Innovations

Chemical Profile, Antitrypanosomal, Antiplasmodial and Antibacterial Activities of the Volatile Oil from the Seed of *Callistemon Citrinus*

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Abstract

Callistemon citrinus, often known as C. citrinus, is a member of the Myrtaceae family and has a number of medicinal uses. For example, the aerial parts of this plant are frequently used to treat respiratory problems including cough and bronchitis, hemorrhoids, and parasite infections. Hydrodistillation was used to extract the volatile oil of the seed (SVO) from the plant under study, and high-resolution gas chromatography-mass spectrometry (GC-MS) was used to analyse the crude extract. The in vitro bioassays of the volatile oil (VO) were conducted utilizing parasite lactate dehydrogenase (pLDH) and Trypanosoma brucei brucei (T. b) against Plasmodium falciparum (P. falciparum) strain 3D7. Agar diffusion was also used to investigate the same VO's antibacterial properties. Utilizing human cervical cancer cells (HeLa cells), the cytotoxicity of the VO was evaluated. For the wet sample, the SVO yield was 0.95% v/w. α-pinene (13.20%) and eucalyptol (37.56%) were the two primary components of the SVO, which was primarily composed of oxygenated monoterpene (61.26%). Trypanosome/plasmodium parasites are inhibited by samples having an IC50 value of less than 0.02 mg/mL. Against the trypanosome, the SVO's activity was moderate at 0.092 mg/mL. P. falciparum's vitality was not considerably reduced by the SVO at a dose of 0.050 mg/mL.

Additionally, it shows no discernible reduction in HeLa cancer cell line proliferation. SVO, on the other hand, showed significant antibacterial action against the six chosen bacterial strains. The study's findings demonstrated that the SVOs have potent bioactive molecules with notable antitrypanosomal and antibacterial qualities, which could provide novel opportunities for the development of antimicrobial and trypanocidal drugs.
Keywords: *Callistemon citrinus, volatile oils, cell cytotoxicity, antitrypanosomal, antiplasmodial.*

Introduction

The Royal Botanic Garden Kew[1] regards *Callistemon citrinus* as a synonym of *Melaleuca citrina*. It was once known as Callistemon lanceolatus and is the bottlebrush variety that is most often grown. When conditions are right, it normally grows into a small tree-proportion and reaches a height of 10 to 15 feet with a fine texture. When the tree is young, its many, long, slender branches have hard, fibrous bark. Usually grey in color, the bark has ridges that overlap[2]. The leaf venation is pinnate, and the leaf arrangement is alternating, simple, sharp-pointed, and lanceolate or linear in shape. This plant is evergreen, with leaf blades that are between two and four inches long, and the veins are plainly apparent on both sides[2]. The spherical, brown fruits have a length of around 0.5 inches and a brown color. Fruit capsules are only opened once the plant components that contain them have died[3, 4]. Australia is home to a large population of *C. citrinus*, which is also widely grown in South Africa, particularly in certain parts of the Eastern Cape Province[5, 6]. The plant's aerial parts are commonly used in traditional medicine to treat gastrointestinal disorders, discomfort, and infectious diseases brought on by bacteria, fungi, viruses, and other pathogens. Due to their pleasant, energizing aroma[7,8], they can also be used as a tea substitute[7]. Its strong anti-inflammatory, fungitoxicity, antinociceptive, and antibacterial properties make it a popular treatment for respiratory disorders such as bronchitis and cough[9, 10]. Adults in Uganda consume about 500 mL of the fresh leaf decoction of *C. citrinus* to treat coughs[11]; in China, it is also frequently used as a traditional Chinese medicine pill to treat hemorrhoids [12]. Furthermore, women use it as a douche to clear the genitourinary tract of excessive menstruation or mucosal discharge known as leucorrhea. It is additionally employed to treat urinary incontinence and bedwetting in children[13]. In Jamaica, the decoction is used as a hot tea remedy for diarrheal infections[14] and gastroenteritis, while in Kenya; it is used to treat toothaches[15].

Numerous studies have been conducted on the volatile oil found in *C. citrinus* leaves, flowers, and stems[16, 17, 18, 19, 20]. The quality, concentration, and kind of phytochemical compounds in the volatile oils are all influenced by environmental factors that cannot be ignored, including irradiance, harvesting time, relative

humidity, photoperiod, location, plant cultivation methods, soil structure, and climate[21]. The aforementioned considerations have been used to demonstrate why it is unacceptable to precisely specify the constituents of volatile oils[21]. As secondary metabolites of plants, volatile oils are made up of a wide range of bioactive substances with distinct chemical and structural properties, which gives them a wide range of functionalities. In addition to their diverse chemical makeup, volatile oils are a special class of innovative, potentially effective antibacterial agents that have attracted particular attention[22].

Trypanosoma brucei is the vector that causes sleeping sickness, often known as HAT. The tsetse fly (Glossina) transmits this protist parasite to humans. Millions of people suffer from sleeping sickness, which, if left untreated, can develop into a lethal nerve disorder affecting around 36 nations in sub-Saharan Africa[23]. Some of the current medications used to treat trypanosomiasis include pentamidine, suramin, melarsoprol, mel B, and arsobal. However, there have been a number of drawbacks to these medications, including insufficient supply, resistance by trypanosoma parasites, high cost, and some adverse effects[24, 25]. These drawbacks have led to the documentation of a few African medicinal herbs with anti-trypanosomiasis properties[26,27, 28]. It has recently been shown that *P. falciparum* is resistant to antimalarial medications like artemisinins. Finding novel, affordable, and highly effective antimalarial medications is therefore crucial[29,30]. The hunt has begun for new lead medications to treat trypanosomiasis and malaria, or for a molecule with antiplasmodial and antitrypanosomal properties, preferably derived from African plants. These medications are required to tackle diseases, as the majority of synthetic medications have been shown to be highly harmful and resistant to treatment[31]. They are no longer as effective when used for treatment due to their increasing antimicrobial resistance[32]. Thus, alternative natural products are required, particularly those derived from volatile oils[33], which have several components with potent antibacterial properties [34]. Recent research reports have indicated that *C. citrinus* organic leaf extracts possess significant potency against Gram positive and Gram negative bacteria, as well as fungi[9, 16, 35]. These extracts can be used as effective antibacterial agents against a wide range of pathogenic bacterial strains, [36, 37, 38]. *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*)[39] are two opportunistic bacteria that cause serious and potentially fatal infections in immune-compromised people. Lower urinary tract infections and septicaemia are caused by the gram-positive bacterium S. aureus, which is present in the human intestine and is responsible for food poisoning, toxic shock syndrome, and wound infections following surgery.[40]. In the current study, the volatile oil from *C. citrinus* seeds gathered in August during the winter months in the Eastern Cape Province of South Africa was evaluated for its chemical composition, antitrypanosomal, antiplasmodial, antibacterial, and cell cytotoxic properties. The volatile oil of *C. citrinus* seeds grown in South Africa has limited information, as far as we know, on

its antitrypanosomal and antiplasmodial properties. Additionally, the plant's volatile oil was tested for its antibacterial properties against six different kinds of bacteria using the agar well diffusion technique, and its subsequent assessment of cell cytotoxicity was conducted.

Materials and Methods

Plant Material and collection

In May 2023, fresh seeds of *C. citrinus*were gathered from the University of Fort Hare campus in Alice, in the Eastern Cape Province of South Africa (sample site GPS coordinates: Latitude: 32° 46' 59.99" S; Longitude: 26° 52' 59.99" E).

Organisms

Listeria Ivanovii ATCC19119, Escherichia coli 0157:H7: ATCC 35150, Vibrio alginolyticus DSM 2171, Salmonella typhiACC, Staphylococcal enteritisACC and *Staphylococcus aureus ACC*

Extraction of Volatile oil

The procedure outlined by Larayetan *et al*.[16] was followed in order to extract the volatile oil. In summary, 250 g of plant seed was extracted using modified Clevenger equipment and the hydro-distillation process for three hours in compliance with European Pharmacopoeia[41]. Before being used, the extracted volatile oil was dried in anhydrous sodium sulphate, sealed in a colored vial, and kept at 4 °C. The seed's volatile oil yield (v/w %) was computed.

Characterization of Volatile Oils Constituents by Gas Chromatography-Mass Spectrometry (GC/MS)

To analyse and identify the components of the volatile oil, GC-MS was utilized. The GC-MS analyses were performed using a Hewlett-Packed HP 5973 mass spectrometer that was interfaced with an HP-6890 gas chromatograph. For this analysis, the following conditions of operation were used: The column temperature was 70°C at the beginning, 240°C at the end, 3 minutes for equilibration, 4 °C min^{-1} for the ramp, and 240°C at the conclusion; the inlet mode was split less, with an initial temperature of 220°C, 8.27 psi of pressure, 30 mL/min of flush out flow, and 0.20 minutes of flush out time. Helium gas brand; capillary column 30 m × 0.25 mm i.d.; coat thickness 0.25 μm; initial flow 0.7 mL/min; linear velocity 32 cm/s; MS: EI technique at 70 eVLarayetan*et al*[16].The retention index (RI) of each component was compared to those in the literature to determine the constituents of the volatile oils, and the peak regions were used to determine each constituent's percentage composition.

Antiplasmodial activity

The malaria parasite strain 3D7 of *P. falciparum* was cultured in RPMI 1640 medium supplemented with 25mMHepes and 2mM L-glutamine (Lonza). In

addition, 5% Albumax II, 20 mM glucose, 0.65 mM hypoxanthine, 60 µg/mL gentamycin, and 2-4% hematocrit human red blood cells were added to the media. The culture conditions for the parasites were maintained at 37 °C in sealed T25 or T75 culture flasks with an environment of 5% CO_2, 5% O_2, and 90% N_2. Using the technique outlined by Makleret *et al*.[42], parasite lactate dehydrogenase (*pLDH*) activity was used to determine parasite vitality. Positive controls included either artemisinin (Sigma-Aldrich) or chloroquine (Sigma-Aldrich). The *C. citrinus* VO was screened at a concentration of 0.050 mg/mL against malaria parasites using a stock solution of VO in dimethyl sulfoxide (DMSO) at a concentration of 20 mg/mL. In a 37 °C CO_2 incubator, the VO was introduced to the parasite cultures in 96-well plates and left for 48 h. The plate was taken out of the incubator after 48 h had passed. In a new 96-well plate, 20 microliters (20 µL) of culture were taken out of each well and added to 125 µL of a combination of Malstat solution and NBT/PES solution. The activity of the parasite lactate dehydrogenase (*pLDH*) enzyme in the cultures can be ascertained with the aid of these solutions. When *pLDH* is present, a purple product is produced. Using an absorbance at 620 nm (Abs_{620}), the product was measured in a 96-well plate reader. Each well's Abs_{620} measurement reveals the presence of *pLDH* activity there. Our earlier research[43, 44] provided a description of this technique.

Antitrypanosomal activity

Trypanosoma brucei brucei, sometimes known as *T. b. brucei*, is typically used for drug screening and has no effect on humans. As previously mentioned in the antiplasmodial experiment, the volatile oil was combined with *T. bruceiin vitro* cultures in 96-well plates at a fixed dose of 0.050 mg/mL derived from the stock solution to evaluate antitrypanocidal efficacy. After these mixes were incubated for roughly 48 h, the number of parasites that could withstand the drug interaction was measured by adding a reagent based on resazurin, which living cells could convert to resorufin. As a fluorophore ($Excitation_{560}/Emission_{590}$), resorufin can be measured using a multi-well fluorescence plate reader.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) Assessment

The seed sample's minimum inhibitory concentration (MIC) was determined using the micro dilution technique. Mueller Hinton broth (MBH) was dispensed into each eppendorf tube in the following amounts: 750, 800, 850, 900, and 950 µL. A single 400 mg dose of the stock (seed and volatile oil) was dissolved in 550 µL of dimethyl sulfoxide (DMSO) and vortexed for each solution. The total volume in each tube was then brought to 1 mL by transferring aliquots of 250, 200, 150, 100, and 50 µL, respectively, to the tubes containing MHB. The resulting mixtures were then carefully vortexed. To ensure adequate mixing of the volatile oils, exactly 20 µL of each bacterial strain's inoculum suspension (0.5 McFarland, ~1 × 108cfu/mL) was added and vortexed to give the SVO and broth enough time to combine.

DMSO and ciprofloxacin, respectively, were employed as the positive and negative controls. Each test was run in duplicate, and they were incubated for 24 h at 37 °C. The lowest concentration of the volatile oils that did not exhibit any visible growth when compared to the control, which contained only MHB, was defined as the minimum bactericidal concentration (MBC). All of the wells that did not exhibit any visible growth in the MIC method above were streaked out onto fresh nutrient agar plates, and the culture was then incubated for 24 h at 37 °C. After a 24 h incubation period, the extracts with the lowest concentration that did not exhibit any colony growth on the solid medium were considered to have the minimum bactericidal concentration (MBC).

Cytotoxicity assay

n 96-well plates containing HeLa (human cervix adenocarcinoma) cells, the SVO volatile oil was incubated at a concentration of 0.050 mg/mL (unless otherwise stated) for 24 hin order to determine the IC50 value for cytotoxicity, as described by Keusch *et al*.[45]. Compounds are typically tested in duplicate wells, and standard deviations (SD) were derived.

Dose response

In the dosage response assay, seed volatile oil (SVO), which significantly decreased parasite viability in the single concentration test, was utilized to calculate IC50 values. The concentration that reduced viability by 50% (IC50) was found by dose-response regression analysis using the Graph Pad Prism 5 for Windows, Version 5.02 (Graph Pad Software, Inc.) program. For the SVO, the percentage viability was obtained versus log (extract concentration). Depending on the kind of test that was performed, pentamidine, chloroquine, or emetine were utilized as positive standard medications for comparison. The concentration range of the volatile oils used in the antitrypanosomal and antiplasmodial assays was 0.25–0.0011 mg/mL (3-fold dilutions), and for the cytotoxic assays, it was 0.125–5.72×10^{-5} mg/mL (also in a 3-fold dilution series).

Statistical analysis

For statistical studies and to resolve IC50 from the dose-response curve[43], Microsoft Excel and version 5.02 of the Graph Pad software, Inc. program were utilized.

Results

Components of the volatile oils extracted

After fresh C. citrinus seeds were hydro distilled, pale yellow oil was produced. In terms of wet sample yield, it was 0.95 v/w. 95.78% of the total oil content, or forty-one (41) components, were found in the SVO. Primarily, the SVO consisted of monoterpene hydrocarbons (26.96%) and oxygenated monoterpenoids

(61.26%). Eucalyptol (37.56%), α-pinene (13.20%), α-terpineol (8.11%), and terpinen-4-ol (5.99%) were the main constituents of the SVO, as indicated in Table 1. It included a few noteworthy ingredients as well, including α-pinene (4.36%), linalool (2.23%), and thymol (0.75%).

Table 1: Chemical compositions of the volatile seed oil of *Callistemon citrinus*

Compound	**Retention Index**	**Percentage**
		SVO
Isoamyl acetate	878	0.18
Isobutyric acid	892	0.37
β-Thujene	920	1.06
α-Pinene	939	13.20
Camphene	955	4.36
(+)-4-Carene	996	0.93
α-Phellandrene	1005	5.15
Eucalyptol	1053	37.56
γ-Terpinene	1062	2.26
Linalool	1098	2.23
Fenchol	1110	0.43
Cis-2-menthenol	1121	0.11`
α-Campholenal	1127	0.17
(E)-Pinocarveol	1137	1.36
Trans-2-menthenol	1140	0.13
(+)-Borneol	1173	1.17
Terpinen-4-ol	1182	5.99
α-Terpineol	1189	8.11
Cis-carveol	1229	0.18
Cis-p-mentha-1(7)-8-diene-2-	1231	0.08
Carvotanacetone	1247	0.27
Cis-Geraniol	1251	0.18
Geraniol	1255	2.03
Sabinyl acetate	1262	0.57
Bornyl acetate	1289	0.04
Thymol	1290	0.75
Eugenol	1356	0.49
Geranyl acetate	1381	0.39
Alloaromadendrene	1462	0.13
α-Selinene	1497	0.50
Globulol	1568	1.36
Spathulenol	1576	2.07
Viridiflorol	1586	0.65
Rosifoliol	1600	0.17
Isoaromandendrene epoxide	1612	0.15
Eudesma-4, 11-dien-2-ol	1690	0.08
Farnesol	1713	0.10
Benzyl benzoate	1750	0.20
Exo-2-hydroxycineole	1836	0.19
Hydroquinone	2693	0.23
Total (%)		**95.78**
Hydrocarbons Monoterpene		26.96
Oxygenated Monoterpenes		61.26
Sesquiterpene Hydrocarbons		0.63
Oxygenated Sesquiterpenes		4.78
Others (%)		2.15
% Yield of EOs (v/w)		0.95

Antiplasmodial activity

The percentage viability of the VO obtained from C. citrinus seeds was 78.01 ± 5.49%, and the antiplasmodial efficacy was ineffective against the malaria parasite at 0.050 mg/mL. Only the sample that was able to significantly lower the level of *pLDH* to less than 20% at a dose of 0.050 mg/mL was deemed to be active against the malaria parasite. The IC50 value of the standard drug chloroquine, which is utilized as a basis of comparison and positive control, is 3.84×10-6 mg/mL.

Antitrypanosomal activity

The SVO that were extracted via hydro-distillation had an impact on *T. b. brucei* viability at 0.050 mg/mL; the proportion of viable parasites was estimated to be 0.51%, indicating that the seeds exhibited antitrypanosomal properties. The SVO exhibited 0.092 mg/mL IC50 antitrypanosomal activity. According to Beroet *al.*[46], samples that have an IC50 value of less than 0.02 mg/mL are regarded as good or very potent, whereas samples that have an IC50 value of 0.02–0.06 mg/mL are regarded as moderate, and samples that have an IC50 value greater than 0.1 mg/mL are viewed as non-active. The IC50 value of the pentamidine utilized as a positive control was 2.24×10^{-6}mg/mL.

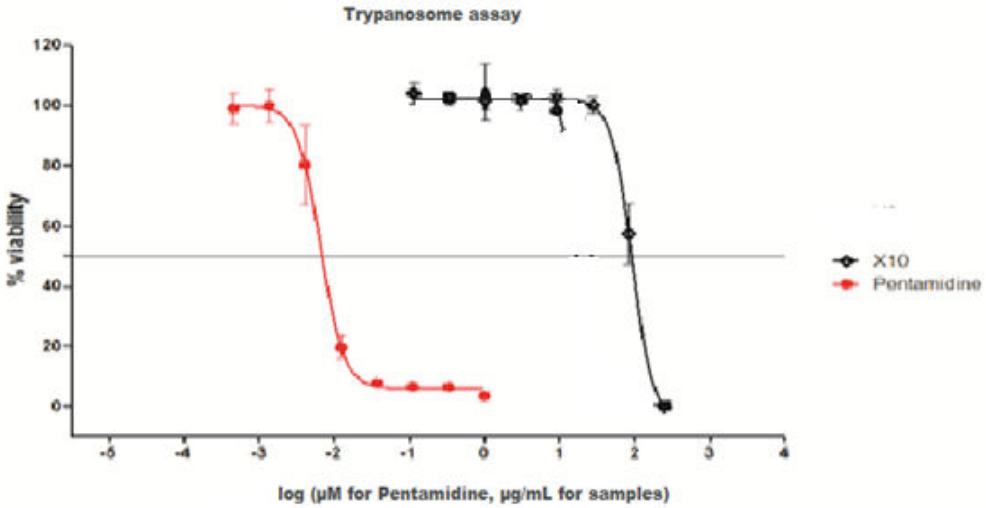

Figure 1: Dose-response curve for trypanosome assay

X10-Seed essential oil

Antibacterial potency of seed, leaf and flower volatile oils of*Callistemon citrinus*

The SVO extracted from *C. citrinus* demonstrated strong inhibitory action against the three multi-drug-resistant reference strains (*Vibrio alginolyticus DSM 217*, *Escherichia coli 0157:H7: ATCC 35150*, and *Listeria ivanovii ATCC 19119*) alongside three other multi-drug-resistant bacteria (*Salmonella typhi ACC*, *Staphylococcal*

enteritis ACC, and *Staphylococcus aureus ACC*). Results from the minimum inhibitory concentration (MIC) assay in Table 2 show that the SVO of this plant has MIC values ranging from 0.05 to 0.10 mg/mL. The SVO was bactericidal against *Staphylococcus aureus ACC* and *Escherichia coli 0157:H7: ATCC 35150*, *Vibro alginolyticus DSM 217*, and *Staphylococcal enteritis ACC* at 0.05 mg/mL. Also, the SVO from *C. citrinus* was bacteriostatic against *Salmonella typhi* ACC and *Listeria ivanovii ATCC 19119*) at MBC values of (0.10-0.20) mg/mL, as shown in Table 3.

Table 2: Minimum inhibitory concentration (MIC) values mg/mL for the volatile oils of seed, leaf and flower of *Callistemon citrinus* against bacteria strains.

Bacteria	**SVO**	**Ciprofloxacin (Positive control)**	**Dimethyl Sulfoxide (DMSO) (Negative Control)**
Gram positive			
***Staphylococcal enteritis ACC*)**	0.10 ± 0.01	0.05 ± 0.01 NG	0.5 mL VG
Staphylococcus aureus ACC	0.05 ± 0.02	0.05 ± 0.01 NG	0.5 mL VG
Listeria ivanovii ATCC 19119	0.10 ± 0.01	0.05 ± 0.01 NG	0.5 mL VG
Gram negative			
Escherichia coli 0157:H7:ATCC 35150	0.05 ± 0.01	0.05 ± 0.01 NG	0.5 mL VG
Vibrio alginolyticus DSM 217	0.10 ± 0.02	0.05 ± 0.01 NG	0.5 mL VG
Salmonella typhi ACC	0.10 ± 0.01	0.05 ± 0.01 NG	0.5 mL VG

ACC: AEMREG culture collection, ATCC: American type collection center, NG: no growth; VG: visible growth, SVO: seed volatile oil. Values are mean ± SD, n = 2

Table 3: Minimum bactericidal concentration (MIC) values mg/mL for the volatile oils of seed, leaf and flower of *Callistemon citrinus* against bacteria strains

Bacteria	SEO	Ciprofloxacin (Positive control)	Dimethyl Sulfoxide (DMSO) (Negative Control)
Gram positive			
***Staphylococcal enteritis ACC*)**	Bacteriostatic. 0.10 ± 0.01 VG	Bactericidal at 0.05 ± 0.01. NVG	0.4 mL VG
Staphylococcus aureus ACC	Bactericidal. 0.05 ± 0.02 NVG	Bactericidal at 0.05 ± 0.01. NVG	0.4 mL VG
Listeria ivanovii ATCC 19119	Bacteriostatic. 0.10 ± 0.01 VG	Bactericidal at 0.05 ± 0.01. NVG	0.4 mL VG
Escherichia coli 0157:H7:ATCC 35150	Bactericidal. 0.05 ± 0.01 NVG	Bactericidal at 0.05 ± 0.01. NVG	0.4 mL VG
Vibrio alginolyticus DSM 217	Bacteriostatic. 0.10 ± 0.02 VG	Bactericidal at 0.05 ± 0.01. NVG	0.4 mL VG
Salmonella typhi ACC	Bacteriostatic. 0.10 ± 0.01 VG	Bactericidal at 0.05 ± 0.01. NVG	0.4 mL VG

ACC: AEMREG culture collection, ATCC: American type collection center, NG: no growth; VG: visible growth, SVO: seed volatile oil, FVO: flower volatile oil, LVO: leaf volatile oil. Values are mean ± SD, n = 2

Cytotoxicity effect

Utilizing HeLa (human cervical adenocarcinoma) cells cultured in stock solutions of the volatile oils at a concentration of 0.050 mg/mL, the cytotoxicity effect of the SVO was examined. Due to the SVO sample's higher than 70% cell viability, HeLa cells treated with it at 0.050 mg/mL did not experience any appreciable cytotoxic effects.

Discussion

Eucalyptol (37.56 %) constituted the majority of the SVO. Certain researchers have indicated that the primary constituent of the aerial sections of the C. citrinus plant volatile oils appears to be eucalyptol.[16, 17, 18, 19, 20]. From our earlier research,

we found that the main constituents of the leaf essential oil component were α-pinene (20.02 %) and eucalyptol (48.98%), while the main constituents of the stem oil were α-pinene (31.03 %) and eucalyptol (56.00 %). Alpha-pinene (25.70 %) and eucalyptol (18.10 %) were discovered to be the primary components of flower volatile oil in another study reported by Jamzad*et al.* [17]; on the other hand, eucalyptol (36.60 %) and α-pinene (29.70 %) were reported to be the primary components of flower volatile oil from the Himalaya[18]. The main component of the volatile oils extracted from leaves in Reunion Island and the lower Himalaya region was eucalyptol, which was found to be 68.0 and 66.30%, respectively. Other minor components were α-pinene (12.80 and 18.70%) and α-terpineol (10.6 and 9.92%)[19,20]. No antiparasitic effect was observed against the *P. falciparum* parasite, despite the fact that the SVO in this study contained thymol and linalool as minor components. This could be because the two components' percentage concentrations were low, as shown in Table 1. The results of earlier studies that identified thymol and linalool [47,48]as parts of terpenoids in the volatile oils under investigation with antiplasmodial activity are in variance with what we observed in this study. Furthermore, a number of comparable observations showing strong antiplasmodial activity in volatile oils derived from several aromatic plants have been reported in the literature [47, 49, 50,51].
With an IC50 value of 0.092 mg/mL, the SVO of C. citrinus showed antitrypanosomal action. The antitrypanosomal activity of SVO may be attributed to the presence of eucalyptol and α-pinene as major components and linalool as a minor component. This is because IC50 values for eucalyptol, α-pinene, and linalool obtained from the volatile oil of Cymbopogon species from the Benin Republic[52] have been reported to possess antitrypanosomal activity (0.047, 0.039, and 0.0832) mg/mL. The essential oils extracted from *Eucalyptus citriodora*, *Eucalyptus camaldulensis*, *Cymbopogon citrates*, and *Citrus sinensis* also exhibited a GC-MS profile that revealed the presence of eucalyptol, cyclobutane, 6-octane, and citral, which may be the cause of their in-vitro antitrypanosomal *brucei brucei* and antitrypanosomal evansi activities53. The hydrophobic character of the hydrocarbons in volatile oils (Table 1), which interact with trypanosomes to change the conformation of their membrane structure and cause a loss of membrane stability, is another factor that may generate antitrypanosomal activity[53]. Table 1 indicates the possible existence of α-pinene, α-phellandrene, terpinen-4-ol, α-eucalyptol, and α-Terpineol as causes of the antibacterial property of the SVO reported in this investigation. Although some of the minor chemical constituents, including nerol, campholenal, sabinyl acetate, geranyl acetate, thymol, and fenchol, may potentially be accountable for their antibacterial action[9], the aforementioned components have been documented to possess antimicrobial properties[19, 53].Due to differences in the composition of their cell walls, researchers have shown that volatile oils tend to be more effective against Gram-positive than Gram-negative bacteria [54,55, 56, 57]. This idea was refuted by reports from earlier investigations on volatile oils, which showed

that depending on the kind of volatile oil and the constituents, certain Gram-negative strains are more susceptible than some Gram-positive ones[58, 59]. Furthermore, there isn't a set guideline regarding gram sensitivity. *Thymus syriacus Boiss* and *Syzygium aromaticum L* volatile oils were reported to be more effective against Gram-negative bacteria, including E. coli 0157:H7 and K. pneumoniae[61], by Preuss*et al.* Our earlier research also revealed that the antibacterial properties of the oils derived from leaves and flowers were more effective against Gram-negative bacteria, like *Vibrio alginolyticus DSM 2171* (67 ± 2.0 & 60 ± 5.0 mm) and *Aeromonas hydrophila ACC* (58 ± 0.3 & 52 ± 1.0 mm), than they were against Gram-positive bacteria, like *Staphylococcal enteritis ACC* (62 ± 0.5 & 55 ± 2.0 mm)[16]. This result is corroborated by Aweke and Yeshanew[62], who found that at a tested dose of 50 mg/mL, the volatile oil of *C. citrinus* from Ethiopia was more effective against the Gram-negative Salmonella typhi (27.93 ± 2.10 mm) than the Gram-positive Staphylococcus aureus (23.83 ± 2.75 mm). Several Gram-negative bacteria (*Escherichia coli* 0157:H7:*ATCC 35150* and *Vibrio alginolyticus DSM 217*) were more susceptible to the volatile oils found in the seeds and leaves, which is in line with the results of this study (Table 2). Our earlier findings were consistent with the result in question. The results of this investigation further substantiated the antimicrobial potential of the VO of this plant, which anticipates it to have an antibacterial action tied to its traditional use. The SVO's non-cytotoxicity against HeLa (human cervix adenocarcinoma) cells at 0.050 mg/mL may indicate that they are safe to use as targeted drugs for mammals. However, more research is required to fully understand the effects of Callistemon citrinus volatile oil, especially in the area of bioassay-guided fractionation on the plant's aerial parts, which will determine which compounds will produce antitrypanosomal lead drugs. The SVO's toxicity to mammalian cells was studied, but further research is required to promote it as a potential therapeutic agent to treat systematic infection. This research should focus on the drug's pharmacokinetics, pharmacodynamics, drug-like qualities, and pathophysiology.
Eucalyptol (37.56 %) constituted the majority of the SVO.Certain researches have indicated that the primary constituent of the aerial sections of the C. citrinus plant volatile oils appears to be eucalyptol.[16, 17, 18, 19, 20]. From our earlier research, we found that the main constituents of the leaves essential oil component were α-pinene (20.02%) and eucalyptol (48.98%), while the main constituents of the stem oil were α-pinene (31.03)16 and eucalyptol (56.00).α-pinene (25.70%) and eucalyptol (18.10%) were discovered to be the primary components of flower volatile oil in another study reported by Jamzad*et al.*[17]; on the other hand, eucalyptol (36.60%) and α-pinene (29.70%) were reported to be the primary components of flower volatile oil from the Himalaya[18]. The main component of the volatile oils extracted from leaves in Reunion Island and the lower Himalaya region was eucalyptol, which was found to be 68.0 and 66.30%, respectively. Other minor components were α-pinene (12.80 and 18.70%) and α-terpineol (10.6 and 9.92%).[20,19,20]. No antiparasitic effect was observed against the P. falciparum

parasite, despite the fact that the SVO in this study contained thymol and linalool as minor components. This could be because the two components' percentage concentrations were low, as shown in Table 1. The results of earlier studies that identified thymol and linalool[47,48]to be parts of terpenoids in the volatile oils under investigation with antiplasmodial activity are in variance with what we observed in this study.Furthermore, a number of comparable observations showing strong antiplasmodial activity in volatile oils derived from several aromatic plants have been reported in the literature[47, 49,50,51.]

With an IC50 value of0.092 mg/mL, the SVO of C. citrinus showed antitrypanosomal action. The antitrypanosomal activity of SVO may be attributed to the presence of eucalyptol and α-pinene as major components and linalool as a minor component. This is because IC50 values for eucalyptol, α-pinene, and linalool obtained from the volatile oil of Cymbopogon species from the Benin Republic[52] have been reported to possess antitrypanosomal activity (0.047, 0.039, and 0.0832) mg/mL. The essential oils extracted from *Eucalyptus citriodora*, *Eucalyptus camaldulensis*, *Cymbopogon citrates*, and *Citrus sinensis* also exhibited a GC-MS profile that revealed the presence of eucalyptol, cyclobutane, 6-octane, and citral, which may be the cause of their in-vitro antitrypanosomal *brucei brucei* and antitrypanosomal evansi activities53. The hydrophobic character of the hydrocarbons in volatile oils (Table 1), which interact with trypanosomes to change the conformation of their membrane structure and cause a loss of membrane stability, is another factor that may generate antitrypanosomal activity[53]. Table 1 indicates the possible existence of α-pinene, α-phellandrene, terpinen-4-ol, α-eucalyptol, and α-Terpineol as causes of the antibacterial property of the SVO reported in this investigation. Although some of the minor chemical constituents, including nerol, campholenal, sabinyl acetate, geranyl acetate, thymol, and fenchol, may potentially be accountable for their antibacterial action[9], the aforementioned components have been documented to possess antimicrobial properties[19, 53]. Due to differences in the composition of their cell walls, researchers have shown that volatile oils tend to be more effective against Gram positive than Gram-negative bacteria[55,56,57]. This idea was refuted by reports from earlier investigations on volatile oils, which showed that depending on the kind of volatile oil and the constituents, certain Gram-negative strains are more susceptible than some Gram-positive ones[58,59]. Furthermore, there isn't a set guideline regarding Gram sensitivity.Preusse*t* al.[60] found that *Thymus syriacus* Boiss and *Syzygiumaromaticum* L volatile oils were more active against the Gram-negative bacteria such as *E. coli* 0157:H7 and *K. pneumoniae*[61]. Our earlier research also revealed that the antibacterial properties of the oils derived from leaves and flowers were more effective against Gram negative bacteria, like *Vibrio alginolyticus DSM 2171* (67 ± 2.0 & 60 ± 5.0 mm) and *Aeromonas hydrophila ACC* (58 ± 0.3 & 52 ± 1.0 mm), than they were against Gram positive bacteria, like *Staphylococcal enteritis ACC* (62 ± 0.5 &

55 ± 2.0 mm)[16].This result is corroborated by Aweke and Yeshanew[62], who found that at a tested dose of 50 mg/mL, the volatile oil of *C. citrinus* from Ethiopia was more effective against the Gram negative Salmonella typhi (27.93 ± 2.10 mm) than the Gram positive Staphylococcus aureus (23.83 ± 2.75 mm). Several Gram-negative bacteria (*Escherichia coli* 0157:H7: *ATCC 35150* and *Vibrio alginolyticus DSM 217*) were more susceptible to the volatile oils found in the seeds and leaves, which is in line with the results of this study (Table 2). Our earlier findings*16* were consistent with the result in question. The results of this investigation further substantiated the antimicrobial potential of the VO of this plant, which anticipates it as having an antibacterial action tied to its traditional use[63].The SVO's non-cytotoxicity against HeLa (human cervix adenocarcinoma) cells at 0.050 mg/mL may indicate that they are safe to use as targeted drugs for mammals. However, more research is required to fully understand the effects of Callistemon citrinus volatile oil, especially in the area of bioassay-guided fractionation on the plant's aerial parts, which will determine which compounds will produce antitrypanosomal lead drugs. The SVO's toxicity to mammalian cells was studied, but further research is required to promote it as a potential therapeutic agent to treat systematic infection. This research should focus on the drug's pharmacokinetics, pharmacodynamics, drug-like qualities, and pathophysiology.

Conclusion

The present study's results indicate that the SVO of C. citrinus has potent antibacterial and antitrypanosomal properties without causing any harm, making it a promising alternative to synthetic antimicrobial and antitrypanosomal agents currently used to treat bacterial infections and nagana disease in cattle. It is crucial to isolate the pure chemicals and associated mechanisms of action that are responsible for these biological functions. These aspects were outside the purview of the current study, and they are now the subject of on-going research as part of a broader project we are working on at our lab.

Abbreviations

SVO: seed volatile oil; ATCC: American type collection centre; GC-MS: Gas chromatography-mass spectrometry; IC50: Inhibitory concentration at 50%; pLDH: Parasite Lactate Dehydrogenase; HAT: Human African Trypanosomiasis, UFH: University of Fort Hare; SD: Standard Deviation; RPMI medium: Roswell Park Memorial Institute medium. HeLa: Henrietta Lacks

Availability of data and materials

All data and materials used in this study are in the manuscript as well as in the supporting files attached.

Competing interests

The author(s) assert that they have no opposing interests.

Consent for publication

All authors mentioned agreed to the publication of the manuscript.

Ethics approval and consent to participate

Not applicable in this study.

Reference

1. *Govaerts, R.H., and Faden, R.B. 2013. World checklist of selected plant families. Royal Botanic Gardens, Kew.*
2. *Mabhiza, D., Chitemerere, T., and Mukanganyama, S. 2016.Antibacterial Properties of Alkaloid Extracts from Callistemon citrinusandVernoniaadoensis against Staphylococcus aureus and Pseudomonas aeruginosa. International journal of medicinal chemistry.*
3. *Brophy, J.J., Goldsack, R.J., Forster, P.I., Craven, L.A., and Lepschi, B.J. 1998. The leaf essential oils of the Australian members of the genus Callistemon (Myrtaceae). Journal of essential oil research 1; 10(6):595-606.*
4. *Gardens, Royal Botanic, and Sydney Domain Trust. "The NSW Plant Information Network System (PlantNET)." (2004).*
5. *Gilman, E.F. Calistemonrigidus, Fact sheet FPS-93, 1999, Environmental Horticulture Department, Institute of Food and Agricultural Sciences, University of Florida, USA..*
6. *Nel, J.L., Richardson, D.M., Rouget, M., Mgidi T.N., Mdzeke, N., Le Maitre, D.C., Van Wilgen, B.W., Schonegevel, L., Henderson, L., and Neser S. 2004. A proposed classification of invasive alien plant species in South Africa: towards prioritizing species and areas for management action: working for water. South African Journal of Science 1; 100(1-2):53-64.*
7. *Sutar, N., Sutar, R., and Kumar, M. 2014. Callistemon citrinus (bottle brush) an important medicinal plant: a review of its traditional uses, phytocontituents and pharmacological properties. Ind Res J Pharm &Sci1:68-77.*
8. *Goyal, P.K, Jain, R., Jain, S., and Sharma, A. A. 2012. Review on biological and phytochemical investigation of plant genus Callistimon. Asian Pacific Journal of Tropical Biomedicine. 1; 2(3):S1906-9.*
9. *Oyedeji,O..O, Lawal, O.A., Shode, F.O., and Oyedeji, A.O. 2009. Chemical composition and antibacterial activity of the essential oils of Callistemon citrinus and Callistemon viminalis from South Africa. Molecules 2; 14(6):1990-8.*
10. *Sudhakar, M., Rao, C.V., Rao, A.L., Ramesh, A., Srinivas, N., Raju, D.B., and Murthy, B.K. 2004. An antinociceptive and anti-inflammatory effect of the standardized oil of Indian Callistemon lanceolatus leaves in experimental animals. East and Central African Journal of Pharmaceutical Sciences 7(1):10-5.*
11. *Namukobe, J., Kasenene, J.M., Kiremire, B.T., Byamukama, R., Kamatenesi-Mugisha, M., Krief, S., Dumontet, V., and Kabasa, J. D. 2011. Traditional plants used for medicinal purposes by local communities around the Northern*

sector of Kibale National Park, Uganda. Journal of ethno pharmacology 14; 136(1):236-45.

12. *Ji, T. 2009. Traditional Chinese medicine pills for treating hemorrhoid. CN 101352524 A; 20090128.*
13. *Abd, A.J. 2012. Studying of antibacterial effect for leaves extract of Callistemon viminalis in vitro and vivo (urinary system) for rabbits. Journal of Kerbala University 10(2):246-54.*
14. *Salem, M.Z., Ali, H.M., El-Shanhorey, N.A., and Abdel-Megeed, A. 2013. Evaluation of extracts and essential oil from Callistemon viminalis leaves: Antibacterial and antioxidant activities, total phenolic and flavonoid contents. Asian Pacific journal of tropical medicine 1; 6(10):785-91.*
15. *Kamau, L.N., Mbaabu, P.M., Mbaria, J.M., Gathumbi, P.K., and Kiama, S.G. 2016. Ethnobotanical survey and threats to medicinal plants traditionally used for the management of human diseases in Nyeri County, Kenya. TANG 31; 6(3):21-1.*
16. *Larayetan, R.A., Okoh, O.O., Sadimenko, A., and Okoh, A.I. 2017. Terpene constituents of the aerial parts, phenolic content, antibacterial potential, free radical scavenging and antioxidant activity of Callistemon citrinus (Curtis) Skeels (Myrtaceae) from Eastern Cape Province of South Africa. BMC complementary and alternative medicine 17(1):292.*
17. *Jamzad, M., Kazembakloo, A., Tehrani, A.D., and Rostami, F.2015. Essential oil composition and antioxidant activity of hydromethanolic extract from the flowers leaves and stems of Callistemon citrinus (Curtis) Skeels. Indian Journal of Natural Products and Resources (IJNPR)[Formerly Natural Product Radiance (NPR)]19;5(4):308-12.*
18. *Kumar, D., Sukapaka, M., Babu, G.K., and Padwad, Y. 2015. Chemical composition and in vitro cytotoxicity of essential oils from leaves and flowers of Callistemon citrinus from western Himalayas. PloS one 26; 10(8):e0133823..*
19. *Chane-Ming, J., Vera, R.R., and Fraisse, D.J. 1998. Chemical composition of essential oil of Callistemon citrinus (Curtis) Skeel from Reunion. Journal of essential oil research 1; 10(4):429-31.*
20. *Srivastava, S.K., Ahmad, A., Syamsunder, K.V., Aggarwal, K.K., Khanuja, S.P. 2003. Essential oil composition of Callistemon viminalis leaves from India. Flavour and Fragrance journal 18(5):361-3.*
21. *Panizzi, L., Flamini, G., Cioni,andP.L., Morelli, I. 1993. Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceaes. Journal of ethno pharmacology 1; 39(3):167-70.*
22. *Yap, P.S., Yiap, B.C., Ping, H.C., and Lim, S.H. 2014. Essential oils, a new horizon in combating bacterial antibiotic resistance. The open microbiology journal 8:6.*
23. *World Health Organization. 2012. Research priorities for Chagas disease, human African trypanosomiasis and leishmaniasis. World Health Organization technical report series (975).*

24. *Welburn, S.C., Maudlin, I., and Simarro, P.P. 2009. Controlling sleeping sickness–a review. Parasitology 136(14):1943-9.*
25. *Nwodo, N.J., Ibezim, A., Ntie-Kang, F., Adikwu, M.U., and Mbah, C.J. 2015. Anti-trypanosomal activity of Nigerian plants and their constituents. Molecules 28; 20(5):7750-71.*
26. *Atawodi, S.E., Ameh, D.A., Ibrahim, S., Andrew, J.N., Nzelibe, H.C., Onyike, E.O., Anigo, K.M., Abu, E.A., James, D.B., Njoku, G.C., and Sallau, A.B. 2002. Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. Journal of Ethno pharmacology 1; 79(2):279-82.*
27. *Peter, S., Nandal, P.N., Prakash, S.O., Rao, J., and Kumar, S.R. 2012. In vitroantitrypanosomal evaluation of Picrorhizakurroarhizomes. Int Res J Pharm 3:205-8.*
28. *Dyary, H.O., Arifah, A.K., Sharma, R.S., Rasedee, A., Mohd-Aspollah, M.S., Zakaria, Z.A., Zuraini, A., Somchit, M.N. 2014. Antitrypanosomal screening and cytotoxic effects of selected medicinal plants. Trop. Biomed 31 (1):89-96.*
29. *Mbacham. W., Roper, C., Targett,G.,andGrenwood, B. 2004, Antimalarial drug resistance in Cameroon: therapeutic efficacy and biological markers of resistance. Gates malaria partnership annual report 13.*
30. *Titanji, V.P., Zofou, D., and Ngemenya, M.N. 2008. The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. African Journal of Traditional, Complementary, and Alternative Medicines 5(3):302.*
31. *Hoet, S., Stevigny, C., Block, S., Opperdoes, F., Colson, P., Baldeyrou, B., Lansiaux, A., Bailly, C., and Quetin-Leclercq, J. 2004. Alkaloids from Cassythafiliformis and related aporphines: antitrypanosomal activity, cytotoxicity, and interaction with DNA and topoisomerases. Planta medica1; 70(5):407-13.*
32. *Schelz, Z., Molnar,andJ., Hohmann, J. 2006. Antimicrobial and antiplasmid activities of essential oils. Fitoterapia1; 77(4):279-85.*
33. *Prabuseenivasan, S., Jayakumar, M., and Ignacimuthu, S. 2006. In vitro antibacterial activity of some plant essential oils. BMC complementary and alternative medicine 6(1):39.*
34. *Langeveld, W.T., Veldhuizen, E.J., Burt, S.A. 2013. Synergy between essential oil components and antibiotics: a review. Crit. Rev. Microbiol28; 10.*
35. *Islam, M.R., Ahamed, R., Rahman, M.O., Akbar, M.A., Al-Amin, M., Alam, K.D., Lyzu, F.2010. In vitro antimicrobial activities of four medicinally important plants in Bangladesh. Eur J Sci Res 39(Suppl 2):199-206.*
36. *Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. International journal of food microbiology 1; 94(3):223-53.*
37. *Hulin, V., Mathot, A.G., Mafart, P., and Dufossé, L. 1998. Les proprietés anti-microbiennes des huilesessentiellesetcomposésd'arômes. Sciences des aliments 18(6):563-82.*

38. *Jirovetz, L., Buchbauer, G., Denkova, Z., Stoyanova, A., Murgov, I., Schmidt, E. and Geissler, M., 2005. Antimicrobial testing and gas chromatographic analysis of pure oxygenated monoterpenes 1, 8-cineole, α-terpineol, terpinen-4-ol and camphor as well as target compounds in essential oils of pine (Pinuspinaster), rosemary (Rosmarinusofficinalis), tea tree (Melaleucaalternifolia). Scientiapharmaceutica, 73(1), pp.27-38.*
39. *Lestari, ES. 2004. Antimicrobial resistance among Staphylococcus aureus and Escherichia coli isolates in the Indonesian population inside and outside hospitals. In14th European congress of clinical microbiology and infectious diseases. Prague/Czech Republic.*
40. *Jose, B., Reddy, L.J. 2010. Evaluation of antibacterial activity of the leaf and flower essential oils of Gliricidiasepiumfrom south India. Intl J App Pharm 2:20-2.*
41. *European Pharmacopoeia Commission. 2004. European Pharmacopoeia. 5th Ed. Council of Europe: Strasbourg Cedex, France.*
42. *Makler, M.T., Ries, J.M., Williams, J.A., Bancroft, J.E., Piper, R.C., Gibbins, B.L., and Hinrichs, D.J. 1993. Parasite lactate dehydrogenase as an assay for Plasmodium falciparum drug sensitivity. The American Journal of Tropical Medicine and Hygiene 1; 48(6):739-41.*
43. *Larayetan, R., Mike, O.O., Omobola, O.O.,andAnthony, I.O. 2018. Silver nanoparticles mediated by Callistemon citrinus extracts theirantimalaria, antitrypanosoma and antibacterial efficacy J. Molliqdoi: 10.1016/ j.molliq.2018.10.020.*
44. *Rotimi L, Ojemaye MO, Okoh OO, Sadimenko A, Okoh AI. Synthesis, characterization, antimalarial, antitrypanocidal and antimicrobial properties of gold nanoparticle. Green Chemistry Letters and Reviews. 2019 Jan 2;12(1):61-8.*
45. *Keusch, G.T., Jacewicz, M., Hirschman, S.Z. 1972. Quantitative micro assay in cell culture for enterotoxin of Shigelladysenteriae 1. Journal of Infectious Diseases 1; 125(5):539-41.*
46. *Bero, J., Beaufay, C., Hannaert, V., Hérent, M.F., Michels, P.A., and Quetin-Leclercq, J. 2013. Antitrypanosomal compounds from the essential oil and extracts of Keetialeucantha leaves with inhibitor activity on Trypanosomabrucei glyceraldehyde-3-phosphate dehydrogenase. Phytomedicine15; 20(3-4):270-4.*
47. *Babili, F.E., Bouajila,J.,Souchard, J.P., Bertrand, C., Bellvert, F., Fouraste, I., Moulis, C.,and Valentin, A.2011. Oregano: chemical analysis and evaluation of its antimalarial, antioxidant, and cytotoxic activities. Journal of food science 76(3):C512-8*
48. *Goulart, H.R., Kimura, E.A, Peres, V.J., Couto, A.S., Duarte, F.A., Katzin, A.M. 2004. Terpenes arrest parasite development and inhibit biosynthesis of isoprenoids in Plasmodium falciparum. Antimicrobial agents and chemotherapy 1; 48(7):2502-9.*

49. *Tchoumbougnang, F., Zollo, P.A., Dagne, E., and Mekonnen Y. 2005. In vivo antimalarial activity of essential oils from Cymbopogoncitratus and Ocimumgratissimum on mice infected with Plasmodium berghei. Plantamedica71(01):20-3.*
50. *Valentin, A., Pélissier, Y., Benoit, F., Marion, C., Kone, D., Mallie, M., Bastide, J.M., and Bessière, J.M. 1995. Composition and antimalarial activity in vitro of volatile components of Lippiamultiflora. Phytochemistry1; 40(5):1439-42.*
51. *Milhau, G., Valentin, A., Benoit, F., Mallié, M., Bastide, J.M., Pélissier, Y., and Bessière, J.M. 1997. In vitro antimalarial activity of eight essential oils. Journal of Essential Oil Research 1; 9(3):329-33.*
52. *Kpoviessi, S., Bero, J., Agbani, P., Gbaguidi, F., Kpadonou-Kpoviessi, B., Sinsin, B., Accrombessi, G., Frédérich, M., Moudachirou, M., and Quetin-Leclercq, J. 2014a. Chemical composition, cytotoxicity and in vitro antitrypanosomal and antiplasmodial activity of the essential oils of four Cymbopogon species from Benin. Journal of ethno pharmacology 10; 151(1):652-9.).*
53. *Habila, N., Agbaji, A.S, Ladan, Z., Bello, I.A, Haruna, E., Dakare, M.A., Atolagbe, T.O. 2010. Evaluation of in vitro activity of essential oils against Trypanosomabruceibrucei and Trypanosomaevansi. Journal of parasitology research.*
54. *Riaz, M., and Chaudhary, F.M. 1990. The chemical composition of Pakistani Callistemon citrinus oils. Journal of Essential Oil Research 1; 2(6):327-8.*
55. *Ratledge, C., and Wilkinson S.G. 1988. An overview of microbial lipids. Microbial lipids 1:3-22.*
56. *Mith, H., Dure, R., Delcenserie, V., Zhiri, A., Daube, G., and Clinquart, A. 2014. Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria. Food science & nutrition 2(4):403-16.*
57. *Lambert, R.J., Skandamis, P.N., Coote, P.J., and Nychas, G.J. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of applied microbiology 12; 91(3):453-62.*
58. *Tyagi, A.K., and Malik, A. 2011. Antimicrobial potential and chemical composition of Eucalyptus globulus oil in liquid and vapour phase against food spoilage microorganisms. Food Chemistry 1; 126(1):228-35.*
59. *Maida, I., Lo Nostro, A., Pesavento, G., Barnabei, M., Calonico. C., Perrin, E., Chiellini, C., Fondi, M., Mengoni, A., Maggini, V., and Vannacci, A. 2014. Exploring the anti-Burkholderiacepacia complex activity of essential oils: a preliminary analysis. Evidence-Based Complementary and Alternative Medicine 2014.*
60. *Preuss, H.G., Echard, B., and Enig, M. 2005. Brook I, Elliott TB. Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram-*

positive and gram-negative bacteria. Molecular and cellular biochemistry 1; 272(1-2):29-34.

61. *Al-Mariri, A., and Safi, M. 2014.In vitro antibacterial activity of several plant extracts and oils against some gram-negative bacteria. Iranian journal of medical sciences 39(1):36.*
62. *Aweke, N., and Yeshanew, S. Chemical Composition and Antibacterial Activity of Essential Oil of Callistemon citrinus from Ethiopia.*
63. *Doughari, J.H., Human, I.S., Bennade. S., and Ndakidemi, P.A. 2009. Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. Journal of Medicinal Plants Research. 3(11):839-48.*