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Magar SS
M.Sc. Scholar, Department of PHM Medicinal, Aromatic, Plantation, Spices and Forest Crops, PGI-PHTM, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Shirke GD
Professor and Head, Department of PHM Medicinal, Aromatic, Plantation, Spices and Forest Crops, PGI-PHTM, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Kadam JH
Professor (CAS), Department of PHM, Medicinal, Aromatic, Plantation, Spices and Forest Crops, PGI-PHTM, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Bodake PS
Dean and Head, Department of Agronomy, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India

Kawade SA
M.Sc. Scholar, Department of PHM Medicinal, Aromatic, Plantation, Spices and Forest Crops, PGI-PHTM, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Corresponding Author:
Magar SS
M.Sc. Scholar, Department of PHM Medicinal, Aromatic, Plantation, Spices and Forest Crops, PGI-PHTM, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Studies on extraction of essential oil from patchouli (*Pogostemon cablin*)

Magar SS, Shirke GD, Kadam JH, Bodake PS and Kawade SA

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Abstract
There are several aromatic herb plants available today that produces essential oils, the plant known as patchouli (*Pogostemon cablin*) is one among these. The experiment was conducted in Factorial Completely Randomized Design (FCRD) for different parameters. The present research was carried out to identify the correct stage and season of harvesting of patchouli for better recovery of essential oil. The experiment consists of two treatments, which include plant parts of patchouli and sub treatments which include different seasons. The plant parts of patchouli consist the leaves and stems and three seasons i. e. rainy season, winter season and summer season were studied. The variation of different seasons on the recovery of essential oil of patchouli leaves and stems was calculated. From the present investigation, it was observed that the maximum essential oil recovery was observed in leaves as compared to stems, also the winter season gives maximum essential oil yield followed by rainy season and minimum in summer season. The major chemical compound of patchouli is patchouli alcohol. The patchouli alcohol was present higher amount in leaves essential oil as compared to stem essential oil of patchouli. The winter season gives maximum amount of patchouli alcohol chemical compounds than rainy and summer season.

Keywords: Patchouli, essential oil, rainy season, winter season, summer season

Introduction
Patchouli (*Pogostemon cablin*) is a widely grown aromatic crop that holds significant commercial value. It is a member of the Lamiaceae family, with delicate oval leaves and square stems, this bushy herb has a strong scent. When the leaves are rubbed, it produces a weird scent that is somehow distinctively patchouli. It grows to a height of two to three feet. Southern climates are ideal for the plant's growth (Kongkathip *et al*. 2009) [14]. Patchouli is also known guanghuoxiang in Chinese, Pachi in Sanskrit, Pachauli in Hindi, Pachapat in Bengali, Pachila in Malayalam, Pachetene in Kannada, Pacha in Gujarati, Panch in Marathi and Patchouli in French etc. 'Patchouli' is a name that means green leaves in Tamil (Yadav *et al*. 2017) [31]. In many locations around the world, it grows wild. Additionally, it grows naturally in Singapore, Malaysia and Indonesia. In Madhya Pradesh, Tamil Nadu, Kerala and Karnataka patchouli was first brought to India during the year 1941. A vital plant that supports the foreign exchange market is the patchouli plant (*Pogostemon cablin*). Indonesia provides 90% of the patchouli oil used worldwide (Ramya *et al*. 2013) [23].

Tropical plants like patchouli can also be grown in subtropical climates, up to a height of 800–1000 meters above mean sea level. It favors warm, muggy weather. Rainfall between 150 and 300 cm per year, which is very substantial yet evenly distributed, can be used to cultivate the crop. Patchouli needs loamy, rich in humus and nutrients, deep, well-drained, fertile, and somewhat acidic soil. It grows most readily in loose, deep loamy soils that are high in organic matter and have a friable, loose texture. For optimal growth, the pH of the soil should be between 5.5 and 7.5. It grows best in coastal regions with 80–90% relative humidity, well-drained sandy loam soil, pH 6.0–6.8, and temperatures between 20 and 35 °C (Anon, 2010) [4].

Patchouli oil is very slow to volatilize and is thick, viscous and sticky. The top note of premium oils is elusive, wine-like, flowery and sweet. As the oil ages (about one year after distillation), its top note becomes more dominant. The body note is extremely earthy, woodsy, balsamic, profoundly sweet and thick (Anon. 2005) [3]. It is discovered that 92% of the oil is made up of substances that barely affect its odor.

Sesquiterpenes make about 40-45% of the oil, of which 35-47% is made up of patchouli camphor, patchouli alcohol or patchoulol. Additionally, odorless is patchouli alcohol. However, it has been discovered that one or several patchoulol satellite compounds may be to blame for the distinctive smell (Sarwar *et al*. 1982) [27].

Essential oils are concentrated liquids that include a variety of substances, including hormones, vitamins, chemical components, aromatic compounds and other natural substances. Due to its antifungal and antibacterial qualities, patchouli oil is used to treat eczema, dandruff and skin infections. Additionally, it is a component of insect repellent products. Due to its antidepressant, anti-inflammatory, cytophylactic and deodorant characteristics, the oil is also utilized in aromatherapy. It is also utilized in a vast array of toilet soaps, fragrances, lotions, pre and post-shave products and detergents. Due to its high tenacity, it is especially well suited for heavy scents and for giving lesser perfumes a lasting character and power (Parganiha *et al*. 2016) [22].

Steam distillation, Soxhlet extraction, supercritical fluid extraction and pressured fluid extraction methods have all been used to extract patchouli oil (Shah *et al*. 2017) [28]. Commercially, fragrances and cosmetics mainly contain its oil (Hasegawa *et al*. 1992) [11]. It has bacteriostatic, anti-fungal and anti-insecticidal effects (Kukreja *et al*. 1990) [15]. In China, the decoction of the leaves is used with various medications to cure headaches, colds, diarrhea, nausea and vomiting. Patchouli's market potential has expanded due to its appealing aroma, adaptability and widespread use in the food, perfume, beverage, agarbathi, soap and cosmetics industries (Ambrose *et al*. 2013) [2].

Patchouli oil is extracted from fresh or dried leaves. When the plant's seeds, flowers and leaves crushed it release even more of the distinct patchouli aroma. The cultivation of the patchouli in Konkan region is increasing day by day. However, to identify the correct stage of harvesting of patchouli for better recovery of oil it is a prime need to standardize season and plant part.

Materials and Methods

The Patchouli plant material was collected from KVK field, Killa-Roha. Experiment was carried out in the laboratory of Department of Post-Harvest Technology and Management of Medicinal, Aromatic, Plantation, Spices and Forest Crops of Post Graduate Institute of Post-Harvest Management, Killa-Roha and field of KVK, Roha. The plant samples were collected as per the treatment and were dried in tray dryer at 45±5 ℃ up to constant weight and extraction of essential oil was carried out using hydro distillation method (Clevenger apparatus) for leaf and stem parts of the plant separately in rainy, winter and summer season for 6 hrs. The fresh weight of 250 g each of leaves and stems was taken for different treatments and seasons. The chemicals were procured from the Department of Post-Harvest Management of Medicinal, Aromatic, Plantation, Spices and Forest Crops of the Post Graduate Institute of Post-Harvest Technology and Management, Killa-Roha, Dist.-Raigad.

Research Design

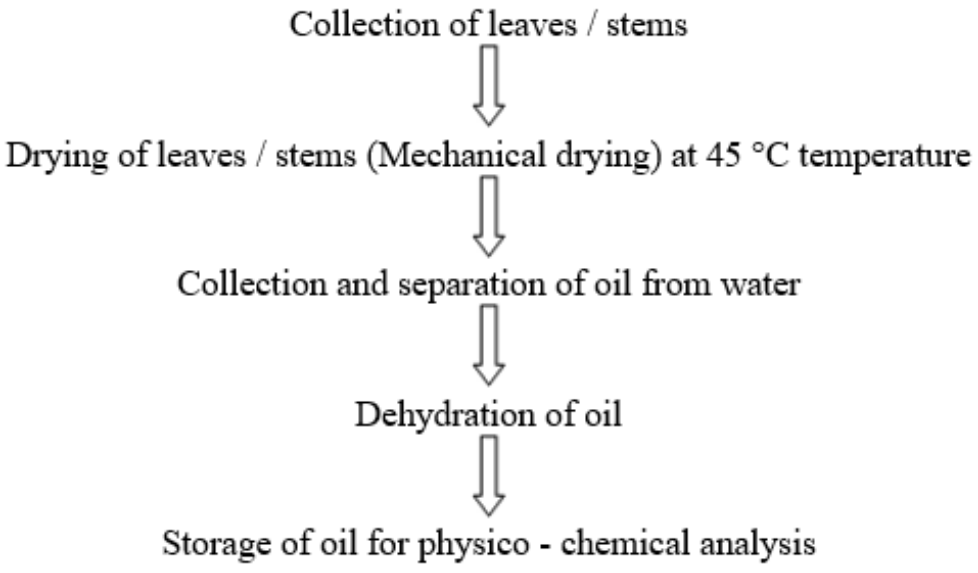
Main treatments (plant part used)	:	2 (Two) T_1: Leaves T_2: Stem
Sub – treatments (seasons of harvesting)	:	3 (Three) T_1: Rainy season T_2: Winter season T_3: Summer season
Replication	:	4 (Four)
Treatment combinations	:	2×3 = 6
Statistical design	:	FCRD

Methods adopted

Extraction of essential oil by hydro distillation

Patchouli oil was extracted from dried patchouli leaves and stems by hydro distillation method. Hydro distillation tests were performed in a circulatory Clevenger-type apparatus with a 3 L capacity, a round bottom flask, and a water condenser. In order to isolate the essential oil by hydro distillation, the plant material was kept in a round-bottom flask with adequate water to prevent burning and charring. It was warmed up and heated till boiling. The essential oil was released from the oil glands in the plant tissue as a result of the heat of the water. Then, using a condenser, the mixture of water and oil vapor was condensed. After condensing, the mixture was directed into the collector so that the water and essential oil could be separated there. Using anhydrous sodium sulfate, oil and water were separated from one another and dehydrated (Shah *et al*. 2017) [28].

Flow chart (hydro distillation of leaves / stems in Clevenger apparatus)



Physical and Chemical analysis methods

Recovery of dried leaves and stems (%)

The fresh leaves and stems were dried in tray dryer and after drying it was weighted. The recovery was expressed in percentage (Charles and Simon 1990) [7].

$$\text{Recovery of dried leaves and stems (\%)} = \frac{\text{Weight of dried leaves and stems}}{\text{Weight of fresh leaves and stems}} \times 100$$

Yield of oil extracted from leaves and stems (%)

The oil was extracted from leaves by hydro distillation method and after that yield was calculated (Ying chen *et al*. 2014) [32].

After extraction of oil from leaves, the yield of essential oil was calculated by following formula

$$\text{Essential oil (\%)} = \frac{\text{Volume of oil obtained (ml)}}{\text{weight of the dried leaves and stems taken (gm)}} \times 100$$

Specific gravity of oil (g/ml @ 25 ℃)

The specific gravity of oil was determined by using pycnometer. Weigh the empty pycnometer (m), fill the pycnometer with distilled water. Dip the pycnometer into a water bath at a temperature of 25 ℃ ± 2 °C for 30 minutes, then weigh the contents (m1). Empty the pycnometer, washed and cleaned, then the pycnometer was filled with patchouli oil samples, then put in the bath under the same conditions (Abram *et al*. 2021) [1].

Refractive index of oil (g/ml @ 20 ℃)

The refractive index of oil was determined by using Abbe Refractometer. The refractometer was kept in sufficient light and noted the temperature and then open the prism of Refractometer and clean with cotton dipped in alcohol. A drop of the patchouli essential oil sample was placed on the carefully cleaned fixed prism and slowly the two prisms of the refractometer were brought together and clamped. Wait for some time. The refractive index reading of the patchouli essential oil was read directly on the side scale through the other eyepiece. After taking the reading, the prisms were opened and carefully cleaned with a piece of lens paper (Pallavi, 2006) [20].

Acid Value (mg of KOH/g)

The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. Weigh accurately appropriate amount of the cooled oil sample in a 250 ml conical flask and add 50 ml to 100 ml of freshly neutralized hot ethyl alcohol and about one ml of phenolphthalein indicator solution. Boil the mixture for about five minutes and titrate while hot against standard alkali solution shaking vigorously during the titration. The weight of the oil taken for the estimation and the strength of the alkali used for titration so that the volume of alkali required for the titration does not exceed 10 ml (Hamid and Hamid, 2015) [10].

$$\text{Acid value} = \frac{56.1VN}{W}$$

Where,

V = Volume in ml of standard potassium hydroxide or sodium hydroxide used,

N = Normality of the potassium hydroxide solution or Sodium hydroxide solution

W = Weight in g of the sample

Ester value (mg of KOH/g)

The saponification value is the number of mg of potassium hydroxide required to saponify 1gram of oil/fat. Melt the sample if it was not already liquid and filter through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample was completely dry. Mix the sample thoroughly and weigh about 1.5 g of dry sample into a 250 ml Erlenmeyer flask. Pipette 25 ml of the alcoholic potassium hydroxide solution into the flask. Conduct a blank determination along with the sample. Connect the sample flasks and the blank flask with air condensers, keep on the water bath, boil gently but steadily until saponification is complete, as indicated by absence of any oily matter and appearance of clear solution. Clarity may be achieved within one hour of boiling. After the flask and condenser have cooled somewhat wash down the inside of the condenser with about 10 ml of hot ethyl alcohol neutral to phenolphthalein. Titrate the excess potassium hydroxide with 0.5N hydrochloric acid, using about 1.0 ml phenolphthalein indicator (Hamid and Hamid, 2015) [10].

$$\text{Ester value} = \frac{56.1\,(B-S)N}{W}$$

Where, B = Volume in ml of standard hydrochloric acid required for the blank, S = Volume in ml of standard hydrochloric acid required for the sample, N = Normality of the standard hydrochloric acid and W = Weight in gm of the oil taken for the test.

GC-MS analysis of patchouli essential oil

Volatile compounds, Patchouli alcohol (%)

Gas chromatography (GC), a method for measuring the volatile chemical constituents, was used to determine the composition of the essential oil. It is one of the four objective tests done to determine the quality, identity and purity of every essential oil. The Gas-Liquid Chromatography analysis of the patchouli essential oil samples were conducted. The GC-MS data analysis gives the chemical composition of the patchouli oil sample along with identification and tentative percentage composition of the constituents present in the oil sample (Yadav *et al*. 2017) [31].

Analysis condition: MS5970 column: HP-5MS 5% Phenyl Methyl Sibs:30m ×250µm×0.25µm.

Oven Program

50 ℃ for 4 min ⟶ 3 ℃/min to 230 ℃ for 4 min ⟶ 10 ℃/min to 280 ℃ for 7 min

Carrier gas: He:12.348 psi/MS

Sample injection/ split: 1µl of 5% solution in Acetone

Mass range: 35 to 450

Statistical analysis

As a part of this experiment, the storage treatment and storage time was estimated by using Factorial completely randomized design (FCRD) to improve experimental and statistical accuracy. Analysis and interpretation of data was carried out in accordance with Panse and Sukhatme (1985) [21] using Factorial Completely Randomized Design and valid conclusions were drawn only on significant differences between treatment mean at 5% level of significance.

Result and Discussion

Table 1: Variation between different treatments on physical parameters of patchouli influenced by different seasons

Parameters	Treatments	Rainy season	Winter season	Summer season
Recovery of dried leaves and stems (%)	T_1	37.79	37.77	37.89
	T_2	37.84	37.85	37.96
Yield of oil from leaves and stems (%)	T_1	2.198	3.333	1.905
	T_2	0.105	0.105	0.173
Specific gravity of oil (g/ml at 25 ℃)	T_1	0.972	0.978	0.972
	T_2	0.963	0.971	0.964
Refractive index of oil (g/ml at 20 ℃)	T_1	1.509	1.516	1.504
	T_2	1.336	1.365	1.325

T_1: Leaves, T_2: Stems, S1: Rainy season, S2: Winter season, S3: Summer season

Variation between recovery of dried leaves and stems (%) of patchouli influenced by different seasons

The data regarding variation between different treatments on recovery of dried leaves and stems of patchouli influenced by different seasons is given in Table no. 1. The higher recovery of dried leaves and stems recorded in stems (37.96%) and the lower in leaves (37.77%). The recovery of stem was higher due to the low moisture content in patchouli stem than leaves. Kulathilaka *et al*. (2016) [16] noticed the identical result in patchouli leaves and stems. During the different seasons, the highest recovery of dried leaves and stems was observed in the summer season, followed by rainy and winter season. The highest recovery of dried leaves and stems in summer due to low moisture content in the leaves and stems, followed by rainy and winter season. Similarly, Blank *et al*. (2007) [6] reported similar results in *Cymbopogon winterianus Jowitt* leaves.

Variation between yield (%) of essential oil extracted from leaves and stems of patchouli influenced by different seasons

The yield of essential oil extracted from leaves and stems was highest in the leaves (3.333%) as compared to the stems (0.105%) (Table 1). The yield of oil extracted from the leaves was higher due to the presence of large amount of oil glands in the leaves than the stems. The similar result was reported by Ying chen *et al*. (2014) [32] in patchouli leaves and stems. Jaafar *et al*. (2007) [13] noticed identical results in leaves and stems of *Etlingera elatiorn*. The highest yield of oil extracted from leaves and stems was observed in the winter season, followed by the rainy and the summer season. Winter season yields the maximum amount of oil in the leaves and the stems because of a number of factors, including climate, storage duration, cultivation pattern and extraction method. During winter season the temperatures were low enough to minimize oil loss from the plant part as well as during the extraction process. Because of the longer photoperiod and higher temperatures throughout the summer, the oil output was decreased. The similar result was mentioned by Luo *et al*. (2000) [19] in patchouli leaves and stem indicates higher oil yield during winter season. The identical results were mentioned by Rathore *et al*. (2022) [24] in *Rosmarinus offcinalis L*. Wang *et al*. (2012) [30] observed similar results in *Schefflera heptaphylla* in leaves and stem oil and Hussain *et al*. (2008) [12] in basil leaves.

Variation between the specific gravity (g/ml at 25 ℃) of patchouli essential oil extracted from leaves and stems influenced by different seasons

The specific gravity of essential oil extracted from patchouli was higher in the leaves (0.978 g/ml at 25 ℃) and lower in the stem (0.964 g/ml at 25 ℃) oil (Table 1). The yield and technique of essential oil extraction had significant impact on the quality of the extracted oil as well as the specific gravity. The specific gravity increases with increasing patchouli oil component content. Abram *et al*. (2021) [1] noticed the same result in patchouli. The identical result was observed by Espino *et al*. (2002) [9] in patchouli. The specific gravity of oil was higher in the winter season, followed by the rainy season and the summer season. Specific gravity increases with decreasing temperature. Bhardwaj *et al*. (2019) [5] showed the identical result in *Hedychium spicatum* rhizomes.

Table 2: Variation between different treatments on chemical parameters of patchouli influenced by different seasons

Parameters	Treatments	Rainy season	Winter season	Summer season
Acid value (mg of KOH/g)	T_1	3.767	3.867	2.747
	T_2	3.428	3.615	2.525
Ester value (mg of KOH/g)	T_1	13.568	13.937	12.626
	T_2	13.435	13.385	11.826
Patchouli alcohol (%)	T_1	50.206	53.245	47.525
	T_2	42.235	44.325	40.434

T_1: Leaves, T_2: Stems, S1: Rainy season, S2: Winter season, S3: Summer season

Variation between the refractive index (g/ml at 20 ℃) of patchouli essential oil extracted from leaves and stems influenced by different seasons

The refractive index of leaves and stems oil was highest in leaves oil (1.516 g/ml at 20 ℃) and lowest in the stems oil (1.325 g/ml at 20 ℃) (Table 1) In order to produce purer leaf oil, the refractive index technique evaluates the speed at which light passes through the essential oil and reflects back. Patchouli oil's density and viscosity have a significant impact on its refractive index value. The higher is the density, the higher is the oil's refractive index value. The identical result was reported by Singh *et al*. (2020) [29] in *Curcuma longa L*. and Sandhyavali *et al*. (2011) [26] in *Saussurea simpsoniana*. The refractive index of leaves and stems oil was higher in winter season, followed by the rainy season and summer season. Temperature and wavelength

affect the refractive index. Bhardwaj *et al*. (2019) [5] showed the similar result in *Hedychium spicatum* rhizome. Hussain *et al*. (2008) [12] also reported identical result in basil leaves.

Variation between acid value (mg of KOH/g) of patchouli essential oil extracted from leaves and stems influenced by different seasons

The data regarding Variation between different treatments on acid value of patchouli essential oil influenced by different seasons is given in Table no. 2. The acid value of essential oil was higher in leaves oil (3.867 mg of KOH/g) and the lower in stem oil (2.525 mg of KOH/g). The leaves oil has greater purity, quantity and quality. The presence of free acid compounds in the oil sample can be determined the acid number. Abram *et al*. (2021) [1] noticed the same result in patchouli leaves. The identical result was observed by Dutta *et al*. (2014) [8] in lemongrass leaves. As regards seasonal effect, the highest acid value of leaves and stems oil was observed in the winter season, followed by rainy and summer season. The high acid content of patchouli oil may have an effect on its quality. This is due to the fact that the chemical components of patchouli oil that come into direct contact with the air or that are present in humid conditions produce a large amount of free acid compounds, which lead to an oxygen-catalyzed oxidation process with the air. Bhardwaj *et al*. (2019) [5] reported the identical result for acid value of oil in *Hedychium spicatum* rhizome.

Variation between the ester value (mg of KOH/g) of patchouli essential oil extracted from leaves and stems influenced by different seasons

The ester value of leaves and stems oil was highest in leaves (13.937 mg of KOH/g) and lowest in stems (11.826 mg of KOH/g) (Table 2). The ester number is influenced by the length of distillation; the greater the ester number, the longer the distillation period. Patchouli oil's strong and enduring scent is a result of its high ester number, which denotes its resistance to oxidation. The identical result was reported by Abram *et al*. (2021) [1] and Lubis *et al*. (2021) [18] in patchouli leaves. The ester value of leaves and stems oil was higher in the winter season, followed by in the rainy season and the summer season. The ester value is responsive to the increased temperature. Bhardwaj *et al*. (2019) [5] noticed the similar results in *Hedychium spicatum* rhizome.

Variation between patchouli alcohol (%) component of patchouli essential oil extracted from leaves and stems influenced by different seasons

The patchouli alcohol of oil was highest in leaves oil (53.245%) and lowest in stem oil (40.434%) (Table 2). The alcohol found in patchouli, known as sesquiterpenes, was greatly affected by a number of variables, including temperature, sun exposure, precipitation and insect impact. Ying chen *et al*. (2014) [32] noticed the identical results in patchouli leaves and stems oil. The patchouli alcohol of leaves and stems oil was higher in winter season, followed by the rainy season and the summer season. The observed variances in the contents could be the result of various chemical kinds, plant nutrition, environmental and genetic variables. Hussain *et al*. (2008) [12] reported similar result in basil. Patchouli alcohol was main component of patchouli. Luo *et al*. (2000) [19] reported the identical result in patchouli in that patchouli alcohol was higher in November and lower in other month. The same result was found by Rawat *et al*. (2016) [25] in *Valeriana jatamansi* Jones in roots wherein he reported that patchouli alcohol was higher in winter season than other seasons. Lakusic *et al*. (2012) [17] also noticed the same result in rosemary in that the main content was higher in the winter season.

Conclusion

The oil was extracted from the patchouli plant parts by hydro distillation method. The patchouli leaves yield higher amount of oil as compared to patchouli stems that yields a lower amount of oil. The patchouli is a perennial plant of which oil quality and yield was higher in the winter season, followed by rainy and summer. During winter season, the oil yield was higher as compared to the rest of the seasons. As compared to leaves and stems essential oil, the chemical compound present in winter season oil had better quality having better recovery, as compared to other seasons. The leaves oil of patchouli had higher essential chemical component than stem oil of patchouli.

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