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Article**Influence of Post-Harvest Drying Processes on the Composition and Biological Activities of Essential Oils from Leaves of Camphor Tree from Uttarakhand Himalaya, India****Bhawana Kanyal¹, Chitra Pande^{1*}, Geeta Tewari¹, Aabha¹, Lata Rana¹, Rajendra C. Padalia², Tanuja Kabdal³, Om Prakash³, Shalini Singh⁴**¹ Department of Chemistry, Kumaun University, Nainital-263002, Uttarakhand, India² CSIR- CIMAP, Research Center, Pantnagar- 263149, Uttarakhand, India³ Department of Chemistry, College of Basic Sciences and Humanities, GBPUAT, Pantnagar- 263145, Uttarakhand, India⁴ Department of Chemistry, Uttarakhand Open University, Haldwani 263139, India**Corresponding Author: chitrapande2861@gmail.com (Chitra Pande)*

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Abstract: *Cinnamomum camphora* L. belonging to family Lauraceae, is an aromatic evergreen plant which has been traditionally used to heal several health problems such as cold, fever, pneumonia, diarrhea and various infections. In this study, four drying treatments (sun, shade, blower and oven) were applied on the leaves of *C. camphora* collected in summer season and their effect on the yield, composition and biological properties of the essential oil was evaluated. Hydro distillation of fresh and dried samples was carried out by Clevenger apparatus and essential oils obtained were analyzed by GC and GC/MS. Alpha-pinene, camphene, myrcene, limonene and camphor were the common major constituents of the essential oils. The highest essential oil yield was obtained by blower drying technique. Shade dried sample possessed the best antioxidant, anti-inflammatory and α -amylase inhibition activities. The highest percentage of camphor was obtained in sun dried plant material. Despite of comparatively low essential oil yield in sun dried *C. camphora*, sun drying technique is less energy consuming and increased the content of major component of *camphora* leaves.

Keywords: Drying, *Cinnamomum camphora*, essential oil, camphor, bioactivity, GC/MS.

Introduction

Since ancient times, plant medicines have been used for curing human and animal health. Aromatic and medicinal plants are used in a variety of beauty products, fragrance, food products, aromatherapy and pharmaceutical industry. They are valuable for their therapeutic properties and bioactive compounds found in them¹. There are lots of opportunities for new drugs to be devel-

oped using natural products such as Tulsi, Garlic, Turmeric, Cinnamon and Curry leaves².

There are 55 genus and over 2000 species in the Lauraceae family worldwide³. Camphor tree (*Cinnamomum camphora* L.), belonging to the family Lauraceae, an evergreen fragrant tree, is native to China, India, Mongolia, Japan and Taiwan. The tree is about 20-30 m in height and the leaves are glossy and waxy^{4,5}.

The main component of *C. camphora* leaf oil is camphor along with 1,8-cineole, linalool, eugenol, limonene, safrole, α -pinene, myrcene, p-cymene, nerolidol and camphene as the other dominant components^{3,6-7}. The different parts of *C. camphora* L. such as leaves, twigs and bark have been traditionally used for treating diseases like rheumatism, muscle pain, sprains, bronchitis, indigestion and asthma⁸. Moreover, the plant has been reported to exhibit allelopathic⁹, nematocidal⁹, antibacterial^{10,11}, antitumor¹¹, antifertility¹², anti-hyperlipidemic¹², antioxidant^{8,11-16}, insecticidal^{9,11,17-18}, anti-inflammatory^{8,11-12,19}, antimicrobial^{9,12-13,20-22}, antifungal^{4,23-24} and larvicidal²⁵ properties. Chemical composition and essential oil yield of fresh (leaf) *C. camphora* have been investigated from Nepal⁹, Guangzhou (China)^{10,26}, Assam (India)²⁰, Australia²⁶ and Nainital (India)³.

Fungal contamination decreases medicinal properties of herbal drugs by changing their chemical composition²⁷. Raw materials of herbal drugs have been reported to be contaminated by microbes, which is one of the reasons behind the decline in their demand globally. Since camphor oil is used extensively in traditional medicine, it is suggested as a food additive for herbal products²⁷.

There are many ways to preserve food, among which drying is one of the most common and oldest methods²⁸. Drying process is an easy way of preserving food by removing excess water from agricultural raw materials to a level that stops the growth of microbes and slow down unwanted biochemical reactions²⁹ and also increases shelf life of plant material³⁰. It prevents enzymatic degradation and microbial deterioration for herbs and plants. Consequently, raw material will remain fresh for longer periods of time³¹. There are some recent reports on the chemical composition and bioactive potential of the essential oil of *C. zeylanicum* from India³²⁻³³.

However, to the best of our knowledge, no work has been reported on the impact of various drying conditions on the quality of essential oil of *C. camphora* L. The composition of shade dried leaves of *C. camphora* L. has been explored by Bhandari *et al.*³⁴ from Doon Valley, Uttarakhand

(India). Therefore, the present study is the first attempt to investigate the effect of four drying methods on content, composition and biological activities of *C. camphora* L. leaf essential oil and examine the best drying technique particularly for summer season collection.

Materials and methods

Collection site and identification of plant specimen

Fresh leaves of *C. camphora* (CC) plant (6 kg) were collected in the month of July (Summer) from Naukuchiatal, district Nainital (Longitude 79° 59' E; Latitude 29° 32' N; Altitude 1200 m), Uttarakhand. The plant specimen was botanically identified at the Botanical Survey of India in Dehradun (Voucher no. 736).

Drying treatments

Initially, all collected plant material was separated into seven parts (800 g each). One part for fresh (CCF) analysis while the rest of parts were dried by various drying methods like sun drying (CCSN) (30°C \pm 5; 41 hrs.); shade drying (CCSH) (22°C \pm 5; 22 days); blower drying (CCB) (for 45 hrs) and hot air oven drying (in SANCO hot air oven 220-230 V) (at 30°C for 45 hrs. (CC30); at 40°C for 36 hrs. (CC40), and 50°C for 43 hrs. (CC50)) until constant weight.

Extraction of essential oil

The leaves of fresh and dried *C. camphora* were hydro distilled in a glass Clevenger apparatus for four hours to obtain the essential oil. Anhydrous Na₂SO₄ was added to the oil samples after extraction and stored in glass vials at 4°C. All experiments were repeated three times.

Analysis of the essential oil

Essential oil samples were analyzed at the Advanced Instrumentation Research Facility (AIRF), JNU (New Delhi) using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

Gas chromatography (GC)-FID method

A Shimadzu 2010 GC equipped with Rtx-5 capillary column (30 m \times 0.25 mm inner diameter

with 0.25 μm film coating) and connected to a flame ionization detector (FID) was used to analyze the oil samples. Temperature for the column was programmed at 50°C for 2 min hold time then increased by 3°C min⁻¹ to 210°C with 2 min hold time and left at 280°C (8°C min⁻¹) for 6 min hold time. Nitrogen was used as the carrier gas with a flow rate 76.7 mL min⁻¹. The injector port temperature and the detector temperature were 280°C and 290°C respectively. An injection volume of 0.5 μL oil with solvent (*n*-hexane) was injected through split sampling technique using a split ratio of 1/60 with each sample.

Gas chromatography/mass spectrometry (GC/MS) method

GC/MS analysis of the essential oil was conducted on a Shimadzu QP 2010 plus coupled with a TD-20 thermal desorption system and a Rtx-5 (30 m \times 0.25 mm and 0.25 μm film thickness) fused silica capillary column. The column temperature was programmed at 50°C for 2 min then increased by 3°C min⁻¹ to 210°C for 2 min hold time and finally at 280°C (6°C min⁻¹) for 6 min hold time and helium was used as a carrier gas with a flow rate 16.3 mL min⁻¹. Temperatures at the injector port and ion source were set to 260°C and 220°C respectively. For the analysis, 0.2 μL injection volume was prepared in *n*-hexane and normal injection mode with split ratio 1/10. MS measurements were performed at 70 eV using a mass scan (m/z) range of 40-650 amu.

Identification of the compounds and their quantification

In order to identify the individual components of essential oils, the retention index (RI) was calculated based on homologous *n*-alkane C₉-C₃₃ series under similar experimental conditions and matching with mass spectral WILEY (7th edition) and NIST (version 4.1) libraries. The quantity of each compound was determined by the method applied by Zheljzakov *et al.*³⁵. Finally, each component was identified by analyzing and comparing the fragmentation pattern of the mass spectral data with those published in the literature³⁶.

Bioactivities

Antioxidant activity

To evaluate the antioxidant activity of the essential oils, various *in vitro* experiments were carried out, and the results were displayed as mean \pm SD of three experimental data.

DPPH free radical scavenging activity

To determine DPPH (2,2'-diphenyl-1-picrylhydrazyl) antioxidant potential, the procedure used by Bhatt *et al.*³⁷ and Rani *et al.*³³ was followed. Each sample was treated with 5 mL DPPH in a 0.004% methanol solution (5-25 $\mu\text{L}/\text{mL}$). Butylated hydroxy toluene (BHT) was tested as an antioxidant standard in the same concentrations (5 $\mu\text{L}/\text{mL}$ -25 $\mu\text{L}/\text{mL}$) as in the essential oil samples. At 517 nm, the absorbance was taken in triplicate using UV-visible spectrophotometer (Thermo Scientific *Evolution-201*), after 30 minutes in the dark at room temperature. The percentage of DPPH that was inhibited was determined using the equation below:

$$\% \text{ DPPH radical scavenging activity (IC}_{50}) = [1 - (A_t / A_o)] \times 100$$

Where, A_o = Absorbance of control; A_t = Absorbance value of the essential oil sample or standards at 517 nm and IC = Inhibitory concentration. To calculate IC₅₀ (half-maximal inhibitory concentration) values, inhibition percentages were plotted against concentration.

Hydrogen peroxide radical scavenging activity

The H₂O₂ radical scavenging activity of the examined samples was determined using the previously reported procedure^{38,39}. The test solution was made up of 0.6 mL of H₂O₂ solution (40 mM) in phosphate buffered saline (PBS; 0.1 M; pH 7.4), along with 0.4 mL of methanolic solution containing essential oils or standard at different concentrations (5-25 $\mu\text{L}/\text{mL}$). Ten minute incubation was conducted at room temperature with the above solution. Methanol was used as a blank and 230 nm absorbance was measured against it. L-ascorbic acid and BHT (5-25 $\mu\text{L}/\text{mL}$) was used as a positive control in this study. Following is the formula for calculating H₂O₂ scavenging percentage:

% H₂O₂ activity (IC₅₀) = [1 - (A_t / A_o)] × 100

Where, A_o = Absorbance of control; A_t = Absorbance value of the essential oil sample or standards and IC = Inhibitory concentration. To calculate IC₅₀ (half-maximal inhibitory concentration) values, inhibition percentages were plotted against concentration.

Iron metal chelating activity

The method described by Karakoti *et al.*⁴⁰ was used to investigate metal chelating activity. We combined various test sample concentrations (5-25 µL/mL); 0.1 mL of 2mM FeCl₂ · 4H₂O; 0.2 mL of 5mM ferrozine and to make up the final volume 5mL, add 4.7 mL of methanol to the solution, then incubated for 10 minutes at room temperature. Shaking the solution and incubating it for 30 minutes at room temperature the absorbance was measured at 562 nm by UV-visible spectrophotometer (Thermo Scientific, Evolution-201). As a standard antioxidant, EDTA dipotassium salt dihydrate was used. The chelation capacity of the test samples with Fe²⁺ ion was determined using the following equation:

% Fe²⁺ metal chelating activity (IC₅₀) = [1 - (A_t / A_o)] × 100

Where, A_o = Absorbance of control; A_t = Absorbance value of the essential oil sample or standards at 562 nm and IC = Inhibitory concentration. To calculate IC₅₀ (half-maximal inhibitory concentration) values, inhibition percentages were plotted against concentration.

Anti-inflammatory activity

***In-vitro* protein denaturation activity**

The inhibition of egg albumin denaturation procedure was used to assess anti-inflammatory activity described in literature⁴¹. The reaction mixture contained different concentrations of essential oils (5 µL/mL – 25 µL/mL) as well as 200 µL (0.2 mL) of fresh albumin protein. To make a total volume of 5 mL; 2.8 mL of phosphate buffered saline (PBS; pH 6.4) was added to the solution. Then the reaction mixture was incubated for 15 minutes at 37°C followed by heating at 70°C for 5 minutes in a water bath. After cooling, the absorbance of the test samples was taken at 660 nm by UV

visible spectrophotometer (Thermo Scientific, Evolution-201) with diclofenac sodium as a standard. By using the following equation, the percentage inhibition of protein denaturation was determined:

% inhibition of protein denaturation (IC₅₀) = [1 - (A_t / A_o)] × 100

Where, A_o = Absorbance of control; A_t = Absorbance value of the essential oil sample or standards at 660 nm and IC = Inhibitory concentration. To calculate IC₅₀ (half-maximal inhibitory concentration) values, inhibition percentages were plotted against concentration.

Antidiabetic activity

***α*-amylase inhibition activity**

The standard acarbose and various concentrations of the essential oils (5 µL/mL - 25 µL/mL) were permitted to react with 200 µL of α-amylase solution and 100 µL of phosphate buffer (2 mM; pH 6.9). Then twenty minutes incubation was conducted on the reaction mixture. The mixture was further incubated at 37°C for 5 minutes with 100 µL of 1% starch solution. Finally, 500 µL of dinitrosalicylic acid reagent (30 g of sodium potassium tartrate tetrahydrate and 20 mL of 2N NaOH added to 1 gm 3,5- dinitrosalicylic acid in 50 mL reagent grade water) was added to the test solution, which was then heated for five minutes in boiling water bath⁴². An UV visible spectrophotometer (Thermo Scientific Evolution-201) was used to measure absorbance at 540 nm.

% inhibition of α- amylase activity (IC₅₀) = [1 - (A_t / A_o)] × 100

Where, A_o = Absorbance of control; A_t = Absorbance value of the essential oil sample or standards at 540 nm and IC = Inhibitory concentration. To calculate IC₅₀ (half-maximal inhibitory concentration) values, inhibition percentages were plotted against concentration.

Statistical data analysis

The analysis was performed three times and the mean of triplicate values was calculated using MS-Excel. All the results were presented as mean ± standard deviation (SD). Statistical package for social sciences (SPSS 16.0) software

was used to perform One-way ANOVA and mean values were compared using Duncan's multiple range tests (significant level of $p < 0.05$). The hierarchical cluster analysis of essential oils was conducted using Ward's method (SPSS 16.0).

Results and discussion

The effect of drying techniques on the oil content and composition

There was a significant influence of drying methods on the essential oil content (Figure 1). In this study, the highest essential oil content (0.11 %) was obtained through CCF as well as CCB samples (Figure 1). No significant difference was observed between the essential oil content obtained from CCSN and those obtained from CC50 sample (0.05 % w/w). The CC30 sample had 0.06% oil content; the CCSH sample had 0.07 % oil content while the sample dried in oven at 40°C (CC40) contained 0.09 % oil content. Low temperature appeared to enhance the essential oil content. In a previous investigation on *O. kilimandscaricum* by Kumar *et al.*⁴³, in summer collection, the essential oil yield was observed to be slightly reduced on shade drying (0.8% - 0.6%). A report from Iran also suggested that the essential oil yield was affected by drying temperature and time taken during drying⁴⁴. Another report by Pandey *et al.*⁴⁵ in which Patchouli (*Pogostemon cablin*) leaves were dried and the results revealed that

the highest essential oil yield was obtained in the oven dried sample at 40°C and the lowest in the sun dried sample. Another finding showed that high temperature of the oven treatment decreased the content of essential oil⁴⁶.

In the CCF sample, twenty-nine compounds were identified, representing 99.33 % of the total oil. The oil sample was dominated by camphor (71.18 %) followed by limonene (5.54 %), α -pinene (4.36 %), camphene (2.78 %) and myrcene (2.64 %) (Table 1; Figure 2). Joshi *et al.*³ identified camphor (81.5 %), 1,8-cineole (2.6 %), p-cymene (2.3 %), α -pinene (1.4 %), α -humulene (1.3 %), and camphene (1.1 %) as major components of fresh *C. camphora* L. Report from Guangzhou (China) showed that the essential oil of fresh *C. camphora* Chvar. Borneol, was characterized with the presence of d-borneol (81.58 %), camphor (2.96 %), α -pinene (2.03 %), d-limonene (1.64 %), 1,8-cineole (1.60 %) and camphene (1.54%) as major compounds¹³. The essential oil of fresh *C. camphora* L. Presl collected from Hefei (China) showed the presence of camphor (18.48 %), eucalyptol (16.46 %), linalool (11.58 %) and 3,7-dimethyl-1,3,7-octatriene (11.07 %) as the major constituents¹⁸. According to Chen *et al.*⁴⁷, major compounds of *C. camphora* were linalool (26.6 %), eucalyptol (16.8 %), α -terpineol (8.7 %), isoborneol (8.1 %), β -phellandrene (5.1 %), and camphor (5.0 %).

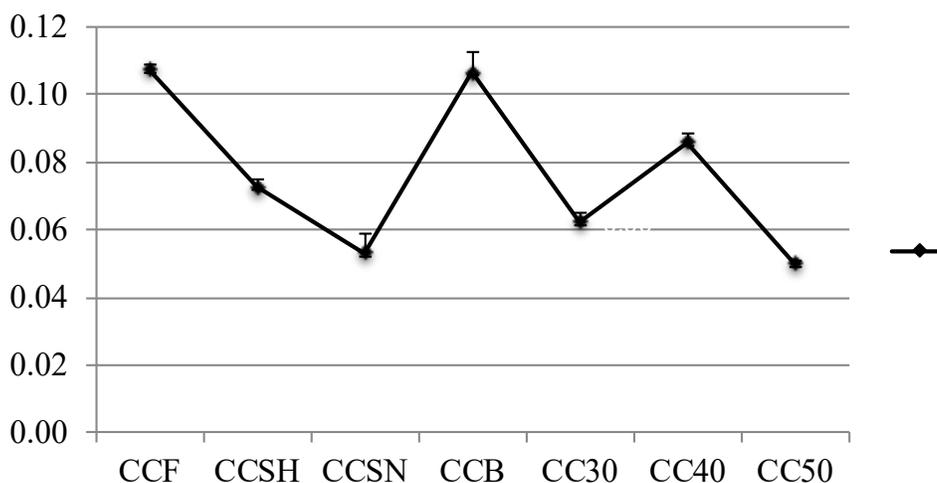


Figure 1. Essential oil content obtained at different drying conditions of *C. camphora* L. collected from Nainital district of Uttarakhand

Thirty-four compounds were identified in CCB sample where the percentage of camphor decreased from 71.18 % to 40.65 %. The content of other major components like α -pinene (from 4.36 to 11.00 %), camphene (2.78 to 5.39 %), myrcene (2.64 to 6.09 %) and limonene (5.54 to 13.02 %) were observed to increase (Table 1; Figure 2).

In the oil from the CCSH material; thirty-four compounds representing 99.23 % of the total volatiles were identified. Camphor (53.32 %), limonene (8.92 %), α -pinene (8.44 %), camphene (5.03 %) and myrcene (4.53 %) were the major compounds of the oil. At the same time, the percentage of limonene (from 5.54 to 8.92 %), α -pinene (from 4.36 to 8.44 %), camphene (2.78 to 5.03%) and myrcene (2.64 to 4.53 %) was found to increase while that of camphor (from 71.18 to 53.32 %), decreased (Table 1; Figure 2). Bhandari *et al.*³⁴ identified twenty-compounds in the shade dried leaves of *C. camphora* and d-Camphor (46.06 %) was the major compound followed by limonene (9.74%), α -pinene (9.71%) and β -myrcene (4.91 %).

Number of compounds identified in the essential oil extracted from CCSN sample was thirty-four and under the influence of sun drying method, camphor content was observed to increase from 71.18 % to 72.53 %. On the other hand, α -pinene (from 4.36 to 3.93%), camphene (2.78 to 2.14%), myrcene (2.64 to 1.81%), and limonene (5.54 to 3.58%) had a significant decline in their percentage after drying (Table 1; Figure 2).

Twenty-seven compounds were identified in the oven dried sample at 30°C (CC30) having 66.14 % of camphor, 8.35 % of limonene, 6.50 % of α -pinene, 4.18 % of myrcene and 3.68 % of camphene as major compounds. Total 48 and 41 compounds were present in the essential oils of oven dried samples (CC40 and CC50) of which thirty compounds were identified, constituting 94.75 % and 98.88% of the total oil respectively. The content of camphor ranged from 61.76 % to 60.51 %, α -pinene 4.33 % to 5.45 %, limonene 3.84 % to 6.21 %, camphene 2.36 % to 3.15 % and myrcene 2.04 % to 2.94 % in the CC40 and CC50 dried leaves of *C. camphora* respectively (Table 1; Figure 2).

The present work revealed that the mean percentage of camphor decreased significantly from 71.15 % to 40.65 % in CCB, 71.15 % to 53.32 % in CCSH samples and 71.15 % to 66.51 % - 66.14 % in oven dried samples while for the CCSN material, camphor percentage increased from 71.18 to 72.53. Additionally, significant increase in the content of limonene, camphene, α -pinene and myrcene observed in the essential oil of CCSN and CC40 leaves of *C. camphora*. In a previous study on *Ocimum kilimandscharicum*, the percentage of camphor showed slight degradation during drying. The other major constituents such as limonene, camphene and myrcene showed minor quantitative changes⁴³. Bisht *et al.*⁴⁸ noted an increase in the percentage of camphor from 33.41 % in the essential oil fresh to 49.74 % in the essential oil of sun dried *Ocimum americanum*.

Two minor components namely germacrene A and δ -elemene which were absent in CCF sample, appeared in the essential oil of dried samples except CC30 and CC50. Additionally, β -selinene and caryophyllene oxide appeared in the CCB and CCSN samples. β -bourbonene which was absent in CCF, CCSH, CC30 and CC50 samples appeared (0.05 % to 0.13 %) in the essential oil of CCB, CCSN and CC40 samples of *C. camphora*. Bulnesol was present in traces only in the CCSH sample. Guaiol was not detected in the CCF but appeared in the CCSH, CCSN, CC40 and CC50 samples with the percentage range of 0.11 % to 0.15 %. The percentage of p -cymene varied from 0.16 % to 0.58 % and was present in the CCB, CCSN and CC40 samples (Table 1). Hamrouni-Sellami *et al.*³⁰ and Pirbalouti *et al.*⁴⁴ reported that some essential oil components might appear after drying in aromatic plants. Moreover, some of the minor constituents such as γ -elemene and humulene epoxide II (CC30 sample), bicyclogermacrene (CCB and CC40 samples), and spathulenol (CCB, CCSN and CC40 samples) disappeared on drying. Results of the experiment also showed that monoterpene hydrocarbons decreased in CCSN as well as in CC40 samples. Percentage of oxygenated monoterpenes remained approximately same in CCSN sample while in other samples, its amount decreased as compared to the CCF sample. The

Table 1. Effect of drying treatments on essential oil composition of *C. camphora* L. collected in summer season

No.	Compounds	RI ^c	RI ^l	RT (min)	Concentration ^{AB} (% of oil \pm SD)									
					CCF	CCB	CCSH	CCSN	CC30	CC40	CC50			
1	Tricyclene	919	921	8.3	t	0.07	0.06	t	t	t	t	t	t	
2	α -Thujene	923	924	8.4	0.28 ^{ab}	0.74 ^c	0.58 ^d	0.22 ^a	0.46 ^c	0.22 ^a	0.22 ^a	0.22 ^a	0.32 ^b	
3	α -Pinene	931	932	8.8	4.36 ^b \pm 0.14	11.00 ^b \pm 0.19	8.44 ^b \pm 0.06	3.93 ^b \pm 0.07	6.50 ^b \pm 0.02	4.33 ^b \pm 0.02	4.33 ^b \pm 0.03	5.45 ^c \pm 0.03	0.03	
4	Camphene	946	946	9.4	2.78 ^b \pm 0.05	5.39 ^b \pm 0.11	5.03 ^b \pm 0.06	2.14 ^a \pm 0.01	3.68 ^b \pm 0.02	2.36 ^b \pm 0.02	2.36 ^b \pm 0.08	3.15 ^d \pm 0.02	0.02	
5	Sabinene	970	969	10.3	0.95 ^c	2.70 ^f	1.77 ^e	0.55 ^a	0.68 ^b	0.64 ^{ab}	0.64 ^{ab}	1.20 ^d	0.02	
6	β -Pinene	975	974	10.5	1.66 ^c	3.71 ^g	3.15 ^f	1.35 ^a	2.22 ^c	1.50 ^b	1.50 ^b	2.06 ^d	0.02	
7	Myrcene	990	988	11.1	2.64 ^a \pm 0.03	6.09 ^a \pm 0.01	4.53 ^a \pm 0.08	1.81 ^a \pm 0.02	4.18 ^a \pm 0.03	2.04 ^b \pm 0.01	2.04 ^b \pm 0.01	2.94 ^d \pm 0.02	0.02	
8	α -Phellandrene	1005	1002	11.7	0.47 ^c	1.26 ^g	1.16 ^f	0.35 ^a	1.04 ^c	0.41 ^b	0.41 ^b	0.66 ^d	0.02	
9	α -Terpinene	1015	1014	12.2	0.33 ^f	0.17 ^e	0.14 ^d	0.05 ^a	0.12 ^c	0.06 ^a	0.06 ^a	0.11 ^b	0.02	
10	p -Cymene	1024	1020	12.5	ND	0.58	ND	0.16	ND	0.17	0.17	ND	0.02	
11	Limonene	1030	1024	12.8	5.54 ^a \pm 0.02	13.02 ^a \pm 0.01	8.92 ^a \pm 0.05	3.58 ^a \pm 0.10	8.35 ^a \pm 0.03	3.84 ^b \pm 0.28	3.84 ^b \pm 0.28	6.21 ^d \pm 0.01	0.01	
12	1,8-Cineole	1032	1026	13.1	1.86 ^e	1.85 ^e	1.45 ^d	1.08 ^b	0.63 ^a	1.16 ^c	1.16 ^c	3.04 ^f	0.01	
13	(<i>E</i>)- β -Ocimene	1046	1044	13.5	0.17 ^d	0.14 ^c	0.25 ^c	0.06 ^a	0.09 ^b	0.08 ^b	0.08 ^b	0.19 ^d	0.01	
14	γ -Terpinene	1057	1054	14.0	0.17 ^a	0.44 ^d	0.32 ^c	0.16 ^c	0.36 ^c	0.19 ^a	0.19 ^a	0.25 ^b	0.01	
15	(<i>Z</i>)-Sabinene hydrate	1072	1065	14.7	0.23 ^d	0.25 ^c	0.19 ^c	0.08 ^a	0.20 ^c	0.10 ^b	0.10 ^b	0.33 ^f	0.01	
16	Terpinolene	1084	1086	15.3	0.53 ^c	1.16 ^g	1.08 ^f	0.38 ^a	1.00 ^c	0.43 ^b	0.43 ^b	0.63 ^d	0.01	
17	Camphor	1160	1141	18.7	71.18 ^a \pm 0.51	40.65 ^a \pm 0.62	53.32 ^b \pm 1.05	72.53 ^b \pm 0.97	66.14 ^d \pm 0.02	61.76 ^e \pm 0.51	61.76 ^e \pm 0.51	60.51 ^e \pm 0.93	0.93	
18	Borneol	1174	1165	19.5	0.63 ^c	0.26 ^a	0.43 ^b	0.74 ^d	0.26 ^a	0.78 ^e	0.78 ^e	0.84 ^f	0.93	
19	Terpinen-4-ol	1185	1174	19.9	0.58 ^d	0.44 ^c	0.27 ^b	0.27 ^b	0.57 ^d	0.22 ^a	0.22 ^a	0.65 ^e	0.93	
20	α -Terpineol	1202	1186	20.6	0.91 ^e	0.79 ^d	0.52 ^a	0.68 ^b	0.74 ^c	0.53 ^a	0.53 ^a	1.08 ^f	0.93	
21	δ -Elemene	1335	1335	26.6	ND	t	0.13	t	ND	0.06	0.06	ND	0.93	
22	α -Copaene	1376	1374	28.5	t	0.07	t	0.05	t	0.11	0.11	t	0.93	
23	β -Bourbonene	1383	1387	28.8	ND	0.06	ND	0.05	ND	0.13	0.13	ND	0.93	
24	β -Elemene	1390	1389	29.1	0.14 ^b	0.56 ^f	0.35 ^d	0.51 ^e	0.06 ^a	1.05 ^g	1.05 ^g	0.27 ^c	0.93	
25	(<i>E</i>)-Caryophyllene	1415	1417	30.4	0.63 ^a	1.45 ^d	1.24 ^c	1.85 ^f	0.74 ^b	3.09 ^g	3.09 ^g	1.54 ^e	0.93	
26	γ -Elemene	1426	1434	30.8	0.05	0.13	0.11	0.14	ND	0.34	0.34	0.13	0.93	
27	α -Humulene	1453	1452	31.9	0.85 ^b	2.80 ^f	1.95 ^d	2.44 ^e	0.18 ^a	5.17 ^g	5.17 ^g	1.81 ^c	0.93	

table 1. (continued).

No.	Compounds	RI ^c	RI ^l	RT (min)	CCF	CCB	Concentration ^{A,B} (% of oil ± SD)					
							CCSH	CCSN	CC30	CC40	CC50	
28	Germaacrene D	1476	1484	32.9	0.36	1.18	0.86	t	t	t	2.67	0.90
29	β-Selinene	1484	1489	33.3	ND	0.83	ND	1.03	ND	ND	ND	ND
30	Bicyclogermacrene	1495	1500	33.6	1.33	ND	1.90	0.68	0.52	0.52	ND	2.87
31	Germaacrene A	1502	1508	34.0	ND	t	t	1.15	ND	ND	t	ND
32	Hedycaryol	1550	1546	35.7	0.06	0.05	0.07	0.14	0.13	0.13	ND	0.10
33	Germaacrene B	1558	1559	36.0	0.12	0.24	0.20	0.15	t	t	0.55	0.23
34	Spathulenol	1580	1577	36.8	0.25	ND	0.16	ND	0.11	0.11	ND	0.75
35	Caryophyllene oxide	1577	1582	37.0	ND	0.08	ND	0.15	ND	ND	ND	ND
36	Guaiool	1596	1600	37.6	ND	ND	0.13	0.15	ND	ND	0.14	0.11
37	Humulene epoxide II	1605	1608	38.0	t	0.18	0.08	0.10	ND	ND	0.23	0.10
38	neo-Intermedeol	1659	1658	39.9	0.27 ^b	0.28 ^b	0.44 ^d	0.28 ^b	0.06 ^a	0.06 ^a	0.39 ^c	0.45 ^d
39	Bulnesol	1664	1670	40.2	ND	ND	t	ND	ND	ND	ND	ND
Total compound identified					29	34	34	34	27	27	30	30
Total identified oil (%)					0.10	0.10	0.07	0.05	0.06	0.06	0.08	0.05
% Monoterpene hydrocarbons (S. No. 1-11, 13-14, 16)					19.88	46.47	35.43	14.74	28.68	28.68	16.27	23.17
% Oxygenated monoterpenes (S.No. 12, 15, 17-20)					75.39	44.24	56.18	75.38	68.54	68.54	64.55	66.45
% Sesquiterpene hydrocarbons (S. No. 21-31, 33)					3.48	7.32	6.74	8.05	1.50	1.50	13.17	7.75
% Oxygenated sesquiterpenes (S. No. 32, 34-39)					0.58	0.59	0.88	0.82	0.30	0.30	0.76	1.51
Total % composition					99.33	98.62	99.23	98.99	99.02	99.02	94.75	98.88

The digits in bold indicate the major compounds; RI^c= Calculated retention indices with respect to *n*-alkane standard (C₉-C₃₃) series; RI^l = Retention indices found in literature³⁶; RT= Retention time; CCF= Fresh; CCB= B lower dried; CCSH= Shade dried; CCSN= Sun dried; CC30= Oven dried at 30°C; CC40= Oven dried at 40°C; CC50= Oven dried at 50°C; SD = Standard deviation; t = trace amount (i.e. <0.05 %); ND= Not detected; Mean values of specific component (in rows) followed by different alphabets (a-g) are significantly different at p<0.05 according to Dunnett test. Concentration^A= Calibration curves determined the quantity of each compound, which was obtained by running GC analyses on each compound (S. No. 3, 7, 11, 17, 25); Concentration^B= The use of standard compounds in Co-injection by using standard compounds (4, 6, 28).

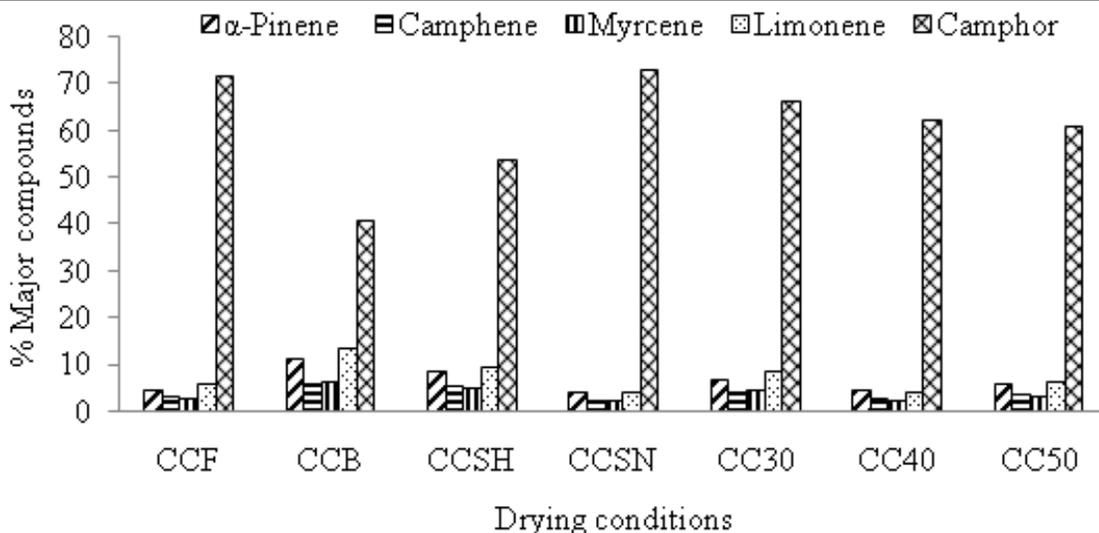


Figure 2. Percentage of major compounds present in essential oil samples of *C. camphora* L. at different drying conditions

percentage of sesquiterpene hydrocarbons as well as oxygenated sesquiterpenes increased in all samples except in CC30 sample (Table 1).

Antioxidant activities

DPPH radical scavenging activity

All essential oil samples were evaluated for their ability to scavenge free radicals in comparison to standard antioxidant, BHT in terms of IC_{50} value as $7.82 \mu\text{g/mL}$ (Table 2). The essential oils extracted from CCF and CCSH had strong antioxidant activity ($IC_{50}=8.44$ and $9.62 \mu\text{g/mL}$ respectively) equivalent to the standard. While the minimum activity was shown by CC50 and CC40 samples with the IC_{50} values of 14.65 and $12.19 \mu\text{g/mL}$ respectively. The total amount of antioxidants needed to inhibit DPPH free radicals by 50% is known as the IC_{50} value or inhibitory concentration. The IC_{50} values ($\mu\text{g/mL}$) of the samples varied in the following order: BHT (7.82) < CCF (8.44) < CCSH (9.62) < CCB (10.75) < CCSN (11.42) < CC30 (11.84) < CC40 (12.19) < CC50 (14.65). The IC_{50} values for CC40 and CC50 were high (low activity) which might be due to the lowest monoterpene content in their essential oils. Monoterpenes are considered as active antioxidants while sesquiterpenes including hydrocarbons and oxygenated derivatives are reported to show low antioxidant activity ⁴⁹.

Although, in the present study, no correlation was found between any class of compounds or major components with the biological activity, there may be antagonistic effect of the various major and minor components on the biological activity of the essential oils.

H₂O₂ radical scavenging activity

In hydrogen peroxide scavenging activity, the antioxidant standard BHT exhibited the maximum scavenging activity followed by CCF and the lowest activity was displayed by CC50. The IC_{50} values ($\mu\text{g/mL}$) of samples were in the following order: BHT (11.08) < CCF (11.60) < CCSH (13.65) < CCB (13.96) < CCSN (14.45) < CC30 (16.41) < CC40 (16.72) < CC50 (17.26) (Table 2).

Metal chelating activity of Fe²⁺

The chelation capacity of the samples increased with concentration. The metal chelating ability of EDTA and ascorbic acid was greater than that of the samples of essential oils. In this technique more powerful activity was also observed for CCF ($IC_{50} = 15.80 \mu\text{g/mL}$) in comparison to EDTA ($IC_{50} = 15.64 \mu\text{g/mL}$) and ascorbic acid ($IC_{50} = 15.77 \mu\text{g/mL}$). The order of IC_{50} values in different samples was given as: EDTA (15.64) < AA (15.77) < CCF (15.80) < CCSH (15.90) < CCB (16.47) < CCSN (16.53) < CC30 (18.53) < CC40 (21.43) < CC50 (23.79) (Table 2).

Table 2. Antioxidant activities of *C. camphora* L. collected in summer season

S. No.	Samples / Standards	Mean value of antioxidant activity in terms of IC ₅₀ with SD (µg/mL±SD)		
		DPPH activity	Metal chelating activity	H ₂ O ₂ activity
1	CCF	8.44 ^b ±0.02	15.80 ^c ±0.02	11.60 ^b ±0.02
2	CCSH	9.62 ^c ±0.01	15.90 ^d ±0.01	13.65 ^c ±0.01
3	CCB	10.75 ^d ±0.01	16.47 ^e ±0.01	13.96 ^d ±0.01
4	CCSN	11.42 ^e ±0.01	16.53 ^f ±0.02	14.45 ^e ±0.01
5	CC30	11.84 ^f ±0.01	18.53 ^g ±0.01	16.41 ^f ±0.01
6	CC40	12.19 ^g ±0.01	21.43 ^h ±0.01	16.72 ^g ±0.01
7	CC50	14.65 ^h ±0.01	23.79 ⁱ ±0.01	17.26 ^h ±0.01
8	BHT	7.82 ^a ±0.01	-	11.08 ^a ±0.02
9	AA	-	15.77 ^b ±0.02	-
10	EDTA	-	15.64 ^a ±0.02	-

Mean values of specific activity followed by different alphabets (a-i) are significantly different at $p < 0.05$ according to Duncan test for each column; SD= Standard deviation; CCF= Fresh; CCSH= Shade dried; CCB= Blower dried; CC30= Oven dried at 30°C; CC40= Oven dried at 40°C; CC50= Oven dried at 50°C; BHT= Butylated hydroxytoluene; AA= Ascorbic acid; EDTA= Ethylene diaminetetraacetic acid; IC₅₀ = Half maximal inhibitory concentration.

***In vitro* anti-inflammatory activity**

Protein denaturation is a phenomenon in which proteins lose their secondary and tertiary structures due to external stress or factors like heat, strong acids or bases, concentrated inorganic salts and organic solvents. Most natural proteins lose their biological functions when denatured, which is one of well-documented reasons of inflammation⁵⁰. All samples in the current investigation displayed mean inhibition of protein denaturation at various concentrations (5–25 µg/mL). Significant anti-inflammatory activity was demonstrated by CCF (IC₅₀= 8.87) and CCSH (IC₅₀= 9.80) in terms of percentage inhibition. A comparison of the IC₅₀ of the results with the standard diclofenac sodium (IC₅₀= 7.23) was also performed. Table 3 illustrates the IC₅₀ values of samples that inhibit inflammation. The order of IC₅₀ values (µg/mL) in different samples was given as: DFS (7.23) < CCF (8.87) < CCSH (9.80) < CCSN (10.55) < CC30 (10.72) < CCB (11.34) < CC40 (11.63) < CC50 (14.76). The anti-inflammatory properties of substances like camphor, borneol, and 1,8-cineole have been reported⁵¹.

Antidiabetic activity

The essential oils of *C. camphora* were tested for their *in-vitro* α-amylase inhibitory actions at concentrations ranging from 5 µg/mL-25 µg/mL. All of the samples of essential oils had considerable α-amylase inhibitory action, however to a lesser level than the standard drug acarbose. The order of IC₅₀ values (µg/mL) in different samples was given as: Acarbose (7.17) < CCF (9.79) < CCSH (10.19) < CCB (11.07) < CCSN (11.52) < CC30 (12.41) < CC40 (13.25) < CC50 (15.52) (Table 4). No significant correlations were observed between major compounds and bioactivity and the CCSH (*C. camphora* shade dried) sample showed the highest bioactivity which might be due to the synergistic effect of the oil profile under this condition. Similarly, previous report on *Rosmarinus officinalis* L. essential oil also revealed synergistic effects of the constituents on biological activity⁵².

Cluster analysis

Using hierarchical cluster analysis, four distinct clusters were identified based on the major components (percentage > 5.00 %) of *C. camphora* L. essential oils collected in the

Table 3. *In vitro* protein denaturation activity of *C. camphora* L. collected in summer season

S. No.	Samples / Standards	Mean value of antiinflammatory activity in terms of IC ₅₀ with SD (µg/mL±SD)
1	CCF	8.87 ^b ±0.03
2	CCSH	9.80 ^c ±0.02
3	CCB	11.34 ^f ±0.02
4	CCSN	10.55 ^d ±0.03
5	CC30	10.72 ^e ±0.02
6	CC40	11.63 ^g ±0.03
7	CC50	14.76 ^h ±0.02
8	DFS	7.23 ^a ±0.02

Mean values of specific activity followed by different alphabets (a-h) are significantly different at $p < 0.05$ according to Duncan test; SD= Standard deviation; CCF= Fresh; CCSH= Shade dried; CCB= Blower dried; CC30= Oven dried at 30°C; CC40= Oven dried at 40°C; CC50= Oven dried at 50°C; DFS= Diclofenac sodium; IC₅₀= Half maximal inhibitory concentration.

Table 4. *In vitro* α-amylase inhibition activity of *C. camphora* L. collected in summer season

S. No.	Samples / Standards	Mean value of antidiabetic activity in terms of IC ₅₀ with SD (µg/mL±SD)
1	CCF	9.79 ^b ±0.01
2	CCSH	10.19 ^c ±0.01
3	CCB	11.07 ^d ±0.01
4	CCSN	11.52 ^e ±0.01
5	CC30	12.41 ^f ±0.02
6	CC40	13.25 ^g ±0.01
7	CC50	15.52 ^h ±0.02
8	Acarbose	7.17 ^a ±0.02

Mean values of specific activity followed by different alphabets (a-h) are significantly different at $p < 0.05$ according to Duncan test; SD= Standard deviation; CCF= Fresh; CCSH= Shade dried; CCB= Blower dried; CC30= Oven dried at 30°C; CC40= Oven dried at 40°C; CC50= Oven dried at 50°C; IC₅₀ = Half maximal inhibitory concentration.

summer season. As part of cluster I, there were CCF and CCSN samples. There were three samples in cluster II: CC30, CC40 and CC50. Cluster III contained CCSH sample while in cluster IV, CCB sample was found (Figure 3). Bhatt *et al.*⁵³ categorized *Ocimum americanum* L. essential oil into three clusters. The clusters obtained in the present study along with their

composition are:

Cluster I (CCF and CCSN)

Camphor (71.18 to 72.53 %), limonene (3.58 % to 5.54 %).

Cluster II (CC30, CC40 and CC50)

Camphor (60.51 % to 66.14 %), limonene (3.84

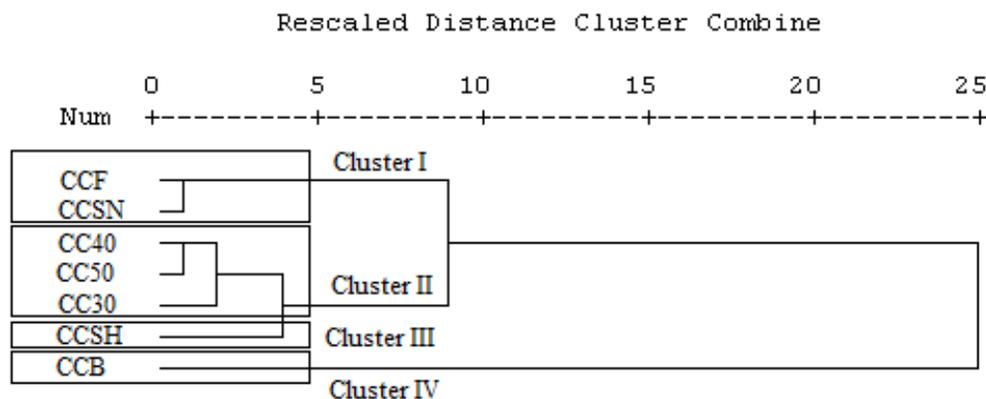


Figure 3. Cluster analysis of essential oil samples of *C. camphora* L. at different drying collected from Nainital district of Uttarakhand

% to 8.35 %), α -pinene (4.33 % to 6.50 %).

Cluster III (CCSH)

Camphor (53.32 %), limonene (8.92 %), α -pinene (8.44 %), camphene (5.03 %).

Cluster IV (CCB)

Camphor (40.65 %), limonene (13.02 %), α -pinene (11.00 %), myrcene (6.09 %), camphene (5.39 %).

Conclusion

The results of the present study showed that there was significant qualitative and quantitative variation in the essential oil composition of *C. camphora* after drying. The retention of the marker component (camphor) was the highest in CCSN leaves of plant. CCSH sample exhibited the best bioactivities which might be due the synergic effects of the major components. Blower drying method resulted in the highest oil yield while sun drying is a simple and inexpensive method which helped in retention of the characteristic components of the oil.

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