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RESEARCH ARTICLE

Drying Potential of Leaves Oil of *Zanthoxylum armatum* DC from North India

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Abstract:

Background:

The study on drying and its social acceptance has increased rapidly. Among different drying techniques, shade drying is one of the most feasible methods to keep intact from the decay of the main active components of the plant materials. Shade drying is an ancient drying method that increases durability, major constituents and activity of the plant material.

Aims:

Research was conducted to examine the drying potential of aromatic leaves oil of *Zanthoxylum armatum* DC.

Methods:

The fresh plant material was collected from Lohaghat, Champawat district of Uttarakhand and hydrodistilled before and after shade drying to assess the changes in the quality of volatile constituents by GC and GC-MS techniques. A two-tailed paired t-test was executed to assess the difference between drying treatments using MS-Excel.

Results:

The major components in the fresh oil were 2-undecane, linalool, (*E*)-β-ocimene, α-pinene and β-phellandrene. In the oil from dried material, the three predominant compounds were noted. A significant increase was observed in the percentage of β-phellandrene, undecanal and myrcene after shade drying ($p<0.01$). Five components absent in the fresh plant material appeared and one disappeared during the drying process.

Conclusion:

Shade drying significantly influenced the essential oil composition of *Z. armatum*.

Keywords: *Zanthoxylum armatum*, Drying, Linalool, β-Phellandrene, 2-Undecane, Undecanal.

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1. INTRODUCTION

The drying technique has been playing a successful role in the storage and food safety since ancient times. Efforts on drying methodology should be made to raise the active components of the aromatic herbs. These active components are the major part of various drugs, spices, natural beauty products, aroma profiles and are responsible for anticancer, antiaging, anti-oxidant, anti-microbial, anti-inflammatory and other various activities [1]. Shades drying, sun-drying, air-drying, oven-drying, microwave-drying, fluidized-drying and freeze-drying are the most useful methods which influence drying technology. Shade drying represents the most traditional and more commonly used method to preserve the colour, quality and activity response of the major compounds of essential oils as compared to other drying methods. Dried herbs have been good sources of essential micronutrients, the main ingredient of the dishes and a small amount is responsible for colour, sweet aroma and tangy flavors [2]. Dried herbs become more valuable worldwide with beneficial health impacts. Therefore, different drying methods need to be part of the research.

Zanthoxylum armatum DC, is one of the most famous winged prickly aromatic shrubs belonging to the family Rutaceae, which comprises about 150 genera distributed worldwide [3]. It is native of India, China, Korea and Japan,

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mostly grow in wastelands, mountains, valleys, forests at an altitude range of approximately 1000-2500m. This wild aromatic spiny shrub is often locally known as Tejphal, Timur, Tejowati, Nepal pepper and Toothache tree [4]. *Zanthoxylum armatum* leaves, fruits, bark, seeds and young sticks are well known for their therapeutic potential as a medicinal component and widely used for carminative, stomachache, rheumatism, toothache, antipyretic, sudorific and anthelmintic properties [5]. Earlier studies show that the complete plant parts, *i.e*., bark, seeds, leaves, root and fruits, are a great source of alkaloids, steroids, flavonoids, amides, lignans, terpenes, carbohydrates, proteins and essential oils [6]. The fruits and young sticks of *Z. armatum* are the main components of traditionally used herbal toothpaste and to keep gum strengthen and hygienic. The barks, as well as fruits, are also useful in various types of skin disease, wounds, eye and ear disease, cough, fever, etc. Dried ground leaves and seeds of *Zanthoxylum* species are used in spice, soup and flavor products [4, 7]. The chemical composition of the essential oil of the same species might be different due to different environmental, seasonal, geographical distribution (*i.e*., different longitude and latitude), different physicochemical properties of soils, different time of harvesting and different plant parts at different stages.

There are several reports on antimalarial [8, 9], essential oil composition and anti-inflammatory [10], anthelmintic [11], antifungal, antibacterial, antimicrobial [12 - 14], anticancer [15, 16] and antioxidant free radical scavenging [17] activities of extract and essential oil of *Z. armatum*. Variation in the essential oil composition of different drying methods on some aromatic plants of family Rutaceae has been reported on *Murraya koenigii* L [18, 19]. from north India, *Citrus reticulata* (Kinnow), *C. sinensis* (Mussami) and *C. paradisii* (Grapefruit) from Pakistan [20]. To the best of our knowledge, no earlier reports on the drying effect of the leaves oil of *Z. armatum* have been found. Therefore, this study was undertaken to investigate the effect of drying on *Z. armatum* leaves essential oil. For the effective production of essential oil, knowledge of the suitable post-harvest technique is needed for high-quality products. This study reflects on the potential of a shade drying approach on *Z. armatum*.

2. MATERIALS AND METHODS

2.1. Collection and Identification of *Z. armatum*

Fresh leaves of *Zanthoxylum armatum* were collected from the wild field of Lohaghat (Latitude 29.404178; Longitude 80.0943366; Altitude 5869 ft), Champawat district, Uttarakhand in the month of September 2018. A fraction of plant material was shade dried (20±5°C) until a constant weight was obtained. The identification of the plant was done at Botany Department, D. S. B. Campus, Nainital (Acc. No. AR01).

2.2. Extraction of Essential Oil

Fresh and shade dried plant materials were sliced into small parts and 500 g and 200 g of fresh (ZFL) and dried (ZDL) samples were extracted by using the hydrodistillation method in a Clevenger apparatus for 5 hours [18] and 2.5 mL and 0.6 mL oils were obtained, respectively. The oils were dried over anhydrous sodium sulphate and stored in glass vials in Biological Oxygen Demand (BOD) incubator prior to the analysis [19]. All the experiments were performed in three replicates.

2.3. Analysis of Extracted Oil

GC conditions are summarized in Table **1**.

The GC/MS used was 2010 GC coupled with Shimadzu QP 2010 plus with thermal desorption system TD 20 having Rtx-5 capillary column (30m x 0.25mm with film thickness 0.25µm). The GC-MS was programmed in similar conditions to those of GC. Helium was used as a carrier gas and the injector temperature was 230°C. The injection volume was 0.2µL diluted oil in n-hexane with split mode (split ratio 1:40). MS was taken at 70eV with a mass range of 40-650amu [21].

Table 1. GC Shimadzu 2010 conditions.

Injection Port	Column Information and Detector	Column Oven
Injection Mode: Split Temperature: 260°C Carrier Gas: N_2/Air Split Ratio: 1:40	Column Name: RXi-5 Sil MS Film Thickness: 0.25 µm Column Length: 30.0 m x 0.25mm column head pressure: 30.0mL/min Detector: FID FID temperature: 270°C	Initial Temperature: 50.0 C Total Program Time: 60 min Column Oven Temperature Program Rate(C/min) Temperature(C) Hold Time(min) 3°C min^{-1} 210°C 2 minute 8°C min^{-1} 210°-280°C 14 minutes

2.4. Identification of the Volatile Components

The identification of the oil components was done on the basis of their Retention Index (RI) which was calculated with respect to n-alkane series (C_9-C_{33}; Polyscience Corp., Niles IL) under similar GC conditions, MS Library (NIST:NIH version 2.1 and WILEY: 7th edition), comparison with the existing MS literature data [22] and the relative amount of individual volatile component was calculated on the basis of GC peak area without using any response factor [21].

2.5. Statistical Analysis

The mean and standard deviation of triplicate values were calculated using MS-Excel and the analyzed data was presented as mean ± standard deviation (SD). Two-tailed paired t-test was executed to compare mean values of percentage of constituents between fresh and shade dried *Z. armatum* at a probability level of $p<0.01$ and $p<0.05$ using MS-Excel [2].

3. RESULTS AND DISCUSSION

3.1. Essential Oil Yield of *Z. armatum*

The oil yield of fresh *Z. armatum* leaves (0.5%; v/w) was higher as compared to the shade dried (0.3%) plant material. The obtained essential oils were found to be pale yellow in colour. According to Weyerstahl *et al.* (1999), oil yield was acquired 0.5% from the fresh leaves of *Z. alatum* [23]. Luong *et al.* (2003) from Vietnam reported 0.52% oil yield of dried *Z.*

alatum leaves [24]. In a report from China, among six air-dried plant materials (leaves and branches) of the genus *Zanthoxylum*, the highest oil yield (0.53%) was observed for *Z. armatum* DC [25].

3.2. Essential Oil Composition of Fresh and Dried Leaves

GC (Figs. **1** and **2**) and GC/MS analysis revealed the presence of 42 and 51 out of which twenty-six and thirty compounds were identified representing 90.6% and 96.9% of the total oil in fresh and shade dried leaves of *Z. armatum*, respectively (Table **1**). The major components in the fresh oil were 2-undecane (30.0%), linalool (15.9%), (*E*)-β-ocimene (14.9%), α-pinene (7.4%) and β-phellandrene (6.7%) (Table **2**).

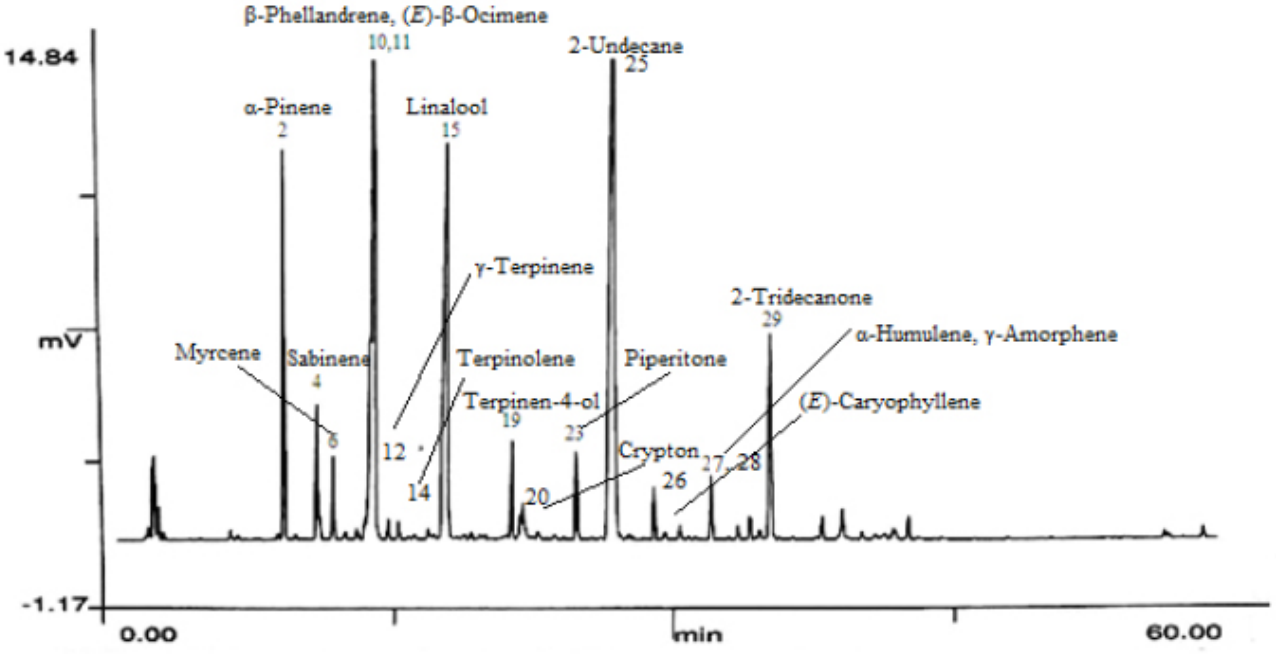

Fig. (1). Gas Chromatogram of ZFL essential oil.

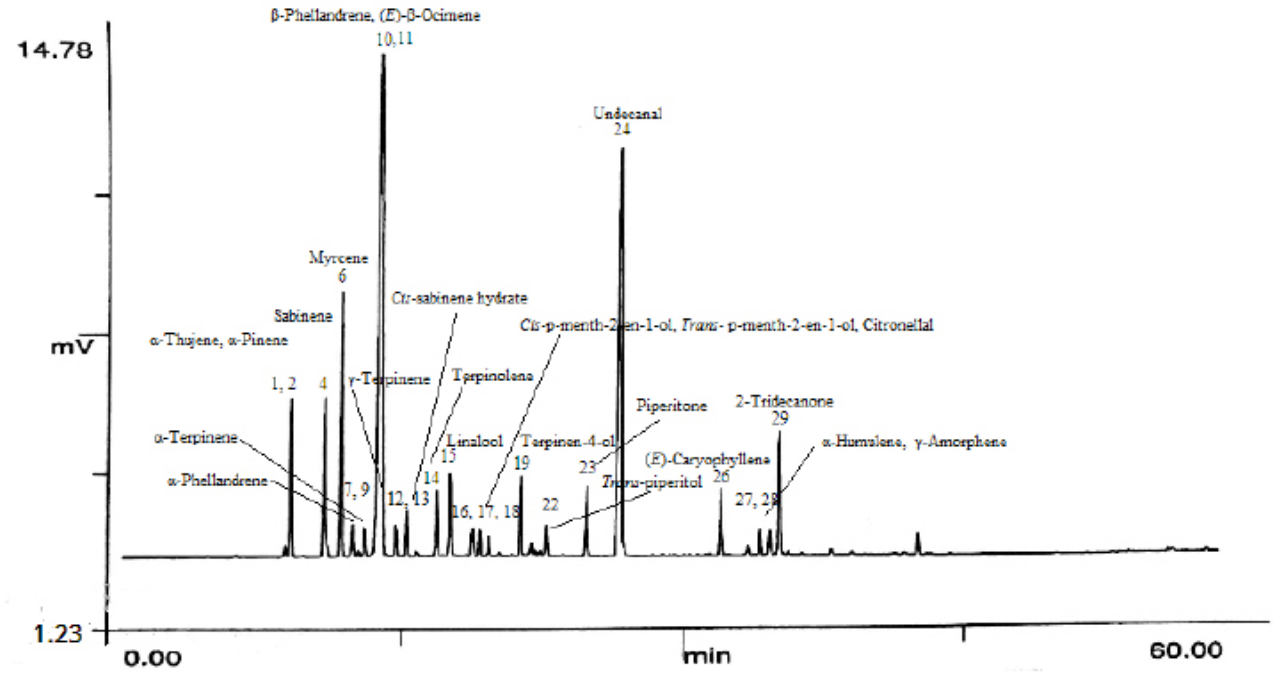

Fig. (2). Gas Chromatogram of ZDL essential oil.

Table 2. Effect of shade drying on the essential oil composition of *Z. armatum*.

S. N.	Compound[a]	$RI_{Calculated}$	RI [21]	RT_{ZFL} (min)	Mean Percent of Oil (ZFL) ± SD	RT_{ZDL} (min)	Mean Percent of Oil (ZDL) ± SD
1.	α-Thujene	930	924	8.8	0.1	8.8	0.1
2.	α-Pinene	932	932	9.2	7.4±0.6	9.2	3.6^{**}±0.7
3.	Camphene	946	946	9.8	0.1	-	ND
4.	Sabinene	972	969	10.9	2.2±0.3	10.9	3.9^{NS}±0.8
5.	β-Pinene	975	974	11.1	0.3	11.1	0.2
6.	Myrcene	993	988	11.8	1.4±0.5	11.9	7.6^{**}±0.5
7.	α-Phellandrene	1005	1002	12.4	0.1	12.4	0.8
8.	δ-3-Carene	1008	1008	-	ND	12.7	0.1
9.	α-Terpinene	1016	1014	13.1	0.2	13.1	0.9
10.	β-Phellandrene	1038	1025	13.8	6.7±0.4	13.9	35.5^{**}±4.3
11.	(*E*)-β-Ocimene	1048	1044	14.1	14.9±0.9	14.1	1.7^{**}±0.3
12.	γ-Terpinene	1058	1054	14.7	0.3	14.8	0.8
13.	*Cis*-sabinene hydrate	1069	1065	-	ND	15.8	0.2
14.	Terpinolene	1085	1086	16.9	0.1	16.9	1.8
15.	Linalool	1105	1095	17.7	15.9±0.6	17.6	2.7^{**}±0.3
16.	*Cis*-p-menth-2-en-1-ol	1126	1118	-	ND	18.8	0.8
17.	*Trans*- p-menth-2-en-1-ol	1143	1136	19.2	0.1	19.2	0.7
18.	Citronellal	1152	1148	19.7	0.1	19.7	0.4
19.	Terpinen-4-ol	1183	1174	21.4	1.6±0.5	21.4	2.4^{*}±0.7
20.	Crypton	1187	1183	21.9	0.5	21.9	0.3
21.	Piperitol	1197	1195	22.4	Trace	22.4	0.1
22.	*Trans*-piperitol	1210	1207	22.7	0.1	22.7	0.8
23.	Piperitone	1255	1249	24.8	1.8±0.3	24.8	1.7^{NS}±0.3
24.	Undecanal	1308	1305	-	ND	26.7	22.5^{**}±0.5
25.	2-Undecane	1310	1316	26.9	30.0±1.0	26.9	0.1^{**}
26.	(*E*)-Caryophyllene	1419	1417	32.1	1.2±0.3	32.1	1.7^{NS}±0.6
27.	α-Humulene	1452	1452	33.5	0.2	33.5	0.3
28.	γ-Amorphene	1498	1495	34.7	0.2	34.7	0.7
29.	2-Tridecanone	1502	1495	35.3	4.7±0.6	35.2	4.4^{NS}±0.7
30.	(*E,E*)-α-Farnesene	1506	1505	-	ND	35.7	Trace
31.	Germacrene B	1554	1559	37.9	0.4	37.9	0.1
Total					90.6	-	96.9
Class of compounds					-	-	-
Monoterpene hydrocarbons					33.8	-	57.2
Monoterpene alcohols					17.7		7.0
Oxygenated monoterpenes					2.4	-	2.9
Aliphatic compounds					34.7	-	27.0
Sesquiterpene hydrocarbons					2.0	-	2.8

[a]Mode of identification: Retention Index on RTx-5 column; ND = Not Detected; SD=Standard Deviation; RT = Retention time; Mean values ±SD (standard deviation) followed by **, * and ^{NS} indicate significance difference between pairs (fresh and sun dried) at $p < 0.01$, $p < 0.05$ and not significant respectively.

An Indian report suggested that the essential oil of mature dried seeds of *Z. armatum* was found to be rich in linalool [26]. In contrast, Waheed *et al.* (2011) from Pakistan reported that linalool was totally absent in the seeds oil of *Z. armatum*. However, 3-borneol was the major constituent [3]. Seeds contained the highest amount of linalool (71.0%) as compared to the other plant parts [26]. Reported data from Himachal Pradesh and Uttarakhand resemble with the presence of 2-undecanone as the major component in the leaf oil [5, 9]. On the other hand, *Z. alatum* from Vietnam has shown that 1,8-cineol (41.0%) was the major component [24] (Table **3**).

Table 3. Different species of *Zanthoxylum* along with major constituents of the essential oil.

S.N.	Collection	Plant part	Species	Major Constituents	Reference
1	**India** (north-west)	Aerial part (Fresh)	*Z. alatum*	1,8-Cineol (15.7%), linalool (18.8%) and 2-undecanone (17.0 %)	[23]
	(Northern India)	Seed	*Z. alatum.*	Linalool (71.0%), limonene (8.2%) and β-phellandrene (5.7%)	[26]
	India	Seeds	*Z. armatum*	Linalool (57-87.7%)	[8]
	Uttarakhand	Leaves	*Z. armatum*	Linalool (10.00-35.57%), limonene (1.59- 6.46%)	[29]
	Uttarakhand (Kumaun Himalaya)	Leaves	*Z. armatum*	2-Undecanone Rich	[9]
	India (Uttarakhand)	Leaves	*Z. armatum*	Bornyl acetate (16.61-22.66%), cymene (8.25-12.50%), α-copaene (7.54-7.59%) and γ-terpinene (5.33-7.66%)	[13]
	Himachal Pradesh	Leaves	*Z. armatum*	2-Undecanone (5.1-80.1%).	[5]
2	**Vietnam**	Leaves	*Z. alatum*	1,8–Cineole (41.0%), sabinene (8.4%) and terpinen-4-ol (5.2%)	[24]
3	**China**	Dried leaves and branches	*Z. armatum*	□-Terpinene (45.56%), piperitone (33.47%), and 3-carene (8.88%)	[25]
4.	**Pakistan**	Seeds	*Z. armatum*	3-Borneol (9.718%), iso- bornylacetate (9.574%) and dihydro carveol (8.816%)	[3]

Different extraction techniques, plant origin, genetic traits and other environmental factors might be responsible for these differences in chemical composition. In the oil from dried material, the predominant compounds were β-phellandrene (35.5%), undecanal (22.5%) and myrcene (7.6%). Reported data from China revealed the presence of *β*-terpinene (45.56%), piperitone (33.47%), and 3-carene (8.88%) as the major components in dried *Z. armatum* [25]. The mean percentage of α-pinene (7.4-3.6%), (*E*)-β-ocimene (14.9-1.7%), 2-undecane (30.0-0.0%) and linalool (15.9-2.7%) decreased significantly ($p<0.01$) while that of myrcene (1.4-7.6%), β-phellandrene (6.7-35.5%) and undecanal (0.0-22.5%) increased significantly ($p<0.01$) on shade drying of *Z. armatum*. Five compounds, namely, δ-3-carene, *cis*-sabinene hydrate, *cis*-p-menth-2-en-1-ol, undecanal and (*E,E*)-α-farnesene, which were absent in fresh plant material, appeared during the drying process. Only one component, *i.e*., camphene, disappeared in the oil of shade-dried plant material. The major composition of fresh *Z. armatum* leaves was aliphatic compounds (34.7%) followed by monoterpene hydrocarbons (33.8%) while monoterpene hydrocarbons (57.2%) and aliphatic compounds (27%) were the predominant compounds in the dried plant (Table **2**).

The main components of dried plant material, particularly *β*-phellandrene and myrcene, are reported to show antimicrobial and antifungal activity [27, 28]. During the drying process, the changes in the percentage of various volatile components are probably due to chemical reactions such as the breakdown of glycosylates, esterification, dehydration reactions, or oxidation reactions or may be the consequences of cell rupture [29].

CONCLUSION

In the present study, the essential oil composition of leaves of *Z. armatum* subjected to natural shade drying was compared with fresh oil using a two-tailed t-test. Finally, it can be concluded that shade drying showed a significant effect on essential oil yield and components. Shade drying decreased the oil content, percentage of α-pinene, (*E*)-β-ocimene and linalool. Therefore, efforts should be taken to illustrate the proper drying method for a particular plant species to increase the emerging market of drying.

ETHICAL STATEMENT

Fresh leaves of *Zanthoxylum armatum* were collected from the wild field of Lohaghat (Latitude 29.404178; Longitude 80.0943366; Altitude 5869 ft), Champawat district, Uttarakhand in the month of September 2018.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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