BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 12, Number 2, October 2023 | Pages: 485-498 | DOI: 10.14421/biomedich.2023.122.485-498 ISSN 2540-9328 (online)

Determination of Oil Quality and Antifungal Effect of Selected Citronella Accessions (*Cymbopogon nardus*, *Cymbopogon winterianus*) to Formulate an Anti-Dandruff Shampoo

Rathnayaka Mudiyanselage Nipuni Wijerathna[1], Achini Anuradha Wijeweera[2], Anushi Madushani Wijethunga[1], Mapa Mudiyanselage Sumudu Tharangani Mapa[1,*]
[1]Department of Biosystems Technology, Faculty of Technology, University of Sri Jayewardenepura, Sri Lanka.
[2]National Cinnamon Research and Training Center, Sri Lanka.

Corresponding author*
sumudu.mapa@sjp.ac.lk

Manuscript received: 28 March, 2023. Revision accepted: 21 August, 2023. Published: 23 August, 2023.

Abstract

Citronella is an aromatic grass of the family Poaceae which can be classified into two categories Ceylon Citronella (*Cymbopogon nardus*) and Java Citronella (*Cymbopogon winterianus*). The Citronella oil was extracted from five selected Ceylon Citronella (HP T1, HP T2 and HP T3) and Java Citronella (MP T1 and MP T2) accessions using steam distillation and hydrodistillation methods. Citronella oil quantity extracted by hydrodistillation with Xylene from Ceylon Citronella was higher (2.45-2.67 mL/100 g) than the Java Citronella (1.57-1.64 mL/100 g). The oil quantity of Ceylon Citronella (HP T1-5.52 %, HP T2- 1.40 %, HP T3- 1.05 %) and the quantity of Java Citronella (MP T1- 1.25%, MP T2- 1.79%) extracted by hydrodistillation showed a significant difference ($P<0.0001$) and there was no significant difference ($P=0.7055$) between the oil quantity of Ceylon (HP T1- 1.07%, HP T2- 1.18 %, HP T3- 1.19%) and Java (MP T1- 1.16%, MP T2- 1.23%) oils extracted by the steam distillation. Both Java and Ceylon Citronella oils showed organoleptic properties with pale yellow to pale brownish yellow colour and a strong citrusy aroma which meets the ISO 3848 and ISO 3849 standards. The oil of Ceylon Citronella accessions showed refractive index (1.465-1.487), relative density (0.893-0.910), and ethanol solubility (1:2 mL) within the ranges specified in SLS 170 standards. Java Citronella oil exhibited the refractive index (1.4660-1.4730), relative density (0.880-0.892), ethanol solubility (1:2 mL), and optical rotation (-5˚ to 0˚) which meets the specifications of ISO 3848 standards. Geraniol, Citronellol, and Citronellal were identified as the major constituents using the gas chromatography-mass spectrometry (GC-MS) where Java Citronella oil showed high Geraniol content (48.60-49.17%) than Ceylon Citronella oil (16.93-26.49%). All types of tested Citronella oil showed inhibition against *Candida albicans* where HP T3 (1.9 cm) and MP T1(2.0 cm) oils showed the highest promising antifungal activity among Ceylon oils and Java oils respectively. Therefore, these two oils were selected for the antidandruff shampoo formulation. The two antidandruff shampoo samples were formulated with 2% v/v concentrations of HP T3 and MP T1 Citronella oil which were determined as MIC for the inhibition of *C. albicans*. Antidandruff shampoo tested against C. *albicans* showed greater antifungal activity (HP T3 - 2.5±0.05 cm; MP T1 - 2.5±0.05 cm) than the crude Citronella oil (HP T3- 1.9±0.11 cm; MP T1-2.0±0.1 cm), also attained the organoleptic and physical properties such as pH (4.0-8.0), foam height (>100 mL), dirt dispersion (no ink in foam), viscosity, low wetting time and solid content (HP T3-14.75±0.12%; MP T2-12.33±0.19%) in acceptable specification range. This study exhibits that Ceylon Citronella oil HP T1 has the highest oil quantity from all selected accessions. Hydrodistillation can be used to extract high oil quantity than the steam distillation method from both Java and Ceylon Citronella types. Compared to Ceylon Citronella oil, Java oil has significant potential industrial applications with high Geraniol content and with the highest antifungal activity against C. *albicans*. Also, the tested Citronella oil of all selected accessions of both Java and Ceylon types meet the organoleptic and physiochemical requirements specified by the ISO and SLS quality standards with excellent antifungal activity against C. *albicans*, which provides prospective to use Citronella oil as a natural, safe, and eco-friendly fungicide in future product formulations.

Keywords: Antidandruff shampoo; Antifungal activity; Citronella oil; *Cymbopogon nardus*; *Cymbopogon winterianus*.

Abbreviations: International Standard Organization (ISO); Sri Lanka Standards (SLS); Minimal Inhibitory Concentration (MIC); Saburound Dextrose Agar (SDA); Potato Dextrose Agar (PDA); Dimethyl sulfoxide (DMSO).

INTRODUCTION

Citronella is an important type of aromatic grass belonging to the family Poaceae (Devi et al., 2016). Citronella can be classified into two categories as Ceylon Citronella (*Cymbopogon nardus*) and Java Citronella (*Cymbopogon winterianus*). The essential oil produced from Citronella, enhance its economic value as an herb. A number of studies have found that Citronella oil has therapeutic value due to the major components Geraniol, Citronellol, and Citronellal (Ameliana, Almawadah and Wulandari, 2019) and shows certain anti-fungal

properties, which can weaken or destroy certain fungi (Li et al., 2012). It also contains many possible bioactive components with great pharmaceutical and medicinal consequences which can act as anti-inflammatory, anticancer, antioxidant, and anticonvulsant agents (Devi et al., 2016). These properties of Citronella oil enhance its value and the applications in many fields such as cosmetics, food, medicinal, ayurvedic, and herbal products. Therefore, there is a huge potential to use Citronella oil in herbal cosmetic products to cure common fungal diseases like dandruff.

Dandruff is a major dermatological problem for many people, and it is observed as white scales, which cause scalp itching and hair loss. Excessive sweat gland secretion and the appearance of microorganisms on the scalp can also lead to the formation of dandruff. The most common causes of dandruff are the fungi such as *Candida albicans* (Ameliana, Almawadah and Wulandari, 2019) and *Malassezia furfur* (Pingili, Vanga and Raparla, 2016). Antidandruff shampoos and other treatment options contains various anti-dandruff substances, including pyrithione, salicylic acid, imidazole derivatives, selenium sulfide, tar derivatives, ketoconazole (Pingili, Vanga and Raparla, 2016) zinc, menthol, and thymol (Ameliana, Almawadah and Wulandari, 2019), etc. as key ingredients. However, the use of these chemicals can cause hair loss and adverse skin reactions such as rashes, itching, and dermatitis. These cannot prevent the recurrence of dandruff and the side effects caused by these chemicals cannot be ignored. Therefore, there is a demand for new antimicrobial agents and alternative therapies with natural ingredients. Citronella oil will be a highly effective instinctive remedy for this purpose.

Introducing the Citronella oil from the selected five Citronella accessions belonging to *Cymbopogon nardus* and *Cymbopogon winterianus* which are grown in Sri Lanka, will be a new approach to use Citronella oil in the herbal cosmetics industry. Direct application of Citronella oil on the skin is an impractical and less effective method. Therefore, a special formulation incorporated with Citronella oil should be prepared to treat *Candida albicans*. Suitable topical preparation for fighting dandruff is an anti-dandruff shampoo which is a liquid, gel, lotion, or aerosol that contains surfactants to generate foam and cleanse the scalp and hair.

This study focused to determine the antifungal effect of Citronella oil (*Cymbopogon nardus*, *Cymbopogon winterianus*) against dandruff causing fungi *Candida albicans* to formulate an anti-dandruff shampoo. The quality of Citronella oil, extraction methods, and shampoo preparations were determined according to the to criteria of SLS and ISO standards. The Minimum Inhibitory Concentration (MIC) of Citronella oil was determined by antifungal assay of agar well diffusion method.

MATERIALS AND METHODS

Study area

The plants of *Cymbopogon nardus* (Ceylon Citronella) and *Cymbopogon winterianus* (Java Citronella) were selected from the farm of the National Cinnamon Research and Training Center of Sri Lanka, located in Palolpitiya, Matara, Sri Lanka (6° 2' 2.94" N Latitude, 80° 33' 38.448" E Longitude).

Figure 1. A - *Cymbopogon nardus* (Ceylon Citronella), B- *Cymbopogon winterianus* (Java Citronella).

Citronella oil Extraction

- *Sample collection and processing*

The matured Citronella leaves from selected accessions *Cymbopogon nardus* (HP T1, HP T2, HP T3) and *Cymbopogon winterianus* (MP T1, MP T2) were harvested, dried under shade for 3-5 days, and cut into small pieces.

- *Determination of moisture content*

The Entrainment method (SLS 86 part 5) was used to measure the moisture content of Citronella samples by

using Dean and Stark apparatus (Witeg Germany). The moisture content of approximately 10 g of the Citronella leaves was measured with 60 mL of saturated toluene for about one and a half hours and refluxing was continued for two intervals of 15 minutes revealing no change. Then, the whole unit was left to cool for about 15 minutes, and the readings of moisture amount were taken from the bottom of the Dean and Stark arm.

The percentage moisture content was calculated by the following equation.

$$Moisture\ Content\ (\%) = \frac{(Arm\ Reading)}{(Sample\ Weight)} \times 100$$

- *Hydro distillation of Citronella oil with Xylene and without Xylene*

A modified Clevenger method with hydro distillation was used to determine the oil quantity of Citronella samples (ASTA Method No 5, Fourth Edition, 1997). Approximately 50 g of Citronella leaves with 500 mL of clean water were added to the flask and connected with the Clevenger arm where the graduated tube was filled with clean water up to the level of 5 mL and the unit was heated by a heating mantle (WHM 12393, Wisd Laboratory Instruments, German) while condensing a cooling system for 5 hours. After distillation, the system was allowed to cool, and the extracted Citronella oil was collected into a cleaned and oven-dried glass bottle by removing the water layer of the Clevenger arm. Then samples were kept for about 24 hours to settle. The minute water particles suspended in oil were removed by a cleaned glass syringe. The purified oil samples were subjected to GC-MS analysis. The oil percentage in 100 g of sample in dry weight basis was calculated by following equations.

$$Moisture\ mass\ of\ the\ sample = \frac{(Moisture\ \%)}{100} \times Mass\ of\ the\ sample$$

$$Dry\ weight\ of\ the\ sample = Mass\ of\ the\ sample - Moisture\ mass\ of\ the\ sample$$

$$Oil\ sample\ weight = Bottle\ with\ oil\ weight - Empty\ bottle\ weight$$

$$Oil\ \%\ (g\ in\ 100\ g\ of\ sample) = \frac{(Oil\ sample\ weight)}{(Dry\ weight\ of\ the\ sample)} \times 100$$

The hydrodistillation with Xylene was carried out by adding 1 mL of Xylene to the Clevenger arm. The dry weight of the samples and the oil percentage in 100 g of sample (dry weight) by hydrodistillation with Xylene were calculated by following equations.

$$Oil\ volume = Arm\ Reading - Xylene\ Volume$$

$$Oil\ \%\ (mL\ in\ 100g\ of\ sample) = \frac{(Oil\ volume)}{(Dry\ weight\ of\ sample)} \times 100$$

- *Steam distillation of Citronella oil*

A steam boiler setup was used for the steam distillation of Citronella samples. The distillation was run for 5 hours and the oil with moisture was collected and allowed to cool. Samples were subjected to settle for overnight and the oil was separated from the water using a separatory funnel. The remaining minute water particles suspended with oil were removed by adding Anhydrous Sodium Sulfate. Oil percentage in 100 g of sample (dry weight) was calculated by following equations.

$$Oil\ sample\ weight = Bottle\ with\ oil\ weight - Empty\ bottle\ weight$$

$$Oil\ \%\ (g\ in\ 100\ g\ of\ sample) = \frac{(Oil\ sample\ weight)}{(Dry\ weight\ of\ the\ sample)} \times 100$$

Determination of Citronella oil quality

The quality of extracted Citronella oil from selected accessions was evaluated in terms of organoleptic properties, relative density, refractive index, optical rotation, and ethanol solubility (ISO 3848, ISO 3849, SLS 170).

The colour, odor, and texture of the oil samples were observed under the organoleptic properties and the relative density of oil at 25 ℃ was measured using a standardized 10 mL pycnometer. The refractive index of the Citronella oils at 20 ℃ was measured by a J-57WR

refractometer and P-8000 digital Polarimeter (Germany) was used to measure the Optical rotation at 20 ℃.

The 80% Ethanol was used to determine the ethanol solubility of Citronella oil samples at 20 ℃. The Burette was filled with 50 mL alcohol and 1 mL of the oil sample was added to the Erlenmeyer flask. Then the oil was titrated with 80% alcohol and the burette reading of the endpoint at which the turbidity of the solution disappeared was recorded.

The alcohol solubility of the 1 mL oil sample was determined by the following equation.

$$Alcohol\ solubility = Final\ burette\ reading - Initial\ burette\ reading$$

Determination of Citronella oil composition

The GC-MS analysis to determine the composition of Citronella oil was carried out Trace 1300 ISQ QD Gas Chromatography Mass Spectrometer (Thermo Fisher Scientific, USA) equipped with TGWAXMS column (30 m long × 0.25 mm inner diameter, 0.25 µm film thickness). The sample was prepared by diluting 50 µL of the Citronella oil with 950 µL methanol in a vial and 0.5 µL from the dilution was injected into the column through the syringe.

The instrument was programmed with a linearly automated oven temperature from 50 ℃ to 220 ℃ in the rate of 3 ℃ min^{-1} and Helium was used as the carrier gas with a carrier flow rate of 1 mL / min. and the pressure was set as 5 BAR. The mass spectra were acquired in E1 mode (70ev), in a mass range of 50 to 500 amu and the transfer line and the ion source temperatures were maintained at 220 ℃ and 200 ℃ respectively.

The individual oil components were identified by comparing the retention time with standard substances and a computer search by matching mass spectrum data with the MS library (NIST MS search 2.0).

Determination of antifungal effect and Minimum Inhibitory Concentration (MIC) of Citronella oil

The antifungal effect of Citronella oil from selected accessions was tested using the agar well diffusion method (Balouiri, Sadiki and Ibnsouda, 2016). Saburound Dextrose Agar (SDA) was used as the media for testing and 6.5 g of SDA was suspended in distilled water in an Erlenmeyer flask and warmed until homogenized. The media, Petri plates, and distilled water were autoclaved at 121 ℃ for 15 minutes for sterilization. The sterilized liquid SDA was poured into Petri plates and left until solidified (Ameliana, Almawadah and Wulandari, 2019).

A suspension of *Candida albicans* was made by adding an inoculum of *Candida albicans* to saline water (Silva, Guterres, Weisheimer and Schapoval, 2008) and the mixture was vortexed, and the turbidity was checked with 0.5 McFarland standard (Oliveira et al., 2011). Then, 100 µL of fungal suspension was spread over the solidified SDA medium. After, a hole of 4 mm diameter was punched in the middle of the SDA medium aseptically using a cork borer and 20 µL (Balouiri, Sadiki and Ibnsouda, 2016) of Citronella oil was pipetted and added into the well. The plates were sealed and incubated at 37 ℃ for 24 - 72 hours (Oliveira et al., 2011). The inhibitory zone diameters of each oil type were recorded. The oil types with the highest inhibitory zone diameters were selected to determine the MIC.

The MIC was tested following agar well diffusion for Citronella oil with the highest antifungal activity in several oil concentrations (1%, 2%, 3%, 4%, and 5% v/v) diluted with Dimethyl sulfoxide (DMSO) solution. The same procedure also was followed using Potato Dextrose Agar (PDA) as the growth media. The DMSO and Captan (N-trichloromethylthio-4- cyclohexane-1,2-dicarboximide) were assessed as the negative and positive controls, respectively. The lowest concentration shows an inhibitory zone with no visible growth of fungus during the incubation was considered as the MIC value of the Citronella oil (Ameliana, Almawadah and Wulandari, 2019).

Formulation of Citronella oil antidandruff shampoo

The Citronella oil shampoo was made with the modified formula shown in Table 1. The Sodium Lauryl Sulfoacetate (SLSA), Sorbic acid, and Sodium chloride were measured and added to a 500 mL beaker and the Cocamidopropyl betaine, Glycerin, Tween 80, and the oil were pipetted and added to the beaker and topped up with the distilled water. The mixture was stirred with a magnetic stirrer at 1500 rpm until homogenous. A shampoo sample without Citronella oil was formulated as the negative control. The pH of formulated shampoo samples was measured.

Table 1. Citronella oil antidandruff shampoo formulation.

Material	Function	Concentration in 100 mL (%)
Sodium Lauryl Sulfoacetate (SLSA)	Primary surfactant	10
Cocamidopropyl Betaine	Co surfactant	5
Sorbic acid	Preservative	0.3
Sodium chloride (NaCl)	Viscosity adjuster	1
Glycerin	Humectant	3
Polysorbate 80 (Tween 80)	Oil solvent	0.5
Citronella oil	Active Ingredient (Antidandruff agent)	2
Distilled water	Solvent	89.5
Total Volume		100 mL

Determination of antifungal effect of Citronella antidandruff shampoo

The antifungal effect of formulated shampoo against *Candida albicans* was examined by following the previous method of agar well diffusion. The well of the medium was filled with 20 µL of Citronella oil shampoo. The Citronella oil and Captan were tested as the positive control and the shampoo without Citronella oil was tested as the negative control.

Evaluation of Citronella antidandruff shampoo

Formulated shampoo samples were tested for quality under different types of organoleptic and physiological parameters. The organoleptic properties were evaluated based on their clarity, colour, odor, and texture (AlQuadeib, Eltahir, Banafa and Al-Hadhairi, 2018).

- *Foaming ability and foam stability*

The forming ability of the shampoo was evaluated by the cylinder shake method (Al Badi and Khan, 2014). A 50 mL of 1% v/v shampoo solution was added to a 250 mL graduated cylinder and the cylinder was covered by hand and shaken for 10 times. The total volume of that foam that appeared after 1 min shaking was measured and recorded. The foam stability was tested by measuring the foam volume in 1min intervals for 4 min.

- *Determination of pH*

The pH of the formulated shampoo samples and 10% v/v diluted shampoo samples were directly measured using calibrated CyberScan PC 650 pH meter (EUTECH INSTRUMETS, Thermo Scientific, Singapore) and a strip of pH paper at room temperature 27±2 ℃ (SLS 1346:2018) (Al Badi and Khan, 2014).

- *Rheological evaluations (Viscosity)*

The viscosity of the shampoo samples was evaluated by using BDV-1S Digital Viscometer (BIOBASE Meihua, China) with the spindle L1 at speed of 60 rpm. The temperature and the size of the container were kept constant during the study (AlQuadeib, Eltahir, Banafa and Al-Hadhairi, 2018).

- *Dirt dispersion*

A drop of India ink was added to a large test tube with 2 drops of shampoo in 10 mL of distilled water. Then, the test tube was stoppered and shaken 10 times. The amount of ink in foam was evaluated as none, light, moderate or heavy (Al Badi and Khan, 2014).

- *Wetting time*

Drave's test was used to determine the wetting time of the formulated shampoo samples (AlQuadeib, Eltahir, Banafa and Al-Hadhairi, 2018). A canvas fabric was cut into 1.0 inch diameter disc with an average weight of 0.44 g. The smooth surface of the disc was placed on the surface of 1% v/v shampoo solution and the time required for the disc to begin sinking was measured by a stopwatch as the wetting time.

- *Percentage solid content*

A cleaned and dried dish was weighed, and 4 g of shampoo was added to the dish The dish with shampoo was weighed and the exact initial weight of shampoo was calculated. The dish was placed on a hot plate until the liquid portion of the shampoo was evaporated. After the remaining solid content was weighed and the percentage (%) of solid content was calculated (Al Badi and Khan, 2014).

Data analysis

Data were subjected to one-way ANOVA and two-way analysis of variance according to the GLM (General Linear Mode) at a 0.05 significance level using SAS OnDemand for Academics online version. The mean value, standard deviation, calculations, and figures with graphical representations of mean values were performed by Microsoft Excel for Microsoft 365 MSO (Version 2205 Build 16.0.15225.20028).

RESULTS AND DISCUSSION

Quantity and quality of Citronella oil

Organoleptic properties (colour, odor and texture) of the Java and Ceylon Citronella oil obtained from the selected accessions were determined. The colour measurements were carried out by direct eye observation at a distance of 30 cm (Fitri et al., 2022) and the observed colours of each oil type ranged between pale yellow to pale brownish yellow as specified in the ISO standard (ISO 3849:2003). The characteristic Citronella oil aroma (strong Citrusy) of each 5 types of oil was sensed at a distance of 5 cm (Fitri et al., 2022) as the odor and the oil from Java Citronella (MP T1 and MP T2) and Ceylon Citronella HP T3 oils have a stronger aroma than HP T1 and HP T2 due to their high Citronellal content (Table 3). According to the physical appearance and texture, each type of oil is an oily and viscous liquid that is volatile in the open atmosphere.

The moisture contents of the selected Citronella accessions shown in Table 2 are significantly different ($P < 0.0001$). The MP T2 Java Citronella accession showed the highest significant moisture content whereas the HP T3 Ceylon Citronella accession showed the least significant moisture content. Both Java Citronella accessions showed high amounts of moisture content above 50% compared to the moisture content of Ceylon Citronella accessions.

According to the results of Table 2, the % oil content obtained by hydrodistillation with Xylene from each accession showed a significant difference ($P = 0.0001$). The oil quantity (mL) per 100 g of Citronella sample from Ceylon Citronella is significantly higher than the Java Citronella. The Ceylon Citronella accession HP T3

showed the highest oil quantity of 2.67 mL/100 g whereas oil from MP T1 Java Citronella accession shows the least oil quantity of 1.57 mL/100 g. Extracted Citronella oil quantities from each accession by hydro distillation without Xylene showed a significant difference (P<0.0001) where the HP T1 accession showed the highest oil quantity of 5.52 % where oil from HP T3 accession shows the least oil quantity of 1.05% (Table 2). The % oil contents of selected Citronella accessions extracted by steam distillation does not show a significant difference (P = 0.7055) and ranged from 1% to 1.25% (Table 2).

Table 2. Citronella oil content and quality under different parameters.

Quality parameter	Accession of Citronella					Probability	CV%
	HP T1	HP T2	HP T3	MP T1	MP T2		
Moisture (%)	46.16 ^{c}	42.89 ^{d}	28.46 ^{e}	52.71 ^{b}	56.40 ^{a}	<0.0001	2.23
Hydro distillation with xylene - % Oil v/w (mL/100g)	2.67 ^{a}	2.45 ^{a}	2.53 ^{a}	1.57 ^{b}	1.64 ^{b}	0.0001	13.67
Hydro distillation - % oil w/w (g /100 g)	5.52 ^{a}	1.40 ^{bc}	1.05 ^{c}	1.25 ^{bc}	1.79 ^{b}	<0.0001	14.14
Steam distillation- % oil w/w (g /100 g)	1.07 ^{a}	1.18 ^{a}	1.19 ^{a}	1.16 ^{a}	1.23 ^{a}	0.7055	9.43
Refractive Index	1.47 ^{c}	1.49 ^{a}	1.48 ^{b}	1.47 ^{c}	1.47 ^{c}	<0.0001	0.06
Optical Rotation	-2.87°^{a}	-9.89°^{b}	0.72° ^{a}	-0.82°^{a}	-0.75°^{a}	0.0003	-94.40
Relative density	0.90 ^{b}	0.91 ^{a}	0.90 ^{b}	0.88 ^{c}	0.88^{c}	<0.0001	0.55
Ethanol Solubility (mL)	1.25^{a}	1.25^{a}	1.25^{a}	1.25^{a}	1.25^{a}	1.0000	4.61

Note: Probability<0.05 have a significant difference within mean values. Mean within each column followed by the same letter are not significantly different. Mean values from highest significant difference have been denoted with "a"

The relative density resulted for the oils of selected Citronella accessions shows a significant different (P < 0.0001). Ceylon Citronella oil from accessions HP T1, HP T2 and HP T3 shows a significantly high relative density compared to the Java Citronella oils of MP T1 and MP T2. According to the relative density of Ceylon Citronella oil samples, HP T1, HP T2 and HP T3 have been resulted as 0.90, 0.91 and 0.90 respectively, with highest significant difference in HP T2, and in the range of 0.893-0.910, the relative density for Ceylon Citronella essential oil in 25 ℃ specified in the SLS standards (SLS 170). Also, the relative density values resulted for Java Citronella oil samples MP T1 and MP T2 are same (0.88) and within the range of 0.880-0.892 specified for the relative density of Java Citronella essential oil in 25 ℃ (Table 2).

The measured refractive index values of Citronella oil from selected Citronella accessions were significantly different (P < 0.0001). The Citronella oil of accession HP T2 resulted with the highest significant refractive index, while HP T1, MP T1 and MP T2 shows the lowest significant refractive index (Table 2). According to the SLS quality standards, the refractive index of Ceylon Citronella should be in the range of 1.465-1.487. Therefore, only the oil from accession HP T2 shows a slightly higher refractive index out of the given range where the oil from HP T1 and HP T3 rely on the given refractive index range in the SLS standards (SLS 170). Although there is no specified refractive index range for the Java Citronella in SLS standards (SLS 170), the ISO standard have been specified the refractive index for Java Citronella in the range of 1.4660-1.4730 (ISO 3848). The refractive index values of both selected Java Citronella accessions MP T1 and MP T2 resulted as 1.47 (Table 2), which is in the range specified in the ISO standard.

The optical rotation of the oil samples from selected accessions of Java and Ceylon Citronella has resulted in a significant difference (P < 0.0001). The sample of HP T3 oil has the highest significance positive value (+0.72˚) which indicates that the oil a has dextrorotary polarized light plane. The HP T1 and HP T2 oils are in negative values -2.87˚ and -9.89˚ respectively indicating levorotary of the light plane. However, the oil samples from selected Ceylon Citronella accessions are not lie down within the ISO specified range of -22 ˚ to -12 ˚ (ISO 3849:2003) showing an odd optical rotation which might be a result of irregular distribution of oil composition (St-Gelais, 2021). Optical rotation results of Java Citronella oil samples MP T1 and MP T2 are -0.82˚ and -0.75˚ respectively are negative values that indicates a levorotary light plane and are not significantly different to each other (Table 2).

Both ISO and SLS standards have specified that the alcohol solubility of Java or Ceylon Citronella should be determined by using 80% (v/v) ethanol in 20 ℃ and high-quality Citronella oil should have 1:2 ethanol solubility (ISO 3848, ISO 3849, SLS 170). All the extracted oil samples were tested for ethanol solubility have been resulted the same value 1.25 mL of ethanol for the solubility of 1 mL of oil and does not show a significant difference (Table 2). This illustrate that the oil of selected Citronella accessions has their ethanol solubility at an acceptable level by international and local standards.

Chemical composition of Citronella oil

The Citronellal, Geranyl acetate, Citronellol, Geraniol, and Geranyl iso butyrate are the main compounds identified from the oil samples of selected Citronella accessions according to the GC-MS results (Table 3). The Citronellal contents available in each oil type are significantly different. The oil from accession HP T3 has the highest significant Citronellal content (20.29%), whereas the oil of HP T1 has the least significant Citronellal content (2.43%). The oil of Java Citronella accessions MP T1 and MP T2 have a Citronellal content of around 6-7% and which is lesser than the Citronellal content of HP T3 oil. The percentage availability of the Geranyl acetate has a significant difference among the five oil types of selected accessions where the oil of HP T1 has the highest significant Geranyl acetate content (13.48%) and in contrast, the oil HP T2 has the least Geranyl acetate content of 0.63% (Table 3). Citronellol contents identified by the GC-MS showed a significant difference among the tested oil samples. The Java Citronella oil samples MP T1 and MP T2 have high Citronellol content compared to Ceylon Citronella oil where MP T1 oil has the highest significant Citronellol content of 11.41%. The Ceylon Citronella oil HP T1 has the least significant Citronellol content of 2.06% and the high Citronellol content of HP T3 (9.63%) compared to the other Ceylon Citronella oil types of HP T1 and HP T2, is exceptional as it does not have a significant difference to the Citronellol content of Java Citronella oil MP T2. The available proportions of the compound Geraniol show a significant difference in tested oil samples. Java Citronella has very high Geraniol content around 50% of its total composition compared to Ceylon Citronella where Java oil MP T1 and MP T2 have the highest significant Geraniol contents 48.60% and 49.17% respectively with no significant difference to each other. The Ceylon citronella oil HP T1 and HP T3 have Geraniol contents 2.49% and 26.12% respectively with no significant difference to each other and Ceylon oil HP T2 has the least significant Geraniol content of 16.93% (Table 3). Geranyl iso butyrate is another type of the main compound identified in Citronella essential oil in minor quantities. The percentage content of Geranyl iso butyrate available in the tested samples has resulted in a significant difference. However, the Ceylon oil HP T2 has significantly higher Geranyl iso butyrate compared to other types of oil samples (Table 3).

Table 3. Chemical composition of the Citronella oil.

Chemical Compound	Accession of Citronella					Probability	CV%
	HP T1	HP T2	HP T3	MP T1	MP T2		
Citronellal (%) RT-14.73	2.43^{e}	3.46^{d}	20.29^{a}	6.57^{c}	7.39^{b}	<.0001	4.83
Geranyl acetate (%) RT-24.84	13.48 ^{a}	0.63 ^{d}	3.48 ^{c}	4.95 ^{bc}	5.54 ^{b}	<.0001	18.59
Citronellol (%) RT-25.24	2.06 ^{d}	3.16 ^{c}	9.63 ^{b}	11.41 ^{a}	10.11^{b}	<.0001	8.80
Geraniol (%) RT-28.23	26.49 ^{b}	16.93 ^{c}	26.12 ^{b}	48.60 ^{a}	49.17 ^{a}	<.0001	7.09
Geranyl iso butyrate (%) RT-29.39	0.99 ^{b}	2.75 ^{a}	0.44 ^{c}	0.23 ^{c}	0.21 ^{c}	<.0001	17.84

Note:RT- Retention Time, Probability<0.05 have a significant difference within the mean values.Mean within each column followed by the same letter are not significantly different. Mean values from highest significant difference have been denoted with "a"

Antifungal effect of Citronella oil

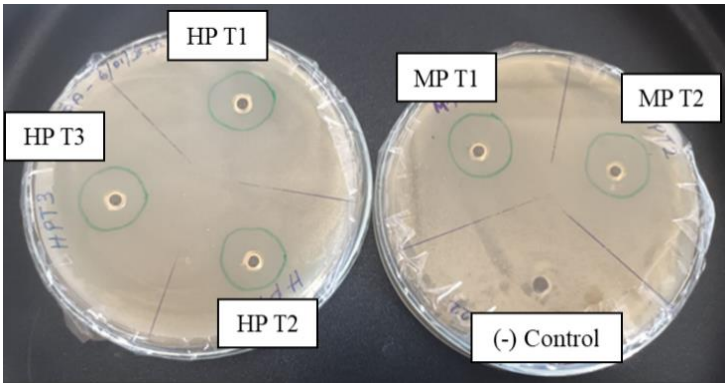

Figure 2. Inhibition zones of Citronella oil against *Candida albicans* on SDA.

All five types of non-diluted Ceylon Citronella (HP T1, HP T2, HP T3) and Java Citronella (MP T1, MP T2) oil samples that tested positive for the inhibition of *Candida albicans* with evidently visible inhibition zones compare to the negative control (Figure 1). The diameters of the inhibition zones that appeared on the

plates with Citronella oil (Figure 1) showed that the antifungal activity of the tested oil samples is different from each other. The Citronella oil of MP T1 accession of Java Citronella showed the highest inhibition zone diameter whereas oils of accessions HP T1 and HP T2 showed the least inhibition zone diameter.

Table 4. Inhibition zone diameters (Mean) of Citronella oil against *Candida albicans.*

Accession of the oil sample	Inhibition zone diameter (cm)	Probability	CV%
HP T1	1.8		
HP T2	1.8		
HP T3	1.9	0.1556	5.31
MP T1	2.0		
MP T2	1.9		

Minimum Inhibitory Concentration of Citronella oil

Table 5. Inhibition zone diameters of HP T3 and MP T1 oil concentrations (1%-5% v/v) against *Candida albicans* on PDA and SDA media.

Factor	Level	Diameter of Inhibition Zone (cm)	
		PDA	SDA
Factor 1 – Citronella accession			
	HP T3	1.267^{a}	0.722^{a}
	MP T1	1.361^{a}	0.672^{b}
Probability		0.0988	<0.0001
Factor 2 – Concentration (%)			
	1	-0.00^{d}	-0.00^{d}
	2	1.30^{bc}	-0.00^{d}
	3	1.25^{c}	1.00^{c}
	4	1.46^{b}	1.03^{b}
	5	1.40^{bc}	1.15^{a}
	Positive control	2.47^{a}	1.00^{c}
Probability		<0.0001	<0.0001
Interactions – Accession * Concentration (%)			
	HP T3*1	-0.00^{d}	-0.00^{d}
	HP T3*2	1.43^{bc}	-0.00^{d}
	HP T3*3	1.23^{c}	1.00^{b}
	HP T3*4	1.27^{c}	1.03^{b}
	HP T3*5	1.20^{c}	1.30^{a}
	Positive control	2.47^{a}	1.00^{b}
	MP T1*1	0.00^{d}	-0.00^{d}
	MP T1*2	1.17^{c}	-0.00^{d}
	MP T1*3	1.27^{c}	1.00^{b}
	MP T1*4	1.67^{b}	1.03^{b}
	MP T1*5	1.60^{b}	1.00^{b}
	Positive control	2.47^{a}	1.00^{b}
Probability		0.0122	<0.0001
CV%		12.55	3.38

Note: Probability<0.05 has a significant difference in mean values. The mean within each column followed by the same letter is not significantly different. Mean values from the highest significant difference have been denoted with "a"

The 1% v/v concentration of HP T3 and MP T1 oil samples were not showed clearly visible zones against the fungi *Candida albicans* on PDA media (Table 5). The clearly visible inhibition zones were observed starting from the 2% v/v concentration corresponding to the commercial antifungal agent, Captan which was used as the positive control. The results of the same antifungal assay carried out on SDA show that there were no inhibition zones for both 1% and 2% v/v concentration and the inhibition zones were observed afterward at the 3% v/v concentration. The positive control also indicated smaller inhibition zones on SDA than the inhibition zones for the positive control in PDA.

Formulation of Citronella oil antidandruff shampoo

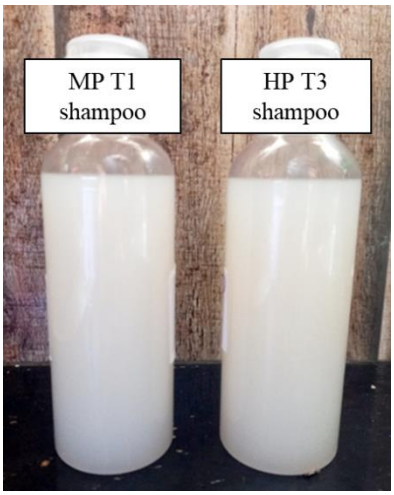

Figure 3. Formulated antidandruff shampoos with Citronella essential oil HP T3 and MP T1.

Antifungal activity of Citronella oil antidandruff shampoo

Citronella oil shampoo samples with MP T1 Java Citronella and HP T3 Ceylon Citronella showed a high antifungal activity than shampoo without Citronella oil (Table 6). However, shampoo samples without Citronella oil also showed substantial antifungal activity corresponding to the commercial fungicide used as the positive control and the crude Citronella oil.

Table 6. Inhibition zone diameters (Mean± SD, n=3) of HP T3 and MP T1 oil and shampoo with 2% v/v concentration of oil against Candida albicans

Selected accession	Inhibition zone diameter (cm)				
	Antidandruff shampoo	Shampoo without oil	Crude Citronella oil	Distilled Water	Positive control
HP T3	2.5±0.05	2.1±0.1	1.9±0.11	0	1.9±0.1
MP T1	2.5±0.05	2.0±0.2	2.0±0.1	0	2.1±0.1

Quality of Citronella oil antidandruff shampoo

The pH of the shampoo formulations with Citronella oil of HP T3 and MP T1 accessions resulted within 4-5 while the pH of negative control was higher than 5. The diluted shampoo samples formulated by incorporating Citronella oil of HP T3 and MP T1 accessions showed pH values of more than 5 while the diluted negative control also showed a high pH value than the initial pH (Table 7).

The shampoo formulations with Citronella oil of HP T3 and MP T1 and the negative control shampoo samples showed viscosity within 30-50 mPa.s which are significantly different from each other (Table 7). Although all samples resulted in low viscosity values in high rpm like 60 rpm, the shampoo solution with MP T1 Citronella oil shows significantly low viscosity compared to shampoo solution with Citronella oil HP T3 and negative control.

All shampoo samples with Citronella oil HP T3 and MP T1 and negative control samples showed similar results for dirt dispersion indicating no remain of ink in the foam (Table 7)

The wetting time of the shampoo solutions with Citronella oil HP T3 and MP T1 and negative control resulted in very low values of 6.59±0.14 seconds, 5.91±0.19 seconds, and 5.59±0.42 seconds and the wetting time of HP T3 shampoo was higher compared to the other tested samples (Table 7).

The percentage solid content of the formulated shampoo with Citronella oil HP T3 and negative control were lower than the acceptable range of 20-30% and, they were not significantly different from each other (Table 7).

Table 7. Physiological properties (Mean ±SD, n=3) of Citronella oil antidandruff shampoo.

Parameter	HP T3	MP T1	Shampoo without oil (Negative control)
pH	4.53±0.03	4.49±0.03	5.20±0.54
pH of diluted shampoo solutions	5.51±0.02	5.48±0.03	5.53±0.03
Viscosity (mPa.s)	46.4±1.08	30.23±0.56	41.99±0.17
Dirt dispersion (Ink in foam)	None	None	None
Wetting time (s)	6.59±0.14	5.91±0.34	5.59±0.42
% Solid content	14.75±0.12	12.33±0.19	13.47±0.23

The shampoo with MP T1 Citronella oil produced significantly high foam volume (170 mL) than foam volumes of shampoo with HP T3 Citronella oil (141 mL) and shampoo without Citronella oil (negative control).

As the graph illustrates, the foam volume of each shampoo sample reduced within 3 minutes time and remained constant at certain levels above 100 mL (Figure 3).

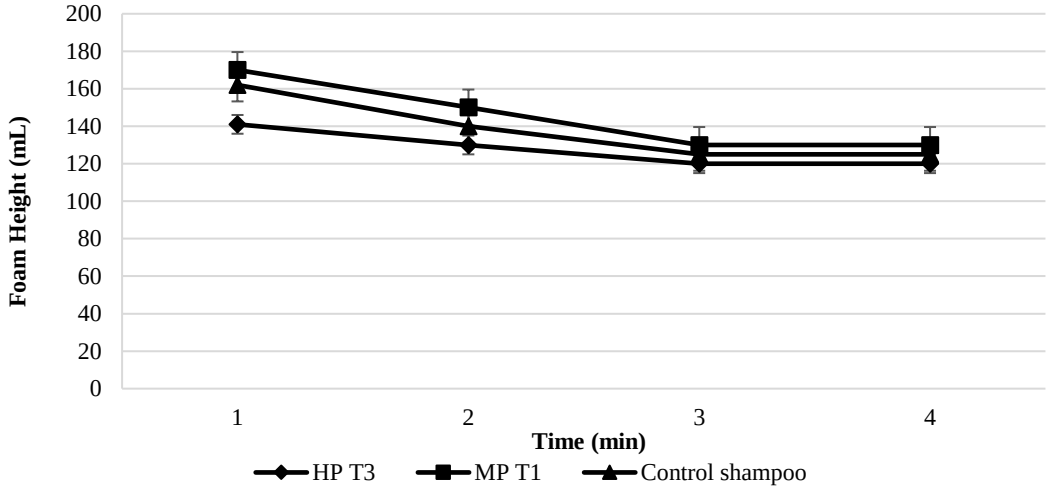

Figure 4. The foam volumes of the shampoo samples with time.

Discussion

Quantity and quality of Citronella oil

The Citronella plant leaves were harvested before the flowering period to obtain a high oil yield, as flowering causes the aging of plants (Blank et al., 2007). Shade drying was used to dry the plant leaves without leading to a reduction of oil quality and quantity. Direct drying in hot sunlight should be prevented as far as avoiding chemical transformations of this volatile essential oil. Drying of Citronella leaves aimed to reduce the moisture content with minimum quality reduction. The moisture content should be reduced literally to be at an equilibrium level that is defined for certain relative air humidity and temperature with a minimum quality reduction in terms of active ingredients, colour, flavor and aroma (Özgüven, Gülseren and Müller, 2019). Therefore, Citronella leaves should be spread out under a shade for drying. In the process of drying, the Citronella leaves under the shade should be frequently turned to avoid the formation of molds and the fermentation of leaves which will lead to the oil quality reduction. After the completion of the drying period, the overdried parts and the foreign matters should be removed. The Citronella leaves were cut into small pieces, to enhance the even distribution in the still which prevent steam channeling as well as for the easy insertion of plant leaves to the sample container (still) as well as to the round bottom flask of Clevenger distillation apparatus.

The moisture content of the harvested Citronella leaves was measured after shade drying, to calculate the dry weight of the sample. Citronella leaves were distilled with toluene which is less concentrated than water and immiscible with water. Method of hydro distillation with xylene was performed to determine the oil content that could be obtained from each type of Citronella sample and hydro distillation without xylene was used to extract the Citronella oil for the GC-MS analysis. The steam distillation was carried out to obtain the Citronella oil samples where the higher oil yields can be obtained for further analysis such as determination of refractive index, alcohol solubility, optical rotation, and relative density.

The oil yield of Citronella plants depends on several factors, including the presence of climatic differences, soil fertility, plant age, and the separation of Citronella oil (Ameliana, Almawadah and Wulandari, 2019). However, the study was carried out in the same location and same time period, and the above differences in oil content is due to the genetic variations of each accession. Hydrodistillation with the organic compound xylene showed the highest efficiency in Citronella oil extraction among the three extraction methods used and hydro distillation with or without an organic compound resulted with high oil quantity than steam distillation in most oil accessions (Table 2). However, steam distillation is one of the most common and main methods used for the extraction of Citronella oil in bulk quantities for industrial and commercial purposes.

All the oil samples from selected Java and Ceylon Citronella accessions that were tested are showing required quality standards for relative density, refractive index, ethanol solubility and can be considered as high-quality samples (Table 2). Essential oils are composed of many different compounds, most of which are optically active. Their different compositions allow them to be distinguished by measuring their optical rotation. These measurements also provide comprehension of the quality and purity of essential oil, as any change in its optimal composition can affect the optical rotation (Nandapure et al., 2016). Although the optical rotation of Ceylon

Citronella oil samples was not in the ISO-specified range, Java Citronella oil samples resulted within the ISO-specified range (-5˚ to 0˚) for optical rotation (ISO 3848). Therefore, the results prove that the oil samples of selected Java Citronella are within the acceptable range (Table 2).

Chemical composition of Citronella oil

The composition of the oil samples extracted from the selected Citronella accessions was determined by subjecting to the GC-MS and the constituents available in each oil type were identified by comparison of retention time with the library search of mass spectra for fragmentation pattern of reference standard compounds. The proportions of each compound present in five tested oil types were calculated from the GC peak areas using the normalization method.

The main constituents identified from the oil samples of selected Citronella accessions were Geraniol, Citronellol, and Citronellal. Geraniol is the main compound that has been identified in the highest proportions from each oil sample. Citronellal and Citronellol are the other two types of constituents identified in moderately high amounts in the composition of tested Citronella oil. Therefore, the GC-MS results of the tested Citronella oil samples comparatively correspond to the literature that indicates Citronella oil (*Cymbopogon nardus*) contains different types of chemical components, including Citronellal, Citronellol, and Geraniol, which have antifungal and antibacterial activities (Ameliana, Almawadah and Wulandari, 2019).

According to Aguiar et al., 2014, the high Citronellal content of C. *nardus* (Ceylon Citronella) oil extremely performs as a potent inhibitor of various fungi at ambient temperatures. Therefore, the Citronella essential oil from HP T3 accession shows a potential to inhibit the fungi *Candida albicans* with its significantly very high amount of Citronellal content (Table 3) and also comparatively high amounts of Geraniol and Citronellol contents. Along with this, the oil of selected Java Citronella accessions MP T1 and MP T2 are also considered to have a high potential antifungal activity due to the availability of high Citronellal, Citronellol, and Geraniol contents in their composition.

According to the GC-MS results, there are differences in the chemical composition and the percentage content of the constituents in the Citronella oil samples of selected accessions. These changes in the essential oil composition may possibly occur due to several environmental (climatic, seasonal, or geographic) factors and genetic differences (Aguiar et al., 2014).

Antifungal effect of Citronella oil

The oil of HP T3 resulted the highest inhibition zone among the tested Ceylon Citronella samples and MP T1 oil showed the highest inhibitory action among tested Java Citronella samples (Table 4). In addition, HP T3 oil showed unique high proportions of the main antifungal constituents Citronellal, Citronellol, and Geraniol (Table 3) in its composition (Ameliana, Almawadah and Wulandari, 2019). Therefore, the oils of accessions HP T3 and MP T1 were selected as the active ingredients with antifungal properties for the formulation of antidandruff shampoo. And further, the selected oil samples were subjected to antifungal analysis for the determination of Minimum Inhibitory Concentration against *Candida albicans.*

Identification of the volume of Citronella oil that should be incorporated into the shampoo formulation to perform antidandruff function was significant. Therefore, the MIC of selected Citronella essential oils for the inhibition of *Candida albicans* was identified across five concentrations (1%, 2%, 3%, 4%, and 5% v/v) by performing agar well diffusion assay (Ameliana, Almawadah and Wulandari, 2019). The oil samples were evaluated in both SDA and PDA media to compare the inhibitory activity of selected Citronella oil samples in different media (Table 5). The positive control also indicated smaller inhibition zones on SDA than the inhibition zones for the positive control in PDA. Therefore, the results of the MIC antifungal assay on PDA media were considered to be more accurate and precise.

Table 5 demonstrates that there is no significant difference between the antifungal activity of the Citronella oils of HP T3 and MP T2 accessions on PDA against *Candida albicans*. In contrast, the antifungal activity of the two oil types shows a significant difference in SDA against *Candida albicans*.

In the PDA media, the positive controls show the highest significant inhibition whereas the 1% v/v concentration of both HP T3 and MP T1 oils show the least significance with no inhibition against the tested fungi. The second least significant inhibition (1.17 cm) of MP T1 oil in PDA shows in the concentration of 2% v/v. Therefore, the 2% v/v concentration of MP T1 Citronella oil is the MIC that performs antifungal activity against *Candida albicans*. Also, the least concentration of HP T3 oil that shows an inhibition zone diameter is 2% v/v (Table 5). Therefore, the MIC values of both oil samples MP T1 and HP T3 were assumed to be more than 1%v/v concentration and less or equal to 2%v/v concentration ($1\% < MIC \leq 2\%$ v/v). Consequently, the 2% v/v concentration was determined as the MIC of both oils HP T3 and MP T1 against *Candida albicans* and referred to as the concentration of Citronella oil to be incorporated in the antidandruff shampoo formulation.

Formulation of Citronella oil antidandruff shampoo

The Citronella antidandruff shampoos formulated with selected Citronella oil HP T3 and MP T1 with the highest antifungal activity are shown in Figure 2. The Sodium Lauryl Sulfoacetate (SLSA; $C_{14}H_{27}NaO_5S$) has been registered in Drug Bank (Accession Number - DB13157) as a surfactant which also functions as a wetting agent. According to the Final Report on the Safety Assessment

of SLSA by the American College of Toxicology, it is a hydrophilic, skin-friendly, mild surfactant derived from coconut and palm plants which use in cleansing products for both skin and hair to remove surface oil, dirt, and bacteria effectively. The hydrophilic nature of the SLSA was beneficial for the liquid shampoos as well as it improves the rinsing ability of the product. SLSA is a bulky molecule that penetrates the skin and is free from sulfate ions causing less irritation to the skin. This can be effectively used in cosmetic products with pH 5-8.5, which will give better performance for the liquid shampoo prepared for pH 4.0-8.0 (SLS 1346:2018).

Further study of the Final Report on the Safety Assessment of SLSA by the American College of Toxicology (1987), it is a cosmetic ingredient approved by U.S. Food and Drug Administration and used primarily for bath preparations in 5-50%, but the actual concentration contacts with the skin surface are much lesser due to product dilution with water. Therefore, it was considered a much safer and more efficient ingredient for the Citronella antidandruff shampoo formulation.

A commonly used cosmetic ingredient, Cocamidopropyl betaine was used as a co-surfactant to increase the function of the SLSA (Hunter and Fowler, 1998), and Sorbic acid with low toxicity was used as the natural preservative (Dorko et al., 2000). Sodium chloride was effectively used as a salt work as a thickening agent (Penfield, 2005) whereas Polysorbate 80 was used as an emulsifier for the Citronella essential oils which are non-soluble in water (Ariff, Jai, Jamaludin and Ibrahim, 2022). The distilled water was used as the most abundant and common universal solvent in the preparation of shampoo samples, continuous stirring was performed to obtain a homogenized mixture of both the aqueous and the oil phases. Two shampoo samples for MP T1 oil and HP T3 were prepared by adding 2 mL of each oil type separately to obtain a 2% v/v oil concentration in a 100 mL shampoo formulation.

Antifungal activity of Citronella oil antidandruff shampoo

According to the inhibition zone diameters tabulated in Table 6, both shampoo samples with Citronella oil show higher antifungal activity than the crude Citronella essential oils used in their formulations, commercial fungicide, and the shampoo without Citronella oil. However, in this study shampoo formulated as a negative control without Citronella oil also showed inhibitory action against *Candida albicans*. This can be justified as the preservative used in shampoo formulations has increased the antimicrobial activity of the products (Sofos and Busta, 1981).

Quality of Citronella oil antidandruff shampoo

The attractiveness of physical appearance and organoleptic properties are vital factors to be considered in a cosmetic formulation that will enhance consumer preference (Chavan et al., 2019). The physical appearance of the formulated shampoo samples by incorporating Citronella oil of HP T3 and MP T1 accessions and the shampoo without Citronella oil was identified by the visual inspection. According to that, all shampoo mixtures were opaque, and white in colour as shown in Figure 2 due to the absence of any synthetic or natural colouring agent. The natural fragrance of the Citronella oil blended in both formulations MPT1 and HP T3 had given a citrusy aroma in to the shampoo samples which expresses the availability of Citronella oil as the active ingredient. In terms of texture, all shampoo samples were in a viscous liquid form similar to the shampoo formulations in the market and acceptable to the general requirements for the shampoo in SLS 1346:2018 standard. According to the resulting organoleptic properties except for negative control shampoo, both Citronella antidandruff shampoo samples have no significant difference from each other with good characteristics corresponding to the target market preference.

The pH level of a shampoo is accountable for improving and enhancing the hair quality with minimum eye irritation and stabilizing a balanced ecology on the scalp. Generally, most of the shampoo formulations are neutral or slightly acidic in pH. The cuticle (outer layer) of the hair is shrink away and lie flatter on the hair shaft with acidic blends (AlQuadeib, Eltahir, Banafa and Al-Hadhairi, 2018). Also, the mild acidity prevents the swelling and promotes strengthening of the scales, which leads to a shine and smoothness while basic solutions make hair triggers the hair to be frizzier. Therefore, the promoting the shampoo formulations with low pH to minimize the hair damage is an emerging trend in the industry (Krunali et al., 2013). The acidity of Citronella oil has been affected for the low pH of shampoos blended with Citronella oil and the formulations were not required to be acid balanced after initial formulation as both shampoo formulations were ranged in SLS specified pH levels 4.0-8.0 for the shampoo (SLS 1346:2018). The pH of diluted shampoo samples formulated by incorporating Citronella oil of HP T3 and MP T1 accessions were in the pH range of 5.5-5.9 near to the skin pH (Krunali et al., 2013). Also, both shampoo formulations show no significant difference in pH (Table 7).

The viscosity can be defined as the thickness or the stickiness of a liquid (AlQuadeib, Eltahir, Banafa and Al-Hadhairi, 2018). Viscosity plays a vital role on a shampoo product in defining and controlling characteristics such as shelf-life stability and product aesthetics such as clarity, ease of flow in removal from packing, ease spreading in application to hair and product reliability in the package (Chavan et al., 2019). Shampoos are non-Newtonian products with plastic or pseudoplastic flow behaviors which shows high viscosity

in low rpm values and low viscosity in increased shear rates.

Dirt dispersion is a key criterion for evaluating the cleaning action of shampoo formulations (Al Badi and Khan, 2014). Determination of the amount of ink present in the foam phase of the column after shaking the shampoo solution and estimate the dirt dispersion of the shampoo formula (Chavan et al., 2019). The shampoos which cause the ink to be concentrated in the foam phase are considered to be poor-quality formulations as the ink used to represent the dirt that remains in the foam is difficult to rinse away and also will be redeposited on the hair. Therefore, the ink or the dirt should be always remained in the water phase to accomplish a better performance in cleansing (Krunali et al.2013). The results of dirt dispersion ensured that the formulations achieved the cleaning ability and effectiveness in market satisfactory level (Al Badi and Khan, 2014).

The wetting time of a surfactant is a function of its concentration which is used to determine the efficacy of the surfactant (Krunali et al., 2013). The performance of wetting action is dependent on various factors such as diffusion, surface tension, the concentration of the wetting agent, and the nature of the surface being wetted. An acceptable wetting agent should have the ability to reduce surface tension (AlQuadeib, Eltahir, Banafa and Al-Hadhairi, 2018). A low wetting time indicates a greater wetting efficiency with a maximum concentration of detergents (Al Badi and Khan, 2014). All shampoo samples formulated in this study can be categorized as shampoos with acceptable wetting times due to the maximum concentration of surfactants with high efficacy (AlQuadeib, Eltahir, Banafa and Al-Hadhairi, 2018).

The percentage solid content of a shampoo formulation is an important criterion to be considered in terms of quality and performance. Too many solids in a shampoo, makes it difficult to work on hair or to wash out where, the absence of enough solids, leads to be too watery and will wash away rapidly. Therefore, the solid content of a good shampoo should be in the range of 20-30 % (AlQuadeib, Eltahir, Banafa and Al-Hadhairi, 2018). Although, the solid contents of both shampoo formulations are slightly lower than the specified range, are expected to perform cleaning and wash out easily (Table 7).

As a promising fungal inhibitor, Citronella oil can be widely used as a strong fungicide in different industrial applications such as in medical field, food industry agricultural industry and etc. The significant antifungal activity of Citronella oil against *C. albicans* shows that Citronella oil can be used as a remedy for the infections of *Candida* on human skin such as Candidiasis which may cause symptoms including rashes, scaling, itching, and swelling. Therefore, Citronella oil has the potential of used as an active ingredient in herbal cosmeceutical formulations such as soap, creams, lotions, and ointments etc.

CONCLUSIONS

The oil extracted from the selected Citronella accessions were in high quality under the tested physiochemical parameters and meet the organoleptic quality requirements with acceptable colour, aroma, and texture. The Ceylon oil HP T1 has the highest oil content (mL/100 g) and the oil quantity of Ceylon Citronella extracted by hydrodistillation with xylene is higher than the oil content of Java Citronella. Compared to steam distillation, hydro distillation can be used to extract high oil quantities from both Java and Ceylon types. The results of the quality tests performed for the Citronella oil samples in terms of refractive index, relative density, optical rotation, and ethanol solubility followed the acceptable ranges under the ISO and SLS standards. The significant differences in oil quantity and quality of tested Citronella accessions show that these accessions should be genetically different from each other. However, clearly, the oil extracted from all the selected Citronella accessions are of high quality under the physiochemical parameters.

Results of GC-MS reveal that the main constituents of the oil samples of selected Citronella accessions are Citronellal, Geranyl acetate, Citronellol, Geraniol, and Geranyl iso butyrate. The oil HP T3 has high contents of the main constituents Geraniol, Citronellal, and Citronellol. The highest Geraniol contents are available in MP T1 and MP T2 oil samples. The oil of the HP T3 accession has a unique chemical composition compared to the other tested Ceylon Citronella accessions.

All the oil samples have antifungal activity against *Candida albicans* and MP T1 and HP T3 oils have the highest inhibitory action with a minimum inhibitory concentration (MIC) of 2% v/v. Antidandruff shampoos formulated blending MP T1 and HP T3 oils show antifungal activity against *Candida albicans* with evidence that both tested oils can be effectively used as an antidandruff agent in hair shampoo. Also, both shampoo samples are aligned with the quality standards of the organoleptic properties, and physiological properties such as foaming, pH, viscosity, wetting time, dirt dispersion, and solid content (%) which conclude that the use of Citronella oil as an ingredient does not affect to change the acceptable levels of physiochemical parameters of shampoo.

***Acknowledgements*:** This work was supported by National Cinnamon Research and Training Center of Sri Lanka and Department of Biosystems Technology, Faculty of Technology, University of Sri Jayewardenepura, Sri Lanka. The authors wish to thank Ms. W.U.M. Pramodani and laboratory staff of biotechnology laboratory of Faculty of Technology, University of Sri Jayewardenepura for their support.

***Authors' Contributions*:** R.M.N.Wijerathna, A.A. Wijeweera & M.M.S.T. Mapa designed the study. R.M.N.Wijerathna, A.A. Wijeweera, & A.M. Wijethunga carried out the laboratory work. R.M.N.Wijerathna, A.A. Wijeweera & M.M.S.T. Mapa analyzed the data. R.M.N.Wijerathna & M.M.S.T. Mapa wrote the manuscript. All authors read and approved the final version of the manuscript.

***Competing Interests*:** The authors declare that there are no competing interests.

***Funding*:** The authors declare that the research was funded by National Cinnamon Research Institute and Training Center of Sri Lanka.

REFERENCES

Al Badi, K., & Khan, S. A. (2014). Formulation, evaluation and comparison of the herbal shampoo with the commercial shampoos. *Beni-Suef University Journal of Basic and Applied Sciences*, *3*(4), 301–305. https://doi.org/10.1016/j.bjbas.2014.11.005

AlQuadeib, B. T., Eltahir, E. K. D., Banafa, R. A., & Al-Hadhairi, L. A. (2018). Pharmaceutical evaluation of different shampoo brands in local Saudi market. *Saudi Pharmaceutical Journal*, *26*(1), 98–106. https://doi.org/10.1016/j.jsps.2017.10.006

Ameliana, L., Almawadah, A., & Wulandari, L. (2019). The effect of Citronella Oil Concentration (cymbopogon nardus (L.) Rendle) on the quality of shampoo and antifungal activity of candida albicans. *Indonesian Journal of Pharmaceutics*, *1*(2). https://doi.org/10.24198/idjp.v1i2.21551

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A Review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Blank, A. F., Costa, A. G., Arrigoni-Blank, M. de, Cavalcanti, S. C., Alves, P. B., Innecco, R., Ehlert, P. A., & Sousa, I. F. (2007). Influence of season, harvest time and drying on Java Citronella (cymbopogon Winterianus Jowitt) volatile oil. *Revista Brasileira De Farmacognosia*, *17*(4), 557–564. https://doi.org/10.1590/s0102-695x2007000400014

Devi, K., Mishra, S. K., Sahu, J., Panda, D., Modi, M. K., & Sen, P. (2016). Genome wide transcriptome profiling reveals differential gene expression in secondary metabolite pathway of Cymbopogon Winterianus. *Scientific Reports*, *6*(1). https://doi.org/10.1038/srep21026

Fitri, N., Riza, R., Akbari, M. K., Khonitah, N., Fahmi, R. L., & Fatimah, I. (2022). Identification of citronella oil fractions as efficient bio-additive for Diesel Engine Fuel. *Designs*, *6*(1), 15. https://doi.org/10.3390/designs6010015

Li, W.-R., Shi, Q.-S., Ouyang, Y.-S., Chen, Y.-B., & Duan, S.-S. (2012). Antifungal effects of citronella oil against Aspergillus niger ATCC 16404. *Applied Microbiology and Biotechnology*, *97*(16), 7483–7492. https://doi.org/10.1007/s00253-012-4460-y

Nandapure, S. P., Wankhade, S. G., Jadhao, S. M., Bhoyar, S. M., Wanjari, S. S., & Sarode, R. B. (2016). Quality Parameters of Java Citronella Oil as Influenced by Nutrient Management Under Inceptisols. *International Journal Of Tropical Agriculture*, *34*(3), 585–593.

Oliveira, W. A., Pereira, F. de, Luna, G. C., Lima, I. O., Wanderley, P. A., Lima, R. B., & Lima, E. de. (2011). Antifungal activity of Cymbopogon Winterianus Jowitt ex Bor against candida albicans. *Brazilian Journal of Microbiology*, *42*(2), 433–441. https://doi.org/10.1590/s1517-83822011000200004

Pingili*, M., Vanga, S., & Raparla, R. K. (2016). Antifungal activity of plant extracts against dandruff causing organism Malassezia furfur. *International Journal of Bioassays*, *5*(11), 5047. https://doi.org/10.21746/ijbio.2016.11.0010

Silva, C. de, Guterres, S. S., Weisheimer, V., & Schapoval, E. E. S. (2008). Antifungal activity of the lemongrass oil and citral against candida spp.. *Brazilian Journal of Infectious Diseases*, *12*(1). https://doi.org/10.1590/s1413-86702008000100014

Özgüven, M., Gülseren, G., & Müller, J. (2019). Investigation of the efficiency of drying conditions for essential oil produc-tion from aromatic plants. *Makara Journal of Science*, *23*(3). https://doi.org/10.7454/mss.v23i3.11262

Aguiar, R. W., Ootani, M. A., Ascencio, S. D., Ferreira, T. P., Santos, M. M., & Santos, G. R. (2014). Fumigant antifungal activity of corymbia citriodora and cymbopogon nardus essential oils and citronellal against three fungal species. *The Scientific World Journal*, *2014*, 1–8. https://doi.org/10.1155/2014/492138

Hunter, J. E., & Fowler, J. F. (1998). Safety to human skin of cocamidopropyl betaine: A mild surfactant for personal-care products. *Journal of Surfactants and Detergents*, *1*(2), 235–239. https://doi.org/10.1007/s11743-998-0025-3

Ariff, S. B., Jai, J., Jamaludin, S. K., & Ibrahim, N. (2019). Release of encapsulated citronella oil in tween 80 solution. *Journal of Physics: Conference Series*, *1349*(1), 012130. https://doi.org/10.1088/1742-6596/1349/1/012130

Chavan, V. M., J., K., Kiran, T., Suryavanshi, A., & Bhor, A. S. (2019). Formulation and evaluation of Herbal Shampoo. *American Journal of PharmTech Research*, *9*(5), 88–96. https://doi.org/10.46624/ajptr.2019.v9.i5.008

Dorko, C. L., Ford, G. T., Baggett, M. S., Behling, A. R., & Carman, H. E. (2000). Sorbic acid. *Kirk-Othmer Encyclopedia of Chemical Technology*. https://doi.org/10.1002/0471238961.1915180204151811.a01

SOFOS, J. N., & BUSTA, F. F. (1981). Antimicrobial activity of Sorbate. *Journal of Food Protection*, *44*(8), 614–622. https://doi.org/10.4315/0362-028x-44.8.614