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In vitro antimicrobial activity of vetiver root essential oil *Chrysopogon zizanioides* (L.) Roberty, and relationships with their major chemical compounds: A review

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Abstract
The activity of *Chrysopodium zizanioides* root essential oil (CZEO) against bacteria and fungi was first reported in the late 1970s. Since then, numerous studies have been published on the subject. Significant inhibitory effects have sometimes been observed, especially after 2017, against certain strains of Gram-positive bacteria, Gram-negative bacteria, or fungi. Compared to the inhibitory effects of other essential oils (EOs), CZEOs were sometimes highly effective, but sometimes not at all. Very few of the main chemical components of CZEOs are known for their antimicrobial properties. Work in this area is still limited and needs to be continued. The present review provides a compilation of the scientific literature on the antimicrobial properties of CZEO and sheds light on their effects.

Keywords *Chrysopogon zizanioïdes*, vetiver, essential oil, antibacterial, antifungal, antimicrobial

1. Introduction
Vetiver is an ambiguous common name for several species of the genus *Chrysopogon*, including the best-known *C. zizanioïdes* (L.) Roberty [syn. *Vetiveria zizanioïdes* (L.) Nash]. This species of the *Poaceae* family is an herbaceous perennial with an extensive underground root system 1-3 meters long. The roots are fibrous, vertical, and only slightly branched. A pantropical plant, *C. zizanioïdes* is mainly cultivated in India, Haiti, Indonesia, China, Madagascar, Brazil and South Africa. Its crop cycle is annual or biennial.
Chrysopogon zizanioïdes essential oil (CZEO) is obtained by steam distillation from the roots, which are usually dried, with distillation yields ranging from 0.29 to 9.61% w/w on dry basis [1]. One clone, 'Sunshine', is mainly used for the production of vetiver essential oil. In a study of 121 vetiver accessions, 86% belonged to this clone [2].
The complex chemical composition of CZEO has been studied mainly since the 2000s. It consists of over 300 molecules, mostly sesquiterpenoids [3]. The chemical composition of CZEO can vary depending on a number of variables such as geographical origin, soil, climate, essential oil extraction process [4]. Table 1 lists the chemical compounds exceeding 5% w/w in CZEOs identified by simultaneous analysis of samples from different countries.
There are other molecules whose content exceeds 5% in CZEOs, such as dehyro-aromadendrene (7.34% and 9.66%) [7], (5.45%) [8]; β-vatirenene (5.94%) [8], (8.61%) [9]; bicyclovetivenol (10.76%) (South India) [10].
Significant variations in the chemical composition of CZEO can often be observed. An interesting example is cedr-8-en-13-ol, thought to have antimicrobial activity which will be discussed later. This compound is the main constituent (26.5%) of a CZEO distilled in China [11], or in India (14.5%) [12], another in Indonesia (12.67%) [13]; and one with unspecified origin (12.36%) [8]. On the other hand, it's a minor component (1.0 and 2.2%) of a CZEO sample from India [14], and was not reported in nine samples of different origins [5]. Among the major molecules reported in the literature, there are questionable cases. For example, it concerns an interesting study which tested a CZEO with authenticated root against eight bacterial strains [15]. The main compound identified by the authors, *trans-p*-tert-butylcyclohexyl acetate (21.47% of EO) is not a natural molecule and has never been reported as a component of an essential oil.

Table 1: Main chemical constituents of CZEO in studies of samples of different origin

Number of samples analysed (origins)	Countries	Major compounds (>5% w/w)	Reference
27 (of trade)	Brazil, China, Haiti, India, Java, Madagascar, Mexico, Reunion Island, Salvador	α-cadinol (0-6.5%), cyclocopacamphan-12-ol (epimer A) (0-6.7%), β-eudesmol (0-5.2%), khusimol (3.4-13.7%), prezizan-7β-ol (0-9%), vetiselinenol (1.3-7.8%), β-vetivenene (0-5.7%), α-vetivone (2.5-6.4%)	[5]
4 (of trade except 1)	Brazil, Haïti, Reunion Island, Java	isovalencenol (4.4-15.3%), khusimol (6.4-13.3%), γ-vetivenene (0-5.1%), β-vetivone (1.9-8.0), zizanoic acid* (0.5-24.0%)	[6]
21 (various cultivars)	USA (Florida), Nepal, Portugal	(E)-isovalencenol (9.8-16.5%), khusimol (14.5-27.6%), vetiselinenol (2.6-11%), vetivenic acid (0-7.8%), β-vetivone (1.3-7.1%), α-vetivone (2.3-5.7%)	[1]

* Synonym: khusenic acid

It should be noted that plant compounds are routinely classified as 'antimicrobial' on the basis of susceptibility tests that produce minimum inhibitory concentrations (MICs) in the range of 100 to 1000 µg/mL [16]. In the case of essential oils, the following ranges of MIC values are considered worthy of publication: 100-500 µg/mL (strong activity), 500-1000 (moderate activity) [17].

Many essential oils show inhibitory effects in these concentration ranges. For example, in one study, 27 of 29 essential oils tested showed inhibition between 100 and 1000 µg/mL against at least one strain of various *Escherichia coli* [18].

Several reviews have been published on bioactivities of vetiver essential oils [14, 19, 20, 21, 22, 23]. However, with regard to the antimicrobial effects of CZEO, these reviews appear somewhat restricted and lack comparative tables. The aim of this review is to provide a compilation of the scientific literature on the *in vitro* antimicrobial properties of CZEO and highlight its implications.

2. Antimicrobial activity of vetiver essential oils

The first published studies on the antimicrobial activity of CZEO date back to the late 1970s, against bacteria [24], and keratinophilic fungi [25]. Since then, more than twenty studies have been published on the subject. CZEOs have shown significant antibacterial and antifungal activity. Their minimum inhibitory concentrations (MIC) against certain microbial species are comparable to those of traditional antimicrobial essential oils [26, 27]. Taking into account the nature of the germs evaluated, the present review classically divided them into three groups: Gram-positive bacteria, Gram-negative bacteria, and microscopic fungi.

2.1 MIC of CZEOs against Gram-positive bacteria

The following genera of Gram-positive bacteria have been tested against CZEOs: *Arcanobacterium, Bacillus, Brevibacterium, Corynebacterium, Cutibacterium, Enterococcus, Kytococcus, Micrococcus, Mycobacterium, Staphylococcus, and Streptococcus.* The Gram-positive bacteria evaluated were mainly the most common pathogenic bacteria *Staphyllococcus aureus*, and the following commensal bacteria, depending on the organ involved. Foot flora: *Micrococcus luteus, Kytococcus sedentarius, Corynebacterium xerosis, Corynebacterium* sp, *Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus cohnii*. Others the external mucous membranes, especially the armpits (axillary cavities): *Arcanobacterium haemolyticum, Micrococcus luteus Staphylococcus epidermidis Staphyllococcus haemolyticus, Staphylococcus xylosus Corynebacterium xerosis.* Natural skin flora: *Cutibacterium acnes*. Oral cavity flora: *Streptococcus mutans*. Digestive tract flora: *Enterococcus faecalis*. Others Gram-positive bacteria tested come from the soil: *Bacillus subtilis*, *Brevibacterium agri*, *B. epidermidis*, *B. linens*; these last three being involved in foot odor. *Mycobacterium smegmatis*, very rarely pathogenic, was also tested.

As can be seen from the literature data since 1994, most of the published studies on the inhibitory effect of CZEOs against Gram-positive bacteria show MICs values usually between from 31.3 and 1000 µg/mL (see Tables 2 and 3). Another study reported rather modest inhibitory effects with several MICs between 1000 and 2000 µg/mL [28]. The main species studied belong to the genus *Staphyloccocus* including *Staphylococcus aureus, S. epidermis, S. cohnii, S. epidermidis, S. hominis, S. haemolyticus, S. xylosus.* Table 2 summarizes the comparative results of the inhibitory activitiy of CZEOs against Gram-positive bacteria that were evaluated by at least two publications. It shows inhibitions that are often significant.

Table 2: MIC ranges of *Chrysopogon zizanoides* essential oils against Gram-positive bacteria evaluated by at least two publications.

Gram-positive bacteria	CMI (µg/mL)	Reference
Staphyllocucus aureus	39 to 2000	[11, 12, 13, 29, 30, 31, 32]
Staphylococcus epidermis	100 to 2000	[26, 30, 31, 33]
Methicillin-resistant *Staphyllococcus aureus*	≤ 130 to 2000	[13, 28, 33]
Bacillus subtilis	150 to 2000	[11, 28, 31]
Enteroccocus faecalis	31.3 to 600	[13, 32, 34]
Micrococcus luteus	300 to 500	[26, 29, 31]
Cutibacterium acnes	250 and 500	[30, 33]

All of the results for MIC values express in µg/mL are shown in Table 3; those expressed in v/v in Table 4.

Table 3: MIC values (µg/mL) of *Chrysopogon zizanoides* essential oils against Gram-positive bacteria strains, listed in descending year of publication

Data on CZEO tested	Germ tested and strain	MIC (µg/mL)	Reference
1 distilled EO in India, gas chromatography (GC) analysis published	*Micrococcus luteus* ATCC 10240 *Staphylococcus aureus* ATCC 11632 *Streptomyces griseus* ATCC 10137	>500 >500 >500	[29]
1 commercial EO; country not specified, GC analysis published	*Cutibacterium acnes* ATCC 11 827 *Staphylococcus aureus* ATCC 25923 *Staphylococcus epidermidis* ATCC 22 23 *Streptococcus pyogenes* ATCC 12 334	250±0 170±90 100±20 40±0	[30]
1 distilled EO in India, GC analysis published, dried root	*Staphylococcus aureus* MTCC96 *Streptococcus mutans* MTCC497	50±0 100±0	[12]
1 distilled EO in China, GC analysis published, dried root	*Bacillus subtilis* ATCC 14579 *Staphylococcus aureus* ATCC 6538	312.5 39	[11]
1 distilled EO in Turkey, dried root	*Enterococcus faecalis* ATCC 29212	31.3	[34]
1 distilled EO in Indonesia, GC analysis published	*Enterococcus faecalis* ATCC 51299 VREF* *Staphylococcus aureus* ATCC 43300 MRSA*	510 510	[13]
3 Commercial EO from Reunion Island (distilled there ?), GC analysis published (result ranges)	*Bacillus* sp. 15003287301 *Bacillus subtilis* 15000964701 *Corynebacterium striatum* 12572545501 MRSA* 15004850001 and 15004306601 MRSA* 15509530101 and 15004159801 *Staphylococcus epidermidis* MR 15511909903	≤500 to 2000 ≤500 to 2000 ≤500 to 2000 ≤500 to 2000 ≤500 to 2000 ≤1000 to 2000	[28]
1 commercial EO; country not specified	*Cutibacterium acnes* ATCC 11827 *Brevibacterium agri* ATCC 51663 *Brevibacterium epidermidis* DSM 20660 *Brevibacterium linens* DSM 20425 *Staphylococcus aureus* ATCC 25923 *Staphylococcus aureus* MRSA*ATCC 43300 *Staphylococcus aureus* GMRSA* ATCC 33592 *Staphylococcus epidermidis* ATCC 2223	500 50 190 190 500 500 130 130	[33]
1 distilled EO in India, Fresh root	*Mycobacterium smegmatis* Wild type sensitive to quinolones and fluoroquinolones *Mycobacterium smegmatis* resistant to quinolones and ciprofloxacin	125 125	[35]
1 distilled EO in Brazil, analyzed, dried root	*Bacillus cereus* ATCC14576 *Bacillus subtilis* 6633 *Micrococcus luteus* ATCC 9341 *Micrococcus roseus* ATTC 1740 *Staphylococcus aureus* ATCC 25923 *Staphylococcus aureus* ATCC 6538 *Staphylococcus epidermides* ATCC 12229	150 150 300 70 150 150 300	[31]
1 distilled EO in Reunion Island, GC analysis published	*Enterococcus faecalis* ATCC 29213 *Staphylococcus aureus* ATCC 25923	600 600	[32]

*MRSA (methicillin resistant *Staphylococcus aureus*), GMRSA (gentamicin-methicillin resistant *Staphylococcus aureus*), VREF (vancomycin-resistant *Enterococcus faecalis*)

Table 4: Other MIC values of *Chrysopogon zizanoides essential oils* against Gram-positive bacteria strains, listed in descending year of publication

Data on CZEO tested	Germ tested and strain	MIC	Reference
1 distilled EO in India	*Steptococcus agalactiae* (Clinical mastitis in dairy cattle) *Staphyllococcus aureus* (Clinical mastitis in dairy cattle)	25% v/v 12.5% v/v	[36]
1 distilled EO in The Comoros, GC analysis published	*Bacillus subtilis* *Micrococcus luteus* *Staphylococcus aureus*	0.02% v/v 0.02% v/v 0.02% v/v	[37]
1 Commercial EO	*Staphylococcus aureus*	None	[38]
1 distilled EO in India, dried root	*Trichosporon asahii* MTCC 6179 *Trichosporon cutaneum* MTCC 255	100 µL/mL 100 µL/mL	[39]
1 commercial oil, country not specified Note: Organ used according to publication: leaf!	*Enterococcus faecalis* NCTC 8213 *Staphylococcus aureus* NCTC 6571	0.12% v/v 0.06% v/v	[40]
Lack of data	*Arcanobacterium haemolyticum* *Corynebacterium xerosis* *Corynebacterium* sp. B Group *Corynebacterium* sp. C Group *Corynebacterium* sp. D2 Group *Kytococcus sedentarius* *Micrococcus luteus* *Staphylococcus cohnii* *Staphylococcus epidermidis* *Staphyllococcus haemolyticus* *Staphylococcus hominis* *Staphylococcus xylosus*	200 µL/L 100 µL/L 300 µL/L 300 µL/L 400 µL/L 100 µL/L 50 to >1000 µL/L >1000 µL/L 200 to 1000 µL/L >1 000 µL/L 500 µL/L 200 µL/L	[26]

In an *ex vivo* study of root canal infection by *Enterococccus faecalis* at different time intervals (1, 7, 14 days), the percentage reduction of colony forming unit (log10) at 7 and 14 days was between 6.3-14.6% for vetiver root essential oil, 7.0-22.6% for *Matricaria recutita* flower essential oil, compared to the control group [41]. Another study reported a slight sensitivity of the phytopathogenic bacterium *Pectobacterium carotovorum* to a CZEO from Egypt against (13.5% at a concentration of 1000 µg/mL) [42].

2.2 MIC of CZEOs against Gram-negative bacteria

The following genera of Gram-negative bacteria were tested against CZEOs: *Acinetobacter*, *Aggregatibacter*, *Alcaligenes*, *Bartonella*, *Enterobacter*, *Escherichia*, *Fusobacterium*, *Klebsiella*, *Moraxella*, *Porphyromonas*, *Prevotella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Serratia*.

The Gram-negative bacteria studied are mainly from to the flora of the digestive tract: *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*. Others are bacteria found in the foot flora: *Acinetobacter* sp., *Alcaligenes* sp., *Moraxella* sp. Another is the flora of oral cavity: *Prevotella intermedia.*

Between 1997 and 2017, almost all published studies involving the inhibition by CZEO showed a lack of effect with results >1000 µg/mL leading to the conclusion that CZEO may not be effective against Gram-negative bacteria [26, 28, 31, 32, 40]. In 2017, an inhibition at a concentration of 600 µg/mL against *Escherichia coli* was reported [33]. These results were not consistent with those published latter. Between 2019 and 2022, much higher inhibitory effects were reported, ranging from 22 to 312 µg/mL for fifteen Gram-negative bacterial species [11, 12, 34, 43]. Table 5 summarizes the comparative results of the inhibitory effects of CZEOs against Gram-negative bacteria that were evaluated by at least two publications. The ranges of inhibition observed are even wider than in the case of Gram-positive bacteria.

Table 5: MIC ranges of *Chrysopogon zizanoides* essential oils against Gram-negative bacteria evaluated in at least two publications.

Gram-negative bacteria	CMI (µg/mL)	Reference
Escherichia coli	15.6 to >20000	[11, 12, 28, 31, 32, 33, 34]
Pseudomonas aeruginosa	125 to > 30000	[11, 12, 28, 31, 32, 33]
Klebsiella aerogenes	125 to 9620	[12, 28, 31]

All of the results for MIC values expressed in µg/mL are shown in Table 6; those expressed in v/v are show in Table 7.

Table 6: MIC values (µg/mL) of *Chrysopogon zizanoides* essentials oils against Gram-negative bacteria strains, listed in descending year of publication

Data on CZEO tested	Germ tested and strain	MIC (µg/mL)	Reference
1 distilled EO in India, analyzed	*Salmonella typhimurium* ATCC 13311	> 500	[29]
1 distilled EO in Brazil, GC analysis published	*Aggregatibacter actinomycetemcomitans* ATCC 43717 *Fusobacterium nucleatum* ATCC 25586 *Fusobacterium nucleatum* Clinicat Isolate *Porphyromonas gingivalis* ATCC 33277 *Porphyromonas gingivalis* Clinical Isolate *Prevotella intermedia* ATCC 49046 *Prevotella intermedia* Clinical Isolate *Prevotella melaninogenica* ATCC 700524 *Prevotella nigrescens* ATCC 33563	22.0±6.25 50.0±0.0 250.0±100.0 62.5±25.0 100.0±0.0 22.0±6.25 150.0±58.0 50.0±0.0 62.5.0±25.0	[43]
1 distilled EO in India, GC analysis published dried root	*Escherichia coli* MTCC443 *Klebsiella aerogenes* MTCC 111 *Proteus mirabilis* MTCC3310 *Pseudomonas aeruginosa* MTCC96	800±12.5 125±10 75±5 125±2,5	[12]
1 distilled EO in Turkey, dried root	*Enterobacter cloacae* ATCC 13047 *Escherichia coli* ATCC 25922 *Proteus vulgaris* ATCC 13315	15.6 15.6 15.6	[34]
1 distilled EO in China Chine, GC analysis published, dried root	*Escherichia coli* (ATCC 8739) *Pseudomonas aeruginosa* ATCC 27853	312.5 312.5	[11]
3 commercial EO from Reunion Island (distilled there ?), GC analysis published (result ranges)	*Acinetobacter baumannii* RCH *Acinetobacter baumannii* SAN008 *Acinetobacter baumannii* AYE *Escherichia coli* ATCC25922 *Escherichia coli* BLSE 15509082801 *Klebsiella aerogenes* 15509970101 *Klebsiella aerogenes* 15501261101 *Klebsiella pneumoniae* BHR OXA48 *Klebsiella pneumoniae* 15000077501 *Pseudomonas aeruginosa* ATCC27853 *Pseudomonas aeruginosa* 155089996501 *Pseudomonas aeruginosa* 15509942001	> 1000 to > 2000 for all species	[28]
1 commercial EO; country not specified	*Escherichia coli* ATCC 25922 *Pseudomonas aeruginosa* (ATCC 27858)	600 1000	[33]
1 distilled EO in Brazil, GC analysis published, dried root	*Enterobacter cloacae* HMA/FT 502 *Escherichia coli* 8739 *Escherichia coli* ATCC 11229 *Klebsiella aerogenes* ATCC 13048 *Pseudomonas aeruginosa* ATCC 27483 *Pseudomonas aeruginosa* ATCC 9027 *Serratia marcescens* ATCC 14756	9620 for all species	[31]
1 distilled EO in Reunion Island, GC analysis published	*Escherichia coli* ATCC 25922 *Pseudomonas aeruginosa* ATCC 27853	>20000 >30000	[32]

Table 7: Other MIC values expressed in v/v of *Chrysopogon zizanoides* essential oils against Gram-negative bacteria strains, listed in descending year of publication

Data on CZEO tested	Germ tested and strain	MIC	Reference
1 distilled EO in India	*Escherichia coli* (Clinical mastitis in dairy cattle)	12.5% v/v	[36]
1 distilled EO in The Comoros, GC analysis published	*Escherichia coli*	0,02% v/v	[37]
1 Commercial EO	*Escherichia coli* *Salmonella typhi*	None None	[38]
1 Commercial EO	*Bartonella henselae*	0.032-0.063% v/v	[44]
1 Commercial oil; country not specified Note: Organ used according to publication: leaf!	*Acinetobacter baumannii* NCTC 7844 *Aeromonas veronii* biogroup *sobria* ATCC 9071 (*A. sobria*) *Escherichia coli* NCTC 10418 *Klebsiella pneumoniae* NCTC 11228 *Pseudomonas aeruginosa* NCTC 10662 *Salmonella enterica* subsp. *enterica* serotype *typhimurium* ATCC 13311 (*Salmtyphimurium*) *Serratia marcescens* NCTC 1377	>2,0% v/v for all species	[40]
1 commercial EO; country not specified	*Acinetobacter* sp. *Alcaligenes* sp. *Moraxella* sp.	>1 000 µL/L >1 000 µL/L 100	[26]

2.3 MIC of CZEOs against fungi and yeasts

Published studies on the inhibitory effects of CZEOs on fungi have focused on species with different effects. *Microsporum canis*, *M. gypeum*, *Trichophyton equinum*, *T. interdigitale*, *T. mentagrophytes*, *T. rubrum* belong to the dermatophyte group of skin and mucosal pathogens. *Aspergillus brasilienis*, *A. flavus*, *A. fumigatus*, *A. niger*, causing aspergillosis. *Penicillium notatum*, rarely pathogenic, was also evaluated.

Three species of yeast were evaluated, including *Candida albicans*, causing candidosis, *C. glabrata*, also pathogenic, *Cryptococcus neoformans*, causing cryptococcosis, and *Saccharomyces cerevisiae*. More than a dozen phytopathogenic fungi were evaluated. Table 8 summarizes the comparative results of the inhibitory activity of CZEOs against fungi evaluated in at least two publications. The homogeneity of the results is much better than for bacteria. In addition, all the fungi and yeasts tested were sensitive to CZEO, except to a lesser extent *Aspergillus fumigatus* and *Candida albicans* (see Tables 8, 9, 10 and 13).

Table 8: MIC ranges of *Chrysopogon zizanoides* essential oils against fungi and yeast evaluated in at least two publications

Gram-positive bacteria	CMI (µg/mL)	Reference
Candida albicans	200 to 1800	[12, 26-29, 31, 32, 33]
Trichophyton mentagrophytes	100 and 190	[26, 27, 33]
Aspergillus flavus	50 and >500	[12, 29]
Aspegillus fumigatus	>1 000 and 2200	[26, 27]
Aspergillus niger	25 and 78	[12, 45]
Microsporum canis	90 and 200	[27,33]

All of the results for MIC values expressed in µg/mL are shown in Table 9. Other results for MIC values expressed in v/v or ppm are shown in Table 10.

Two additional data are added below: *Rhizoctonia solani* was found to be dose-dependently sensitive (ED_{50} of 297 and 352 µg/mL) to a northern type Indian vetiver essential oil and a southern type Indian vetiver essential oil [10]. *Fusarum oxysporum*, *Alernaria citri* and *Rhizoctonia solani* were sensitive to an analyzed CZEO from Egypt (inhibition of 60.3, 69.2 and 79.2% respectively at a concentration of 1000 µg/mL) [42].

2.4 Minimum bactericidal and fungicidal concentrations of CZEOs

Some publications have reported results for minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) of CZEOs (Table 11). By comparison, MIC and MBC or MFC were quite close for all the germs tested except in one case of *Prevotella intermedia* with a MIC of 22 and 150 µg/mL, and MBC of 400 µg/mL [43].

2.5 Studies comparing the antimicrobial effects of CZEOs from different origins

The numerous results on the inhibitory effects of CZEO microorganisms show marked discrepancies. It seemed worthwhile to investigate whether any studies had been carried out to compare the effects of CZEO samples of different origins. Only one study compared the effects of three essential oils of vetiver [28] (Table 12). This shows slight differences among Gram-negative bacteria and more marked differences for Gram-positive bacteria and fungi.

Table 9: MIC value (µg/mL) of *Chrisopogon zizanoides* essential oils against fungal strains, listed in descending year of publication

Data on CZEO tested	Germ tested and strain	MIC (µg/mL)	Reference
1 distilled EO in India, GC analysis published	*Aspergillus flavus* (ATCC 9643) *Aspergillus brasiliensis* (ATCC 16404) *Candida albicans* (ATCC 66027) *Saccharomyces* cerevisiae* (ATCC 9763)	>500 >500 >500 >500	[29]
1 distilled EO in India, GC analysis published, dried root	*Aspergillus flavus* (MTCC9606) *Aspergillus niger* (MTCC282) *Penicillium notatum* (MTCC2647) *Rhizopus microsporus* (MTCC383)	50±5 25±2.5 12.5±2.5 25±0	[12]
1 commercial EO; country not specified, GC analysis published	*Aspergillus niger* (1250) *Candida albicans* (1250) *Cryptococcus neoformans* (313)	78 313 20	[45]
3 commercial EO from La Réunion (distilled there ?), GC analysis published (result ranges)	*Candida albicans* ATCC66396 *Candida glabrata* LMA901085	200 to 800 100 to 400	[28]
1 commercial EO; country not specified	*Trichophyton mentagrophytes* ATCC 9533 *Microsporum canis* ATCC 36299 *Candida albicans* ATCC 10231	190 90 1000	[33]
1 distilled EO in Brazil, GC analysis published, dried root	*Candida albicans* (NTC 2010)	300	[31]
1 distilled EO in Reunion Island, GC analysis published	*Candida albicans* (ATCC 10231)	1400	[32]
Lack of data	*Aspegillus fumigatus* *Candida albicans* *Trichophyton interdigitale* *Trichophyton mentagrophytes* *Trichophyton rubrum*	>1000 >1000 200 100 50	[26]
1 commercial EO; country not specified	*Aspergillus fumigatus* *Candida albicans* *Microsporum canis* *Trichophyton interdigitale* *Trichophyton mentagrophytes* (2 strains) *Trichophyton rubrum* (2 strains)	2200 1800 200 200 100 50	[27]

* The authors indicated *Streptomyces* in their article

Table 10: Other MIC value *Chrysopogon zizanoides* essentials oils against fungi strains,, listed in descending year of publication

Data on CZEO tested	Germ tested and strain	MIC	Reference
1 distilled EO in The Comoros, GC analysis published	*Aspergillus niger* *Penicillium digitatum* *Penicillium expensum*	0.2% v/v 0.05% v/v 0.2% v/v	[37]
1 distilled EO in The Comoros, GC analysis published	*Coniophora puteana* ATCC 9351 *Coriolus versicolor* ATCC 12679 *Gloeophyllum trabeum* ATCC 11539 *Poria placenta* ATCC 9891	<0.02% v/v <0.02% v/v <0.02% v/v <0.02% v/v	[46]
Commercial oil, country not specified Note: Organ used according to publication: leaf!	*Candida albicans* ATCC 10231	0.12% v/v	[40]
Lack of data	*Microsporum gypeum* *Trichophyton equinum* *Trichophyton rubrum*	400 ppm 400 ppm 400 ppm	[47]

Table 11: Minimum bactericidal and fungicidal concentrations of CZEO against bacteria and fungi, listed in descending year of publication

Data on CZEO tested	Germ tested and strain	MBC (µg/mL)	Reference
1 distilled EO in Brazil, GC analysis published	*Aggregatibacter actinomycetemcomitans* ATCC 43717 *Fusobacterium nucleatum* ATCC 25586 *Fusobacterium nucleatum* Clinicat isolate *Porphyromonas gingivalis* ATCC 33277 *Porphyromonas gingivalis* Clinical isolate *Prevotella intermedia* ATCC 49046 *Prevotella intermedia* Clinicat isolate *Prevotella melaninogenica* ATCC 700524 *Prevotella nigrescens* ATCC 33563	22.0±6.25 50.0±0.0 50.0±0.0 150±58 250.0±100.0 400±0 400±0 400±0.0 62,5±25	[43]
1 distilled EO in India, GC analysis published, dried root	*Aspergillus flavus* MTCC9606 *Aspergillus niger* MTCC282 *Enterobacter aerogenes* MTCC 111 *Escherichia coli* MTCC443 *Proteus mirabilis* MTCC3310 *Pseudomonas aeruginosa* MTCC96 *Penicillium notatum* MTCC2647 *Rhizopus microsporus* MTCC383 *Staphylococcus aureus* MTCC96 *Streptococcus mutans* MTCC497	135 50 150 >1100 150 200 62.5 37.5 100 225	[12]

Table 12: Antibacterial and antifungal effects of several CZEOs in a single study

Number of CZEOs tested	Origins	Germs examined (genus)	MIC (µg/mL)	Reference
3	Commercial EO from Reunion Island (distilled there ?), GC analysis published (result ranges)	*Acinetobacter baumannii* (3 strains)	>1000 or >2000	[28]
		Bacillus sp.	≤500; ≤1000; 2000	
		Bacillus subtilis	≤500; ≤1000; 2000	
		Candida albicans	200 or 800	
		Candida glabrata	100 or 400	
		Corynebacterium striatum	≤500 to 2000	
		Enterobacter aerogenes (2 strains)	>1,000 or >2000	
		Escherichia coli (2 strains)	>1000 or >2000	
		Klebsiella pneumoniae (2 strains)	>1000 or >2000	
		Pseudomonas aeruginosa (3 strains)	>1000 or >2000	
		MRSA (2 strains)	≤500 to 2000	
		MSSA (2 strains)	≤500 to 2000	
		Staphylococcus epidermidis MS	≤1000 to 2000	

2.6 Studies comparing the antimicrobial effects of CZEOs with other essential oils

In addition to the antimicrobial activity studies on CZEO alone, this essential oil has also been the subject of numerous comparative studies. Table 13 gives an overview of the inhibitory capacities of CZEO compared to other essential plant oils against bacteria and fungi. The results vary from strong to weak effects when compared to various other EOs. The table shows that CZEO is often found among the most inhibitory essential oils. However, it appears that fungi and yeasts are more sensitive to *Cymbopogon* species than to CZEO.

Table 13: Overview of the inhibitory capacities of CZEOs *versus* other essential oils against bacteria and fungi, listed in descending year of publication

Number of EOs tested	Bacteria or Fungi examined	Most inhibiting EOs	CZEO inhibitory activity versus other EOs	Reference
2	*Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Penicillium notatum*, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus microsporus*	*Ocimum basilicum*	Strong, except against *E. coli*	[12]
2	*Enterococcus faecalis*	*Matricaria recutita*	Lesser	[41]
32	*Bartonella henselae*	*Cinnamomum zeylanicum*, *Origanum vulgare hirtum*, *Canarium luzonicum*…	Medium	[44]
60	*Aspergillus niger* *Candida albicans*, *C. neoformans*	*Cinnamomum cassia*, *C. zeylanicum*, *Chrysopogon zizanioïdes* EOs	Strong	[45]
59	*Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA), gentamicin-methicillin resistant *S. aureus* (GMRSA), *S. epidermidis*, *Cutibacterium acnes*, *Brevibacterium agri*, *B. epidermidis*, *B. linens*, *Pseudomonas aeruginosa*, *Echerichia coli*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Candida albicans*	*Chrysopogon zizanioïdes*, *Santalum album*, *Cymbopogon citratus*, *C. martinii*, *Litsea cubeba*, *Pogostemon patchouli*	Strong (the strongest!)	[33]
19	*Staphyllococcus aureus*	*Thymus vulgaris*, *Cymbopogon citratus*, *Chrysopogon zizanioïdes*, *Pogostemon Patchouli*	Strong	[48]
2	*Salmonella typhi*, *Proteus vulgaris*, *Escherichia coli*, *Shigella species*, *Enterococcus species*	*Rosa damascena*	Lesser, exept against *Enteroccoccus* sp.	[38]
3	*Gloeophyllum*, *Poria*, *Coniophora*, *Coriolus*	*Piper capense*, *Piper borbonense*	Strong	[46]
24	*Trichosporon asahii*, *T. cutaneum*	*Mentha piperita*	Lesser	[39]
20	*Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus*, *Colletotrichum lindemuthianum*, *Penicillium* sp, *Rhizopus* sp.	*Cymbopogon* sp., *Trachyspermum ammi*	Lesser	[49]
67	*Pyricularia grisea*, *Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora infestans*, *Puccinia recondita*, *Blumeria graminis (=Erysiphe graminis)*	variable according to the EOs	Lesser, except against *Puccinia recondita*	[50]
73	*Streptococcus pneumoniae* (2strains)	*Origanum vulgaris* ssp. hirtum, *Thymus vulgaris*	Lesser	[51]
52	*Acinetobacter*, *Aeromonas*, *Candida*, *Enterococcus*, *Escherichia*, *Klebsiella*,, *Pseudomonas*, *Salmonella*, *Serratia*, *Staphylococcus*	*Cymbopogon citratus*, *C. martini*, *Origanum vulgaris*, *Thymus vulgaris*…	Lesser to strong	[40]
26	20 strains	*Origanum* sp., *Thymus* sp., *Cinnamomum* sp.	Among the strongest	[26]
3	*Aspergillus flavus*, *A. niger*, *Fusarium oxysporum*, *Penicillium* sp.	*Cymbopogon martinii* var. *motia*	Lesser	[52]
3	*Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Corynebacterium ovis*	*Cymbopogon martinii* var. *motia*, *Pimpinella anisum*	Lesser	[53]
7	*Aspergillus fumigatus*, *Candida albicans*, *Microsporum canis*, *Trichophyton interdigitale*, *T. mentagrophytes* (2 strains), *T. rubrum* (2 strains)	*Cymbopogon martinii*, *Pimenta racemosa*	Strong, except against *Candida albicans* and *Aspergillus fumigatus*	[27]
28	*Microsporum gypeum*, *Trichophyton equinum*, *T. rubrum*	*Anethum graveolens*, *Cymbopogon flexuosus*, *C. winterianus*, *Trachyspermum ammi*, *Chrysopogon zizanoïdes*	Strong	[47]

Of note is a study of 247 essential oils tested in combination. According to the authors, the essential oils of *Santalum* spp. and *Vetiveria zizanioides* in combination contributed the most to antimicrobial activity against five reference wound pathogens (*Staphylococus aureus*, MRSA, GMRSA, *E. coli* and *P. aeruginosa*) [54].

3. Antimicrobial activities of CZEOs main compounds

The sesquiterpenes, which are the main constituents of CZEO, are not well known for their antimicrobial properties. Over the last thirty years, some studies have been carried out in an attempt to identify the molecules of CZEO that would be involved in inhibiting bacteria or fungi. Here are the chemical compounds of CZEO and the papers published on the subject.

Cedr-8-en-13-ol (3); 6-isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydro-naphthalen-2-ol; δ-selinene and γ-gurjunenepoxide-(2); solavetivone

Ramírez-Rueda *et al*. were the first to report a bioguided analysis of CZEO. Multi-drug resistant *Staphyllococcus aureus* and *Enterococcus faecalis* were the bacteria tested. Their method was based on TLC bioautography and *in vitro* evaluation. According to their results, the five compounds mentioned above may be responsible for the antimicrobial activity of CZEO [13]. No other study has been published on the antimicrobial activity of these five molecules.

Khusimol and zizanoic acid

These two molecules showed significant antimycobacterial activity against drug-resistant mutants of *Mycobacterium smegmatis* and the virulent strain H37Rv of *M. tuberculosis*. Zizanoic acid was four times and khusimol twice more active than the standard drugs ciprofloxacin and nalidixic acid against the ciprofloxacin (CSC 101) and lomefloxacin (LOMR5) resistant mutants. These two sesquiterpene compounds were tested against the virulent strain H37Rv of *Mycobacterium tuberculosis*, which showed that zizanoic acid was two times more active than nalidixic acid, while khusimol was equally active [55].

Khusimol

This compound was found to be less antimicrobial than a dichloromethane extract against bacterial strains of *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus saprophyticus*; fungi such as *Aspergillus fumigatus*, *Rhizopus* sp, Dermatophytes such as *Microsporum canis*, *Microsporum gypseum*, *Trichophyton metagrophytes*, *T. rubrum* and yeast *Candida albicans*. The CMI of khusimol was between 250 and > 1000 µg/mL compared to between 125 and 1000 µg/ml for the dichloromethane extract [56].

According to a study on cell wall bacteria, fractional distillation of n-hexane extract from CZEO affected cell wall biosynthesis of a *Bacillus subtilis* ASK4744 strain. Compositional analysis (not provided by the authors) would suggest that khusimol may contribute to the bactericidal activity of a vetiver extract from EO [57].

In vitro, khusimol was antifungal against *Alternaria triticina*, *Drechslera oryzae* and *Fusarium moniliforme* with IC_{50} values of 100; 45; and 90µg/mL, respectively [58].

α-cadinol

This sesquiterpene possesses antifungal activities ranging from 11.7-51.9 µg/mL against plant pathogens: *Colletotrichum gloeosporioides* Penzig BCRC35003, *Ganoderma australe* BCRC36246), *Fusarium oxysporum* f. sp. *melonis* BCRC32121, *F. solani* BCRC32458, *Pestalotiopsis funereal* BCRC35266, and *Rhizoctonia solani* BCRC31626 [59].

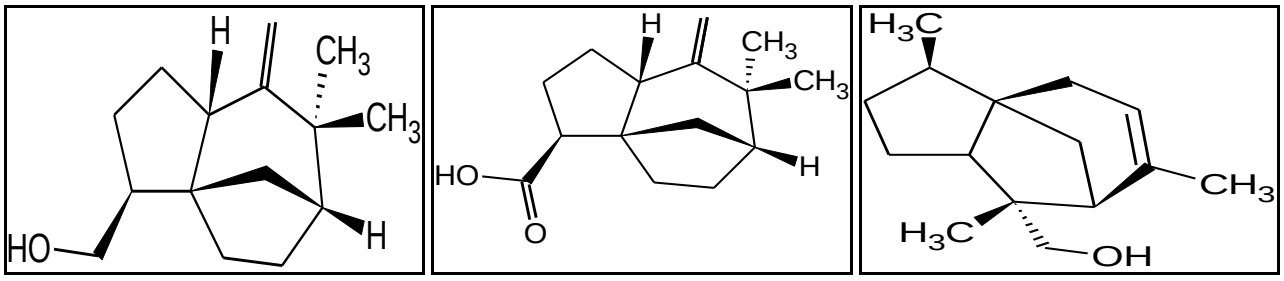
5,10-pentadecadien-1-ol; α-curcumene; hydroxyjunipene; (+)-cycloisosativene; valencene; selino-3,7(11)-diene

All these compounds extracted from CZEO in their pure form or as a mixture show significant antimycobacterial activity against the drug-resistant strains (MDR-R and MDR-40) of *Mycobacterium smegmatis* with MIC values between 31.25 and 62.5 µg/mL. An MIC of 125 µg/mL was reported for vetiver EO from India [35]. Of these six molecules, only α-curcumene has been reported to have significant antibacterial and antifungal effects [60].

Valencene

This compound showed no inhibitory effect (>400 µl/mL) against the following bacteria tested *Escherichia coli*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Penicillium citrinum*, and *Aspergillus niger* [61].

Note on 'vetiverol'. The study by Viollon C and Chaumont JP (1994) [26] on twenty bacteria and fungi reported that vetiverol had MIC values very close to those of a CZEO tested. However, no single chemical structure should be attributed to the name 'vetiverol' [3].



Khusimol Zizanoic acid Cedr-8-en-13-ol

Fig. 1: Chemical structures of three antimicrobial sesquiterpenes in vetiver essential oil

4. Conclusion

The studies reported in this review show that the antimicrobial effects of CZEOs sometimes differ greatly from one study to another. Inhibitory activities against Gram-positive bacteria were often significant, especially against *S. aureus*. Effects against Gram-negative bacteria appeared weak in the papers published up to 2017. From that year onwards, strong inhibition results were obtained. It is known that Gram-negative bacteria are more resistant to essential oils than Gram-positive bacteria. The cell wall lipopolysaccharides of Gram-negative bacteria could prevent the active components of essential oils from reaching the cytoplasmic membrane [62].

The interest of CZEO as an antifungal agent was underlined in studies against many of the germs tested. However, the inhibitory activity against *Candida albicans*, the most frequently tested germ, was variable.

The chemical compounds in vetiver essential oils vary, particularly according to origin. Could there be a link between

origin and antibacterial and antifungal activity? The literature does not provide an answer. It's a pity that various studies have been carried out on commercial CZEOs without specifying the country of origin and sometimes without analyzing the chemical composition. This raises the question of the profile of these EOs. In many cases of vetiver root essential oils analyzed, the number of chemical compounds identified appears too limited or unidentified compared to the chemical complexity of CZEOs. With so many components and so many unidentified components, who knows what synergistic or antagonistic interactions might be occurring? New research is needed to compare the chemical composition of different CZEOs and the results of inhibition tests. Furthermore, the nature of the antibacterial compounds in CZEOs remains unclear. None of the major chemical components of CZEOs are known to inhibit microorganisms. Some preliminary work to identify the antimicrobial compounds of CZEOs has opened up new avenues. However, the effects demonstrated by a few compounds, such as khusimol or khusimic acid, are modest in magnitude and not sufficient to explain CZEOs antimicrobial activity. The most potent antimicrobial molecules in commonly used essential oils are mainly monoterpenes (e.g. cinnamaldehyde, eugenol, thymol, citral...), which are absent in CZEOs.

Studies comparing the antimicrobial activity of CZEO and other EOs clearly show that CZEO is often among the most effective. In addition, unlike most essential oils, CZEO has the advantage of containing none or only trace amounts of the skin-allergenic substances listed in European Regulation (EU) 2023/1545 [63].

Overall, vetiver essential oil exhibits antimicrobial properties against several Gram-positive bacteria, Gram-negative bacteria fungi, and holds promise for various applications in pharmaceuticals and cosmetics. For example, its activity against pathogens such as *Cutibacterium acnes*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* makes this EO a promising candidate for anti-acne treatments [30]. However, its efficacy is certainly influenced by its chemical composition and origin, making it a versatile natural product for antimicrobial use. Although CZEO shows attractive antimicrobial properties, further research is needed to re-identify the active molecules in order to consolidate the potential of CZEO to combat pathogenic microorganisms.

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