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Application Details

APPLICATION NUMBER	202241054127
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	21/09/2022
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TITLE OF INVENTION	Chemical Composition and Biological activity of Vanilla palmarum and Lippiaalba
FIELD OF INVENTION	CHEMICAL
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ADDITIONAL-EMAIL (As Per Record)	esdiyeminfotech@gmail.com
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	14/10/2022

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(57) Abstract :

[014] Vanilla palmarum and Lippia alba are plants that can be found in the semiarid region of Bahia. Both are used for medicinal purposes by local populations in different ways. However, V. palmarum is not studied much in several aspects, such as its chemical composition and the biological activities it performs. L. alba, in turn, is one of the most studied plant species, but, due to its geographic variability, the chemical composition of its essential oil is quite variable. Therefore, the objective of this work was to analyze the chemical composition, antioxidant activity and antidermatophytic activity of the crude extracts of V. palmarum, as well as the chemical composition and antidermatophytic activity of the essential oil of L. alba. The dermatophytes used were Trichophyton mentagrophytes, Trichophyton rubrum and Microsporum gypseum. Inhibition of mycelial growth of all fungi was evaluated and minimum inhibitory and minimum fungicidal concentrations of extracts and oil were determined. The antioxidant activity of the extracts was evaluated by free radical scavenging methods. The extracts of V. palmarum showed antioxidant activity. Both extracts and essential oil showed antidermatophytic activity. Carvone and limonene are the major compounds in the essential oil analyzed. Accompanied Drawing [FIG. 1] [FIG. 2][FIG. 3] [FIG. 4][FIG. 5] [FIG. 6][FIG. 7]

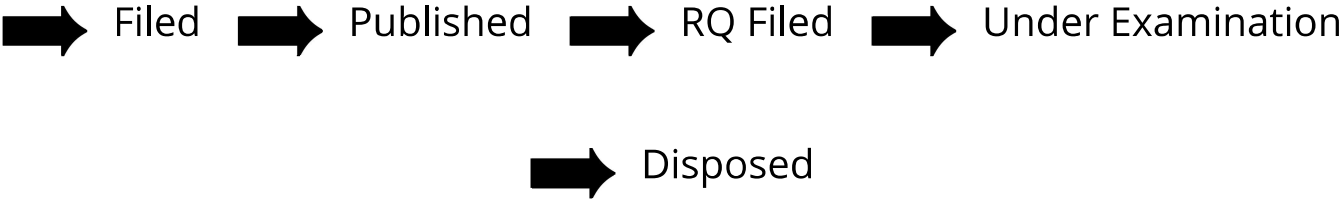
No. of Pages : 28 No. of Claims : 6

Application Status

APPLICATION STATUS

Awaiting Request for Examination

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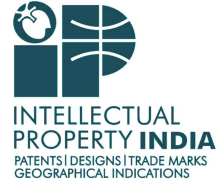
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[See Rule 22(1)]
RECEIPT



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Date/Time 2022/09/21 22:21:38

To
Harish Sharma

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A-2, Sect.-60, Noida, Uttar Pradesh

CBR Detail:

Sr. No.	Ref. No./Application No.	App. Number	Amount Paid	C.B.R. No.	Form Name	Remarks
1	E-12/7190/2022/CHE	202241054127	2500	38137	FORM 9	
2	202241054127	TEMP/E-1/62162/2022-CHE	1600	38137	FORM 1	Chemical Composition and Biological activity of Vanilla palmarum and Lippiaalba

TransactionID	Payment Mode	Challan Identification Number	Amount Paid	Head of A/C No
N-0001026508	Online Bank Transfer	2109220032975	4100.00	1475001020000001

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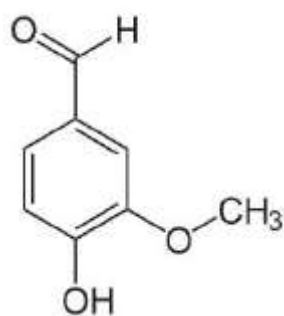


FIG 1: Chemical structure of vanillin

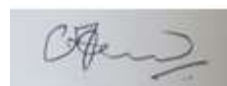


FIG 2: Vanilla palmarum



FIG 3: Lippia alba

Signature:



A handwritten signature in black ink, appearing to read 'K. Muthuchelian'.

Applicant(s) Name: Prof. Dr. K. Muthuchelian et. al.

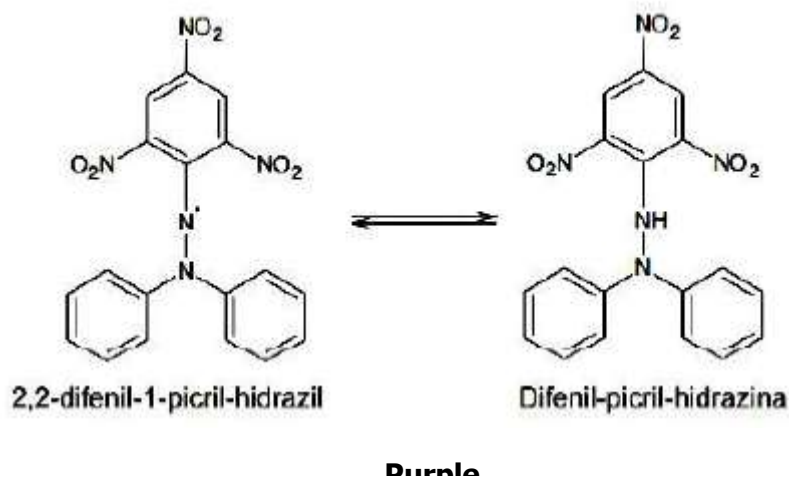


FIG 4: Radical and non-radical forms of DPPH, respectively

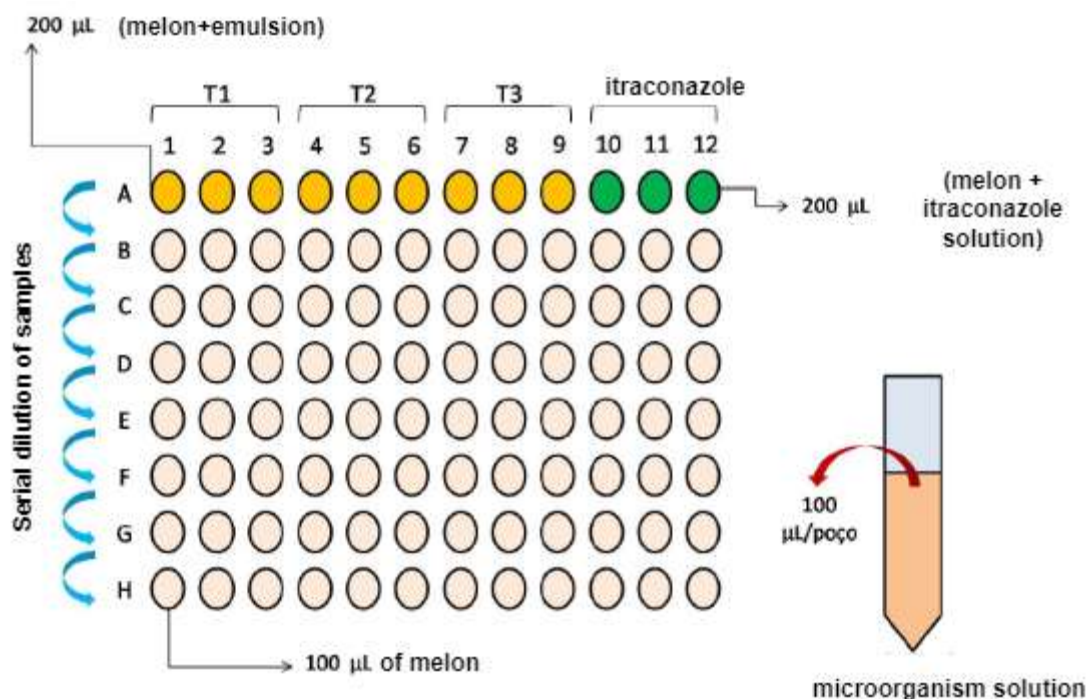


FIG 5: Procedures for testing the minimum inhibitory concentration of *L. alba* essential oil, where the letter "T" represents the triplicate

Signature:

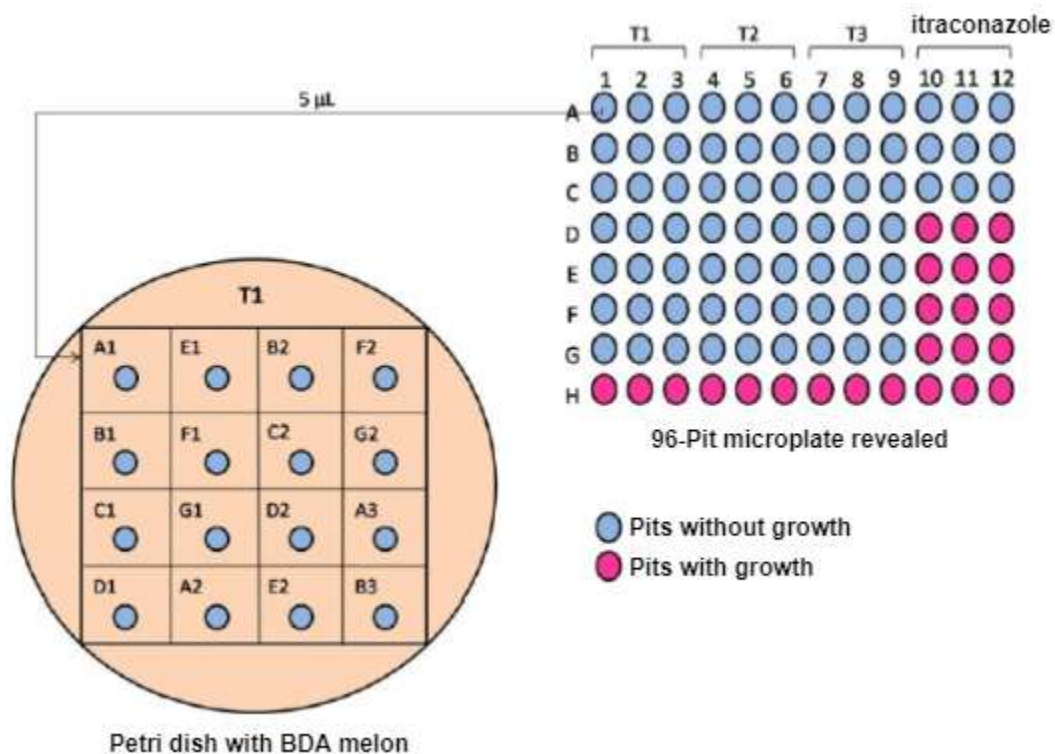


FIG 6: Procedures for the minimum fungicide concentration test.

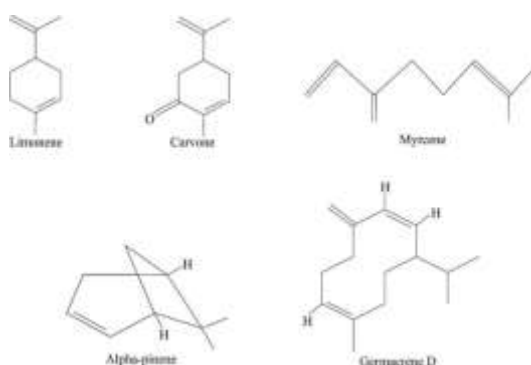


FIG 7: Major compounds in the essential oil of *L. alba*, chemotype III.

Dated this 21st day of September 2022

Signature:

Applicant(s) Name: Prof. Dr. K. Muthuchelian et. al.

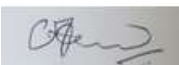

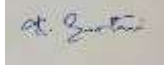





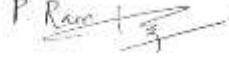
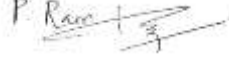
FORM 1 THE PATENTS ACT 1970 (39 of 1970) and THE PATENTS RULES, 2003 APPLICATION FOR GRANT OF PATENT (See section 7, 54 and 135 and sub-rule (1) of rule 20)				(FOR OFFICE USE ONLY)	
				Application No.	
				Filing date:	
				Amount of Fee paid:	
				CBR No:	
				Signature:	
1. APPLICANT'S REFERENCE / IDENTIFICATION NO. (AS ALLOTTED BY OFFICE)					
2. TYPE OF APPLICATION [Please tick (✓) at the appropriate category]					
Ordinary (✓)		Convention ()		PCT-NP ()	
Divisional ()	Patent of Addition ()	Divisional ()	Patent of Addition ()	Divisional ()	Patent of Addition ()
3A. APPLICANT(S)					
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10. Mr. P. Ram Kumar	INDIAN	India	Assistant Professor, PG and Research Department of Chemistry, V.O.Chidambaram College, Thoothukudi – 628008, Tamil Nadu, India	
Natural Person (✓)	Other than Natural Person			
	Small Entity ()	Startup ()	Others ()	
4. INVENTOR(S) [Please tick (✓) at the appropriate category]				
Are all the inventor(s) same as the applicant(s) named above?	Yes (✓)		No ()	
If “No”, furnish the details of the inventor(s)				
Name in Full	Nationality	Country of Residence	Address of the Inventor	
Same as Applicant				
5. TITLE OF THE INVENTION				
“Chemical Composition and Biological activity of Vanilla palmarum and Lippiaalba”				

6. AUTHORISED REGISTERED PATENT AGENT(S)			IN/PA No.		
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8. IN CASE OF APPLICATION CLAIMING PRIORITY OF APPLICATION FILED IN-CONVENTION COUNTRY, PARTICULARS OF CONVENTION APPLICATION					
Country	Application Number	Filing date	Name of the applicant	Title of the invention	IPC (as classified in the convention country)
9. IN CASE OF PCT NATIONAL PHASE APPLICATION, PARTICULARS OF INTERNATIONAL APPLICATION FILED UNDER PATENT CO-OPERATION TREATY (PCT)					
International application number			International filing date		
10. IN CASE OF DIVISIONAL APPLICATION FILED UNDER SECTION 16, PARTICULARS OF ORIGINAL (FIRST) APPLICATION					
Original (first) application No.			Date of filing of original (first) application		
11. IN CASE OF PATENT OF ADDITION FILED UNDER SECTION 54, PARTICULARS OF MAIN APPLICATION OR PATENT					
Main application/patent No.			Date of filing of main application		
12. DECLARATIONS					
<p>(i) Declaration by the inventor(s)</p> <p>(In case the applicant is an assignee: the inventor(s) may sign herein below or the applicant may upload the assignment or enclose the assignment with this application for patent or send the assignment by post/electronic transmission duly authenticated within the prescribed period).</p> <p>I/We, the above named inventor(s) is/are the true & first inventor(s) for this Invention</p>					

and declare that the applicant(s) herein is/are my/our assignee or legal representative.

(a) Date 21/09/2022

(b) Name	(c) Signature
1.Prof. Dr. K. Muthuchelian	
2.Dr. S. Alwin David	
3.Dr. K. Gayathri	
4.Mr. S. Nagul Dev	
5.Dr. S. Mohamed Rabeek	
6.Dr. R. Gokulan	
7.Divya S	
8.Mr. R. G. Padmanabhan	
9.Dr. G. Baskar	
10.Mr. P. Ram Kumar	

(ii) Declaration by the applicant(s) in the convention country

~~(In case the applicant in India is different than the applicant in the convention country: the applicant in the convention country may sign herein below or applicant in India may upload the assignment from the applicant in the convention country or enclose the said assignment with this application for patent or send the assignment by post/electronic transmission duly authenticated within the prescribed period)~~

~~I/We, the applicant(s) in the convention country declare that the applicant(s) herein is/are my/our assignee or legal representative.~~

~~(a) Date~~

~~(b) Signature(s)~~

~~(c) Name(s) of the signatory~~

(iii) Declaration by the applicant(s)

I/We the applicant(s) hereby declare(s) that: -

- ☐ I am/We are in possession of the above-mentioned invention.
- ☐ The provisional/complete specification relating to the invention is filed with this application.
- ☐ ~~The invention as disclosed in the specification uses the biological material from India and the necessary permission from the competent authority shall be submitted by me/us before the grant of patent to me/us.~~
- ☐ There is no lawful ground of objection(s) to the grant of the Patent to me/us.
- ☐ I am/we are the true & first inventor(s).
- ☐ ~~I am/we are the assignee or legal representative of true & first inventor(s).~~
- ☐ ~~The application or each of the applications, particulars of which are given in Paragraph 8, was the first application in convention country/countries in respect of my/our invention(s).~~
- ☐ ~~I/We claim the priority from the above mentioned application(s) filed in~~

~~convention country/countries and state that no application for protection in respect of the invention had been made in a convention country before that date by me/us or by any person from which I/We derive the title.~~

~~☐ My/our application in India is based on international application under Patent Cooperation Treaty (PCT) as mentioned in Paragraph 9.~~

~~☐ The application is divided out of my /our application particulars of which is given in Paragraph 10 and pray that this application may be treated as deemed to have been filed on DD/MM/YYYY under section 16 of the Act.~~

~~☐ The said invention is an improvement in or modification of the invention particulars of which are given in Paragraph 11.~~

13. FOLLOWING ARE THE ATTACHMENTS WITH THE APPLICATION

(a) Form 2

Item	Details	Fee	Remarks
Complete/ Provisional specification)#	No. of pages : 26		
No. of Claim(s)	No. of claims : 06 No. of pages: 01		
Abstract	No. of pages: 01		
No. of Drawing(s)	No. of drawings: 07 No. of pages: 03		

In case of a complete specification, if the applicant desires to adopt the drawings filed with his provisional specification as the drawings or part of the drawings for the complete specification under rule 13(4), the number of such pages filed with the provisional specification are required to be mentioned here.

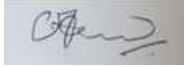
- (b) Complete specification (in conformation with the international application)/as amended before the International Preliminary Examination Authority (IPEA), as applicable (2copies).
- (c) Sequence listing in electronic form
- (d) Drawings (in conformation with the international application)/as amended before the International Preliminary Examination Authority (IPEA), as applicable (2copies).
- (e) Priority document(s) or a request to retrieve the priority document(s) from DAS (Digital Access Service) if the applicant had already requested the office of first filing to make the priority document(s) available to DAS.
- (f) Translation of priority document/Specification/International Search Report/International Preliminary Report on Patentability.
- (g) Statement and Undertaking on Form 3
- (h) Declaration of Inventorship on Form 5
- (i) Power of Authority
- (j) **Total fee ₹.....in Cash/ Banker's Cheque /Bank Draft bearing No.....**

Date on..... Bank.

I/We hereby declare that to the best of my/our knowledge, information and belief the fact and matters stated herein are correct and I/We request that a patent may be granted to me/us for the said invention.

Dated this 21st day of September, 2022

Signature:



Name: Prof. Dr. K. Muthuchelian et al.

To,
The Controller of Patents
The Patent Office, at Kolkata

Note: -

- * Repeat boxes in case of more than one entry.
- * To be signed by the applicant(s) or by authorized registered patent agent otherwise where mentioned.
- * Tick (/) / cross (x) whichever is applicable / not applicable in declaration in paragraph-12.
- * Name of the inventor and applicant should be given in full, family name in the beginning.
- * Strike out the portion which is / are not applicable.
- * For fee: See First Schedule";

FORM 2

THE PATENTS ACT, 1970(39 of 1970)

&

The Patent Rules, 2003

5

COMPLETE SPECIFICATION

(See section 10 and rule 13)

TITLE OF THE INVENTION

**“Chemical Composition and Biological activity of Vanilla palmarum and
Lippiaalba”**

10

We, applicant(s)

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7. Divya S	INDIAN	Department of Textiles and Apparel Design, Bharathiar University, maruthamalai Road Coimbatore-641 046 Tamil Nadu, India.
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10. Mr. P. Ram Kumar	INDIAN	Assistant Professor, PG and Research Department of Chemistry, V.O.Chidambaram College, Thoothukudi – 628008, Tamil Nadu, India

5

The following specification particularly describes the nature of the invention and the manner in which it is performed:

[01] FIELD OF THE INVENTION

The present invention relates to analyze the chemical composition, antioxidant activity and antidermatophytic activity of the crude extracts of *V. palmarum*, as well as the chemical composition and antidermatophytic activity of the essential oil of *L. alba*.

5 [02] BACKGROUND OF THE INVENTION

The Vanilla genus

One of the generations of Orchidaceae is Vanilla, which belongs to the subfamily Vanilloideae, tribe Vanilleae, subtribe Vanillinae and consists of approximately 110 species.

This genus is very important economically, as it is from the *Vanilla planifolia* beans that
10 vanilla is obtained, the second most expensive fragrance in the world. The largest producers of vanilla are India, Indonesia, Madagascar, Mexico, Reunion Islands and Comoros. Vanilla is used in the manufacture of cosmetics and in the food industry.

In addition to having economic importance on the world stage, Vanilla species are popularly used for medicinal purposes. However, there is not much scientific research that
15 proves the beneficial actions of the extract of plant parts of different species of this genus. *V. planifolia* is, so far, the most studied species in this regard. It is known that vanilla is a mixture of compounds, and vanillin (FIG 1) is the phenolic found in the highest concentration in this mixture. However, there are other phenols of equal value in vanilla, such as p-hydroxybenzaldehydes, p-hydroxybenzoic acids and vanillic acid, all of which
20 are used in the pharmaceutical industry.

Due to the large amount of phenolics present in vanilla extract, it is considered a powerful antioxidant, helping to prevent cancer and acting as a antineoplastic. Researchers have also detected a cholesterol-lowering effect on vanillin, which is related to its hypotriglyceridemic effect or its regulatory effect on cholesterol metabolism. Other
25 researchers have identified an antibacterial effect in vanillin against *Escherichia coli* and

Listeria innocua.

Vanilla palmarum (FIG 2) was first described in 1832 with the basionym *Epidendrum palmarum*, but in 1840 it was transferred to the genus *Vanilla*. In Brazil, it can be found, among other states, in Bahia, where it is often seen in the caatinga, on *Syagrus coronata*,
5 a plant popularly known as licuri.

There are not many studies related to *V. palmarum* in the literature, but there are records that this species is used in folk medicine for the treatment of spine, kidneys and ear pain, in addition to being used as an ornament, food and cosmetic (hair tonic). In the municipality of Milagres, Bahia, *V. palmarum* is used by some residents to combat
10 dermatophytosis, but there is no research that proves the antifungal action of *V. palmarum*.

Lippia

The *Lippia* genus is one of the 36 genera of the Verbenaceae family and the second largest of them, with 200 species distributed across South and Central America and
15 tropical Africa, with most of these species (75%) in Brazilian territory. In nature, the species of this genus are characterized as shrubs or sub-shrubs, usually branches, or perennial herbs.

Popularly known as alecrim do mato, alecrim do campo, citron, alecrim de tray and alecrim, *Lippia* species are often used in cooking and traditional medicine. In the latter,
20 *Lippia* is used as a decoction or infusion, for the oral treatment of respiratory and gastrointestinal disorders and in the fight against infections in general. Based on this information, many researchers have investigated the potential of *Lippia* species to inhibit microbial growth, *L. alba* being the most studied. Several studies have confirmed, through in vitro tests, the antifungal activity of essential oils from *Lippia* sp. against fungi of several
25 species, such as *Aspergillus niger*, *Trichophyton rubrum* and *Candida albicans*, for example.

Lippia alba (lemon balm) (FIG 3) is an aromatic shrub widely distributed in Central and South America, also occurring in India and Australia. It is one of the most used Lippia species in traditional medicine, with reports for the treatment of gastrointestinal disorders, cough, cold, pain and skin problems, among others. Regarding its biological activity, it is also one of the most studied species, especially the antimicrobial action of its essential oil against bacteria such as Staphylococcus aureus, Escherichia coli, Listeria innocua, Pseudomonas aeruginosa and Salmonella choleraesuis and fungi, including dermatophytes. The activity against Trichophyton rubrum, Epidermophyton floccosum and Microsporum gypseum of the linalool-rich essential oil, as well as its possible mechanism of action, was previously described.

However, due to its wide geographic distribution and genetic variability, the essential oil of L. alba presents a wide variation in its chemical composition, giving rise to different chemotypes, classified according to their major components. Seven chemotypes have already been described: chemotype I (citral and/or linalool and/or E-caryophyllene), II (tagetone), III (limonene and carvone or dihydrocarvone, piperitone, piperitenone), IV (myrcene), V (γ -terpinene), VI (camphor/1.8 cineole) and VII (stragol), which may have different biological actions.

[03] SUMMARY OF THE PRESENT INVENTION

The presence of phenolics and flavonoids in V. palmarum extracts suggests their pharmacological potential, since these metabolites act as antimicrobials and antioxidants.

Both the crude methanolic extracts of V. palmarum and the essential oil of L. alba showed antidermatophytic activity against the three dermatophyte species tested.

From the results presented against the dermatophytes tested in this work, the EO of L. alba chemotype III represents a promising alternative for the treatment of dermatophytosis, mainly because its major components (carvone and limonene) have

already been tested in animal models, in which , for the most part, no adverse reactions or mutations were detected, which indicates the safety of these compounds for use in humans.

[04] BRIEF DESCRIPTION OF THE DRAWINGS

5 The invention will be better understood and objects other than those set forth above will become apparent when consideration is given to the following detailed description thereof.

Such description makes reference to the annexed drawings wherein:

FIG 1: Chemical structure of vanillin

FIG 2: *Vanilla palmarum*

10 FIG 3: *Lippia alba*

FIG 4: Radical and non-radical forms of DPPH, respectively

FIG 5: Procedures for testing the minimum inhibitory concentration of *L. alba* essential oil, where the letter “T” represents the triplicate

FIG 6: Procedures for the minimum fungicide concentration test.

15 FIG 7: Major compounds in the essential oil of *L. alba*, chemotype III.

DETAILED DESCRIPTION OF THE INVENTION

[05] BIOLOGICAL ACTIVITIES OF SECONDARY METABOLITES

Antioxidant activity

The antioxidant activity of a chemical compound is determined by its ability to minimize the
20 damage caused by oxidative stress, preventing the formation of free radicals and other reactive oxygen species (ROS) generated by the electron transfer performed by cells. This damage occurs because free radicals – chemical species that have one or more unpaired electrons in their last electronic layer – are highly reactive and can react with any molecule that comes in contact with them.

25 [06] VEGETABLE EXTRACTS

Secondary metabolites can be produced in different plant organs, in which they remain

stored. To extract these substances, the main extraction techniques that can be used are solvent extraction (solid-liquid), steam extraction or extraction using supercritical fluid.

However, of these three techniques, solid-liquid extraction is the most common in industrial processes. It consists of the extraction of a solid matter, of soluble compounds, by a known liquid solvent. When in contact with plant cells, the solvent dissolves the extract, producing a concentrated solution, formed by solvent and extracted solute. However, it is difficult to obtain the complete extraction of the substances, since part of the extract remains retained in the continuous particles of the vegetable raw material.

[07] ESSENTIAL OILS

Essential oils (EOs) are volatile mixtures of different secondary metabolites – mainly from the terpene class. In its composition, mono and sesquiterpenes predominate, but non-terpene compounds can also be found. The number and concentration of these compounds vary, however, from species to species, so that an oil may have different metabolites in its composition.

The production of EOs occurs in different organs of aromatic plants, where they are secreted and stored in specialized structures that differ in morphology, function and distribution. Such structures are found on the surface of plant organs and/or in their tissues, in which they play the role of minimizing the risk of autotoxicity.

As for the physicochemical properties of EOs, they are insoluble in water, appearing in a liquid state and colorless at room temperature. They have a characteristic odor and, therefore, are widely used in the cosmetics, perfumery and aromatherapy industries.

The variability of OEs depends on several factors, such as seasonality, the degree of plant development, genetics and the environment. All these factors influence not only the content of secondary compounds in EOs, but also the biological functions they will play in the plant. It is known that the SOs act in the defense of plants against microorganisms, as attractants for seed and pollen dispersers, as insecticides, as insect repellents and

reducing herbivory.

Given the many functions that EOs play in the plant organism, the scientific community began to investigate the possibility of these substances producing beneficial pharmacological effects for humans. Thus, EOs are widely studied and, through
5 standardized methods, it has been discovered that EOs produced by different species can act, among other ways, as antioxidants, antibacterials, antifungals and anti-inflammatory agents. *Lippia origanoides* EO, for example, proved to be effective against the fungus *Aspergillus flavus*.

It is worth noting that the term “essential oil” was first used in the 16th century by
10 Paracelsus Von Hohenheim, who named the effective component of a drug as *Quinta essentia*. Currently, EOs can be extracted through different techniques, such as steam distillation, destructive distillation and hydrodiffusion. However, hydrodistillation is the most used extraction technique. In it, the water, in which the biomass is immersed and subjected to boiling, comes into contact with the plant cell, opening its pores and releasing
15 the EO from the glands. The generated water and oil vapors condense by cooling in a condenser and flow to the collection container, in which liquid water and oil can be separated by a hygroscopic substance or by density.

[08] METHODS FOR ANALYSIS OF THE BIOLOGICAL ACTIVITY OF EXTRACTS AND ESSENTIAL OILS

20 Test of antioxidant activity by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging method

2,2-Diphenyl-1-picrylhydrazyl (DPPH•) is a stable purple free radical that reacts with hydrogen donor compounds. This radical, when subjected to the action of an antioxidant agent or a radical species, is reduced to 2,2-diphenylpicrylhydrazine (DPPH-H) (FIG 4),
25 which has a yellow color.

DPPH-H absorbs energy at a shorter wavelength than that at which DPPH• absorbs.

Therefore, the higher the concentration of antioxidants in the medium, the lower the absorbance of the mixture.

Antioxidant activity test by the free radical scavenging method 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

- 5 The antioxidant activity test by the azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging method follows the same principle used by the test performed with the free radical DPPH, that is, it is based on the transfer of an electron to the radical. The visible result of this test is the decrease in color intensity in the reaction medium.

Antimicrobial tests

- 10 Laboratory tests of antimicrobial activity are performed with the aim of predicting the in vitro sensitivity of a given microorganism. The mycelial growth inhibition test is one of the ways to analyze the action of antimicrobials against filamentous fungi. This test, usually carried out in five repetitions, consists of sowing the fungus under study in a solid culture medium containing the substance to be tested at a previously established concentration.
- 15 The test result is calculated in percentage, according to Edington's formula, in which the average of the diameters of the colonies submitted to the antimicrobial is compared to the average of a negative control (means without antimicrobial).

[09] DERMATOPHYTES

- Dermatophytes are a group of keratinophilic fungi that invade keratinized tissues (skin, hair and nails), where they form colonies and transform keratin into a substrate. These fungi are distributed in three genera: Trichophyton, Microsporum and Epidermophyton. Their natural habitat is the soil, but, due to the specificity acquired in the course of evolution, they can infect tissues of animals and humans, which leads them to three different classifications. ecological. The first one is that of geophilic fungi, which includes species exclusively from the soil. The second, in turn, is that of zoophilic fungi, which includes species capable of infecting animals. And the third is that of anthropophilic fungi –
- 25

those that infect humans. In fact, zoophiles and anthropophiles can infect both animals and humans. *T. mentagrophytes* and *M. canis* are examples of zoophiles transmitted via animal-human. *T. rubrum*, *T. tonsurans*, *M. gypseum* and *E. floccosum* are examples of anthropophiles transmitted via human-to-human.

5 **[010] ANTIFUNGAL ACTIVITY OF CARVONA-RICH ESSENTIAL OIL FROM *Lippia alba* AGAINST DERMATOPHYTES**

Considering this chemical diversity, the objective of this work was to analyze the activity against dermatophytes of the essential oil of the chemotype rich in carvone of the species *L. alba*.

[011] MATERIALS AND METHODS

10 **Collection of plant material**

The collection of plant material was carried out in February, in the year 2018. The fertile material was herborized and deposited, in the form of an exsiccate, in the Laboratory. The leaves of the collected material were separated and dried at room temperature.

Essential oil extraction

- 15 For the extraction of essential oils, the dry leaves (1.6 kg) were separated into portions of approximately 200 g, being crushed in a blender with the aid of distilled water. Then, the crushed material was subjected to hydrodistillation in a Clevenger apparatus to extract the essential oil. The oil obtained was collected, dried with anhydrous sodium sulfate and stored at -20 °C until the analysis was carried out. The extracted oil content was
- 20 calculated in mL of oil per 100 g of dry leaves.

Analysis of chemical composition

- The analysis of the chemical composition of the essential oils was performed by gas chromatography, using a gas chromatograph with a flame ionization detector (GC/DIC) for quantification and a gas chromatograph coupled with a mass spectrometer (GC/MS) for
- 25 identification of constituents. For the analysis, 1 µL of an essential oil solution in ethyl

acetate, at 25 mg/mL, with a split ratio of 1:100 was injected.

In the GC/DIC analysis, a Shimadzu® CG-2010 Chromatograph was used with a DB-5 capillary column (30 m x 0.5 mm, with a film thickness of 0.25 µm), inlet temperature of 220 °C and detector temperature. 240 °C, helium as carrier gas (1 mL/min), with oven
5 temperature program from 60 °C to 240 °C (3 °C/min), 240 °C (20 min). GC/MS analyzes were performed on a Shimadzu® CG-2010 Chromatograph coupled to a Shimadzu® CG/MS-QP 2010 Mass Spectrometer, DB-5ms capillary column (30 m x 0.25 mm, with a film thickness of 0.25 µm), injector temperature 220 °C, helium carrier gas (1 mL/min), interface temperature 240 °C, ionization source temperature 240 °C, ionization energy 70
10 eV, ionization current: 0.7kV and oven temperature program similar to the one above.

The identification of the constituents was performed by mass spectrometry and by means of the corrected retention time of each peak (arimetic index - AI), using a n-alkane solution (C8 to C24) as standard. The AI was calculated according to the formula described by Adams (2007): $AI = 100 N + 100 \cdot [(t'_R(A) - R(N)) / (t'_R(N+1) - t'_R(N))]$, where, N = Number of
15 carbon atoms of the alkane standard $t'_R(A)$ = retention time of the calculated peak, $t'_R(N)$ = retention time of the alkane corresponding to the calculated peak and $t'_R(N + 1)$ = retention time retention of the alkane eluting after the calculated peak.

Each peak of the chromatogram was identified by its mass spectrum. The quantification of the relative percentage was obtained based on the areas of the corresponding
20 chromatographic peaks calculated by the normalization method.

Antimicrobial tests

Microorganisms

The dermatophyte species tested (*Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Microsporum gypseum*) were isolated from clinical material and identified as agents of
25 cutaneous mycoses (skin, hair and nails). All dermatophytes mentioned were provided by the URM Culture Collection of the Mycology Department of the Center for Biological

Sciences of the Federal University of Pernambuco, in which the strains are registered with the codes 6272, 4727 and 6199, respectively. Dermatophytes were preserved in potato-dextrose-agar (PDA) medium.

Mycelial growth inhibition test

5 The mycelial growth inhibition test was performed. The essential oil emulsions were prepared in 5% Tween. After being sterilized by means of filtration on a 0.22 µm nylon membrane, 1 mL of the emulsions were dispensed into 99 mL of BDA culture medium and poured into Petri dishes at concentrations of 930, 465, 260 and 130 µg /mL. The plates containing the culture medium were exposed to ultraviolet light for two hours, until the
10 solidification. After this time, a 6 mm diameter mycelial disc was inoculated in the center of each plate.

After inoculation, the dermatophytes were incubated in an oven at 28°C for 48 hours, and the growth of colonies was monitored daily by measuring their diameter (in two directions) until the seventh day after inoculation. The experiment was followed by negative controls
15 (culture medium without oil) and positive controls (culture medium with itraconazole).

On the seventh day, the average diameter of the colonies of each dermatophyte was calculated and the results were used in Edington's formula to calculate the percentage of inhibition of mycelial growth: $I = (MCn - MT) \times 100 / MCn$, where I is the percentage of inhibition, MCn is the mean of the diameters of the negative controls and MT is the mean
20 of the diameters of the treatment under analysis. For each treatment, five replications were performed.

To compare the means of each treatment, analysis of variance was performed using the Tukey test, which was conducted using the statistical analysis program Sisvar.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum 25 Fungicide (CFM)

The broth microdilution susceptibility assay was used to determine the MIC of the EO of L.

alba. The tests were performed in Sabouraud-dextrose broth (SD). For each dermatophyte, three essential oil emulsions were made in 5% Tween 80, at a concentration of 9.3 mg/mL. These emulsions were sterilized by filtration through a nylon membrane (0.22 µm).

- 5 For inoculum preparation, fungal samples were grown in petri dishes containing BDA and incubated at 35°C for seven days. After this time, the fungal colonies were scraped with the aid of a sterile straw and the scraping was transferred to a sterile tube containing 5 mL of 0.85% saline solution. After vortexing, the mixture of sporangiospores and hyphae fragments was allowed to rest for 20 minutes and then the supernatant was transferred to
10 sterile colorimeter tubes. The suspension was adjusted to an absorbance between 0.09 and 0.17, in the colorimeter reading. A volume of 1 mL of this suspension was diluted in 49 mL of Sabouraud-dextrose broth (SD) medium, in order to obtain a final concentration between 0.4×10^4 to 5×10^4 CFU/mL, in a 1:50 suspension of micro -body. This concentration was checked by sowing 10 µL of the inoculum prepared in PDA medium
15 and counting the number of colonies.

In the 96-well microplates, 100 µL of SD broth was placed in each well. Then, the sterile emulsions were tested in triplicate, so that 300 µL of each emulsion were distributed in the first wells (100 µL in each). From the first wells, serial dilutions were made for the following wells. Finally, each well received 100 µL of microorganism suspension (FIG 5). The EO
20 concentration in the first wells was about 2400 µg/mL.

Pelleted Itraconazole, prepared in a solution with 400 µL of dimethylsulfoxide (DMSO) and 600 µL of autoclaved distilled water, was used as a positive control, at an initial concentration (first well) of 550 µg/mL. Viability controls of the tested microorganisms and the culture medium were also performed. The MIC of the 5% Tween 80 solution was also
25 tested to eliminate the possibility of Tween interfering with the results. The sterility of the emulsions and SD broth used was verified by inoculating all of them in a microplate. The

weighing of itraconazole in pellets was performed by multiplying the desired initial concentration by the correction factor 4.55, a calculation necessary to obtain the real value of the active ingredient present in the pharmaceutical form in which it is found.

The plates were incubated at 35°C for 72 hours. After this period, for qualitative analysis of microbial growth in the test wells, 50 µL of the resazurin developer were added to each well, at a final concentration of 0.005 mg/well. The MIC was defined as the lowest concentration, in triplicates, in which no microbial growth was observed. The final MIC was obtained by calculating the arithmetic mean and standard deviation of all triplicates.

To confirm the fungicidal activity, the minimum fungicidal concentration (CFM) of the tested EO solution, and also of itraconazole, was determined for each well of the MIC test that did not show microbial growth. For this, Petri dishes containing PDA culture medium were divided into small squares identified with the letter and number corresponding to the well from which the sowing was performed. A volume of 5 µL of the contents of each well was seeded within the limits of the respective square (FIG 6). The plates were incubated under the same conditions used in the MIC test. CFM was defined as the lowest concentration without microbial growth, calculated as the arithmetic mean and standard deviation of all triplicates.

All measurements were performed in quadruplicate and with three independent experiments.

[012] RESULTS

Chemical composition of the essential oil

The essential oil was obtained with a content of 1.96±0.15% (V/m). By chemical analysis (Table 1) it was possible to identify 34 chemical compounds, corresponding to 98.32% of the essential oil, of which 58.82% belong to the class of monoterpenes and 41.18% are sesquiterpenes. Carvone was the major compound (48.17%), followed by limonene

(14.6%), germacrene-D (12.09%), β -myrcene (7.1%) and α -pinene (3.68 %) (FIG 7). Except for D-germacrene (sesquiterpene), all the major compounds detected are monoterpenes.

Table 1: Chemical composition of *Lippia alba* essential oil.

5

Compound	IA _{lit}	IA _{calc}	Mean \pm SD (%)
α -thujene	924	928	0.14 \pm 0.01
α -pinene	932	938	3.68 \pm 0.12
camphene	946	957	T
Sabinene	969	978	0.12 \pm 0.01
β -pinene	974	981	0.33 \pm 0.02
β -myrcene	988	991	7.1 \pm 0.41
d-3-carene	1008	1013	0.17 \pm 0
limonene	1024	1036	14.6 \pm 0.35
E- β -ocimene	1044	1049	0.47 \pm 0.02
linalool	1095	1106	0.77 \pm 0.03
cis-verbenol	1137	1154	0.20 \pm 0
trans-verbenol	1140	1158	1.31 \pm 0.01
Myrthenol	1194	1209	0.35 \pm 0.02
Mirtenal	1195	1212	1.53 \pm 0.07
trans-carveol	1215	1233	0.17 \pm 0.05
carvone	1239	1263	48.17 \pm 1.15
piperitone	1249	1272	0.72 \pm 0.02
trans-mirtanol	1258	1281	0.36 \pm 0.03
myrtenyl acetate	1334	1324	0.22 \pm 0.01
piperitenone	1340	1359	0.86 \pm 0.13
eugenol	1356	1368	0.14 \pm 0.05
α -copaene	1374	1385	0.14 \pm 0.01
β -bourbonene	1387	1394	0.80 \pm 0.09
β -cubebene+ β -elemene	1387/1389	1397	0.67 \pm 0.13
E-caryophyllene	1417	1432	0.24 \pm 0.02
β -copaene	1430	1442	0.22 \pm 0.03
β -gurjunene+(Z)- β -farnesene	1431/1440	1458	0.24 \pm 0.01
allo-aromadendrene	1458	1474	0.38 \pm 0.05
germacrene D	1484	1495	12.09 \pm 1.90
Bicyclgermacrene+ α -Muurolene	1500	1510	0.65 \pm 0.09
δ -cadinene	1522	1531	0.57 \pm 0.09
(E)-nerolidol	1561	1569	0.75 \pm 0.1
spathulenol	1577	1597	0.12 \pm 0
α -cadinol	1652	1678	0.12 \pm 0
Total identified compounds			98.32

IA_{lit}, arithmetic index of the literature; IA_{calc}, calculated arithmetic index; SD, standard deviation

Antimicrobial tests

In the mycelial growth inhibition test (Table 2), all dermatophytes showed a considerable decrease in their growth, at all EO concentrations tested. It is important to note that the

10

5% Tween solution was also evaluated and did not inhibit microbial growth. *T. rubrum* was the most sensitive fungus, being inhibited in 61.97% by the lowest oil concentration (130 µg/mL), with no significant difference between the concentrations of 465 µg/mL and 260 µg/mL of EO, and between these concentrations and itraconazole. There is a dose-dependent relationship in all results, as the microbial inhibition by EO decreases as the concentration decreases, and the highest concentration used in the tests (930 µg/mL) inhibited the growth of dermatophytes by 100%.

Table 2: Inhibition of mycelial growth of dermatophytes under different concentrations of carvone-rich *L. alba* essential oil (p < 0.05).

Inhibition of mycelial growth (%)					
Dermatophyte	930 µg/mL	465 µg/mL	260 µg/mL	130 µg/mL	Itraconazole 32 µg/mL
<i>T. mentagrophytes</i>	100a	79.49b	63.37c	40.80d	71.81e
<i>T. rubrum</i>	100a	77.99b	74.96b	61.97c	71.94b
<i>M. gypseum</i>	100a	60.54b	52.09c	37.54d	24.86e

The results for the MIC and CFM test are described in Table 3, in which it is possible to observe that the dermatophyte most resistant to the action of the studied EO and of itraconazole is *M. gypseum*, which requires a higher MIC of both (72 µg/mL and 137.5 µg/mL, respectively), in relation to the other dermatophytes tested. The CFM data of the EO of *L. alba* indicate that, for all these dermatophyte strains, it has a fungistatic effect, as well as itraconazole, since the CFM values were higher than the MIC.

Table 3: Minimum inhibitory concentration (µg/mL) and minimum fungicidal concentration (µg/mL) of *Lippia alba* essential oil from itraconazole against dermatophytes (mean and standard deviation).

Species	CIM-LA	CFM-LA	CIM-I	CFM-I
<i>T. mentagrophytes</i>	36 ± 0	240 ± 48	6.87 ± 0	244.44 ± 26.46
<i>T. rubrum</i>	36 ± 0	116 ± 48	55 ± 0	129.87 ± 52.92
<i>M. gypseum</i>	72 ± 0	256 ± 99	137.5 ± 0	213.89 ± 190.82

MIC-LA, *L. alba* minimal inhibitory concentration; CFM-LA, minimum fungicidal concentration of *L. alba*; MIC-I, minimum inhibitory concentration of itraconazole; CFM-I,

minimum fungicidal concentration of itraconazole.

[013] DISCUSSION

The chemical composition of the EOs of the same species can vary due to the influence of factors intrinsic to the plants that produce them, such as their growth stage and their health, as well as due to external factors (climate, environment, composition and characteristics). soil, among others), thus interfering with the biological activity of this material.

According to the results obtained by the chemical analysis, the EO of *L. albad* in this study fits, in chemotype III, which is characterized by the large amount of carvone and limonene.

Many researchers report the pharmacological potential of these two monoterpenes – both can act as antimicrobials, antioxidants and immunomodulators, thus suggesting that the observed antidermatophytic activity may be observed. be attributed to these substances.

The oxygenated monoterpene carvone can be found in EOs of several plant species, such as *Mentha spicata* L. and *Mentha viridis* L. A series of carvone-rich EOs were tested for different types of biological activity, thus proving that this monoterpene has pharmacological potential to combat pathogenic fungi and bacteria, such as *Candida* sp. and *Listeria monocytogenes*, for example, and to prevent cancer. Furthermore, Aggarwal et al. (2002) detected the antidermatophytic activity of carvone- and limonene-rich EOs from *M. spicata* and *Anethum sowa* against *T. rubrum* and *M. gypseum*. Another point worth mentioning when it comes to carvone is its low cytotoxicity, which makes it suitable to be used as a complement to clinical protocols used in antifungal therapy.

Limonene, in turn, is a low toxicity monoterpene widely used as a flavoring and flavoring agent and is considered safe by the Food and Drug Administration. In addition to being widely used in the cosmetics and food industries, limonene can be applied clinically, as several studies prove its therapeutic and preventive potential against certain diseases – it has been proven to be a chemopreventive against various types of cancer. As for their

antimicrobial activity, the number of studies that attest to this activity is extensive and EOs that present limonene as the major compound proved to be excellent antimicrobials against pathogenic fungi and bacteria. In an in vitro study carried out with commercial limonene, it was detected that this compound has an inhibitory action with a fungicidal effect against *T. rubrum*.

Regarding β -myrcene, it is another monoterpene considered safe by the Food and Drug Administration, with low oral and dermal toxicity, being frequently used as a flavoring and flavoring agent in the cosmetics and food industries. It can be found in OEs of many plants, presenting itself as one of the major compounds of several of them. However, research carried out with myrcene extracted from natural sources revealed that it does not have antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* species. This information highlights the need to further investigate the antimicrobial property of this isolated substance against other pathogenic microorganisms, including dermatophytes.

When it comes to α -pinene, the major compound found in smaller amounts in the sample, it is known that, in plants, it has fungicidal properties.

Germanacrene-D is the only sesquiterpene that appears as a major component of the analyzed chemotype. It is one of the five isomers of germacrene and can be found in OEs of species from other genera of the Verbenaceae family.

The antimicrobial potential of EOs can be associated with the major compounds, however, it is possible that the components present in smaller amounts in the oil contribute to its antimicrobial action through some synergism with the predominant components. According to the results of the antimicrobial tests carried out in this work, the carvone chemotype of *L. alba* has anti-dermatophytic fungistatic activity against all dermatophytes tested, with a MIC of 72 $\mu\text{g/mL}$ against *M. gypseum* and 36 $\mu\text{g/mL}$ for *T. mentagrophytes* and *T. rubrum*. Another linalool-rich chemotype of this species inhibited the growth of *T. rubrum* and *M. gypseum* with MIC values of 39 $\mu\text{g/mL}$ and 312 $\mu\text{g/mL}$, respectively, with

lower efficiency than here observed. The EO *L. gracilis* also inhibited the *T. rubrum* isolate, but with MIC values of 46.87 and 93.75 µg/mL. However, variations in strains and methodology make a direct comparison between these works difficult. It is also important to highlight the fact that the results of antimicrobial tests can be influenced by the methods used, as they do not have the same sensitivity or are not based on the same principles.

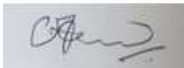

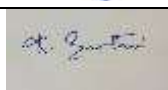

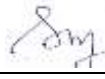


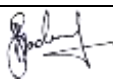
Regarding the usefulness of EOs in the treatment of dermatophytosis, the lipophilic characteristic of the components of these oils can facilitate their distribution through the stratum corneum of the skin, so that their topical application may, depending on the severity of the infection, be sufficient, but further tests to determine the mechanism of action and toxicity must be conducted.


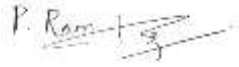
We Claim:

1. The objective of this work was to analyze the chemical composition, antioxidant activity and antidermatophytic activity of the crude extracts of *V. palmarum*, as well as the chemical composition and antidermatophytic activity of the essential oil of *L. alba*.
2. The dermatophytes used were *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Microsporum gypseum*. Inhibition of mycelial growth of all fungi was evaluated and minimum inhibitory and minimum fungicidal concentrations of extracts and oil were determined.
3. The antioxidant activity of the extracts was evaluated by free radical scavenging methods.
4. The extracts of *V. palmarum* showed antioxidant activity.
5. Both extracts and essential oil showed antidermatophytic activity.
6. Carvone and limonene are the major compounds in the essential oil analyzed.

Dated this 21st day of September, 2022

Signature(s):

1.	
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10.	

Applicant(s):

1.	Prof. Dr. K. Muthuchelian
2.	Dr. S. Alwin David
3.	Dr. K. Gayathri
4.	Mr. S. Nagul Dev
5.	Dr. S. Mohamed Rabeek
6.	Dr. R. Gokulan
7.	Divya S
8.	Mr. R. G. Padmanabhan
9.	Dr. G. Baskar
10.	Mr. P. Ram Kumar

5

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ABSTRACT

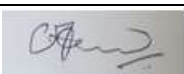
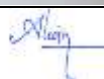
Chemical Composition and Biological activity of *Vanilla palmarum* and *Lippia alba*

[014] *Vanilla palmarum* and *Lippia alba* are plants that can be found in the semiarid
5 region of Bahia. Both are used for medicinal purposes by local populations in different
ways. However, *V. palmarum* is not studied much in several aspects, such as its
chemical composition and the biological activities it performs. *L. alba*, in turn, is one of
the most studied plant species, but, due to its geographic variability, the chemical
composition of its essential oil is quite variable. Therefore, the objective of this work
10 was to analyze the chemical composition, antioxidant activity and antidermatophytic
activity of the crude extracts of *V. palmarum*, as well as the chemical composition and
antidermatophytic activity of the essential oil of *L. alba*. The dermatophytes used were
Trichophyton mentagrophytes, *Trichophyton rubrum* and *Microsporum gypseum*.
Inhibition of mycelial growth of all fungi was evaluated and minimum inhibitory and
15 minimum fungicidal concentrations of extracts and oil were determined. The
antioxidant activity of the extracts was evaluated by free radical scavenging methods.
The extracts of *V. palmarum* showed antioxidant activity. Both extracts and essential
oil showed antidermatophytic activity. Carvone and limonene are the major
compounds in the essential oil analyzed.

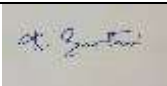

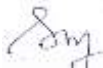




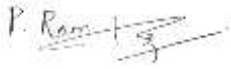
20 Accompanied Drawing [FIG. 1] [FIG. 2][FIG. 3] [FIG. 4][FIG. 5] [FIG. 6][FIG. 7]

Dated this 21st day of September, 2022

Signature(s):

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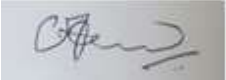
Applicant(s):

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1.	Prof. Dr. K. Muthuchelian
2.	Dr. S. Alwin David
3.	Dr. K. Gayathri
4.	Mr. S. Nagul Dev
5.	Dr. S. Mohamed Rabeek
6.	Dr. R. Gokulan
7.	Divya S
8.	Mr. R. G. Padmanabhan
9.	Dr. G. Baskar
10.	Mr. P. Ram Kumar

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FORM 3 THE PATENTS ACT, 1970 (39 of 1970) and THE PATENTS RULES, 2003 STATEMENT AND UNDERTAKING UNDER SECTION 8 (See section 8; Rule 12)					
1. Name of the applicant(s).		I/We, Prof. Dr. K. Muthuchelian et al., all are citizen of India, Address of one of the Applicant: Advisor, St. Peter's Group of Institutions & Adiyamaan Group of Institutions, Chennai and Hosur.			
2. Name, address and nationality of the joint applicant.		(i) that I/We have not made any application for the same/substantially the same invention outside India Or (ii) that I/We who have made this application No... dated alone/jointly with made for the same/ substantially same invention, application(s) for patent in the other countries, the particulars of which are given below:			
Name of the Country	Date of Application	Applicatio n No.	Status of the Application	Date of Publication	Date of grant
-	-	-	-	-	-
3. Name and address of the assignee		(iii) that the rights in the application(s) has/have been assigned to none that I/We undertake that upto the date of grant of the patent by the Controller, I/We would keep him informed in writing the details regarding corresponding applications for patents filed outside India within six months from the date of filing of such application. Dated this 21st day of September 2022			

4. To be signed by the applicant or his authorized registered patent agent.	Signature: 
5. Name of the natural person who has signed.	Prof. Dr. K. Muthuchelian et. al. Name of the Applicant(s)
	To The Controller of Patents, The Patent Office, at Chennai
Note.- Strike out whichever is not applicable;	

FORM- 5
THE PATENTS ACT, 1970
(39 of 1970)
&
The Patents Rules, 2003
DECLARATION AS TO INVENTORSHIP
[See Section 10(6) and Rule 13(6)]

1. NAME OF THE APPLICANT(S)

I/We, Prof. Dr. K. Muthuchelian et al., all are citizen of India, Address of one of the Applicant: Advisor, St. Peter's group of Institutions & Adiyamaan group of Institutions, Chennai and Hosur.

hereby declare that the true and first inventor(s) of the invention disclosed in the complete specification filed in pursuance of my-/ our application numbered _____ dated 21-09-22 is/are

2. INVENTOR(S)

(a) NAME	(b) NATIONALITY	(c) ADDRESS
1. Prof. Dr. K. Muthuchelian	INDIAN	Advisor St. Peter's group of Institutions & Adiyamaan group of Institutions Chennai and Hosur.
2. Dr. S. Alwin David	INDIAN	Assistant Professor, PG and Research Department of Chemistry, V.O.Chidambaram College, Thoothukudi – 628008, Tamil Nadu, India
3. Dr. K. Gayathri	INDIAN	Assistant Professor Department of Physics Amet Deemed To Be University ECR, Kanathur, Chennai – 603112.
4. Mr. S. Nagul Dev	INDIAN	Assistant Professor Department of Chemical Engineering Hindusthan College of Engineering and Technology Coimbatore-32
5. Dr. S. Mohamed Rabeek	INDIAN	Assistant Professor, PG and Research Department of Chemistry, Jamal Mohamed College (Autonomous), Tiruchirappalli – 620 020
6. Dr. R. Gokulan	INDIAN	Assistant Professor, Department of Civil Engineering,

		GMR Institute of Technology, Rajam – 532 127
7. Divya S	INDIAN	Department of Textiles and Apparel Design, Bharathiar University, maruthamalai Road Coimbatore-641 046 Tamil Nadu, India.
8. Mr. R. G. Padmanabhan	INDIAN	Assistant Professor, Department of Automobile Engineering, Arasu Engineering College, Kumbakonam
9. Dr. G. Baskar	INDIAN	Professor, Department of Biotechnology, St. Joseph's College of Engineering, Chennai – 600119.
10. Mr. P. Ram Kumar	INDIAN	Assistant Professor, PG and Research Department of Chemistry, V.O.Chidambaram College, Thoothukudi – 628008, Tamil Nadu, India
3. DECLARATION TO BE GIVEN WHEN THE APPLICATION IN INDIA IS FILED BY THE APPLICANT(S) IN THE CONVENTION COUNTRY:-- N.A. We the applicant(s) in the convention country hereby declare that our right to apply for a patent in India is by way of assignment from the true and first inventor(s).		
Dated this 21 st day of September, 2022 To, The Controller of Patents The Patent Office, Kolkata		
Prof. Dr. K. Muthuchelian et al. Applicant(s)		

FORM 9

THE PATENT ACT, 1970
(39 of 1970)
&
THE PATENTS RULES, 2003

REQUEST FOR PUBLICATION

[See section 11A (2) rule 24A]

I/We **Prof. Dr. K. Muthuchelian, Dr. S. Alwin David, Dr. K. Gayathri, Mr. S. Nagul Dev, Dr. S. Mohamed Rabeek, Dr. R. Gokulan, Divya S, Mr. R. G. Padmanabhan, Dr. G. Baskar, Mr. P. Ram Kumar** hereby request for early publication of my/our [Patent Application No.] TEMP/E-1/62162/2022-CHE

Dated **21/09/2022 00:00:00** under section 11A(2) of the Act.

Dated this(Final Payment Date):-----

Signature

Name of the signatory

To,
The Controller of Patents,
The Patent Office,
At Chennai

This form is electronically generated.