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<u>Chemical composition analysis of Himalayan</u> <u>cedar essential oils and its effect on Stress-</u> <u>Induced Skin-Barrier Disruption</u>



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Introduction:

Results & Discussion:

Aromatherapy is a holistic healing treatment that uses aromatic essential oils to enhance both physical and emotional health. Currently, fragrance-related products are very popular with consumers, and the application of aromatic essential oils has become increasingly widespread in healthcare industries^[1]. Previous studies suggest that psychological stress can link to the onset or aggravation of multiple skin diseases, and inhalation of certain anxiolytic-like essential oil can be a potential strategy for prevention or relief of chronic stress-induced skin-barrier disruption^[2,3,4]. *Cedrus deodara* (also known as Himalayan cedar, Pinaceae family), is a large evergreen coniferous tree natively growing in Himalayan area from the altitude of 1300 to 3300 meters. The crude oils prepared from steam distillation are often yellowish or darker in color, and its applications include aromatic medicine, soap perfumes, household sprays, cleaning oils, etc. ^[4, 5, 6]. However, few studies have investigated the stress relief effects of CEOs and their potential skin benefits. The purpose of this research is to screen a kind of CEO and to evaluate its effects on stress-induced skin-barrier disruption.

1. Chemical composition of CEOs

Table 2 Chemical composition comparison of

Materials & Methods:

1. Preparation of Modified CEOs

All the CEOs were collected from available commercial suppliers, their information (botanic source, plant parts and origins) were listed in Table 1. All the CEOs were produced by hydro-distillation process. The crude Himalayan cedar essential oil was modified by molecular distillation method using KDL2 molecular distillation apparatus (UIC Corporation, Germany). Then the modified CEOs was prepared by combining above heavy fraction (80%) and *Pelargonium roseum* essential oil (20%).

NO.	Botanic source	Origin	Plant parts	Main composition	
1	Cedrus deodara	Nepal	Wood	α-Himachalene (13.67%), γ-Himachalene (8.99%), β-Himachalene (33.32%), (E)-Atlantone (3.44%).	
2	Cedrus deodara	India	Wood	 α-Himachalene (15.34%), γ-Himachalene (9.07%), β-Himachalene (36.01%), (E)-Atlantone (9.91%). 	
3	Cedrus deodara	China	Needles	Terpinen-1-ol (4.06%) β-terpineol (7.64%) α- terpineol (56.96%) γ- terpineol (17.66%)	
4	Juniperus virginiana	North America	Wood	α-Cedrene (26.65%) β-Cedrene (5.93%) cis-Thujopsene (19.56%) Cedrol (23.49%)	
5	Juniperus virginiana	America	Wood	α-Cedrene (23.02%) β-Cedrene (4.71%) cis-Thujopsene (22.26%) Cedrol (24.71%)	
6	Cedrus atlantica	Morocco	Wood	α-Himachalene (15.76%), γ-Himachalene (10.19%), β-Himachalene (48.38%), (E)-Atlantone (2.80%).	
7	Cedrus atlantica	China	Wood	 α-Himachalene (16.69%), γ-Himachalene (10.48%), β-Himachalene (48.55%), (E)-Atlantone (2.40%). 	
8	Unidentified cedar tree	China	Seeds	Isolongifolene (26.72%) (+)- δ -Cadinene (3.90%) α -Cadinol (3.31%) Cedryl acetate (6.14%)	
8	Unidentified cedar tree	China	Twigs	Isolongifolene (5.77%) Longifolene (10.86%) γ-Cadinene (7.56%) (+)-δ-Cadinene (9.77%)	

Results in Table 1 showed that essential oils extracted from wood of *Cedrus deodara* and *Cedrus atlantica* were similar, mainly were α -Himachalene, γ -Himachalene, β -Himachalene, and (E)-Atlantone, Unlike *Cedrus* genus, essential oils extracted from wood of Juniperus virginiana were α -Cedrene, β -Cedrene, cis-Thujopsene, and Cedrol.

Results in Table 2 showed that the relative content percentage of alkenes were decreased, and on the contrary ketones and alcohols were increased in the heavy fraction. And the heavy fraction was selected for the further studies due to its soft and long lasting odor.



Fig 2 Comparison of α/β brain wave ratio before and after modified CEO inhalation. The y-axis were expressed as value of α/β ration ($\mu V2/\mu V2$). Himalayan CEO before and after molecular distillation

NO.	Compounds	Relative percentage (%)				
		Light fraction	Cedrus deodara essential oils	Heavy fraction		
1	4-Acetyl-1-methylcyclohexene	5.10	1.11	0.17		
2	α-Himachalene	21.24	12.56	10.28		
3	γ-Himachalene	10.96	7.89	7.04		
4	Himachala-1,4-diene	1.88	1.40	1.24		
5	β-Himachalene	36.82	31.20	28.84		
6	(Z)-γ-Atlantone	0.81	4.63	5.34		
7	Himachalol	0.18	0.83	1.05		
8	(E)- γ-Atlantone	0.77	5.90	6.76		
9	Deodarone	0.19	1.11	1.37		
10	Allhimachalol	0.27	1.29	1.56		
11	(Z)-α-Atlantone	0.28	2.11	2.61		
12	(E)-Atlantone	0.79	10.87	12.40		

2. Influence of EEG by inhalation of modified CEO

Typically, the alpha wave (8-13 Hz) change is attributed to attentional states, increases of this electrical activity is considered as indicator of relaxational а psychophysiological state, while increase of beta wave (13-30 Hz) activity has been seen in the presence of mental stress. It was reported that the alpha/beta ratio increased after inhaling the stabilizing fragrance. As presented in Fig 2, the α/β ratio showed an increase trend in all the regions after the participants inhaled modified CEO, these changes are consistently visible but statistically significant was only observed in average value of 8 channels. It was suspended that inhalation of modified CEO might have an instant stress relief effect.

2. Gas Chromatography – Mass Spectrometry Analysis (GC-MS)

GC-MS analysis was performed with Agilent 7890B GC system coupled directly to a 5977A MSD and equipped with a DB-WAX capillary column.

Table1Informationandchemicalcomposition analysis of commercial CEOs

3. Electroencephalogram (EEG) Analysis

The EEG readings were recorded using Eegomini EE-401 system (ANT Neuro, Germany). Total eight silver/silver chloride electrodes were placed on the scalp at positions of Fz, Cz, Pz, F3, F4, Fpz, C3, C4 according to the International 10-20 System. During the inhalation, the EEG measurements were performed for 3 minutes per period per participant before or after inhalation.

4. Electroencephalogram (EEG) Analysis

Procedure	1	2	3	4	5	6	7	8
Action	Enter the room and sit for 30 min	Collect Saliva (S1) and test TWEL (Tb)	Tape stripping	Test TWEL (TO)	Stroop color-word test foir 30 min	Collect Saliva (S2)	Sit for 1h	Collect Saliva (S3) and test TWEL (T1.5)
					CEO			

Fig 1 The procedure of stress-induced skin-barrier disruption experiment

The sequence of the experimental procedures was shown in the Fig 1. The modified CEO was supplied as 1% concentration diluted with jojoba oil, while the jojoba oil was used in placebo control. Skin barrier disruption was achieved by sequential applications of standard cellophane tape (D100, Cuderm, USA) stripping by 20 times in the area of volar forearm. Acute mental stress was induced by a classic version of the Stroop Color-Word Test using a softwar Encephalapp. Trans Epidermal Water Loss value (TEWL) was measured from the skin of the forearm using a Multi Probe Adapter CK-MPA10 (Courage-Khazaka, Germany). The salivary concentration of cortisol was measured using a salivary cortisol enzyme immunoassay kit (#1-3002, SALIMETRICS, USA). The TEWL recovery rate was calculated by the following formula: (T0-T1.5)/(T0-Tb)*100%. The salivary cortisol change rate was calculated by the following formula: (Sn-S1)/S1*100% (Sn=S2 or S3).

3. Effect of modified CEO inhalation on the salivary cortisol level and the recovery rate of TEWL

As showed in Fig 3, in the placebo exposure group, 30-min acute mental stress by Stroop Color-Word Test evoked an increase trend in concentration of salivary cortisol and this effect lasted for the 1 hour post (vs. before stressed). On the contrary, the salivary cortisol concentration in modified CEO inhalation group under stress condition was decreased, which was significantly lower compared to the placebo group, both at the time point of S2 (just after 30-min mental stress) or S3 (1 hour post period). This result indicated that inhalation of modified CEO could lead to relaxation effect under the acute mental stress.

In this experiment, the skin barrier function before or just after stripping was considered as 100% or 0%, respectively. In the placebo group, the skin barrier function recovered to around 40% under a acute mental stress at 1.5 h after stripping. Whereas, in the modified CEO inhalation group, the recovery rate accelerated significantly to 53% (Fig 3). This result indicated the inhalation of modified CEO could help repair the skin barrier.





Fig 4 Effect of modified CEO inhalation on recovery rate of TEWL under acute mental stress.



In conclusion, the above results suggested that repair progress of stress-induced skin-barrier disruption could be accelerated by modified CEO inhalation, possibly through regulating the mental function. This could be a potential application of CEO in cosmetic products to bring both mood and skin benefits for consumers.

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