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Physico-Chemical Characteristics and Stability of Essential Oil from Dried Leaves of *Pogostemon heyneanus* Benth.

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Abstract

Essential oils were extracted from air-dried leaves of *Pogostemon heyneanus* Benth. (Indian patchouli) by steam distillation using a Clavenger tube. Physico-chemical properties of the oil like acid value, ester value, optical rotation, refractive index, saponification value, solubility in 90% ethanol, specific gravity, % alcohol, and % aldehyde was deter-mined. Stability studies of the essential oil included the determination of the effect of light, length of storage and temperature. Based on the set standards, the physico-chemical properties of *Pogostemon heyneanus* Benth. oil do not fall within the specifications for the oils of patchouli. However, these properties may improve later because the quality of the oil could improve through time. Percent alcohol of the oil samples exposed to both light and dark conditions decreased while their percent aldehyde increased. Thin-layer chromatograms showed that middle spots of polar components were not detected under low temperature after 2 hours and more of exposure. At room temperature, at 800C, 1 IOOC, and 1400C, the same number of spots were observed after 2 hours and beyond but at 1700C and boiling point temperatures, an increased number of spots was observed. TLC results of samples exposed to light and dark conditions, respectively, showed an increased number of spots on the fifth month of storage while the samples became more viscous and their color changed from light yellow to yellow-orange.

Keywords: *Pogostemon heyneanus* Benth., essential oil, steam distillation, stability, percent alcohol, percent aldehyde, thin-layer chromatography

Introduction

Essential oils are highly concentrated volatile extracts. They are derived directly from leaves, flowers, fruits, stems, wood, mosses, and roots of very specific and special plants. Being used for thousands of years and known for their remarkable healing properties, essential oils are the world's most concentrated form of herbal energy, and are referred to as "the lifeblood of plants" (www.geavision.com).

At present times, the production of essential oils has attracted many people, including farmers, agriculturists and others having little knowledge even of growing the plants; in search for additional economic crops or profitable sidelines due to the depression of the Philippine economy and the competing demands on thése essential oil resources (Ansaldo,1981).

Robbins (1982) stressed that the production of various commercial products in the Philippines is very feasible because it can grow a wide variety of plant species known to contain essential oils. Pogostemon heyneanus Benth. (Indian patchouli) grows wild in several parts of the world (Quisumbingy 1978). It is locally known as kadlum. It is one of the most common plants in the Philippines.

Listed among the plant's purported uses among the Filipinos are the following: the leaves and tops are used as a repellant of cockroaches, moths and ants. Often crushed with "gugo", the leaves are used for washing the hair; In addition, the essential oil of patchouli is used as anti-inflammatory, astringent, and for fungal infections. It' encourages the generation of skin cells; speeds up the healing of wounds and sores; and helps to fade the scars (Ansaldo, 1981).
In the perfume industry, the oil is one of the most important materials available to a perfumer and is used very widely in many types of perfumes; being mixed well with many other aromas due to its Very deep earthy fragrance that has a subtle herbal sweetness (Robbins, 1982; Quisumbing, 1978).

Despite the various uses and importance of patchouli, there are no published researches made locally on the factors affecting the stability of its oil. The effects of temperature, length of storage and light on the stability of the essential oil from patchouli were thus studied. The results are envisioned to provide baseline information on the storage and keeping quality in order to overcome transport problems, and to enhance the export feasibility of this product.

Review of Literature

Essential Oils

Essential oils are the odorous principles found in various plant parts. They are also called volatile oils or ethereal oils because they evaporate when exposed to the air at ordinary temperatures (Santos, 1981). Essential oils, in some cases, are also known as "essences" or odoriferous constituents of the plants (Jenkins et al., 1957). As a rule, they are colorless particularly when they are fresh, but on long standing, they flay oxidize and resinify thus darkening in color.

Depending on the plant family, essential oils may develop in specialized secretory structures such as glandular hairs (Labiatae), modified parenchyma cells (Piperaceae), oil-tubes called vittae (Umbelliferae), or in lyzigenous or schizogenous passages (Pinaeeae, Rutaceae) (Belardo, 1980).

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The traditional methods of extracting essential oils are distillation, expression, extraction with low boiling solvents, and enfleurage (Concise Encyclopedia of Chemical Technology, 1985). de Castro (1980) stated that steam or hydro distillation is the preferred and the most commonly used method for producing essential oils, employing either water or steam.

Generally, essential oils are liquid at room temperature; however, some are semi-solid, and several are solid. When pure and fresh, most of the essential oils are colorless, but when exposed to air, they gain various colors. In addition, their odor is sensibly modified and they possess various tastes such as sweet, earthy, acrid, caustic, hot, and spicy (www.nappies-direct.co.uk).

Almost all of the essential oils can easily penetrate the skin, bronchioles, nasal passages, lungs and gastro-intestinal tracts because they are fat-soluble and do not dissolve in water (Belardo, 1980). They have high refractive indices and are mostly optically active. At ordinary temperatures, they are usually liquid but crystallize on long standing (Tyler et al., 1988).

An essential oil is characterized by an enormous chemical complexity (de Castro, 1980). Almost every class of organic compounds like hydrocarbons, oxygenated, sulfuretted, nitrogenated and even halogenated compounds, is likely to be present in multiple combinations and in varying proportions.

With very few exceptions, the essential oils are generally mixtures of hydrocarbons and oxygenated compounds that differ greatly in chemical compositions. Their volatile components usually contain fifteen carbon atoms or less. The most important chemical components of essential oils are hydrocarbons, alcohols, aldehydes, ketones, phenols, acids, and sulfur compounds (Jenkins et al., 1957; de Castro, 1980; www.benzalco.com).

Patchouli Plant

Patchouli is a fragrant shrubby East Indian plant of the mint family. It particularly belongs to the division Magnoliophyta, class Magnoliopsida, order *Lamiales* and family *Labiatae* (Quisumbing,1978). It is a small shrub which grows in China, Singapore and Madagascar. Indonesian farmers cultivate this plant which originally came from the Philippines (Encyclopedia Britannica, 1973; www.sunspirit.com.au).

In the Philippines, patchouli grows from 0.5 to 1.0 meter in height. It is an aromatic, erect, branched, and hairy herb in which the leaves are ovate to oblong-ovate, 5 to 11 cm long; usually with coarse and doubly-toothed margins and with pointed or blunt tip. Its flowers are pink-purple, crowded, and borne in hairy, terminal, axillary spikes 2 to 8 cm long, and 1 to 1.5 cm in diameter. With obtuse lobes, the corolla is 8 mm long, and its calyx is about 6 mm long (Quisumbing, 1978).

In distilling the material, Valderrama (1988) suggested that leaves be used because the stalks contain a low percentage of oil.

The Philippine patchouli plant oil contains the following chemical components: patchouli alcohol (also known as patchouli camphor), cadinene, coerulein, benzaldehyde, and eugenol (Quisumbing, 1978).

Among the plant's pharmaceutical potentials include the healing of various types of skin conditions such as rough, cracked skin; wounds; and sores. It gives a warming and soothing relief to the skin; a kind of facial oil for dry, tired or aging skin; and is also used to tighten and bind tissues. Having great germicidal properties, patchouli oil makes an excellent topical treatment for athlete's foot and impetigo. As an astringent, patchouli is added to shampoos or rinsing water for the treatment of dandruff and scalp complaints (www.benzalco.com; www.sunspirit.com.au).

Identification, Locale and Sample of the Study

An estimated 65-kg fresh sample of patchouli leaves were obtained from Guilon, Peoaplata, Samal City and transported to the Ateneo de Davao University Chemistry Laboratory where the analyses were conducted.

The leaves were separated from each stem manually and cleaned from soil, dust, dirt and pests by washing in water. These were air-dried for three days, and cut into small pieces before extraction. Oil samples were extracted by batch method. Appendix 1 shows the general flow of analyses.

Some stems, leaves and flowers were separated, pressed overnight and were sent, together with their respective photos, to the Philippine National

Museum, Metro Manila for authentication.

Extraction of the Oil

Oil samples were extracted in the first four months of the study using the steam distillation process. The extracts were collected in a Clavenger tube receiver. Anhydrous sodium sulfate was added to the oil to remove traces of water. The oil was transferred in amber-colored bottles which were tightly covered and kept in the refrigerator prior to the laboratory analyses.

A. Stability Studies of the Essential Oil

Determination of the effect of light and length of storage. Two 2.5-ml patchouli oil samples were placed in amber-colored vials separately. One vial was stored in the dark while the other was kept indoor under ordinary fluorescent light. Thin layer chromatography (TLC) was performed on the oil samples before storage and every two weeks thereafter for five months. Temperature was periodically monitored. The number and size of spots were determined on TLC plates (Merck, silica gel F_{254}) using toluene as solvent and iodine crystals as developer. The number and size of spots were recorded and any change in color was noted. For comparative purposes, any change in the number of spots in the chromatogram served as a gauge of the change in the quality of the oil.

The % alcohol and % aldehyde of the two oils were also • analyzed monthly. The respective temperatures of the two oils were also periodically monitored.

Determination of the effect of temperature. Prior to the determination of the effect of temperature, the boiling point range of the oil was determined.

After establishing the boiling point range, a new set of 25-mL oil samples in stoppered Erlenmeyer flasks were subjected to low temperature (1518^{0}C), room temperature (28-32^{0}C), 80, 110, 140, and 170^{0}C and the boiling point temperature, respectively, at different times (i.e., 2, 4, 6, and 8 hours). TLC was done periodically and compared against the control oil samples.

Analyses on the % alcohol and % aldehyde of the oil were done prior to and after the subjection of oil at 50^{0}C for 2 and 4 hours, respectively.

B. Physico-Chemical Analysis of the Essential Oil

The various physico-chemical properties of the oil such as the acid number, ester value (by saponification with heat), optical rotation (using a polarimeter), refractive index (using an Abbe refractometer)), solubility (APA, 1975), saponification value (Rummel, 1992; Snell and Biffen, 1944), % alcohol methanol; Jenkins et al., 1957), % aldehyde (as bénzaldehyde; AOAC, 1997), and specific gravity (Snell and Biffen, 1944; McMurry, 1996) were determined.

Results and Discussion

Percentage Yield of Patchouli Oil

Pogostemon heyneanus Benth. oil was extracted by steam distillation from the dried leaves of patchouli plant collected from Guilon, Peñaplata, Samal City. Steam distillation method gave a yield of 0.2% (v/w, on a wet basis) from dried samples.

Physico-Chemical Properties

Freshly distilled patchouli oil is clear, yellowish oil possessing a somewhat strong smell that changed considerably with time. Aging of the oil for a prolonged period is reported to result in a full, rich, and almost fruity smell for which the best grades of patchouli oil is known of (APA, 1975). In addition, patchouli oil aged for several years possesses a much finer and fuller odor than one freshly distilled.

Table 1 shows the physico-chemical properties of the oil extracted from patchouli leaves. These properties were compared with the standards used by the Essential Oils Association of the USA, International Organization for Standardization, and British Standards (Robbins, 1982).

Table 1. Mean values of some physico-chemical properties of the essential oil extracted from dried leaves of *Pogostemon heyneanus* Benth. and some standards

Physico-chemical property	Value	Standard value		
		EOA No. 23	ISO 3757: 1978	BS 2999/10: 1965
Acid value	0.66 (±0.04)	Not more than 5	4, maximum	4, maximum
Ester value	18.3 (±0.6)	-	10, maximum	10, maximum
Optical rotation at 20°C	(+72.4) – (+102.1)	–48° to –65°	–40° to –66°	–40° to –68°
Refractive index at 20°C	1.4880	1.5070 to 1.5150	1.5050 to 1.5120	1.505 to 1.512
Saponification value	21.5 (±0.4)	Not more than 20	-	-
Solubility in 90% v/v ethyl alcohol at 20°C	3.52 in 10 volumes	1 in 10 volumes	1 in 10 volumes	1 in 10 volumes
Specific gravity at 25°C/25°C	0.90338 (±0.00001)	0.950 to 0.975	-	-
Percent alcohol	37.4 (±1.3)	-	-	-
Percent aldehyde	4.4 (±0.3)	-	-	-

The refractive index and specific gravity of the oil sample were found to be 1.4880 at 2000 and 0.90338(±0.00001), respectively. The usually high specific gravity of this oil may be attributed to evaporation of the lower boiling constituents of the oil in the plant. It is also likely due to oxidation and polymerization, which in turn, affects the properties and the quality of the oil. The lower value of specific gravity of oil, however, implies that it has not undergone oxidation and polymerization since it was freshly distilled. It was also observed that the oil is lighter than water and this usually attributes for high content of alcohol, ester, ketone, and hydrocarbon (Brandares et al., 1987: Robbins, 1982).

The oil gave ester, acid, and saponification values of 18.3(±0.06), 0.66(±0.040), and 21.5(±0.4), respectively. The high saponification value was due to high ester content of the oil, while the low acid value is expected since the sample oil was freshly extracted.

Patchouli oil is soluble in 75-90% ethyl alcohol, completely miscible at 3.52 in 10 volumes. It was observed that a little volume of alcohol is needed to solubilize the oil sample. This resulted in low acid value of the oil. It also showed an optical rotation of +72.4 to +102.1 at 200C.

The boiling point of the oil extract ranged from 180.3-182. I OC, indicating the presence of constituents with high polarity and high molecular weights, predominantly that of alcohol.

The above physico-chemical properties were performed to establish the purity of the oil. Most of the results obtained, however, fell outside the range of standard values set by the EOA No. 23, BS 2999/10:1965. and ISO:375 for the oils of patchouli. These physico-chemical properties, however, may improve through time (Guenther, 1956).

The quality of patchouli oil could also be determined by its aldehyde and alcohol contents. As shown in Table 2, freshly distilled samples gave 4.4(±0.3) and 37.4(±1.3) % aldehyde and % alcohol, respectively. The low % aldehyde may be due to the influence of the site of collection and the climatic conditions during collection. In addition, chemical changes may occur during the distillation, such as the hydrolysis of an ester to the corresponding acid and alcohol (Brandares et al., 1987; Guenther, 1956).

Table 2. Mean values of % alcohol and % aldehyde of essential oil as fresh, or as heated at 50°C for 2 or 4 hours

	Fresh	Heated at 50°C for 2 Hours	Heated at 50°C for 4 Hours
% Alcohol	37.4 (±1.3)	12.8 (±1.3)	10.9 (±1.3)
% Aldehyde	4.4 (±0.3)	79.6 (±0.3)	42.6 (±0.3)

When oil was heated, its aldehyde content drastically increased. This may be influenced by the oxidation of its alcohol content. Prolonged heating, however, reduced the % aldehyde due to further oxidation to acid. Consequently, alcohol content of the oil decreased due to the same reason.

Stability Studies

Essential oils are generally unstable and spoilage may be attributed to such general reactions as oxidation, resignification, polymerization, hydrolysis of ester and to interactions of functional groups. These processes may be influenced by the presence of oxygen, heat, or moisture and catalyzed by exposure to light and possibly by metals. In the storage of the oil, it is very important to exclude air and light and to properly dry the oil before storing in containers 6www.benzalco.com; Encyclopedia Britanica, 1973; www.sunspirit.com.au).

Patchouli oil samples were subjected to different temperatures including its boiling point range at different times. After which, TLC was done. The thin layer chromatograms in Figure 1 show the relative separation of the components and the significant spots of the oil sample subjected to the different temperatures.

After 2 hours at low temperature, middle spots of polar components were not detected. This may be due to poor resolution of the samples in the chromatogram plates. At room temperature, 80^0C, 110^0C, and 140^0C, there was a noticeable difference. in the arrangement of 8 spots. However, the same number of spots was observed on the above-mentioned temperatures, for 170^0C and boiling point temperatures, there was a visible change observed. After more than 2 hours of exposure, there were now 9 spots observed, using both the ultraviolet light and the iodine crystal developing solvent. The number of spots in the control samples remained constant in all the chromatograms. Chemical composition of the oil, however, was not identified since this was beyond the scope of the study.

To further investigate the nature and stability of the patchouli oil, comparative studies on the effect of light and length of storage were also undertaken. Figure 2 shows the thin layer chromatograms of the samples.

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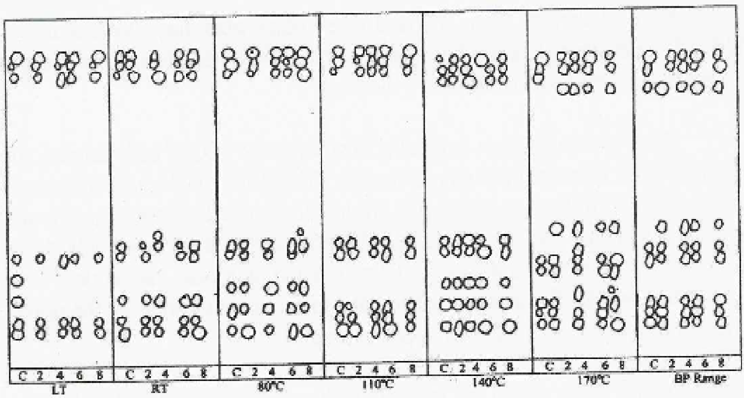
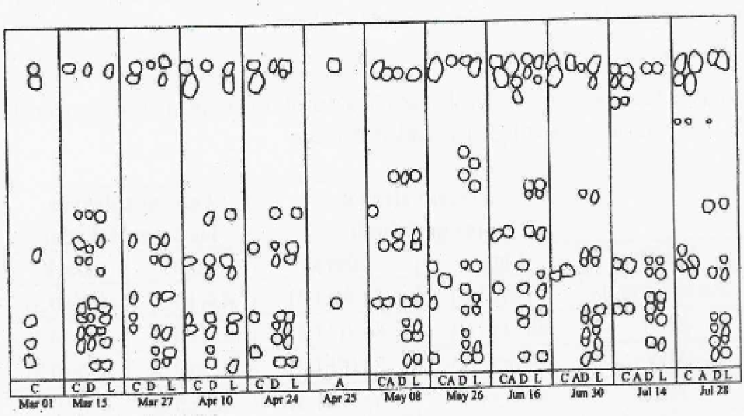


Legend: C = Control oil
2,4,6,8 = Number of hours at which oil samples were subjected
LT = Low temperature (15-18°C)
RT = Room temperature (28-32°C)
BP = Boiling point (180.3-182.1°C)

Figure 1. Thin-layer chromatograms of patchouli oil as subjected to different temperatures at different times.



Legend: C = Control
D = Dark-exposed oil sample
L = Light-exposed oil sample
A = Amber-colored storage vial of fresh oil sample

Figure 2. Thin-layer chromatograms of patchouli oil as subjected to light and dark, monitored every other week for five months

Two oil samples were analyzed every other week for 5 months-using TLC, % aldehyde and % alcohol was also monitored monthly and the storage temperatures were taken weekly for 5 months.

There was an increase in number of spots of the TLC of both samples. It was noted that from the third month onward, TLC plates had 4 samples because of the addition of the new sample (A, freshly distilled) which was stored in an amber-colored vial. In the first 2 months of analyses, there were only three samples because the control sample (C) was stored on a clear vial while it was being refrigerated. It was expected to have undergone oxidation by the direct effect of light because of the kind of container used.

On the fifth month of storage, both samples had almost the same conditions because of the increase in the number of their respective spots. In addition, both samples became more viscous and their color changed from light yellow to yellow-orange. The viscosity and resinosity of the oil may be influenced by dehydration and polymerization of its aldehyde content.

The mean values of % alcohol and % aldehyde of the oil samples which were monitored monthly are summarized in Table 3.

Table 3. Mean values of % alcohol and % aldehyde of essential oil as monitored monthly under light and dark conditions

	Percent alcohol (as methanol)		Percent aldehyde (as benzaldehyde)	
Month	Light	Dark	Light	Dark
March (Fresh)	37.4(±1.3)	37.4(±1.3)	4.4(±0.3)	4.4(±0.3)
April	88.3(±1.3)	86.2(±1.3)	2.9(±0.3)	1.4(±0.3)
May	79.6(±1.3)	74.0(±1.3)	3.2(±0.3)	1.5(±0.3)
June	41.8(±1.3)	62.3(±1.3)	4.2(±0.3)	4.9(±0.3)
July	34.7(±1.3)	42.5(±1.3)	4.2(±0.3)	5.8(±0.3)

The % alcohol of the oil samples increased from 34.4(±1.3) (fresh oil) to 88.3(±1.3) and 86.2(±1.3) for light-exposed and dark-exposed, respectively, after the first month of storage. Low percentage of alcohol on the month of March was due to the presence of water content on the samples since they were still freshly extracted. After the first month of storage, the samples might have been free from •traces of water thus resulting in an increase of % alcohol. This may also be influenced by the sudden change of storage conditions due to the catalytic action of light and heat.

On the month of May and onward, however, % alcohol gradually decreased and finally dropped to 34.7(±1.3) for light-exposed and 42 5(±1.3) for dark-exposed on the fifth month. This decrease of % alcohol was influenced by its oxidation to aldehyde. In effect, there was an increase of the latter on the fifth month.

The % aldehyde of both samples had a significant drop after the first month of storage. This indicates that reduction of aldehyde to alcohol and taken place. However,

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it gradually increased on the succeeding months and finally reached 4.2(±0.3) for light-exposed, and 5.8(±0.3) for dark-exposed samples, respectively, on the fifth month.

Conclusions

Based on the findings of the study, essential oil was extracted from dried leaves of Indian Patchouli (Pogostemon heyneanus Benth.) plant by steam distillation using a Clavenger tube and this has given an average yield of 0.2%.

The physico-chemical properties of the essential oil, except the acid value, were not found within the range of the standard specifications for the oils of patchouli set by the EOA No. 23, BS 2999/10:1965, and ISO:37

Specifically, high ester value 18.3(±0.6) might have resulted from shorter reflux time of the oil; which in turn, also gave a higher saponification value 21.5(±0.4). However, these properties may improve through time. In addition, aged patchouli oil possesses a much finer and fuller odor than one freshly distilled (Guenther, 1956). As a result, the oil can be qualified for export market.

The TLC showed that at low temperature, no conclusion could be drawn because middle spots were not detected. At room temperature, at 800C, 110^0C, Ad 140^0C, no change in number of spots was observed. TLC for 170^0C and boiling point temperatures revealed that an increase in number of spots was present after 2 hours and more of exposure. Control samples showed the same number of spots in all the chromatograms.

Light and length of storage of the essential oil affected its chemical components. This was true because of the increase in the number of spots of both light and dark-exposed samples. In addition, oil samples became more viscous and the color changed from light yellow to yellow orange. These may be due to dehydration and polymerization of the aldehyde content of the oil (Brandares et al., 1987). Furthermore, the % aldehyde increased and consequently, the % alcohol decreased due to the oxidation of the same to aldehyde.

Recommendations

Generally; it is recommended that oil of patchouli should still be industrially produced in the Philippines on large scale because its demand abroad is increasing, and that the plant is easy to cultivate. Further, a follow up study on the oil of Pogostemon heyneanus Benth. is to be done.

Specifically, the following are recommended:

1. Analyze the chemical composition of the same species to further investigate its pharmacological importance;
2. Determine the best method on the processing of oil extracts for a

feasible and acceptable commercial quality; and

3. Determine the best method of oil storage to overcome immediate deterioration and to enhance keeping quality.

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Appendix 1

Flow Chart of the Stability Studies and Physico-Chemical Analyses