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Antitubercular Potential of Essential Oils from Selected Philippine Plants

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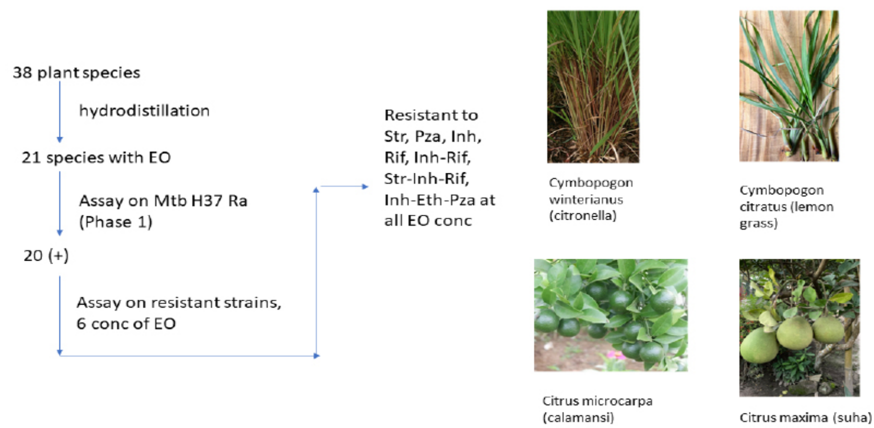
Graphical Abstract



Abstract

Tuberculosis, the disease caused by *Mycobacterium tuberculosis*, has been a global health concern that has plagued the Philippines and other developing countries. The need for continuous search for antitubercular agents is a result of the emergence of drug resistant strains (multi drug- and extensively drug-resistant strains) and other factors. Essential oils are multi-component mixtures that have been used since ancient times for their medicinal properties. This study aimed to determine the in-vitro antitubercular activity of the essential oils from 38 Philippine plants in two phases. Out of 38 species, only 21 yielded essential oils which were assayed against *M. tuberculosis* H37Ra ATCC strain using the submerged disk method. The bioactive essential oils were further assayed, using the agar dilution method, against resistant clinical strains, including mono-, multi-, and poly-drug resistant strains. Those which inhibited the growth of all the resistant strains (the 4 monoresistant strains - Str-, Pza-, Inh-, Rif-; 1 multidrug resistant strain (MDR) - Inh-Rif-; 2 polydrug resistant (PolyDR) strains - Str-Inh-Rif-(SIR), Inh-Eth-Pza-(IEP)) at all concentrations were from *Citrus microcarpa* Bunge (rind), *Citrus maxima* (Burm.) Osbeck (rind), *Cymbopogon citratus* (DC.) Stapf (leaves, stalks) and *Cymbopogon winterianus* Jowitt ex Bor (leaves).

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At all concentrations, the EO from *C. winterianus* stalks inhibited Str-, Inh-, Rif-, and Str-Inh-Rif resistant strains; EO from *Allium sativum* (bulb), against Str-, and Inh-Rif-resistant strains; EO from *Curcuma longa* (leaves), against Inh-Eth-Pza resistant strains. The study shows that the essential oils can be used as a source of antitubercular agents to combat even the multi-drug and poly-drug resistant strains.

Keywords: essential oils, tuberculosis, resistant strains, Philippine plants

Introduction

Tuberculosis has plagued developing countries, including the Philippines, being identified as one of the 30 high TB burden countries which accounted for 87% of new TB cases in 2022 (WHO 2024). TB mortality In the Philippines has been reduced by an average of 2.8% between 2016 and 2020, but was seen to rise by 2021 (National TB Control Program 2024). The number of drugs currently used against *Mycobacterium tuberculosis*, the causative agent in TB, is very limited; most of them were introduced 40 years ago (Lienhardt et al. 2012). The emergence of drug-resistant strains has had significant implications on the efforts to fight tuberculosis. The treatment of multidrug-resistant and extensively drug-resistant tuberculosis is longer, more costly, generally ineffective, and results in more adverse drug reactions (Tupasi et al. 2016). Thus, the need to search for new bioactive compounds that could be potential antitubercular agents.

Essential oils are multi-component mixtures that have been used since ancient times for their medicinal properties. They can be obtained from leaves, bark, flowers and seeds. The constituents are mostly terpenoids and phenolic-derived compounds. Essential oils are extractable by various methods, including steam distillation and hydrodistillation. Though mostly applied in aromatherapy, essential oils are a promising source of bioactive compounds. Some of the reported uses are antibacterial, antifungal, antiprotozoal, antiviral, anti-inflammatory, antimutagenic, cancer preventive, antidiabetic and antioxidant activities (Bakkali et al. 2008; Calo et al. 2015; Edris 2007; Lang and Buchbauer 2012; Raut et al. 2014). Though many studies on the antimicrobial properties of essential oils have been conducted, very few investigations were directed at the antimycobacterial property, in particular, on *Mycobacterium tuberculosis*.

This study aims to determine the antitubercular potential of the essential oils from 38 Philippine plants by testing the activity of the oils in two phases – Phase 1, using *M. tuberculosis* H37Ra ATCC strain, and for confirmation, in Phase 2, using clinical isolates of mono-, multi-, and poly-drug resistant strains.

Materials and methods

Plant material. The plant samples were collected from Bulacan, Pampanga, Batangas, Cavite, Nueva Ecija, Quezon, Rizal, Laguna, Nueva Vizcaya, Manila and Quezon City. Herbarium specimens were collected and identified at the UST Herbarium Botany Laboratory, UST Research Center for the Natural and Applied Sciences.

Extraction. The plant samples (air-dried leaves, bulbs, flowers, stalks, rhizomes, fruits, fresh fruit rinds, and resin) were ground using a blender. The ground samples were subjected to hydrodistillation to extract the essential oils. For each batch, 100 – 200 grams were extracted with one liter of water for 2-3 hours.

Assays

Preliminary Phase (Phase 1)

Disks. Small petri plates using disks with 6mm diameter with thickness that will hold 20 µL assay solution or Whatman® Schleicher & Schuell® antibiotic assay disc ¼ diameter were used.

Microorganism. The Reference strain is *M. tuberculosis* H37Ra ATCC strain.

Method. Preliminary Phase used the submerged disk method, using 5 µg Rifampicin disk (RIF5), 20 µL of EO per disk (computing concentration of disk based on EO weight), with Middlebrook 7H10 with 0.05% Tween 80 as culture medium (M7H10-TW). Separated plates were prepared and attached to RIF5, EO, and drug-free control. In each plate, 5 mL M7H10-TW were dispensed, solidified, placed in plastic bags and refrigerated overnight. The adjusted inoculum was added, taking note of the volume and density. The plates were incubated at 37°C for 2-4 weeks. The endpoint is growth (no activity) or no growth (interpreted as positive or antitubercular activity). Positive and negative controls were included per run.

Secondary Phase (Phase 2)

The secondary phase selected the EO samples that inhibited the growth of Mtb in the Preliminary Phase for further study. Phase 1 is a preliminary assay, while Phase 2 is a secondary assay. The preliminary assay makes use of pure EOs. This will determine if the EO has antimycobacterial activity. Phase 2 makes use of Tween 80: water supplemented EO. This enables the researcher to dilute the oil resulting in final concentrations of 1µg/ml EO to 0.03µg/ml EO.

Microorganisms. Reference strain, seven resistant clinical isolates (4 mono-drug resistant strains: Inh-, Rif-, Str-, Pza- resistant strains; 1 multi-drug resistant strain: Inh-Rif resistant strain; 2 poly-drug resistant strains: Str-Inh-Rif- and Inh-Eth-Pza- resistant strains)

For Phase 2, what was used were stored external quality assessment (EQA) samples. These are those that are used for quality assurance activities and are not identifiable to patients.

Method. The secondary phase used the agar dilution method. The sterile screw-capped 10 mL sterile prescription bottles (6 bots) were filled with 1 mL each of serially two-fold diluted Rif (10 to 0.3 µg/mL). Each prescription bottle was dispensed with 4 mL M7H10-TW. Separate bottles were filled with 1 mL each of serially 2-fold diluted EO. Each bottle was diluted again with M7H10 to a final concentration of 1 µg/mL to 0.03 µg/mL from bottles 1-6 (To obtain the original concentration, each mL of EO was weighed to obtain mg/mL.) The concentrations per essential oil were 5%, 2.5%, 1.25%, 0.625%, 0.312%, 0.156%. The bottles were placed in plastic bags and refrigerated overnight. Adjusted inoculum was placed in each bottle, taking note of the volume and density. The bottles were incubated at 37°C for 2-4 weeks. The endpoint is growth (no activity) or no growth (interpreted as positive or antitubercular activity). Positive and negative controls were included per run.

Table 1. List of Plants and their Percent Yield of Essential Oils.

Botanical name (Sample no.)	Common name	Family	Part used	Source	Yield	Phase 1	Phase 2
Allium sativum L. (1)	Garlic	Amaryllidaceae	Fresh bulb	National Road, Commercial, Bongabon, Nueva Ecija	0.07%	+	+
Annona reticulata Linn. (2)	Anonas	Anonaceae	Dried leaves	Salaban, San Jose Batangas	0.09%	+	+
Bixa orellana L. (3)	Atsuete	Bixaceae	Dried leaves	Brgy. Aya, San Jose, Batangas	0.41%	+	+
Cananga odorata (Lam.) Hook.f.& Thomson (4)	Ylang-Ylang	Annonaceae	Dried flowers	Salaban, San Jose Batangas	1.97%	+	+
Canarium luzonicum (Blume.) A. Gray (5)	Elemi	Burseraceae	Resin	Poblacion 2, Alabat, Quezon	13.56%	+	+
Cinnamomum camphora (L.) J. (6)	Camphor tree	Lauraceae	Dried leaves	UST Campus	3.27%	+	+
Citrus maxima (Burm.) Osbeck (7)	Suha	Rutaceae	Fresh rind	San Leonardo, Nueva Ecija	2.89%	+	+
Citrus microcarpa Bunge (8)	Calamansi	Rutaceae	Fresh rind	Brgy. Malusak, Antimonan, Quezon	1.98%	+	+
Clausena anisum-olens (Blanco) Merr. (9)	Dahon ng Anis	Rutaceae	Dried leaves	Salaban, San Jose Batangas	0.73%	+	+
Curcuma longa L. (10)	Turmeric	Zingiberaceae	Fresh rhizomes	Floridablanca, Pampanga	0.23%	+	+
Curcuma longa L. (11)	Turmeric	Zingiberaceae	Dried leaves	Floridablanca, Pampanga	0.89%	+	+
Cymbopogon citratus (DC.) Stapf (12)	Lemongrass	Poaceae	Dried leaves	Enchanted Farm, Angat Bulacan	1.17%	+	+
Cymbopogon citratus (DC.) Stapf (13)	Lemongrass	Poaceae	Dried Stalk	Enchanted Farm, Angat Bulacan	0.90%	+	+
Cymbopogon winterianus Jowitt ex Bor (14)	Citronella	Poaceae	Dried leaves	Enchanted Farm, Angat Bulacan	2.20%	+	+
Cymbopogon winterianus Jowitt ex Bor (15)	Citronella	Poaceae	Dried Stalk	Enchanted Farm, Angat Bulacan	1.09%	+	+
Eucalyptus sp. (16)	Eucalyptus	Myrtaceae	Dried leaves	Brgy. Salaban, San Jose, Batangas	0.78%	+	+
"*Laurus nobilis*"/ *Cinnamomum* sp.[a] (17)	Laurel	Lauraceae	Dried leaves	Batangas, Batangas	1.00%	+	+
Origanum vulgare L. (18)	Oregano	Lamiaceae	Dried leaves	Parada, Valenzuela	0.19%	+	+
Piper nigrum L.[b] (19)	Black pepper	Piperaceae	Dried leaves	Alfonso, Cavite	0.16%	+	+
Piper retrofractum Vahl. (20)	Litlit	Piperaceae	Dried leaves	Alfonso, Cavite	0.09%	+	+
Pogostemon cablin (Blanco) Benth. (21)	Patchouli	Lamiaceae	Dried leaves	UPLB Laguna Quezon Land Grant FAMY Siniloan Mabitac Laguna	1.95%	+	+
Premna odorata Blanco (22)	Alagao	Lamiaceae	Dried leaves	Brgy. Lapu-lapu II, San Jose, Batangas	0.26%	+	+
Psidium guajava L. (23)	Guava	Myrtaceae	Dried leaves	Angat, Bulacan	0.85%	+	+
Zingiber officinale Roscoe (24)	Ginger Rhizome	Zingiberaceae	Fresh rhizomes	Lapu-Lapu, San Jose Batangas	0.16%	+	+

[a]"*Laurus nobilis*"/*Cinnamomum* sp. - pending identification; [b]*P. nigrum* sample from 2nd collection

Table 2. Essential Oil Distribution According to Clinical Isolate Susceptibility (No Growth).

M. tuberculosis strain	5%	2.5%	1.25%	0.625%	0.312%	0.156%
H37Ra strain (Ref)	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 23 24	1 2 3 4 5 6 7 8 9 11 12 13 14 15 16 17 18 19 2023 24	1 2 4 6 7 8 9 11 12 13 14 15 16 17 18 19 20 24	1 2 6 7 8 9 12 13 14 15 16 17 18	1 6 7 8 9 12 13 14 15 17 18	1 6 7 8 12 13 14 15 17
Rif RS	1 2 4 5 6 7 8 9 11 12 13 14 15 16 17 18 19 20 21 22 23 24	1 4 5 6 7 8 9 11 12 13 14 15 16 17 18 19 20 21 22 23 24	1 6 7 8 9 11 12 13 14 15 16 17 18 20 21	6 7 8 9 11 12 13 14 15 16 17 18 21	7 8 11 12 13 14 15 16 17	7 8 12 13 14 15 17
Inh RS	1 2 4 5 6 7 8 9 11 12 13 14 15 16 17 18 19 20 21 23 24	1 2 6 7 8 9 11 12 13 14 15 16 17 18 19 20 21 23	1 6 7 8 9 11 12 13 14 15 16 17 18 20 21 23	6 7 8 9 11 12 13 14 15 16 17 18 23	7 8 9 12 13 14 15 16 17	7 8 12 13 14 15 16 17
Pza RS	1 2 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 23 24	1 4 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 23 24	1 4 6 7 8 11 12 13 14 15 16 17 18 19 20 21 23 24	1 7 8 11 12 13 14 15 16 17 18 20 21 23	7 8 11 12 13 14 15 16 17 18	7 8 12 13 14 17
Str RS	1 2 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 23 24	1 2 4 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 23 24	1 6 7 8 9 12 13 14 15 16 17 18 20 21	1 6 7 8 9 12 13 14 15 16 17 18 20 21	1 6 7 8 12 13 14 15 17 21	1 7 8 12 13 14 15 17
Inh-Rif RS	1 2 5 6 7 8 9 11 12 13 14 15 16 17 18 19 20 21 23	1 2 6 7 8 9 11 12 13 14 15 16 17 18 19 20 21	1 2 6 7 8 9 11 12 13 14 15 16 17 18 19 20 21	1 2 6 7 8 9 11 12 13 14 15 16 17 18	1 6 7 8 11 12 13 14 15 17	1 7 8 12 13 14 15 17
Str-Inh-Rif RS	1 2 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 23 24	1 4 6 7 8 9 10 12 13 14 15 16 17 18 19 20 21 23 24	1 4 6 7 8 9 10 12 13 14 15 16 17 18 19 20 21 24	1 6 7 8 10 12 13 14 15 16 17 18	7 8 12 13 14 15 17 18	7 8 12 13 14 15 17
Inh-Eth-Pza RS	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 23 24	1 2 3 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21	1 7 8 9 10 11 12 13 14 15 16 17 18 20 21	7 8 9 11 12 13 14 15 16 17 20 21	7 8 9 11 12 13 14 15 16 17 21	7 8 11 12 13 14 17

RS – resistant strain; Plant numbers in Table 2 (1 *A. sativum*, 2 *A. reticulata*, 3 *B. orellana*, 4 *C. odorata*, 5 *C. luzonicum*, 6 *C. camphora*, 7 *C. maxima*, 8 *C. microcarpa*, 9 *C. anisum-olens*, 10 *C. longa* (rhizomes), 11 *C. longa* (leaves), 12 *C. citratus* (leaves), 13 *C. citratus* (stalks), 14 *C. winterianus* (leaves), 15 *C. winterianus* (stalks), 16 *Eucalyptus* sp., 17 "*Laurus*", 18 *O. vulgare*, 19 *P. nigrum*, 20 *P. retrofractum*, 21 *P. cablin*, 22 *P. odorata*, 23 *P. guajava*, 24 *Z. officinale*)

Results and Discussion

Of the 38 plants extracted by hydrodistillation, there were 21 plant species where the essential oils were obtained (Table 1). Four species had two plant parts extracted and yielded essential oils – *Cymbopogon citratus* (leaves and stalks), *Cymbopogon winterianus* (leaves and stalks), *Piper nigrum* (leaves and fruits), *Curcuma longa* (leaves and rhizomes).

One batch of 100-200 g material extraction can take 2-3 hours to produce 0.1 mL – 1 mL for leaves and flowers, 0.3 mL - 0.6 mL for rhizomes, 1.3 – 3 mL for rinds, and 20 -28 mL for resin. The average percent yield of essential oils is 1.5%. The highest yield came from *Canarium luzonicum* or "elemi" resin, which gave 13.56%. Except for this, the range for the % yield is 0.07% - 3.27%. The next high yielding plants are *Cinnamomum camphora*, *Citrus maxima*, *Cymbopogon winterianus*, *Citrus microcarpa*, *Cananga odorata*, *Piper nigrum*, and *Pogostemon cablin*.

Table 3. Ranking of More Active Essential Oils.

***Mycobacterium tuberculosis* strains**	**Essential Oils**
I. All strains inhibited at all concentrations (0.156 % - 5%)	**A.** *Cymbopogon winterianus* (leaves) *Cymbopogon citratus* (leaves) *Cymbopogon citratus* (stalks) *Citrus microcarpa* (rind) *Citrus maxima* (rind) *Cinnamomum* sp. (leaves)
II. All strains inhibited at all concentrations except for Pza and IEP resistant strains at 0.156%	**B.** *Cymbopogon winterianus* (stalks)
III. All strains inhibited at higher concentrations:	**C.**
0.312-5%	*Cymbopogon winterianus* (stalks)
0.625% - 5%	*Eucalyptus* sp. (leaves)
1.25-5%	*Allium sativum* (bulb) *Origanum vulgare* (leaves) *Piper retrofractum* (leaves) *Pogostemon cablin* (leaves)
2.5%-5%	*Clausena anisum-olens* (leaves) *Cinnamomum camphora* (leaves)
IV. Str resistant strain inhibited at all concentrations	All EOs in **A**; *Allium sativum* (bulb)
V. Inh resistant strain inhibited at all concentrations	All EOs in **A**; *Cymbopogon winterianus* (stalks) *Eucalyptus* sp. (leaves)
VI. Rif resistant strain inhibited at all concentrations	All EOs in **A**; *Cymbopogon winterianus* (stalks)
VII. Inh-Rif resistant strain inhibited at all concentrations	All EOs in **A**; *Allium sativum* (bulb) *Cymbopogon winterianus* (stalks)
VIII. Inh-Eth-PZA resistant strain inhibited at all concentrations	All EOs in **A**;

With the exception of *Piper nigrum*, all plants that yielded essential oils showed positive results in Phase 1 and Phase 2 (Table 1). *P. nigrum* has been reported to contain essential oils which are antimicrobial. To confirm the initial negative result of *P. nigrum*, a second collection was carried out, and upon assay for Phase 1, it gave a positive result, but in Phase 2, a weak result was obtained (Table 2).

Certain plants that were expected to yield essential oils but did not yield any essential oils were *Areca catechu*, *Jasminum sambac*, *Pandanus amaryllifolius*, and *Piper betel*. Instead of essential oils, hydrosols were obtained from them. Hydrosols are herbal distillates which are obtained during hydrodistillation. They are also called herbal water, hydrolates, and floral water. Fragrance is not always a factor to consider when expecting a plant to yield essential oils.

Table 2 shows the distribution of the essential oils according to their inhibitory activity on the *M. tuberculosis* strains. The strongest activities are exhibited by those, which at the lowest concentration of 0.156%, can inhibit the microbial growth. Table 2 also shows that all *M. tuberculosis* strains were sensitive to the essential oils. These results were confirmed using chi-square (see Suppl. M).

Table 4. Reported Constituents of the Plants with More Active Essential Oils and Plant Uses.

Plant name	Chemical Constituents	Uses
Citrus microcarpa (Rutaceae) "calamansi"	Limonene (94%) in peel oil, hedycaryol (19%) in leaf oil; Dominant phenolic acids are p-coumaric acid and ferulic acid (Cheong et al 2012); elemol (37%) in leaf EO as major component (Nguyen et al 2018); limonene and β-myrcene in whole fruit EO (Ngo 2020)	Leaf EO inhibited *E. coli*, *B. subtilis*, *S. aureus*, *A. niger*, *C. albicans* and *S. cerivisiae*; antioxidant (Nguyen et al 2018); cytotoxic to breast cancer cells (Palma et al 2019); traditional use for TB-related cough, chest ailments, fever (Sieniawaska et al 2020); leaf decoctions for hypertension and diabetes (Chan et al 2021); peel EO cytotoxic to human mammary tumor cell line (Palma et al 2019), leaf EO antiproliferative activity against HeLa cervical cancer cell line (Othman et al 2022)
Citrus maxima (Rutaceae) "suha"	Limonene (90%), sesquiterpene, terpene alcohols, cadinene from peel EO (Chen et al 2018)	Peel used in cough, swelling and epilepsy (Thavanapong et al 2011); antibacterial on G(+) and G(-) (Chen et al 2018); EO inhibited foodborne pathogen bacteria and methicillin-susceptible *S. aureus* strains from cheese, milk and human stools (Settani et al 2012)
Cymbopogon citratus (Poaceae) "lemongrass"	Citral b, citral a, and b-pinene (Elhassan et al 2016); Geranial, neral, myrcene (Bassoloea et al 2011)	EO antitubercular against 9 clinical isolates, Mtb H37Rv and MDR-TB strain at all concentrations up to 15 ul/mL. (Elhassan et al 2016); EO antibacterial on G(+) and G(-) (Bassoloea et al 2011)
Cymbopogon winterianus (Poaceae) "citronella"	Citronellal, geraniol, citronellol (Rajeswara Rao et al 2004; Singh and Kumar, 2017; Singulani et al 2018); limonene, citronellol, geraniol (Kakaraparthi et al 2014)	Geraniol and linalool are antifungal against *Candida* species (Singulani et al 2018); antifungal, anti-parasitic, a potent mosquito repellent, antibacterial (Wany et al 2013)
Origanum vulgare (Lamiaceae) "oregano"	Sabinene, myrcene, γ-terpinene, carvacrol, p-cymene, thymol (Kokkini et al 2004); caryophyllene and spathulenol, germacrene-D, α-terpineol (Sahin et al 2004); carvacrol, β-fenchyl alcohol, thymol, and γ-terpinene (Teixeira et al 2013)	EO antimicrobial on 10 bacteria,15 fungi, yeast (Sahin et al 2004); antiproliferative in human dermal fibroblasts; anti-inflammatory, tissue remodeling, immunomodulatory, and anticancer activities (Han and Parker 2017)
Allium sativum (Amaryllidaceae) "garlic"	2-methylproppenyl trisulfide, methylallyltrisulfide and diallylsulfide (Ascension et al 2018);	Antibacterial on resistant TB isolates (Ndedi et al 2018); allicin in extract inhibited 17 species of *Mycobacteria* (Delaha et al 1985)
Cinnamomum camphora (Lauraceae) "camphor tree"	Linalool, eucalyptol, α-terpineol, isoborneol, b-phellandrene, camphor (Chen et al 2020)	Antimicrobial, anti-inflammatory, anti-oxidative, anti-allergy (Wu et al 2019); antifungal on *Choanephora cucurbitarum* (Pragadheesh 2013)
Clausena anisum-olens (Rutaceae) "dahon anis"	Phenylpropanoid, estragole, anethole in EO of Phil species (Thai et al 2014); Myristicin, terpinolene, p-cymene-8-ol in EO (You et al 2015); Anisucoumaramide, Anisumic acid (Wang et al 2017);	Condiment; decoction as treatment for nausea, cough, fever (Arbab et al 2012); Contact toxicity and repellency on grain pests (You et al 2015).
Pogostemon cablin (Lamiaceae) "patchouli"	Patchouli alcohol (Kusuma et al 2018), α-bulnesene, α-guaiene, and sesquiterpene hydrocarbons (Hussain et al 2011);	Oil as antistress, antiseptic, antidepressant, aphrodisiac, astringent, diuretic, deodorant, fungicide, insecticide, sedative, tonic, for skin problems such as athlete's foot, acne, dermatitis, irritated skin, dandruff, for wound and scar healing (Ramya et al 2013); EO antibacterial on G (+) and G (-) (Hussain et al 2011; Das et al 2016; Wan et al 2016), and ampicillin resistant *E. coli* (Hussain et al 2011), and methicillin-resistant *S. epidermidis* (Wan et al 2011); EO inhibits biofilms of Group A *Streptococcus* (Nithyanand et al 2015)
Piper retrofractum (Piperaceae) "litlit"	Germacrene D, tetramethyltricyclo[5.3.1.0(4,11)] undec-8-ene, ar-turmerone, benzyl benzoate (Jamal et al 2013)	Dried fruit as spice, preservative, pickles (Hieu et al 2014); treatment for body pain, carminative, expectorant, anti-amoebic, laxative, anti-asthma, antibacterial; leaves for carotid artery disease and tendon discomfort, promote digestive and respiratory health, ingredient in medical teas (Salleh and Ahmad 2020); EO of leaves antibacterial and antifungal (Jamal et al 2013); Antilisterial and antifungal (Hieu et al 2014). sensitive to *B. subtilis*, *S. aureus* and *M. luteus*, but not *E. coli* and *S. epidermidis* (Jamal et al 2013).
Curcuma longa (Zingiberaceae) "turmeric"	Eucalyptol, α-pinene, β-phellandrene, β-pinene, limonene, 1,3,8-p-menthatriene, ascaridole epoxide, 2-methylisoborneol, 5-isopropyl-6-methyl-hepta-3,dien-2-ol in leaf EO (Parveen et al 2013); α-turmerone, β-turmerone, ar-turmerone in rhizome oil; α-phellandrene, terpinolene, 1,8-cineole in leaf oil (Raina et al 2005)	Culinary spice (Hewlings & Kalman 2017); anti-inflammatory, anti-oxidant, anti-diabetic (Xu et al 2018); antimicrobial (Gupta et al 2015)

The most active essential oils where all the clinical isolates of *Mycobacterium tuberculosis* were susceptible even at the lowest concentration of 0.156% of the essential oils are those from *Citrus microcarpa* (rind), *Citrus maxima* (rind), *Cymbopogon citratus* (stalks), *Cymbopogon winterianus* (leaves), *Cymbopogon citratus* (leaves), and *Cinnamomum* sp. (leaves) (Table 3, A).

These essential oils have the highest sensitivity activity among all the essential oils. *C. winterianus* (stalks) is almost as good as the above most active (Table 3, B) in that it was only at 0.156% concentration that it did not inhibit the Pza and IEP-resistant isolates.

The next active EOs which inhibited all clinical strains but at different concentrations, can be further categorized (Table 3, C). Moderately active are the essential oils from *Eucalyptus* sp. (leaves), *Allium sativum* (bulb), *Origanum vulgare* (leaves), *Piper retrofractum* (leaves), and *Pogostemon cablin* (leaves), which inhibited the isolates at the concentrations of 0.625% – 5%. Weakly active are the essential oils from *Clausena anisum-olens* (leaves) and *Cinnamomum camphora* (leaves), which required 2.5% - 5% concentrations for inhibition of the organism.

Worth noting also is the essential oils which inhibited at all concentrations the Str resistant strain (*Allium sativum*, aside from those in group A), the Inh resistant strain (*Eucalyptus* sp. (leaves), *C. winterianus* (stalks), aside from group A), Rif resistant strain (*C. winterianus* (stalks), aside from group A), the Inh-Rif resistant strain (*A. sativum* (bulb), *C. winterianus* (stalks), aside from group A), and the Inh-Eth-Pza resistant strain (*Curcuma longa* (leaves), aside from group A),

Ranking of all the essential oils after post-hoc analysis using Duncan confirmed the same pattern presented in Table 3. The analysis even extended the ranges of sensitivities to include the weakly sensitive *Psidium guajava* (leaves), *Piper nigrum* (leaves), *Annona reticulata* (leaves), *Zingiber officinale* (rhizomes), *Cananga odorata* (flowers), *Canarium luzonicum* (resin), *Curcuma longa* (rhizomes). The essential oils from *Premna odorata* (leaves) and *Bixa orellana* (leaves) have the most resistant activity to the microorganisms (Navidad 2021),

Table 4 shows a brief account of the chemical constituents reported for the more active essential oils, together with the known uses. Extensive literature on these essential oils has been reviewed elsewhere (Anmol et al 2021; Arbab et al 2012; Bora et al 2022; Fuloria et al 2022; Huang et al 2023; Ibáñez and Blázquez 2020; Iweala et al 2023; Junren et al 2021; Kamaruddin et al 2022; Kaur et al 2021; Lee S-H et al 2022; Leliqia and Wardani 2021; Lima Santos et al 2022; Musa et al 2022; Swamy and Sinniah 2015; Thai et al 2014; Zhao et al 2024).

For all the more active essential oils in this study, a common property is their being antimicrobial, to varying degrees. The two citrus plants, *C. microcarpa* and *C. maxima* of the family Rutaceae, contain limonene as a major constituent, and their use as antimicrobial among others is well documented. The two species from Poaceae, *C. citratus* and *C. winterianus*, vary in their major constituents, unlike the *Citrus* plants.

Trang et al (2020) reported citral as the major component of the essential oil from *C. citratus* in Vietnam and that its citral content was higher in the essential oil obtained from the leaf than in that from the culm of lemongrass in all regions of Vietnam. In particular, camphene, valerianol, and epi-α-muurolol can be used to differentiate essential oils originating from leaves versus culms (stalks). In this study, the case of *C. winterianus* stalks vs. leaves differing in sensitivity could be due to a slight difference in composition. It is interesting to note that certain families predominate this group of very active essential oils, namely, Rutaceae and Poaceae.

It would be of interest to find other members of these families sharing a similar sensitivity as those reported here.

Conclusion

Essential oils can be a good source of antitubercular agents to combat tuberculosis. Essential oils can be effective in inhibiting the growth of the resistant strains of *M. tuberculosis*: the monoresistant strains – Str- resistant, Pza-resistant, Inh-resistant and Rif-resistant, the multidrug resistant strain (MDR) Inh-Rif-resistant, and the poly-drug resistant strains (PolyDR) strains- Str-Inh-Rif-resistant and Inh-Eth-Pza-resistant. Most significant are the essential oils that at all concentrations inhibited the growth of all resistant strains. These are the essential oils from *Citrus microcarpa* Bunge (rind), *Citrus maxima* (Burm.) Osbeck (rind), *Cymbopogon citratus* (DC.) Stapf (leaves, stalks) and *Cymbopogon winterianus* Jowitt ex Bor (leaves). The results can be used as a platform for complementing the existing drug regimens for tuberculosis, to address drug resistance at different levels, and to reduce the duration for TB therapy.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, DO, AA, CV; data collection BC, GB, AG, MP; analysis and interpretation of data, AG, FN, AA; original draft preparation, CV, BC, GB, AA; review and editing of the draft, AA.

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Supplementary Table 1. Percentages MIC within Essential Oils using Chi-square. ***(cont'd.)***

Essential Oils		Concentrations					
		5%	2.5%	1.25%	0.625%	0.312%	0.156%
Guava	Count	8	6	2	2	0	0
	% within EO	100.0%	75.0%	25.0%	25.0%	0.0%	0.0%
	% within conc	4.6%	3.8%	1.5%	1.9%	0.0%	0.0%
Black Pepper	Count	7	7	4	0	0	0
	% within EO	100.0%	100.0%	57.1%	0.0%	0.0%	0.0%
	% within conc	4.0%	4.5%	3.0%	0.0%	0.0%	0.0%
Atsuete	Count	2	2	0	0	0	0
	% within EO	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%
	% within conc	1.1%	1.3%	0.0%	0.0%	0.0%	0.0%
Turmeric leaves	Count	8	7	6	5	4	1
	% within EO	100.0%	87.5%	75.0%	62.5%	50.0%	12.5%
	% within conc	4.6%	4.5%	4.5%	4.8%	5.1%	1.7%
Turmeric rhizomes	Count	5	4	2	1	0	0
	% within EO	100.0%	80.0%	40.0%	20.0%	0.0%	0.0%
	% within conc	2.9%	2.5%	1.5%	1.0%	0.0%	0.0%
Calamansi	Count	8	8	8	8	8	8
	% within EO	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	% within conc	4.6%	5.1%	6.1%	7.6%	10.3%	13.6%
Suha	Count	8	8	8	8	8	8
	% within EO	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	% within conc	4.6%	5.1%	6.1%	7.6%	10.3%	13.6%
Ylang ylang	Count	7	5	3	0	0	0
	% within EO	100.0%	71.4%	42.9%	0.0%	0.0%	0.0%
	% within conc	4.0%	3.2%	2.3%	0.0%	0.0%	0.0%
Eucalyptus sp.	Count	8	8	8	8	4	0
	% within EO	100.0%	100.0%	100.0%	100.0%	50.0%	0.0%
	% within conc	4.6%	5.1%	6.1%	7.6%	5.1%	0.0%
Ginger	Count	7	5	3	0	0	0
	% within EO	100.0%	71.4%	42.9%	0.0%	0.0%	0.0%
	% within conc	4.0%	3.2%	2.3%	0.0%	0.0%	0.0%
Elemi	Count	8	2	0	0	0	0
	% within EO	100.0%	25.0%	0.0%	0.0%	0.0%	0.0%
	% within conc	4.6%	1.3%	0.0%	0.0%	0.0%	0.0%
Camphor	Count	8	8	7	6	3	1
	% within EO	100.0%	100.0%	87.5%	75.0%	37.5%	12.5%
	% within conc	4.6%	5.1%	5.3%	5.7%	3.8%	1.7%
"Laurel"/ *Cinnamomum* sp.	Count	8	8	8	8	8	8
	% within EO	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	% within conc	4.6%	5.1%	6.1%	7.6%	10.3%	13.6%
"Anis"	Count	8	8	7	6	3	0
	% within EO	100.0%	100.0%	87.5%	75.0%	37.5%	0.0%
	% within conc	4.6%	5.1%	5.3%	5.7%	3.8%	0.0%
Lemon grass leaves	Count	8	8	8	8	8	8
	% within EO	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	% within conc	4.6%	5.1%	6.1%	7.6%	10.3%	13.6%
Patchouli	Count	8	8	8	4	2	0
	% within EO	100.0%	100.0%	100.0%	50.0%	25.0%	0.0%
	% within conc	4.6%	5.1%	6.1%	3.8%	2.6%	0.0%
P. odorata	Count	2	2	0	0	0	0
	% within EO	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%
	% within conc	1.1%	1.3%	0.0%	0.0%	0.0%	0.0%
Lemongrass stalk	Count	8	8	8	8	8	8
	% within EO	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	% within conc	4.6%	5.1%	6.1%	7.6%	10.3%	13.6%

Supplementary Table 1. Percentages MIC within Essential Oils using Chi-square. ***(cont'd.)***

Essential Oils		Concentrations					
		5%	2.5%	1.25%	0.625%	0.312%	0.156%
Citronella stalk	Count	8	8	8	8	8	6
	% within EO	100.0%	100.0%	100.0%	100.0%	100.0%	75.0%
	% within conc	4.6%	5.1%	6.1%	7.6%	10.3%	10.2%
Citronella leaves	Count	8	8	8	8	8	8
	% within EO	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	% within conc	4.6%	5.1%	6.1%	7.6%	10.3%	13.6%
Anonas	Count	8	5	2	2	0	0
	% within EO	100.0%	62.5%	25.0%	25.0%	0.0%	0.0%
	% within conc	4.6%	3.2%	1.5%	1.9%	0.0%	0.0%
Oregano	Count	8	8	8	7	3	0
	% within EO	100.0%	100.0%	100.0%	87.5%	37.5%	0.0%
	% within conc	4.6%	5.1%	6.1%	6.7%	3.8%	0.0%
Garlic	Count	8	8	8	5	3	3
	% within EO	100.0%	100.0%	100.0%	62.5%	37.5%	37.5%
	% within conc	4.6%	5.1%	6.1%	4.8%	3.8%	5.1%
Litlit	Count	8	8	8	3	0	0
	% within EO	100.0%	100.0%	100.0%	37.5%	0.0%	0.0%
	% within conc	4.6%	5.1%	6.1%	2.9%	0.0%	0.0%
Total	Count	174	157	132	105	78	59
	% within EO	100.0%	90.2%	75.9%	60.3%	44.8%	33.9%
	% within conc	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Supplementary Table 2. Percentages MIC within Microorganisms using Chi-square.

MTb Strains		Concentrations					
		5%	2.5%	1.25%	0.625%	0.312%	0.156%
H37Ra strain	Count	23	22	19	13	11	9
	% within strains	100.0%	95.7%	82.6%	56.5%	47.8%	39.1%
	% within conc	13.2%	14.0%	14.4%	12.4%	14.1%	15.3%
Inh-Rif resistant strain	Count	19	17	17	14	10	8
	% within strains	100.0%	89.5%	89.5%	73.7%	52.6%	42.1%
	% within conc	10.9%	10.8%	12.9%	13.3%	12.8%	13.6%
Str-resistant strain	Count	22	21	14	14	10	8
	% within strains	100.0%	95.5%	63.6%	63.6%	45.5%	36.4%
	% within conc	12.6%	13.4%	10.6%	13.3%	12.8%	13.6%
Str-Inh-Rif resistant strain	Count	22	19	18	12	8	7
	% within strains	100.0%	86.4%	81.8%	54.5%	36.4%	31.8%
	% within conc	12.6%	12.1%	13.6%	11.4%	10.3%	11.9%
Inh-Eth-Pza resistant strain	Count	23	19	15	12	11	7
	% within strains	100.0%	82.6%	65.2%	52.2%	47.8%	30.4%
	% within conc	13.2%	12.1%	11.4%	11.4%	14.1%	11.9%
Pza resistant strain	Count	22	20	18	14	10	6
	% within strains	100.0%	90.9%	81.8%	63.6%	45.5%	27.3%
	% within conc	12.6%	12.7%	13.6%	13.3%	12.8%	10.2%
Inh resistant strain Rif resistant strain	Count	21	18	16	13	9	7
	% within strains	100.0%	85.7%	76.2%	61.9%	42.9%	33.3%
	% within conc	12.1%	11.5%	12.1%	12.4%	11.5%	11.9%
Rif resistant strain	Count	22	21	15	13	9	7
	% within strains	100.0%	95.5%	68.2%	59.1%	40.9%	31.8%
	% within conc	12.6%	13.4%	11.4%	12.4%	11.5%	11.9%
Total	Count	174	157	132	105	78	59
	% within strains	100.0%	90.2%	75.9%	60.3%	44.8%	33.9%
	% within conc	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%