that forest bathing in *C. Camphora* environment significantly decrease the level of DBP, increase the levels of  $\text{SpO}_2\%$ , boosted the heart and autonomic nerve system, reduced the inflammation response and promoted the mood state of participates, which might be linked to the biological effectiveness of terpenes. This study has revealed for the first time that *C. camphora* forest bathing had adjunctive therapeutic effects on elderly patients with HTN, indicating that urban dwellers were encouraged to outdoor and interact with the natural environment. Therefore, so it is necessity to conduct a randomized clinical trial with larger sample sizes and longer inter-

vention in the future.

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### Conflict of interests

All authors declare to disclose any conflict of interest.

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