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ANTIBACTERIAL POTENTIALITY OF *Pogostemon cablin* ESSENTIAL OIL IN THE ITASY REGION

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Abstract

Essential oils (EO) are known for the variability of their characteristics depending on several parameters, including location and soil type. *Pogostemon cablin*, Benth belonging to the Lamiaceae family, a herbaceous plant introduced to Madagascar, is our study material. The aim of this work is to develop the EO of *Pogostemon cablin*, Benth, or Patchouli, in the Itasy region, a new production site in the Malagasy highlands. The aerial part of this plant is harvested in June 2022 and dried in the shade for 4 weeks.

After extraction by hydrodistillation using a Clevenger-type apparatus, the HE's physico-chemical parameters were measured in accordance with AFNOR standards. Its composition was determined by gas chromatography/flame ionization detection (GC/FID). Antimicrobial activity was assessed on solid and liquid media against 6 pathogenic bacteria, including 3 gram + and 3 gram -.

According to the results, the HE yield is 1.62% after 3 hours of hydrodistillation. The relative density at 20°C is 0.986, its rotatory power is -55°41, with a refractive index of 1.5109. The acid and ester indices are 2 and 7 respectively. The EO contains 51.16% Patchoulol, β-caryophyllene (10.77%), α-humulene (9.52%) and α-terpineol (8.11%),

Of the 6 strains tested, *Bacillus cereus, Salmonella typhi and Clostridium perfringens were extremely sensitive*. Their inhibition halo diameters are 40mm, 40mm and 21mm respectively. However, *Staphylococcus aureus*, *Vibrio fischeri* and *Escherichia coli* remain insensitive. Among *highly sensitive* strains, *the* BMC/ MIC ratio revealed that HE had a bactericidal effect on *Clostridium perfringens* and *Bacillus cereus*, and a bacteriostatic effect on *Salmonella typhi.*

Pogostemon cablin essential oil *is* endowed with antibacterial activity, an alternative for the treatment of infectious diseases. This plant is well known in perfumery, and its use against *Clostridium perfringens is promising.*

Key words: *Pogostemon cablin*, *Salmonella typhi*, *Bacillus cereus*, bactericide

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1- Introduction

Patchouli essential oil (EO) is widely used in perfumery, pharmacy and aromatherapy, among other fields. Many famous perfumes such as Arpege, Tabu, Miss Dior, Ysatis and Angel use Patchouli for its sensual, woody and voluptuous notes (Singh *et al.*, 2002). No synthetic product can replace it, which makes it valuable and unique in this field. EO also has a fixing property in the perfume of soaps (Farooqui and Streeramu, 2001), thanks to these properties in pharmacy and perfumery in the European Union. In aromatherapy, Patchouli can also be used to calm the nerves, sharpen the intelligence and improve concentration (Shankaranarayan, 2002).

In 1845, Patchouli was first described by botanist Pelletier-Sautelet in the Philippines. The plant is native to the tropical regions of Asia and Oceania, specifically India, Indonesia and the Philippines (Ramya, 2013). To date, Indonesia is the world's leading producer of Patchouli EO, exporting 1,200 to 1,500 tonnes/year to the USA, Singapore, France, Spain, Switzerland and Germany (Ditjenbun, 2021).

Patchouli was introduced to Madagascar at the Mahanorokely Ivoloina test garden created in 1898. It has been cultivated in Toamasina, Moramanga, Nosy-Be and Ambanja (Ravelojaona, 1958). The Soavinandriana Itasy region is a new production site for this species, with climatic and edaphic factors that meet the plant's requirements. This species needs to be developed to preserve the forest, as it is non-timber. However, knowledge of its characteristics and constituents is one of the objectives of this study, as is knowledge of its antimicrobial activity against a number of bacterial strains.

2- Materials and methods

2-1- Plant material

Pogostemon *cablin* was harvested in June 2022 at PK 144 in the Soavinandriana Itasy region, which lies between -19°18°53° South and 46°76°39° East. The extracted EO comes from the aerial part. Patchouli was dried in the shade for four weeks, leaving the air to circulate freely, and the EO was extracted by hydrodistillation.

Botanical characteristics of the species *Pogostemon cablin*

The plant belongs to the Lamiaceae family. It is a perennial, bushy herb that produces fragrant leaves containing a highly scented oil. Leaves are simple, oval to oblong, leathery, with toothed margins. They measure 2 to 4 inches long and 1and ¼ to 3 and ½ inches wide (Guenther, 1964). The leaves appear velvety. Roots are fasciculated, appearing on the stem. They can reach 30 cm in length and the oldest roots die (Minelle,1959). The clump produces stolons, which have nodes bearing buds. Flowers are borne in terminal spikes or whorls at leaf nodes over a large part of the stem. They are more often irregular (zygomorphic), type 5, with bilateral symmetry. The front-lobed corolla has an upper lip with 2 teeth formed by 2 petals, and a lower lip with 3 teeth formed by 3 petals. A total of 5 fused petals. 5 fused sepals form a tubular ovoid calyx, more or less bilabiate (Randriamihanta, 1994 **)**.

2-2- Bacterial strains

EO antimicrobial activity tests are carried out on six bacterial strains from the Laboratoire de Biochimie Appliquées aux Sciences Médicales (LABASM) at the Faculty of Science. They are composed of three Gram-positive bacteria including *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 14579), *Clostridium perfringens* (ATCC 13124), and three Gram-negative bacteria mainly: *Salmonella typhi* (ATCC 14028), *Vibrio fischeri* (ATCC 49387), *Escherichia coli* (ATCC 25922).

2-3- Essential oil extraction

The EO was extracted by Clevenger hydrodistillation. The aerial part of Patchouli is introduced into the flask, where distilled water is added so that it covers the plant material. Extraction takes around 5 hours. The extracted oil is dehydrated with sodium sulfate and stored in a dark bottle.

2-4- Physical characterization methods for HE

Determining the physico-chemical characteristics of an essential oil is the key to its quality. Physico-chemical properties are determined according to the methods described in AFNOR standards.

. **2.4.1. Relative density at 20°C** (AFNOR NF T 75-111, 1986).

Relative density is the ratio of the mass of HE to that of water at a temperature of 20°C. The two liquids are successively weighed. After noting the weight of the empty pycnometer, a

precise volume of distilled water is poured in and weighed on a precision balance fitted with silica gel to avoid moisture. The same operations are carried out with HE.

2.4.2. Refractive index (AFNOR NF T 75-112,1986)

The refractive index of an essential oil at 20°C is the ratio between the sine of the angle of incidence and the sine of the angle of refraction of a light ray of a given wavelength passing from the air into the EO maintained at a constant temperature. This index is used to verify the purity of a product. It is determined using a refractometer from the Centre National de Recherche Environnementale (CNRE).

2.4.3. Rotatory power (AFNOR, NF-T 75-13)

The rotatory power of an essential oil, noted αD, is the angle at which the plane of polarization of sodium D radiation (λ= 589.3nm±0.3nm) rotates as it passes through 100mm of essential oil under the specified temperature conditions. As Patchouli essential oil is viscous, 95° ethanol **is** added**.** In this case,the apparent specific rotatotory power is given by [α].It is measured using a polarimeter.

2-5- Analysis by Gas Chromatography (GC**)**

GPC can be used to separate small quantities of the various components of HE. GPC coupled with a flame ionization detector (FID) is the most suitable method for identifying and determining the relative proportions of compounds in the oil. The sample is analyzed using a chromatograph equipped with a BP5 capillary column (30m x 0.32mm x 0.5µm). The oven temperature was raised from 60°C (4.5 min) to 230°C, with a program setting of 3°C/min. Flame ionization detector (FID) temperature is 280°C and injector temperature is 260°C. The carrier gas is nitrogen U.

2-6- Disc method

The aromatogram is an *in vitro* method for measuring the antibacterial power of EOs. A bacterial suspension of known concentration is spread on the agar surface. A paper disc impregnated with the EO is placed in the center of the agar and the whole is incubated for 24 h. After incubation, the zone of inhibition is measured.

2-7- Determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and MBC/MIC ratio

The MIC corresponds to the minimum concentration capable of inhibiting all visible germ growth. After 24 h incubation, a colony isolated from the strains to be tested is picked up with a sterile loop and suspended in MUELLER-HINTON BROTH (MHB) nutrient broth. The resulting suspension is then standardized to between 0.5 and 1 MacFarland. The HE is diluted with MBH so that the dilution cascades from ½ to 1/1024. Two controls are used, a positive control (95 µl of MBH plus 5 µl of inoculum) and a negative control (100 µl of MBH). Microplates are covered with aluminum foil and incubated for 24 h at 37°C.

After incubation of the test germs, 40 µl of a 0.2 mg/ml solution of p-iodonitrotetrazolium chloride (INT), a color indicator, is added to each well. Incubate for 15 min at 37°C. INT turns purple in the presence of viable microbial strains (Kuete *et al.*, 2009).

The Minimum Bactericidal Concentration (MBC) is the concentration of EO required for full bactericidal action. It is the antimicrobial concentration that leaves no more than 0.01% of germs surviving.

For BMC determination, 5 µl of each of the non-turbid wells was inoculated onto MUELLER-HINTON AGAR (MHA) solid medium. The BMC corresponds to the lowest concentration at which no microbial colonies grow after incubation for 24 h at 37°C.

The CMB/CMI ratio is interpreted according to the standards used by (Dalmarco *et al.*, 2010). The type of action of HE is bactericidal when the CMB/CMI ratio is less than or equal to 4, and bacteriostatic when this ratio is greater than 4.

3- Results

Patchouli de Soavinandriana Itasy essential oil has its own physico-chemical properties.

Physical properties of essential oil from the aerial part of *P. cablin* species

- **Performance**

The yield of essential oil from the aerial part of *Pogostemon cablin* is 1.62%.

Its relative density at 20°C is 0.986 with a refractive index of 1.510 9 and apparent rotatory power at -55°41.

Analysis by Gas Chromatography

GPC/FID analysis of *Pogostemon cablin* essential oil detected 44 chemical compounds, of which only 8 were identified: α - pinene (0.16%), β- pinene (0.44%), Linalol (0.11%), β-

caryophyllene, (10.77%), α- humulene, (9.52%), Terpenyl acetate (1.73%), α- terpineol (8.11%), Patchoulol (51.16%).

The major constituents identified are β-caryophyllene (10.77%), α-humulene (9.52%) and α-terpineol (8.11%), but Patchoulol is in the majority (51.16%).

Antimicrobial activity of *Pogostemon cablin essential oil*

Among the six strains tested, *Bacillus cereus, Clostridium perfringens,Salmonella typhi* were all extremely sensitive to 10µ EO according to the PONCE *et al.* scale, with inhibition halo diameters respectively greater than 40mm for *Bacillus cereus and Salmonella typhi*, and 21mm for *Clostridium perfringens.* The results of the aromatogram test for *Pogostemon cablin* EO are presented in Table I.

CMI and CMB

The MIC of the strains tested is estimated at 0.961 µg/ml.

The BMC of *Bacillus cereus* and *Clostridium perfringens is equal to* 0.961 µg/ml. The results are shown in Table II.

4- Discussions

Physical characterization of *Pogostemon cablin* EO

Knowledge of the physico-chemical characteristics of an EO is important for its characterization and identification (Lazouni, *et al.*, 2007). In this study, only physical characteristics were investigated.

The Clevenger hydrodistillation method was used to extract HE from *Pogostemon cablin*. The extraction yield was 1.62%. This is lower than in India for the same species, with an oil content of between 2.5 and 3.5% for shade-dried leaves (Vijyakumar, 2004). The reason for this drop in yield can be explained by the fact that the plant was harvested before it was fully mature. Harvesting before the leaves are mature reduces the yield of essential oil (Randriambololona, 2006).

The density at 20°C of *Pogostemon cablin* essential oil is equal to 0.986 Relative density is a parameter used to determine the quality of an EO, the higher the density the higher the quality of the essential oil (De Cliff and Harerimana, 2014). According to AFNOR standard

NF T 75-111, the relative density at 20°C of Patchouli should be between 0.955 and 0.983. Moreover, the relative density at 20°C of the HE studied is higher than the standard.

Patchouli's refractive index (Ir) is 1.510 9, i.e. higher than that of water (Ir =1.333). It is also used to verify the purity of EO (AFNOR, 1992). This index follows the AFNOR NF T 75-112 standard, which indicates that the value should be between 1.505 0 - 1.512 0.

For the purposes of this study, patchouli EO has a levogyratory rotatory power (-55°41). The majority, however, stated that one of the chiral oil molecules present in EO deviates the plane of light polarization to the left. Rotatory power is one of the physical properties used to confirm or question the quality of an EO. However, the literature states that an oil is of good quality if it has a dextrorotatory rotatory power (Ranaivoarisoa and Randriamiharisoa, 1989). The Patchouli EO studied is not added to any synthetic product. The smaller the rotatory power value, the better the quality of the oil (De Cliff and Harerimana, 2014). According to ISO 592, the rotatory power of Patchouli should be between -40° and -60°.

Analysis by Gas Chromatography

The main components identified are β-caryophyllene (10.77%), α-humulene (9.52%) and α-terpineol (8.11%), Patchoulol (51.16%). According to ISO 3757, patchoulol content should be between 27 and 35%.

Patchoulol is the main component responsible for Patchouli's characteristic odour (Naf *et al.*, 1981). One of the parameters used to qualify Patchouli EO is its Patchoulol content. In this study, the level of this alcohol was 51.16%.

Antimicrobial activity of *Pogostemon cablin* EO

The sensitivity of bacterial strains to EO was assessed by aromatogram. *Salmonella typhi (* X> 40 mm*), Clostridium perfringens* (X=21 mm) and *Bacillus cereus* (X> 40 mm) are extremely sensitive to Patchouli EO according to (Ponce *et al.*, 2003). These strains are both Gram-positive and Gram-negative, so Patchouli essential oil has a broad spectrum of activity on bacteria. Gram-positive bacteria are more sensitive than gram-negative bacteria (Huang *et al*, 2012). Aromatic molecules have more difficulty penetrating the membrane of gram-negative bacteria due to its rigidity and the presence of large quantities of lipopolysaccharide.

Staphylococcus aureus, *Vibrio fischeri* and *Escherichia coli*, *on the* other hand, are insensitive to HE because their inhibition halo diameter is less than 8 mm.

The MIC of the strains was 0.961 µg/ml. The CMB/CMI ratio of bacterial strains of *Clostridium perfringens, Bacillus cereus, Staphylococcus aureus and Vibrio fischeri was less than or equal to 4.* According to Dalmarco *et al*. 2010, EO had a bactericidal effect, except for *Salmonella typhi* and *Escherichia coli*, for which EO had bacteriostatic activity.

5- Conclusion

The study carried out on the *Pogostemon cablin* species enabled us to provide more information, such as data on physical characteristics, analysis of EO composition by CPG/DIF and the antibacterial activity of Patchouli from the Soavinandriana Itasy region. Climatic and edaphic factors meet the plant's needs. The physical characteristics obtained justify the quality of the essential oil studied, such as its density at 20°C, better than the AFNOR standard. A characteristic majority component, Patchoulol, is very high, indicating its quality. The EO has bactericidal activity on *Bacillus cereus*, *Clostridium perfringens* and *Salmonella typhi*, *making* it an alternative treatment for infectious diseases. In the future, we could study the action of Patchouli EO on other germs and determine its chemical properties. Patchouli is a non-woody species, so its use has no negative impact on the environment.

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Figure: Chromatographic profile of Patchouli constituents

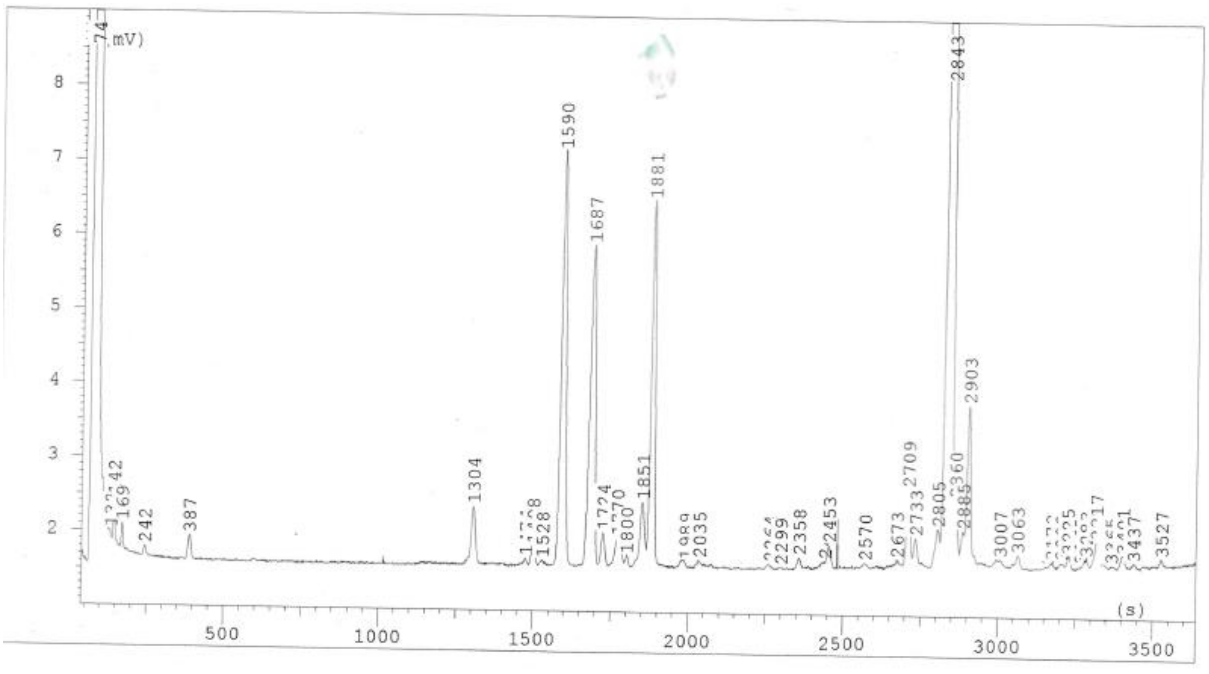

Table I: Inhibition halo diameter of strains tested

Bacteria	**Strains**	**Diameter of inhibition halo (mm)**	**Sensitivity**
	Staphylococcus aureus	7 mm	Insensitive

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GRAM POSITIVE	*Bacillus cereus*	X> 40 mm	Extremely sensitive
	Clostridium perfringens	21 mm	Extremely sensitive
NEGATIVE GRAM	*Salmonella typhi*	X> 40 mm	Extremely sensitive
	Vibrio fischeri	8 mm	Insensitive Insensitive
	Escherichia coli	6 mm	- Insensitive

Table I: Inhibition halo diameter of strains tested

Table II: Reference product BMC value

Germs used	**CMB (µg/ml)**	**Reference (mg/ml)**
Staphylococcus aureus	3, 847	6,5
Bacillus cereus	0,961	-
Clostridium perfringens	0,961	-
Salmonella typhi	15,389	-
Vibrio fischeri	3, 847	-
Escherichia coli	7,694	2

Xian Yang *et al*, 2013

Table III: CMB/CMI ratio values

Germs used	**CMB / CMI**
Staphylococcus aureus	4
Bacillus aureus	1
Clostridium perfringens	1
Salmonella typhi	16,01

Vibrio fischeri	4
Escherichia coli	8

Dalmarco *et al*, 2010