Review

Chemical Variability and Biological Activities of Eucalyptus spp. Essential Oils

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Abstract: Several plant species produce mixtures of odorous and volatile compounds known as essential oils (EOs). These mixtures play important roles in nature and have been utilized by man for pharmaceutical and agrochemical purposes. There are more than 3000 EOs reported in the literature with approximately 300 having commercial use, including the oils from *Eucalyptus* species. Such oils are rich in monoterpenes and have found applications as pharmaceuticals, agrochemicals, food flavorants and in the perfume industry. Such applications are related to their diverse biological and organoleptic properties. In view of their importance, we review in this article up to date information concerning chemical composition and biological activities of essential oils from different species of *Eucalyptus*. Among the 900 species and subspecies of the *Eucalyptus* genus, we examined 68 species. The studies associated with these species were conducted in 27 countries. We have focused on the antimicrobial, acaricide, insecticide and herbicide activities, hoping that such information will contribute in the advances of the research in this field. It is also intended that the information herein described can be useful in the rationalization of the use of *Eucalyptus* EOs as components for new pharmaceutical and agrochemical applications as well as food preservatives and flavorants.

Keywords: Essential(s) oils; monoterpenes; insecticidal activity; antimicrobial activity; acaricide activity; herbicidal activity.

1. Introduction

Nature is a precious reservoir of substances that can be explored towards the development of pharmaceuticals. Several drugs to treat a variety of diseases have been disclosed via screening of natural compounds obtained from animals, microorganisms, marine organisms and plants. These drugs can be natural products *per se* or semi-synthetic derived from a natural product. Also, they can be a fully synthetic compound designed using natural product pharmacophores as model [1-6].

Natural products have also been utilized directly as pest control agents. Moreover, they have also served as models for the development of new pesticides with potential commercial application [7-13].

Although there is an impressive number of plant species, only around 10% produce mixtures of odorous and volatile compounds, collectively called essential or volatile oils [14]. Such oils can be produced by all parts of plants (buds, gums, blossoms, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark), according to the producing species. Essential oils are stored in several secretory structures such epidermic cells, secretory hairs, secretory ducts, secretory cavities, glandular trichomes or resin adducts [15-19]. These oils are hydrophobic in nature, liquid, soluble in alcohol, nonpolar or weakly polar solvents, waxes and oils. They are slightly soluble in water and usually are

colorless or pale yellow [15,17,20]. From the chemical standpoint, they are composed of hydrocarbons and oxygenated compounds collectively known as terpenoids.

EOs are important chemical defense tools that plants use against the attack of insects, fungi, bacteria and virus. EOs are also relevant as herbivorous feed deterrent [15,21-24].

It is known that EOs are also involved in allelopathic interactions inhibiting seed germination and the growth of plants [25-28]. This property has been explored towards the development of herbicides [29-31]. Within this context and considering the favorable biodegradability of EOs components, they can be considered attractive alternative tools for the control of weeds [32]. Essential oils from a variety of plants are also endowed with antibacterial activities [33-36], anti-inflammatory and antioxidant properties [37]

There are more than 3000 EOs described in the literature and approximately 300 of them are commercially available [15,17,38,39], including the oils from various *Eucalyptus* species.

The *Eucalyptus*¹ genus is represented by 900 species and subspecies. It corresponds to one of the principal genus of the Myrtaceae family, native from Australia and cultivated in several countries worldwide [17,40-43]. *Eucalyptus* trees have perennial leaves that are odorous due to the presence of EOs which are produced and stored in secretory cells. These oils are aromatic, spicy and can be colorless or pale yellow, although there are reports describing some as brownish or greenish [43].

The EOs from *Eucalyptus* are rich in monoterpenes and have found industrial applications for pharmaceutical purposes and in perfumery [44]. The oils utilized as pharmaceuticals are rich in 1,8-cineole. In the perfumery industries, oils rich in citronellal, citral and geranyl acetate are the most employed [45].

Considering the versatility of *Eucalyptus* EOs in terms of bioactivities as well as their industrial importance, the purpose of this review is to provide the readers with up to date information concerning chemical composition and biological activities of essential oils from different species of *Eucalyptus*. Two reviews about *Eucalyptus* EOs and biological activities have been published recently. One of them by Vuong et al. [47] focused on anticancer properties of *Eucalyptus* EOs. The other by Zhang et al. [48] described advances in terms of several biological activities until 2010. In this review, among the 900 species and subspecies of the *Eucalyptus* genus, we examined 68 species (3 of them are hybrids). The studies associated with these species were conducted in 27 countries and the literature survey covers recent advances in the field. The review is focused on the antimicrobial, acaricide, insecticide and herbicide activities. We hope that this article will contribute in the advances of the research in this field. It is also intended that the information herein described can be useful in the rationalization of the use of *Eucalyptus* EOs as components of pharmaceuticals and agrochemicals as well as food preservatives.

2. Chemical variability of essential oils of Eucalyptus

Although the essential oils are found in the leaves of more than 300 species of *Eucalyptus*, less than 20 species have been commercially explored for essential oil production [45,49]. In terms of chemical composition, these oils are complex mixtures of substances usually containing 20 to 80 compounds differing in their concentrations. Terpenes and terpenoids are the major components found in EOs from the leaves of *Eucalyptus* [38,50-55] as illustrated in Figure 1.

¹Based on morphological and molecular characteristics, in 1995 the *Eucalyptus* was reclassified by Ken Hill and Laurie Johnson [46]. The *Corymbia*, which previously was classified as a subgenus, was integrated to the *Eucalyptus* genus. There are 113 known *Corymbia* species, being *Corymbia citriodora*, *C. maculata*, *C. ficifolia*, *C. ptychocarpa* and *C. torelliana* the best well known species. However, the classification found in the references used for the preparation of the manuscript was preserved so that to facilitate the discussions.

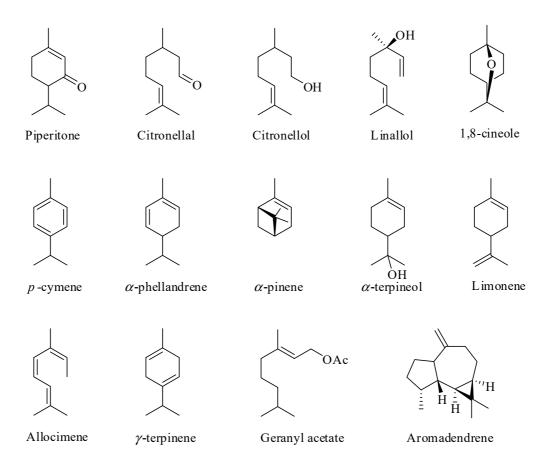


Figure 1. Some of the major constituents of the essential oils of *Eucalyptus* leaves.

The methods used to extract EOs are variable and include the use of hydrodistillation, supercritical carbon dioxide or microwaves, and mainly low or high pressure distillation employing boiling water or hot steam. The composition of the oil can vary according to the method and the drying conditions applied to the vegetal material prior to extraction and also to the storage conditions [56-59]. The method of choice for a particular application depends on the material from which the oil will be extracted. Another important aspect to be considered in the choice of the extraction method is the oil application. For instance, extraction with lipophilic solvents or with supercritical carbon dioxide is preferred for oils to be utilized in the perfumery.

Concerning the extraction of oils from *Eucalyptus*, hydrodistillation is typically the method of choice employed. The extraction yields range from 0.06% to 7% [60] and the chemical composition of the EOs is dependent on the plant species and varieties, and within the same variety, from geographical region, as can be seen in the results of several studies depicted in Table 1 [15,17,39,61,62].

Table 1. Major chemical components for *Eucalyptus* spp. essential oils.

Eucalyptus spp.	Origin	Components of Eucalyptus EOs	EOs yields (%)	Reference
E. camaldulensis	Egypt	1,8-cineole (60.3%), α -pinene	-	[63]
		(13.6%), γ-terpinene (8.8%)		
	Northern	1,8-cineole (19.0%), β -	-	[64]
	Cyprus	caryophyllene (11.6%),		
		carvacrol (9.1%)		
	Pakistan	linalool (17.0%), 1,8-cineole	1.90	[65]
		(16.1%), <i>p</i> -cymene (12.2%)		
	Democratic	1,8-cineole (58.9%), myrtenol	0.30 1	[53]

	Republic of	(4.3%), myrtenal (3.5%)		
	Congo			
	India	1,8-cineole (8.7%), α-	1.97 1	[66]
		phellandrene (27.5%), β -pinene		
		(23.5%), <i>m</i> -cymene (9.5%)	2.24	F. ()
	Nigeria	1,8-cineole (70.4%), β-pinene	0.26	[67]
		(9.0%) , α -pinene (8.8%)	2.40	[(0]
	Taiwan	1,8-cineole (29.6%), limonene	3.48	[68]
		(15.2%), β -pinene (9.9%), α -		
	Inon	pinene (9.7%)		[60]
	Iran Brazil	1,8-cineole (74.7%)	0.62	[69]
	Drazii	1,8-cineole (52.8%), limonene (14.2%), γ -terpinene (6.8%), α -	0.63	[70,71]
		pinene (6.1%)		
	Taiwan	α -pinene (22.5%), p -cymene	0.57	[72]
	Taiwan	(21.7%), α -phellandrene	0.37	[/2]
		(20.1%), 1,8-cineole (9.5%)		
	Tunisia	1,8-cineole (20.6%), α -pinene	0.76-1.42	[73,74]
	ransia	(16.5%)	0.70-1.42	[/0,/4]
	Argentina	1,8-cineole (19.1%), <i>p</i> -cymene	0.38	[75-77]
		(17.9%), β -phellandrene		
		(16.3%)		
	Kenya	1,8-cineole (18.9%), α -cadinol	-	[78]
		(6.4%), β -phellandrene (2.6%)		
	Spain	spathulenol (41.5%), <i>p</i> -cymene (21.9%)	0.71	[79]
E. cinerea	Tunisia	1,8-cineole (70.4%), α-terpineol	3.90	[80]
	D:1	(10.3%)	(07	[01]
	Brazil	1,8-cineole (75.7%), α -terpineol	6.07	[81]
	Brazil	(9.7%), α -pinene (6.2%) 1,8-cineole (83.6%), α -terpinyl	3.56-5.02	[82]
	Diazii	acetate (5.4%), α -pinene (5.0%)	3.30-3.02	[02]
	Tunisia	1,8-cineole (79.2%), α -terpinyl	3.00	[83]
	Turnsia	acetate (5.4%), α -pinene (4.1%)	5.00	[00]
	Argentina	1,8-cineole (88.5%), α -terpineol	_	[84]
	riigentiitu	(9.0%), α -pinene (2.0%)		[01]
	Argentina	1,8-cineole (79.8%), <i>α</i> -terpinyl	2.48	[75,76]
	111801111111	acetate (8.2%)	=. 10	[, 0), 0]
	Argentina	1,8-cineole (56.9%), α -pinene	-	[85]
		(6.4%)		
	Argentina	1,8-cineole (62.1%), <i>p</i> -cymene	-	[86]
E citriodore	Palsistan	(11.2%)	1 90	[45]
E. citriodora	Pakistan	citronellal (22.3%), citronellol (20.0%)	1.82	[65]

Australia	citronellal (68.9%), citronellol	-	[87]
Brazil	(7.6%), isopulegol (7.4%) citronellal (61.8%), isopulegol	-	[88]
	(15.5%), β -citronellol (7.9%)		
Indonesia	citronellal (90.1%), citronellol (4.3%)	-	[89]
Democratic	citronellal (72.7%), citronellol	1.63 ¹	[53]
Republic of Congo	(6.3%), eugenol (3.5%)		
Morocco	1,8-cineole (54.1%), <i>α</i> -pinene (23.6%)	3.30	[49,90]
Brazil	citronellal (82.3%), citronellyl	4.00	[91]
DIUZII	acetate (7.8%), neothujan-3-ol	1.00	[>+]
	(6.8%)		
South Korea	citronellal (73.0%), isopulegol	-	[92]
	(6.7%)		
Taiwan	citronellal (49.5%), citronellol	1.89	[68]
	(11.9%), iso-isopulegol (10.4%)		
Brazil	citronellal (67.5%), citronellol	-	[93]
	(6.9%), menthol (6.1%)		
Brazil	β -citronellal (71.8%), (-)-	-	[94]
	isopulegol (7.3%), isopulegol		
	(4.3%)		
Brazil	citronellal (94.9%), citronellyl	-	[95]
	acetate (2.6%), trans		
	caryophyllene (2.5%)		
China	citronellal (65.9%), citronellol	-	[96,97]
	(10.5%), 1,8-cineole (3.0%)		
China	citronellal (55.3%), citronellol	-	[98]
	(8.3%)		
Brazil	citronellal (76.0%), neo-iso-3-	0.66	[70,71]
	thujanol (11.8%)		
Benin	citronellal (52.8%), citronellol	4.60	[99]
	(20.0%), citronellyl acetate		
	(9.0%)		
Brazil	citronellal (71.8%), isopulegol	-	[100]
	(4.3%)		
Brazil	citronellal (89.6%), citronellyl	-	[101]
	acetate (3.3%), 1,8-cineole		
	(2.9%)		
Colombia	citronellal (49.3%), citronellol	0.70	[102]
	(13.0%), isopulegol (12.9%)		
Brazil	citronellal (71.1%), citronellol	-	[103]
	(8.8%)		

	Kenya	1,8-cineole (11.2%), <i>β</i> -pinene	-	[78]
	Benin	(3.2%), terpinen-4-ol (3.1%) citronellal (52.8%), citronellol	4.60	[104]
		(20.0%), citronellyl acetate (9.0%)		
	Colombia	citronellal (40.0%), isopulegol (14.6%), citronellol (13.0%)	-	[105,106]
	Argentina	citronellal (76.0%), iso- isopulegol (9.0%), citronellyl	-	[86]
		acetate (7.3%)		
	India	citronellal (52.2%), citronellol (12.3%), isopulegol (11.9%)	0.60	[26]
	Brazil	citronellal (64.9%), iso- isopulegol (10.2%), citronellol	2.10	[107]
	T., J:,	(8.3%)	2 27 4 80	[54]
	India	citronellal (48.3%), citronellol (21.9%), <i>iso</i> -isopulegol (12.7%)	2.36-4.80	[54]
E. globulus	Iran	1,8-cineole (84.5%), limonene (8.50%)	-	[108]
	Ethiopia	1,8-cineole (63.0%), α -pinene (16.1%)	-	[109]
	Spain	1,8-cineole (63.8%), α -pinene (16.1%)	-	[110]
	Pakistan	1,8-cineole (56.5%), limonene (28.0%)	1.89	[65]
	Algeria	1,8-cineole (55.3%), spathulenol (7.4%), α -terpineol	2,53	[111]
		(5.5%)		
	Indonesia	1,8-cineole (86.5%), α-pinene (4.7%)	-	[89]
	Democratic	1,8-cineole (44.3%), camphene	1.87 1	[53]
	Republic of	(23.1%), α -pinene (9.3%),		
	Congo	globulol (7.3%)	1 00 1	[110]
	Montenegro	1,8-cineole (85.8%), α -pinene (7.2%), β -myrcene (1.5%)	1.80 1	[112]
	Morocco	1,8-cineole (22.4%), limonene	1.21	[113]
		(7.0%), solanone (6.1%), β- pinene (5.2%)		
	Italy	1,8-cineole (84.9%), α-pinene (5.6%), <i>p</i> -cymene (5.3%)	-	[114]
	India	1,8-cineole (81.9%), limonene (6.6%)	-	[115]
	Brazil	1,8-cineole (83.9%), limonene (8.2%), α -pinene (4.2%)	-	[116]

	Iran	1,8-cineole (47.2%),	-	[117]
		spathulenol (18.1%), α -pinene		
		(9.6%)		
	India	1,8-cineole (44.4%), limonene	-	[42]
		(17.8%), <i>p</i> -cymene (9.5%)		
	Brazil	1,8-cineole (90.0%), tricyclene	-	[118]
		(3.0%)		
	Brazil	1,8-cineole (85.8%), α -pinene	-	[95]
		(9.9%)		
	Argentina	1,8-cineole (52,3-62.1%)	1.31-1.49	[119]
	Kenya	1,8-cineole (17.2%), α -pinene	-	[78]
		(7.1%), spathulenol (6.5%)		
	India	1,8-cineole (33.6%), α -pinene	-	[120]
		(14.2%), limonene (10.1%)		
	Australia	1,8-cineole (81.1%), limonene	-	[121]
		(7.6%), α-pinene (4.0%)		
	Argentina	1,8-cineole (77.9%), α -terpineol	2.25	[75,76]
		(6.0%)		
	Brazil	1,8-cineole (83.9%), limonene	-	[100]
		(8.2%), α-pinene (4.2%)		
	Brazil	1,8-cineole (77.5%), α -pinene	3.10	[122]
		(14.2%)		
	India	1,8-cineole (68.8%), α -pinene	-	[123]
		(2.8%)		
	India	1,8-cineole (66.3%), cis-	-	[124]
		ocymene (21.3%), α -terpinyl		
		acetate (3.4%)		
	Argentina	1,8-cineole (76.7%), α -pinene	1.66	[75,125]
		(11.1%)		
	Egypt	1,8-cineole (21.4%), o-cimene	-	[126]
		(21.4%), α -pinene (6.7%),		
		spathulenol (6.3%)		
	Australia	1,8-cineole (90.0%), α-pinene	-	[127]
		(2.2%)		
E. grandis	Brazil	γ -terpinene (16.8%), o -cymene	2.00	[91]
		(16.7%), β -pinene (11.5%)		
	Taiwan	1,8-cineole (19.8%), α -terpinyl	3.01	[68]
		acetate (12.8%), α -pinene		
		(11.4%)		
	Argentina	α -pinene (52.7%), 1,8-cineole	0.36	[77,128,129]
		(18.4%), p-cymene (8.7%)		
	Brazil	$lpha$ -pinene (40.6%), γ -terpinene	0.31	[70,71]
		(16.3%), p-cymene (13.1%)		
E. saligna	Democratic	1,8-cineole (61.3%), limonene	0.78^{1}	[53]

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	Republic of Congo	(10.1%), <i>p</i> -cymene (7.2%)		
	Brazil	<i>p</i> -cymene (25.6%), α -terpineol (9.3%), α -camphonelal (8.0%),	0.50	[91]
		1,8-cineole (6.2%)		
	Nigeria	α -thujene (63.8%), 1,8-cineole (12.3%)	0.30	[67]
	Brazil	α -pinene (45.1%), p -cymene (22.5%), α -pinene oxide (11.3%)	0.40	[130]
	Argentina	1,8-cineole (93.2%)	_	[131]
	Brazil	<i>α</i> -pinene (25.9%), p-cymene	0.19	[70,71]
	DIUZII	(24.4%), γ-terpinene (24.6%)	0.17	[/0,/1]
	Argentina	1,8-cineole (34.0%), p-cymene	0.36	[75,76]
	0	(21.3%), γ-terpinene (20.10%),		[-/ -]
		α -pinene (13.0%)		
	Brazil	1,8-cineole (45.2%), p-cymene	0.50	[122]
		(34.4%), α-pinene (12.8%)		
	Argentina	1,8-cineole (93.2%), limonene	-	[86]
		(3.3%)		
	Kenya	α -pinene (24.4%), 1,8-cineole	0.38	[132]
		(24.3%), o-cimene (9.9%), α -		
		terpineol (8.8%)		
E. tereticornis	Benin	p-cymene (31.1%), $β$ -	-	[133]
		phellandrene (9.7%)		
	Democratic	p-cymene (28.6%), cryptone	0.45^{1}	[53]
	Republic of	(17.8%), α -pinene (8.3%)		
	Congo			
	Benin	p-cymene (31.1%), $β$ -	-	[133]
		phellandrene (9.7%)		
	Benin	<i>p</i> -cymene (16.7%),	1.00	[99]
		caryophyllene oxide (14.2%),		
		spathulenol (13.5%), cryptone		
		(11.4%)		
	Argentina	β-phellandrene (22.6%), 1,8-	0.60	[75-77]
		cineole (18.6%), p-cymene		
		(14.5%), α -phellandrene (9.4%)		
	Argentina	1,8-cineole (37.5%), p-cymene	-	[86]
		(22.0%), γ-terpinene (10.8%)		

¹ Fresh leaves; (-): not reported

From Table 1, it can be noticed that the species Eucalyptus camaldulensis, E. cinerea, E. citriodora, E. globulus, E. grandis, E. saligna and E. tereticornis are the ones which have received more attention in terms of their essential oil components. A more detailed discussion regarding chemical aspects of EOs of these species is described below.

2.1. Eucalyptus camaldulensis

The reported yields of essential oils for *E. camaldulensis* range from 0.26% to 3.48% being the highest value found for plants cultivated in Taiwan [68]. For most *E. camaldulensis* EOs, 1,8-cineole is the major constituent. This oxygenated monoterpene has been found in quantities superior to 50% in EOs produced by plants cultivated in Egypt (60.3%) [63], the Democratic Republic of the Congo (58.9%) [53], Nigeria (70.4%) [67], Brazil (52.8%) [70,71] and Iran (74.7%) [69]. Plants cultivated in Kenya [78], Northern Cyprus [64] and Argentina [75,76] produce oils with 1,8-cineole accounting for less than 20% (18.9%, 19.0 and 19.1%, respectively). Different chemotypes of *E. camaldulensis* were observed for plants cultivated in Spain and Taiwan. Plants from Spain showed spathulenol (41.5%) and p-cymene (21.9%) as the major components [79], while for the species from Taiwan the principal constituents were α -pinene (22.5%), p-cymene (21.7%) and α -phellandrene (20.1%) [72]. It has been recently reported that the EOs from *E. camaldulensis* cultivated in Tunisia are rich in 1,8-cineole (20.6%) and α -pinene (16.5%) [73]. EOs extracted from plants cultivated in India have shown only 8.7% of 1,8-cineole. In this case, α -phellandrene (27.5%), β -pinene (23.5%) and m-cymene (9.5%) were the other major components [66].

2.2. Eucalyptus cinerea

Among all investigated *Eucalyptus* species, *E. cinerea* is the one that produce a highest amount of essential oils. The lowest extraction yield was found for *E. cinerea* plants cultivated in Argentina (2.48%) [75,76], and the highest for plants cultivated in the State of Paraná in Brazil (6.07%) [81]. Tables 1 and 3 show that 1,8-cineole is the major component found in all the investigated EOs extracted from this species. The lowest reported contents were observed for plants from Argentina, 56.9% [85] and 62.1% [86]. EOs with content of 1,8-cineole superior to 70% were found from plants cultivated in Argentina, 88.5% [84] and 79.8% [75]; in Paraná State, Brazil, 83.6% [82] and 75.7% [81]; and in Tunisia, 79.2% [83] and 70.4% [80].

2.3. Eucalyptus citriodora

One of the most investigated *Eucalyptus* species is *E. citriodora*. In general, it affords good extraction yields of EOs as observed in the studies from plants cultivated in India [54] and in Benin [99] (4.8% and 4.6% yields, respectively). Lower oil yielding species were found, however, in India (0.6%) [26], in São Paulo State, Brazil (0.66%) [70,71] and Colombia (0.70%) [102]. Typically, citronellal is the major constituent in *E. citriodora* EOs. Citronellal percentage lower than 50% of oil content was found in plants from Colombia (40.0%) [105] and 49.3% [102]; in India (48.3%) [54]; and in Taiwan (49.5%) [68].

Essential oils with high content of citronellal (>70%) have been reported in several studies conducted in Brazil, in the states of Minas Gerais (94.9%) [95]; Pernambuco (89.6%) [101]; Goiás (82.3%) [91]; São Paulo (76.0%) [70,71], (71.8%) [94]; Ceará (71.8%) [100]; and Sergipe (71.1%) [103]. Essential oils rich in citronellal have also been found in plants from Democratic Republic of Congo (72.7% of citronellal) [53]; South Korea (73%) [92] and Argentina (76.0%) [86].

As reported to this date, only plants cultivated in Tunisia [49,90] and Kenya [78] do not present citronellal as the major component in their EOs. Therefore, these species of *E. citriodora* represent different chemotypes producing oils rich in oils 1,8-cineole (54.1%) and α -pinene (23.6%) for Tunisian species and 1.8 cineole (11.2%) for species cultivated in Kenya.

2.4. Eucalyptus globulus

E. globulus is the major commercial source of 1,8-cineole, a compound found in almost all the EOs for this species cultivated in several places.

The EOs with 1,8-cineole representing less 50% of the oils were found in Kenya (17.2%) [78], Egypt (21.4%) [126], Morocco (22.4%) [113], India (33.6%) [120], Democratic Republic of the Congo (44.3%) [53], India (44.4%) [42], and Iran (47.2%) [117]. The highest content of 1,8-cineole (> 80%) in EOs of *E. globulus* were reported in studies carried out in Brazil in São Paulo State (83.9-90.0%)

[116,118]; in Minas Gerais State, (85.8%) [95]; and in Ceará State (83.9%) [100]; in Australia (90.0%) [127], 81.1% [121]; in Indonesia (86.5%) [89]; in Montenegro (85.8%) [112]; in Italy (84.9%) [114]; in India (81.9%) [115] and in Iran [108].

A severe limitation on several studies with *E. globulus* EOs is the lack of information on the oil extraction yields. This fact precludes us to evaluate the potential commercial application of such plants as a source of 1,8-cineole. Therefore, the plants that produce oils with high 1,8-cineole content should be further investigated in more details in case of a commercial interest.

2.5. Eucalyptus grandis

As described for other eucalyptus species, different chemotypes are also reported for *E. grandis*. Thus, plant species cultivated in Brazil, Goiás State, represent chemotypes with γ -terpinene (16.8%), o-cymene (16.7%) and β -pinene (11.5%) as the major components of their EOs. In another study conducted in Botucatu (São Paulo State, Brazil) the same chemotypes presented α -pinene (40.6%), γ -terpinene (16.3%) and p-cymene (13.1%) [70,71]. The main components in the EOs from plants found in Taiwan chemotype [68] were 1,8-cineole (19.8%), α -terpinyl acetate (12.8%) and α -pinene (11.4%) while the same chemotypes cultivated in Argentina [129] showed the presence of 52.7% of α -pinene, 18.4% of 1,8-cineole and 8.7% de p-cymene. Concerning EOs extraction yields, species cultivated in Botucatu (São Paulo State, Brazil) and in Argentina are low yielding (0.31% and 0.36%, respectively) while good extraction yields were observed for plants from Goiás State (Brazil) and Taiwan (2.0% and 3.01%, respectively).

In Brazil *E. grandis* is cultivated in an extense area for cellulose pulp production and its leaves represent an important industrial residue. Since this specie produce large amount of oil (2.0%), further investigation to evaluate the use of such residue for oil production could constitute in a good opportunity to maximize the profits.

2.6. Eucalyptus saligna

The specie E. saligna is also cultivated in Brazil for cellulose pulp production and is constituted of several chemotypes, some rich in 1,8-cineole. Example of the 1,8-cineole chemotype is found in plants cultivated in the Democratic Republic of the Congo [53] which presented 61.3% of 1,8-cineole besides limonene (10.1%) and p-cymene (7.2%). Two studies conducted in Argentina [75,76,86], found the same chemotypes producing oils with 1,8-cineole content equal to 93.2% and 34.0%, respectively. In the latter study, the authors also described p-cymene (21.3%), γ -terpinene (20.1%) and α -pinene (12.8%) as other important components of E. saligna EOs. Several studies carried out in Brazil, in different states, revealed different EOs composition of E. saligna. A 1,8-cineole chemotype was found in plants cultivated in Rio Grande do Sul State. The principal components determined in this study were 1,8-cineole (45.2%), p-cymene (34.4%) and α -pinene (12.8%) [122]. In Goiás State [91], the EOs presented p-cymene (25.6%), α -terpinene (9.3%), α -camphonelal (8.0%) and 1,8-cineole (6.2%) as major constituents. Chemotypes presenting α -pinene as major component were found in plants cultivated in São Paulo State, Brazil: α -pinene (45.1%), p-cymene (22.5%) and α -pinene oxide (11.3%) [130]; α -pinene (25.9%), p-cymene (24.4%) and γ -terpinene (24.6%) [70,71]. Finally, species cultivated in Nigeria [67] presented α -thujene (63.8%) as the major component followed by 1,8-cineole (12.3%). In all the aforementioned studies of E. saligna EOs, the extraction yields were low ranging from 0.19% to 0.78%.

2.7. Eucalyptus tereticornis

The yields of essential oils from *E. tereticornis* cultivated in different places varied from 0.45% to 1.0%. Two studies conducted in Benin found EOs presenting *p*-cymene as the main component. In one of these investigations, the plant species had 16.7% of *p*-cymene besides caryophyllene oxide (14.2%), spathulenol (13.5%) and cryptone (11.4%) [99]. In another study, the oils were found to have 31.1% of *p*-cymene and 9.7% of β -phellandrene [133]. The EOs of *E. tereticornis* cultivated in the Democratic Republic of Congo also revealed *p*-cymene (28.6%) as the major component, cryptone (17.8%) and α -pinene (8.3%) [53]. Lucia *et al* [75,76] reported that *E. tereticonis* EOs presented β -

phellandrene (22.6%), 1,8-cineole (18.6%) and p-cymene (14.5%) as the major *constituents*. Toloza and co-workers [86] examined EOs containing 1,8-cineole (37.5%), p-cymene (22.0%), and γ -terpinene (10.8%) as the major components.

From the data reviewed above, it is evident a large chemical variability among *Eucalyptus* essential oil species. Such variation can be ascribed to several factors including climate, soil type, plant age, nature (wet or dried) of the material used in the extraction, vegetative cycle stage, and time of the day when harvesting is made [100,107,134-136].

The chemical composition of EOs also depends upon the organ of the plant from which the oils are extracted [25,36,135,137]. In addition, several other factors may influence oil composition such as the extraction method, seasonal periods of harvesting [35,138-143], and essential oil storage conditions [144].

Having reviewed the chemical composition of essential oils from several *Eucalyptus* species, it is evident that the amount of oil varies from species and places where they are cultivated. A great variation in chemical composition is also observed, with oils rich mainly with monoterpenes and monoterpenoids, as observed for other Myrtaceae species [35,145]. Since the chemical composition of the oils is directly associated with their biological acitivities, we are now going to focus our discussion on such activities and in the multiple applications of such oils.

3. Biological activities of *Eucalyptus* essential oils

Several studies on antioxidant and antimicrobial activities of EOs from eucalyptus have been published in recent years [14,15,17,21,49,146-157]. Significant insecticide, antibacterial and fungicide effects have also been observed for EOs produced by eucalyptus [158,159].

Antimicrobial, acaricide, insecticide and herbicide activities associated with EOs from leaves of *Eucalyptus* have been reported in several articles every year, demonstrating the importance of this research field. Such bioactivities are highly dependent on the oils chemical composition as will be discussed and illustrated in the following section.

3.1. Antimicrobial activity

Eucalyptus EOs have been evaluated against several Gram-positive and Gram-negative bacterial strains as well as against various fungal species (Table 2). The EOs presented different degrees of efficiency against the evaluated species. Among bacterial strains, the pathogenic Gram-positive Staphylococcus aureus was the most sensitive to oils from several Eucalyptus species. From the data available Pseudomonas aeruginosa corresponded to the most resistant bacterial species. The yeast species Candida albicans also exhibited high sensitivity to the oils.

Table 2. Antimicrobial activities for *Eucalyptus* spp. essential oils.

Eucalyptus spp.	Target species	Reference
E. alba	Bacillus subtilis, Citrobacter diversus, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus vulgaris, S. aureus, Salmonella typhimurium, Escherichia coli, P. aeruginosa	[53,67]
E. astringens	Listeria ivanovii, E. coli, Bacillus cereus, Microsporum canis, C. albicans	[83,160,161]
E. bicostata	S. aureus, Streptococcus pneumoniae, C. albicans, L. ivanovii, B. cereus	[83,80,161]
E. botryoides	S. aureus, E. coli	[49,90]
E. camaldulensis	Alternaria alternata, Chaetomium globosum, S. aureus, B. subtilis, E. coli, Aspergillus niger, Rhizopus solani, C. diversus, K. oxytoca, K. pneumoniae, P. vulgaris, P. aeruginosa, Shigella flexneri, Listeria	[53,63-68]
	monocytogenes, C. albicans, B. cereus, Aspergillus clavatus,	

	Cladosporium cladosporioides, Myrothecium verrucaria, Penicillium citrinum, Trichoderma viride, Trametes versicolor, Phaneochaete chrysosporium, Phaeolus schweintizii, Lenzites sulphureu	
E. cinerea	S. aureus, E. coli, Staphylococcus epidermidis, P. aeruginosa, C. albicans, Streptococcus pyogenes, L. ivanovii, B. cereus	[80-83]
E. citriodora	B. subtilis, E. coli, S. aureus, A. niger, R. solani, Phytophthora cactorum, Cryponectria parasítica, Aspergillus spp., Pyricularia grisea, Colletotrichum musae, Enterococcus faecalis, K. pneumoniae, P. aeruginosa, C. diversus, K. oxytoca, P. vulgaris, S. typhimurium, S. flexneri, Salmonella choleraeseus, Botrytis cinerea, Colletotrichum gloeosporioides, Fusarium oxysporum, Pythium ultimum, Rhizoctonia solani, A. clavatus, C. globosum, C. cladosporioides, M. verrucaria, P. citrinum, T. viride, T. versicolor, P. chrysosporium, P. schweintizii, L. sulphureu, Haemonchus contortus	[49,53,65,68,87- 94]
E. cloeziana	S. aureus, E. coli	[91]
E. crebra	B. subtilis, E. coli, S. aureus, A. niger, R. solani	[65]
E. deglupta	S. aureus, C. albicans, B. cereus, E. coli, P. aeruginosa, B. subtilis, K. oxytoca, K. pneumoniae, S. typhimurium, S. flexneri	[53,67]
E. diversifolia	S. aureus, E. faecalis, E. coli	[160,162]
E. dives	Pseudomonas fragi, E. coli, S. typhimurium, L. monocytogenes, S. aureus, Saccharomyces cerevisiae, C. albicans	[17,163]
E. erythrocorys	Bipolaris sorikiniana, B. cinerea	[164]
E. globulus	E. coli, Salmonella typhi, Salmonella paratyphi, S. typhimurium, Shigella, P. aeruginosa, S. aureus, Trichophyton spp., Aspergillus spp., K. pneumoniae, Acinetobacter baumannii, B. subtilis, A. niger, R. solani, Fusobacterium nucleatum, Porphyromonas gingivalis, E. faecalis, C. diversus, K. oxytoca, S. pyogenes, C. albicans, Staphylococcus intermedius, Staphylococcus warneri, Staphylococcus sciuri, Meloidogyne incognita, H. contortus, B. cereus, Pseudomonas fluorescens, Penicillium digitatum, Aspergillus flavus, Mucor spp., Rhizopus nigricans, F. oxysporum, S. cerevisiae, Aspergillus parasiticus	[42,53,65,89,108- 118]
E. gracilis	L. monocytogenes, K. pneumoniae, S. cerevisiae, C. albicans, Mucor ramamnianus, Aspergillus ochraceus	[165]
E. grandis	S. aureus, E. coli, S. choleraeseus, A. clavatus, A. niger, C. globosum, C. cladosporioides, M. verrucaria, P. citrinum, T. viride, T. versicolor, P. chrysosporium, P. schweintizii, L. sulphureu	[68,91]
E. lehmannii	E. coli, S. aureus, B. cereus, P. aeruginosa	[83,160]
E. leucoxylon	E. coli, B. cereus	[83]

E. maidenii	L. ivanovii, E. coli, B. cereus, E. faecalis, S. aureus, Trichophyton soudanense, C. albicans	[80,83,161]
E. melanophloia	B. subtilis, E. coli, S. aureus, A. niger, R. solani	[65]
E. microcorys	S. aureus	[91]
E. microtheca	B. subtilis, E. coli, S. aureus, A. niger, R. solani	[65]
E. odorata	S. aureus, E. faecalis, E. coli, Streptococcus agalactiae, Haemophilus influenzae, S. pyogenes, S. pneumoniae, Trichophyton rubrum, T. soudanense, M. canis, Scopulariopsis brevicaulis, C. albicans	[80,161]
E. oleosa	L. monocytogenes, K. pneumoniae, S. cerevisiae, C. albicans, M. ramamnianus, A. ochraceus	[165]
E. radiata	E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, A. baumannii	[110]
E. robusta	B. subtilis, E. coli, K. oxytoca, K. pneumoniae, P. aeruginosa, S. aureus, S. typhimurium, S. flexneri, C. albicans	[53,130]
E. saligna	B. subtilis, C. diversus, E. coli, K. oxytoca, K. pneumoniae, P. aeruginosa, S. aureus, S. choleraeseus, C. albicans, B. cereus	[53,67,90,130]
E. olida	S. aureus, C. albicans	[17]
E. ovata	E. coli	[49,90]
E. pellita	E. coli, P. aeruginosa, S. aureus, B. subtilis	[166]
E. platyphylla	Deightoniella torulosa	[167]
E. platypus	E. faecalis	[160,161]
E. propinqua	B. subtilis, C. diversus, K. oxytoca, K. pneumoniae, S. aureus, S. typhimurium, S. flexneri	[53]
E. radiata	S. aureus, E. faecalis, K. pneumoniae, E. coli, P. aeruginosa	[89]
E. salmonophloia	L. monocytogenes, K. pneumoniae, S. cerevisiae, C. albicans, M. ramamnianus, A. ochraceus	[165]
E. salubris	L. monocytogenes, K. pneumoniae, S. cerevisiae, C. albicans, M. ramamnianus, A. ochraceus	[165]
E. sargentii	B. subtilis, S. aureus, S. epidermidis, E. coli, P. aeruginosa, K. pneumoniae, P. vulgaris, Shigella dysenteriae, A. niger, C. albicans	[168]
E. sideroxylon	L. ivanovii, B. cereus, M. canis	[80,83,161]
E. smithii	M. canis, Microsporum gypseum, Trichoplyton mentagnophytes, Trichoplyton rubrum	[169]
E. staigeriana	E. faecalis, S. aureus, C. albicans, H. contortus	[17,170-172]
E. tereticornis	Saccharomyces spp., Corynebacteriaceae spp., Sporobolomyces, Torulopsis cândida, Hansenula spp., B. subtilis, C. diversus, E. coli, K. oxytoca, K. pneumoniae, P. vulgaris, P. aeruginosa, S. flexneri	[53,133]

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E. urophylla B. subtilis, E. coli, K. oxytoca, K. pneumoniae, A. clavatus, A. niger, C. [53,68] globosum, C. cladosporioides, M. verrucaria, P. citrinum, T. viride, T. versicolor, P. chrysosporium, P. schweintizii, L. sulphureu

The EOs from *E. staigeriana* presented high antimicrobial activity against all evaluated microorganisms (Table 2). By using the agar diffusion method, *E. staigeriana* EOs presented the highest activity against *S. aureus* with inhibition zone diameter (izd) superior to 90 mm (the growth of the microorganism was inhibited over the entire Petri dish). This value was four times superior to the inhibition zone diameter caused by antibiotic chloramphenicol used as positive control in the biological assays [17]. In the same investigation, it was demonstrated that *E. dives* EOs were also very effective against *S. aureus* (izd 52.3 mm in diameter, a value approximately two times higher than the izd observed for chloramphenicol).

Derwich *et al.* [113] demonstrated the efficiency of *E. globulus* EOs against Gram-negative *E. coli* and Gram-positive *S. aureus* and *S. intermedius*. It was found that *E. globulus* EOs presented excellent activity on *E. coli* in the agar disc diffusion assay (izd = 48.15 mm) compared to *S. aureus* (izd = 13.5 mm) and *S. intermedius* (izd = 10.26). The minimum inhibitory concentration (MIC) for *E. coli* corresponded to 0.15 mg/mL while for *S. aureus* and *S. intermedius* the values were 0.75 mg/mL and 1.08 mg/mL, respectively.

The effects of *E. globulus* EOs on 14 food spoilage microorganisms have been investigated using liquid and vapour fase agar dilution/well diffusion method and disc volatilization method [42]. The MIC found from such methods varied in the range of 2.25-9.0 mg/mL for bacterial and fungal strains. It was observed that MIC obtained for Gram-positive *B. subtilis* and *S. aureus* were lower than MIC values found for Gram-negative *E. coli*, *P. aeruginosa*, and *P. fluorescens* [42]. In general, significantly higher antimicrobial activity was observed in the vapour phase.

As previously mentioned, 1,8-cineole is the main component of *E. globulus* EOs. It has been demonstrated that this compound has antimicrobial activity against several microorganisms including *S. aureus* [173], *E. coli* and *B. subtilis* [174,175]

Vratnica and co-workers [112] investigated the antimicrobial effects of *E. globulus* EOs against 17 microorganisms including food poisoning and spoilage bacteria and human pathogens. In general, the oils were highly active against the evaluated microorganisms. The agar disc diffusion method was utilized and filter paper discs were impregnated with *E. globulus* EOs (5, 10, 15, 20 and 30 μ L). In these assays, the highest izd values were observed for *S. pyogenes* (25-51 mm), *S. aureus* (22-48 mm), and *E. coli* (23-47 mm). The broth microdilution method was used to determine MIC which ranged from 0.09 mg/mL to 3.13 mg/mL. The highest MIC values were found for *P. aeruginosa* and *Salmonella infantis* (3.13 mg/mL) and the lowest for *S. aureus*, *E. coli* and *S. pyogenes* (0.09 mg/mL). In this work no information about the possible compounds responsible for the biological activity was provided.

The essential oils from *E. camaldulensis* were tested against a panel of 12 bacteria strains, and the most sensitive microorganism was *B. subtilis*. For this microorganism, the oils caused izd in the range of 19.3 mm to 29.3 mm at different volumes (20, 30, 40, 50 and 100 μ L) in the agar diffusion method. When tested against *L. monocytogenes* and *S. aureus*, the EOs caused significant growth inhibition of the microorganisms as attested by the izd ranging from 18.0 mm to 25.0 mm for the former and 14.6 mm to 25.0 mm to the latter at the aforementioned volumes of EOs [66].

The biological assays conducted with EOs of *E. odorata* displayed the best results against *S. aureus* (izd = 27.4 mm) as determined in the agar diffusion method, followed by *S. agalactiae* (izd = 19.4 mm), *H. influenzae* (izd = 19.2 mm), *S. pyogenes* (izd = 19.0 mm) and *S. pneumoniae* (izd = 17.4 mm). Moreover, *E. maidenii* exhibited good activity against *S. aureus* (izd = 22.8 mm) [80,161].

Antimicrobial activities of *Eucalyptus* spp. EOs have also been described against resistant bacterial strains. For instance, *P. aeruginosa* is known for its high intrinsic resistance against as antibiotics. This fact has been attributed to the very restrictive outer membrane barrier of the bacteria, being highly resistant even to synthetic drugs [17,49]. The essential oils of *E. camaldulensis* and *E. tereticornis* exhibited relevant activity against *P. aeruginosa*, presenting, respectively, izd = 15.5 mm

and 16.0 mm [53]. The essential oils from *E. cinerea* were less active as shown by the izd = 7.0 mm when tested against *P. aeruginosa* [82].

In general, Gram-positive bacterial strains are more sensitive to *Eucalyptus* EOs than the Gramnegative ones [17,42,82]. This can be rationalized taken into consideration that Gram-negative bacteria possess a lipopolysaccharide membrane which is restrictive to the diffusion of hydrophobic compounds. In addition, the direct contact between the hydrophobic components of the essential oils and the phospholipid bilayer of the cell membrane can occurs in Gram-positive bacteria. As a consequence, the components exert their effects such as increase in the permeability to ions, leakage of vital intracellular components or compromise bacterial enzymes [42,82].

The *Eucalyptus* EOs also caused inhibition on the growth of some fungal species, as in the case of *C. albicans*. Vratnica and co-workers [112] reported that *E. globulus* EOs were two times more effective (izd = 14-46 mm) than nystatin, a drug used to treat fungal infections of the skin, mouth, vagina, and intestinal tract. The authors attributed this effect to the high content of 1,8-cineole in *E. globulus* EOs (85.8%). This information should be taken with care since in another study the correlation between 1,8-cineole content and antifungal activity was not confirmed [68]. Gilles and coworkers [17] reported the effect of *E. staigeriana* (izd = 26.7 mm), *E. dives* (izd = 15.4 mm) and *E. olida* (izd = 12.6 mm) on *C. albicans*, and the superior antifungal activity of *E. staigeriana* was attributed to the presence of 1,8-cineole (34.8%). Low activity on *C. albicans* was observed for EOs extracted from *E. robusta* and *E. saligna*. The monoterpene 1,8-cineole was not detected among the constituents of the EOs of these two species [130]. It should be noted that in none of the studies above bioessays was conducted with 1,8-cineol pure for comparison with EO, which would be important to check synergistic or antagonistic effect on EOs.

Tyagi and Malik [42] investigated the effect of essential oils from *E. globulus* on several fungal species and reported MIC values ranging from 2.25 to 9 mg/mL. The superior limit value was observed for *P. digitatum* and *A.niger*. For *A. flavus*, *R. nigricans* and *F. oxysporum* the value of MIC was 4.5 mg/mL, while for *Mucor* spp. and *C. albicans* MIC of 2.25 mg/mL was reported.

In a recent study, it has been found that essential oils from *E. erythrocorys* significantly reduced the growth of fungal species *B. sorikiniana* (79.6%) and *B. cinerea* (78.5%) [164].

The evaluation antifungal activities of *E. citriodora* EOs, in concentration of 10 mg disc¹, revealed that these oils completely inhibit the growth of *C. cladosporioide*, *M. verrucaria* and *T. viride*. On the contrary, the growth of *A. clavatus*, *A. niger* and *P. citrinum* were partially inhibited (90.7%, 54.6% and 86.0%, respectively). The antifungal activity was ascribed to the main components of *E. citriodora* oils namely citronellal (49.5%) and citronellol (11.9%) [68].

In addition to antifungal, nematicide activity has been described for *Eucalyptus* EOs [176]. *E. citriodora* and *E. staigeriana* EOs presented nematicidade activity against *H. contortus*, a common *parasite* and one of the most pathogenic nematodes to ruminants [93,94,170,171].

Lipid peroxidation and microbial contamination are two important factors related to deterioration of food, an important problem for the food industry [39]. The addition of antioxidants is a well known strategy used to retard or even stop oxidation processes in food. Because of carcinogenicity associated with some synthetic antioxidants, their employment is limited. Within this context, it has been observed an increase interest in the use of natural additives to control food oxidation. The use of essential oils has been considered an alternative to overcome food deterioration problems faced by food industry [157,177]. Natural products presenting antioxidant activity has also been taken into consideration since compounds with antioxidant activity can also be utilized as antimicrobial ones [37,178].

Infections caused by fungi and bacteria represent an important issue considering the continuous increasing of resistance of these species to well known fungicides and antibiotics [179]. Considering the relevant information available in the literature concerning the antimicrobial activity of *Eucalyptus* EOs and other essential oils, the employment of EOs can also be considered a viable alternative to overcome the resistance problem.

Synthetic fungicides are typically employed to prevent the contamination of food commodities from fungal deterioration as well as mycotoxinc contaminations. However, the use of such substances is not free of collateral effects. Most of the synthetic fungicides cause residual toxicity and disturb

food chain, contributing to the development of fungal resistance. This is particularly true when the fungi are exposed to fungicide sub-lethal concentrations. The use of EOs has been considered as an alternative to overcome the problems described associated with synthetic fungicides and protection of food commodities [159,167].

3.2. Acaricide activity

Acaricides can be defined as any substance or mixture of substances intended to prevent, destroy, repel, or mitigate ticks and mites. A number of investigations have demonstrated acaricide effects of essential oils from species of *Eucalyptus* (Table 3).

Eucalyptus spp.	Target species	Reference
E. approximans	Tetranychus urticae	[180]
E. bicostata	T. urticae	[180]
E. camaldulensis	Varroa destructor	[69]
E. citriodora	Boophilus microplus, T. urticae, Neoseiulus californicus,	[95-98]
	Dermanyssus gallinae	
E. globulus	B. microplus	[95]
E. maidenii	T. urticae	[180]
E. sideroxylon	T. urticae	[180]
E. staigeriana	B. microplus, D. gallinae	[95,98]
E. tereticornis	Amblyoma variegatum	[133]

Table 3. Acaricide activities for *Eucalyptus* spp. essential oils.

The effects of essential oils from *E. citriodora*, *E. globulus* and *E. staigeriana* on *B. microplus*, a tick species, were evaluated. The oils from *E. citriodora* caused 100% mortality in an average concentration of 17.5% as compared to *E. globulus* with 15% and *E. staigeriana* with 12.5% [95].

The essential oils from *E. citriodora* are also caused toxic to mite species *T. urticae* and *N. californicus*. A mortality bioassay was used to determine the LD₅₀ of EOs (LD stands for lethal dose; LD₅₀ denotes the dose likely to cause death in 50% of mites). The determined LD₅₀ values were 19.3 μ g/cm³ for *T. urticae* and 21.4 μ g/cm³ for *N. californicus* [96].

Acaricide effects were observed for EOs of *E. approximans*, *E. bicostata*, *E. maidenii* and *E. sideroxylon* on *T. urticae* females. At the concentrations of 0.5% and 1.0%, the observed mortalities can be described as follows: *E. approximans* (67% at 0.5%; 83.1% at 1.0%), *E. bicostata* (67.8% at 0.5% and 82.5% at 1.0%), *E. maidenii* (82.2% at 0.5% and 100.0% at 1.0%), *E. sideroxylon* (78.8% at 0.5% and 79.4% at 1.0%) [180].

The contact toxicity assay was used to evaluate the effects of *E. citriodora* EOs on the mite species *D. gallinae*. Using a dose of 0.21 mg/cm² and after 24h of exposure, it was observed 85% mortality [98]. The effect of *E. citriodora* of EOs, was tested on larvae of mite species *A. cajennense and A. nitens*. In the biological evaluation, the concentrations ranged from 6.25% to 50%. For *A. cajennense*, the acaricide effect varied from 10.8% to 53.1% mortality while *A. nitens* was a more sensitive species (20.1% to 100% mortality) [181].

The acaricide activity of essential oils from *E. camaldulensis* on *V. destructor* mite was also investigated and a LD50 of 1.74 μ L/L of air was found [69].

From the literature surveyed, it was clear that the acaricide effects of essential oils from eucalyptus in some cases are high and could lead to the development of a environmental friendly commercial product to control such parasites. However, the works reported are limited to nine species of eucalyptus, concentrated in five countries. So, considering the large disponibility and diversity in chemical composition of oils from eucalyptus, we believe that oils endowed with more potent and specific acaricidal activities are still to be discovered and converted into commercial products.

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3.3. Insecticide activity

There are more than 1,000,000 species of insects and about 10,000 of them are crop-eating, and of these, approximately 700 species worldwide cause most of the insect damage to man's crop. Moreover, several diseases that affect man are transmitted by insects [182]. Therefore, the control of insects is highly desirable and necessary to improve the quality of life and health for humans. Compounds obtained from natural sources have been investigated for their insecticidal activities [183-185]. Many of such compounds have been used as model for the development of active ingredients to control insects [186-199]. In this regard, essential oils have attracted the attention of researchers as an alternative to insect control [200-206]. As can be seen in Table 4 the essential oils from many *Eucalyptus* species present effects on a variety of insect species.

Table 4. Insecticide activities for Eucalyptus spp. essential oils.

Eucalyptus spp	Target species	Reference
E. astringens	Rhyzopertha dominica, Callosobruchus maculatus,	[73,207]
	Tribolium castaneum, Ephestia cautela, Ephestia	
	kuehniella	
E. badjensis	Haematobia irritans L., Aedes aegypti L.	[208,209]
E. badjensis x E. nitens	H. irritans L., A. aegypti L.	[208,209]
E. benthamii	Sitophilus zeamais	[122]
E. botryoides	H. irritans L., A. aegypti L.	[208,209]
E. camaldulensis	Thyrinteina arnobia, Atta sexdens rubropilosa, A.	[70-78]
	aegypti L., Aedes albopictus, E. cautela, E. kuehniella,	
	Ectomyelois ceratoniae, Pediculus humanus capitis, S.	
	zeamais	
E. cinerea	Musca domestica, A. aegypti L., P. humanus capitis	[75,76,84-86]
E. citriodora	T. arnobia, A. sexdens rubropilosa, Anopheles gambia,	[70,71,78,86,99-106]
	Lutzomyia longipalpis, C. maculatus, A. aegypti L.,	
	Nasutitermes corniger, S. zeamais, T. castaneum, P.	
	humanus capitis	
E. cloeziana	T. arnobia, A. sexdens rubropilosa	[70,71]
E. darlympleana	H. irritans L., A. aegypti L.	[208,209]
E. dorrigoensis	H. irritans L., A. aegypti L.	[208,209]
E. dundasii	R. dominica, Oryzaephilus surinamemsis	[210]
E. dunnii	Blattella germânica L., P. humanus capitis, A. aegypti L.,	[75,76,122,125,128]
	S. zeamais	
E. elata	H. irritans L	[208]
E. fastigata	H. irritans L., A. aegypti L.	[208,209]
E. fraxinoides	H.irritans L.	[208]
E. floribundi	R. dominica, O. surinamensis	[211]
E. globulus	Tribolium confusum, S. zeamais, M. domestica,	[75,76,78,100,119-127]
	Sitophilus oryzae L., A. aegypti L., L. longipalpis,	
	Odontotermes assamensis, T. castaneum, P. humanus	
	capitis	
E. grandis	B. germânica L., A. aegypti L., P. humanus capitis, T.	[70,71,77,128,129]
	arnobia, A. sexdens rubropilosa	

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E. grandis x	A. aegypti L., P. humanus capitis, B. germânica L.	[75-77,128]
E. camaldulensis		
E. grandis x	A. aegypti L., P. humanus capitis, B. germânica L.	[75-77,128]
E. tereticornis		
E. gunnii	A. aegypti L., P. humanus capitis	[75,76,125]
E. lehmannii	R. dominica, C. maculatus, T. castaneum, E. cautela, E.	[73,207]
	kuehniella	
E. leucoxylon	E. cautela, E. kuehniella, E. ceratoniae	[73,74]
E. maculata	T. arnobia, A. sexdens rubropilosa	[70,71]
E. nobilis	H. irritans L., A. aegypti L.	[208,209]
E. oblicua	H. irritans L.	[208]
E. polybractea	H. irritans L., A. aegypti L.	[208,209]
E. radiata	H. irritans L., A. aegypti L.	[208,209]
E. resinífera	H. irritans L., A. aegypti L	[208,209]
E. robertsonii	H. irritans L., A. aegypti L	[208,209]
E. rubida	H. irritans L., A. aegypti L	[208,209]
E. rudis	E. cautela, E. kuehniella, E. ceratoniae	[73]
E. saligna	A. aegypti L., T. arnobia, A. sexdens rubropilosa, S.	[70,71,75,76,86,122,131,132]
	zeamais, P. humanus capitis, T. castaneum,	
	Acanthoscelides obtectus, Sitotroga cerealella	
E. sideroxylon	B. germânica L., A. aegypti L., P. humanus capitis	[75,76,125,128]
E. smithii	H. irritans L., A. aegypti L	[208,209]
E. staigeriana	C. maculatus, L. longipalpis	[100,101]
E. tereticornis	A. gambia, A. aegypti L., P. humanus capitis	[75-77,86,99]
E. urophylla	T. arnobia, A. sexdens rubropilosa	[70,71]
E. viminalis	B. germânica L., A. aegypti L., S. zeamais, P. humanus	[75,76,86,122,128]
	capitis	

The insecticidal activity of essential oils from *E. globulus* was evaluated against the larvae and pupae stages of house fly *M. domestica* (Diptera: Muscidae). The effects of the oils were assessed via fumigation and contact bioassays. Considering larvae stage, in the contact assay the observed lethal concentration (LC50) ranged from 2.73 to $0.60~\mu$ L/cm² for different days of observation, while the lethal time (LT50) varied from 6.0 to 1.7 days. The observed LC50 values in the fumigation test were 66.1 and 50.1 μ L/L after 24 and 48 h, respectively. Pupicidal activity was reported in terms of inhibition percentage rate (PIR) which was 36.0-93.0% for contact assay and 67.9-100% for fumigation test [120]. In another investigation, the EOs of *E.* cinerea were evaluated against adult stage of *M. domestica via* fumigation assays. It was found LC50 of 5.5 mg/dm³ and the mortality of the insects was observed in a period of time lower than 15 minutes [84]. In this work, the major component in the oil was 1,8-cineole (56.9%), a component of several other oils with insecticidal activity.

The effects of essential oils from E. gunnii, E. tereticornis, E. grandis, E. camaldulensis, E. dunnii, E. cinerea, E. saligna, E. sideroxylon, E. globulus ssp. globulus ssp. maidenii, E. viminalis and the hybrids E. grandis x E. tereticornis and E. grandis x E. tereticornis were tested on E. tereticornis tereticornis, tereticornis, tereticornis tereticorni

tereticornis, E. grandis, and E. camaldulensis) had a lower effect on A. aegypti (larval mortality < 50% after 24 h at 40 ppm) [75,76]. The vapor of the EOs of the aforementioned Eucalyptus species were also tested on A. aegypti adults. The toxicity was determined as the number of knockdown mosquitoes as a function of time. The fumigation toxicity was expressed as knockdown effect time (KT50) which varied from 4.2 to 12.0 minutes. The best result was observed for E. viminalis EOs. In this case, a direct correlation was found between the 1,8-cineole EOs contents and toxicity level [76].

The investigation carried out by Cheng and co-workers [72] demonstrated larvicidal activity of *E. camaldulensis* and *E. urophylla* oils against *A. aegypti* and *A. albopictus*. The oils from *E. camaldulensis* presented the best results with LC50 of 31.0 and 55.3 µg/mL, respectively (the corresponding LC90 were 71.8 and 192.4 µg/mL for *A. aegypti* and *A. albopictus*, respectively). The larvicidal activity of individual components of *E. camaldulensis* EOs was also assessed. It was observed that α -terpinene caused the highest larvicidal activity (LC50 of 14.7 µg/mL and LC90 of 39.3 µg/mL for *A. aegypti*; LC50 of 25.2 µg/mL and LC90 > 50.0 µg/mL for *A. albopictus*). The essential oils from *E. citriodora* was toxic to third and fourth instar of *A. aegypti* (LC50 71.2 ppm) [102].

L. longipalpis is the vector of *Leishmania chagasi*, a protozoa species which is responsible for 90% of visceral leishmaniasis in Brazil. The effects of EOs of *E. staigeriana*, *E. citriodora and E. globules* were evaluated on eggs, larva and adult phases of *L. longipalpis*. All oils were active on the evaluated phases being *E. staigeriana* the most effective one followed by *E. citriodora* and *E. globulus* [100]. Although the authors have not assessed individual oil components for their activities, it is worth pointing out that the oils had citronellal (71.8%) as major component, a compound known for its insecticidal activity.

The major pest of maize *S. zeamais* is known to attack both standing crop and the stored cereal. Investigations on the insecticidal and repellent effects of *E. dunnii*, *E. saligna*, *E. benthamii*, *E. globulus* and *E. viminalis* EOs on *S. zeamais* were carried out. By using the contact cytotoxicity assay on filter paper, oils from *E. globulus* and *E. viminalis* caused 100% mortality at concentrations of 0.16 and 0.23 μ L/cm² after 24 h of exposure, respectively. Considering this parameter, the concentration values for other oils were as follows: 0.42 μ L/cm² for *E. dunnii*, 0.65 μ L/cm² for *E. saligna* and 2.60 μ L/cm² *E. benthamii*. A regression analysis allowed the calculation of LC50 values: *E. viminalis* (0.08 μ L/cm²); *E. globulus* (0.10 μ L/cm²); *E. dunnii* 0.16 (μ L/cm²); *E. saligna* (0.25 μ L/cm²) and *E. benthamii* (0.79 μ L/cm²). The analysis of essential oil content and mortality activity resulted in a correlation between 1,8-cineole content and LC50. Thus, it is plausible that this compound can be responsible for the observed activity. Using the calculated LC50, it was possible to determine repellency activity for all *Eucalyptus* EOs [122].

The repellent activity of *E. saligna, E. camaldulensis, E. globulus* and *E. citriodora* EOs were also assayed against *S. zeamais*. Y-shape olphatometer bioassay was utilized and the concentration tested range from 0.002 to 2 μ L/ μ L. EOs were dissolved in hexane and at the highest concentration, *E. camaldulensis* and *E. citriodora* EOs presented the best repellent activity (74.35% and 69.15%, respectively), followed by *E. globulus* (53.68%) and *E. saligna* (40.5%). The repellent activity observed for *E. camaldulensis* EOs was higher than that observed for the positive control DEET (*N-N-diethyl m-toluamide*). Some individual constituents of the essential oils were assayed and the highest repellent activity was associated with 1,8-cineole content (70.87%) [78].

The fumigant toxicity of several EOs was evaluated on *S. oryzae* (also known as the rice weevil). The best activity was associated with *E. globulus* EOs (LD₅₀ of 28.9 μ L/L of air). Individual assessment of 1,8-cineole, the major component of *E. globulus* EOs, revealed a LD₅₀ of 23.5 μ L/L of air for the fumigant toxicity [121].

The essential oils from *E. globulus*, rich in 1,8-cineole, had their antitermite activity evaluated against *O. assamensis*. At the concentration of 2.5 mg/g, *E. globulus* EOs caused 80% mortality while 70% was observed for pure 1,8-cineole [123]. These results suggest that other compounds present in the oil might be enhancing the effect of 1,8-cineole.

P. humanus capitis (head louse) is an obligate ectoparasite responsible for the head lice infestation, also known as pediculosis capitis, nits or cooties. Several reports have described the effects of *Eucalyptus* EOs on *P. humanus capitis*. The fumigant toxicity assay was utilized to evaluate the effect essential oils from *E. sideroxylon*, *E. globulus ssp globulus*, *E. globulus ssp maidenii*, *E. dunnii*, and *E. gunnii* on head lice resistant to permetrin. Among the evaluated oils, the most efficient ones were *E.*

sideroxylon, E. globulus ssp globulus and E. globulus ssp maidenii presenting, respectively, KT50 of 24.75, 27.73, and 31.39 min [125]. A similar investigation conducted with oils from E. cinerea, E. viminalis and E. saligna revealed KT50 values of 12.0, 14.9, and 17.4 min [86]. A comparative investigation on the effect of EOs from hybrids (E. grandis x E. camaldulensis and E. grandis x E. tereticornis) and no-hybrids (E. grandis, E. camaldulensis, and E. tereticornis) eucalyptus species on P. humanus capitis was carried out. The fumigant activity of hybrids was higher than non-hybrid ones. The observed KT50 values for the hybrid were E. grandis x E. tereticornis (12.99 min) and E. grandis x E. camaldulensis (13.63 min). For the non-hybrid the values for KT50 parameter were E. grandis (25.57 min.), E. camaldulensis (35.01 min.) and *E. tereticornis* (31.31 min) [77].

E. citriodora leaves has been traditionally used as insecticide repellent, especially by low income families to protect them against mosquitoes [212].

The red flour beetle *T. castaneum* is a worldwide pest of stored products, particularly <u>food grains</u>. The essential oils of E. citriodora, rich in citronellal, citronellol and isopulegol, present repellent activity against this beetle species (0.084 mL/L dose repellent media after 4 h of exposure). The observed activity was higher than the commercial product [ethyl 3-(*N*-acetyl-*N*butylamino)propionate] used as positive control [105].

The evaluation of fumigant activity of essential oils from E. camaldulensis, E. astringens, E. leucoxylon, E. lehmannii and E. rudis against the pests of stored products E. kuehniella, E. cautella and E. ceratoniae showed that E. camaldulensis oils present high toxicity on E. cautella and E. kuehniella (LC50 = 11.07 and 26.73 μ L/L of air, respectively). Considering *E. ceratoniae*, the most effective EOs were extracted from E. rudis (LC₅₀ = 31.4 μL/L of air) [73]. In another study, the effects of E. camaldulensis and E. leucoxylon EOs on larvae and adult stages of E. ceratoniae were investigated. The oils presented bioactivity on both stages of the insect development. For adult stage, 100% mortality was achieved for both oils after 120 h of exposure at 26.31 μ L/L of air; at higher concentration (131.58 μ L/L of air) the exposure time was reduced to 48 h. The LC50 after 24 h of exposure corresponded to 12.07 μ L/L of air and 21.75 μL/L of air for *E. camaldulensis* and *E. leucoxylon*, respectively. Considering the larvae stage, 100% mortality was observed at 131.58 µL/L of air after 264 h of exposure [74].

The essential oils from *E. tereticornis*, at the concentration of 160 ppm, caused 100% mortality on the larves of Anopheles stephensi [213].

The observed insecticide activity of *E. tereticornis* EOs on *A. gambiae* was associated to *p*-cymene and 1,8-cineole as demonstrated by the biological assays conducted with these individual components [99].

3.4. Herbicide activity

Weeds compete with crops for water, nutrients and light and their control is of fundamental importance in modern agriculture. It is estimated that approximately 10% of all plant species are weeds, corresponding to approximately 30,000 species. Among them, 1,800 cause serious economic losses in crop production [214].

The observation of plant growth regulation effects caused by EOs has attracted the attention of researchers for the possibility of utilizing these natural sources for weed control [215,216]. Such investigations are important in view of the evolution of weed resistance to traditional herbicides. There is a constant need for the development of weed control agents that are environmentally benign, that present low toxicity to mammals, low recalcitry, and that can be applied at low rates [217-219]. In this regard, nature has been considered an important source of compounds that can be explored to afford herbicides that can meet the aforementioned criteria [218,220,221].

As shown in Table 5, several studies have been conducted on the phytotoxic effects of *Eucalyptus* EOs on weeds [31,215,222,223]. It has been demonstrated that these oils inhibit and/or retard the germination of seeds. Effects on crop species have also been described [18].

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Table 5. Herbicides activities for *Eucalyptus* spp. essential oils.

Eucalyptus spp.	Target species	Reference
E. brockwayii	Solanum elaeagnifolium Cav.	[224,225]
E. camaldulensis	Amaranthus hybridus, Portulaca oleracea	[79]
E. citriodora	Cassia occidentalis, Echinochloa crus-galli, Sorghum bicolor	[26,54,107]
	L., Cucumis sativus L., A. viridis L., Triticum aestivum L.,	
	Oryza sativa L.	
E. dundasii	S. elaeagnifolium Cav.	[224,225]
E. erythrocorys	Sinapis arvensis L., Phalaris canariensis L.	[164]
E. melliodora	S. elaeagnifolium Cav.	[225]
E. salubris	S. elaeagnifolium Cav.	[224,225]
E. spathulata	S. elaeagnifolium Cav.	[224,225]
E. urophylla	Lactuca sativa L.	[226]

The phytotoxic effect of *E. citriodora* EOs collected from differents stages of leaves (juvenile and adult leaves) and fallen (senescent leaves and brown leaf litter) has been investigated on two weed species (*E. crus-galli* and *A. viridis*) and two crops (*T. aestivum* and *O. sativa*). As a general trend, the adult leaf oils presented superior phytotoxicity compared to leaf litter oils. In a subsequent investigation, Batish and co-workers [26] examined the phytotoxic effects of EOs extracted from decaying leaves of *E. citriodora* against weed species *C. occidentalis* and *E. crus-galli*. Also, the phytotoxicities of EOs major components, i.e., citronellal and citronellol, were also assessed. The EOs exhibited superior effect on the germination of *C. occidentalis* ($I_{50} = 1.49 \text{ mg/mL}$) compared to *E. crus-galli* ($I_{50} = 1.49 \text{ mg/mL}$). The EOs presented similar effects on root elongation ($I_{50} = 0.31 \text{ mg/mL}$ for *C. occidentalis* and 0.35 mg/mL for *E. grus-galli*). Comparing the effects on germination of the major components, citronellal was more effective in inhibiting the germination ($I_{50} = 0.55 \text{ mg/mL}$ and 0.14 mg/mL for *C. occidentalis* and *E. grus-galli*, respectively). On the contrary, citronellol presented more pronounced effects on root elongation ($I_{50} = 0.13 \text{ mg/mL}$ and 0.09 mg/mL for *C. occidentalis* and *E. grus-galli*, respectively).

Silverleaf nightshade ($S.\ elaeagnifolium$) is a perennial and agressive weed species common in Australia. The phytotoxicity of five selected Eucalyptus EOs from Australia, namely $E.\ brockwayii$, $E.\ dundasii$, $E.\ melliodora$, $E.\ salubris$ and $E.\ spathulata$, was evaluated on germination and root elongation of $S.\ elaeagnifolium$. The EOs from $E.\ salubris$ caused the highest inhibitory effect on germination presenting 73% of inhibition index. This effect was superior to that presented by a commercial eucalyptus oil (38% of inhibition index) purchased from the market and used as positive control in the assays. In terms of root growth inhibition, $E.\ salubris$ was again the most effective oil (reduction of 84% of root elongation when applied at 10 μ L/dish). At the same dose, commercial eucalyptus oil caused only 41% decrease in root length [224]. The phytotoxic effects of aqueous volatile fractions of the aforementioned EOs, i.e, the water soluble volatile fractions obtained along with the essential oils EOs (water insoluble fractions) during the steam distillation process were also assessed. It was also observed strong phytotoxic effects on germination, shoot length and root elongation of $S.\ elaeagnifolium$ [225].

Shingh and co-workers [31] investigated the herbicidal effect of essential oils produced by E. citriodora against the weed species Parthenium hysterophorus. Several parameters were evaluated and germination was completely inhibited at oil concentration of 5.0 nL/mL. Plants of P. hysterophorus (4-week-old) were sprayed with different concentrations of essential oils (0-100 μ L/mL). A week after spraying, it was noticed damage and chlorophyll content and respiratory activity decreased as the oil concentration increased. When sprayed with concentrations up to 50 μ L/mL, plants showed recovery over time. However, when the weed species were sprayed with 75 μ L/mL and 100 μ L/mL, plants died after two weeks. Moreover, plants sprayed with 50 μ L/mL and concentrations higher than that were

dessicated and wilted. *E. citriodora* EOs caused rapid electrolyte leakage at concentrations of 5-75 μ L/mL indicating an effect on membrane integrity.

Phytotoxic effects of *E. citriodora* EOs on the crops *S. bicolor* L. (sorghum) and *C. sativus* L. (cucumber) have been reported. From the biological essays, an allelopathic effect was observed mainly causing germination and radicule growth inhibition of *S. bicolor and C.* sativus seeds. It was also observed that the increase of oil concentration (0 to 5000 ppm) leads to a linear decrease in the germination as well as in the radicule length of *S. bicolor* [107].

4. Commercial relevance of Eucalyptus EOs

The world production and trade of essential oils from several eucalyptus species is dominated by China which is the biggest producer of EOs rich in cineole [227]. Other important producers include South Africa, Portugal, Spain, Brazil, Australia, Chile and Swaziland [44]. There are important aspects to be considered with respect to cultivation of *Eucalyptus* spp. aimed for production of EOs such as environmental, genetic variation and leaf type.

In the international market, the oils are categorized into medicinal, perfumery and industrial according to their composition and main end-use. Among them, the most important in terms of volume of production and trade is the medicinal type.

Similar to other essential oils, all species of *Eucalyptus* are rich in monoterpenes. For medicinal purposes, the value of *Eucalyptus* EOs rely on 1,8-cineole content (minimal 70% in mass). The amount of 1,8-cineole is directly related to the price of oils intended to medicinal applications. It should be mentioned that medicinal oils are designed in terms of 1,8-cineole content. '*Eucalyptus* oil China 80%', '*Eucalyptus* oil 70/75% Spain/Portugal' and '*Eucalyptus* oil 80/85% Spain/Portugal' are typical descriptions. The highest price is associated with an essential oil know as 'eucalyptol' which contains about 98% of 1,8-cineole [44,228]. In Table 6 are presented the main *Eucalyptus* species that have been used for the extraction of medicinal oils. 1,8-cineole is the main component in the oils and the % of this component is described [229,230].

Eucalyptus species	1,8-cineole (%)
E. camaldulensis	80-90
E. cneorifolia	40-90
E. dumosa	33-70
E. elaeophora	60-80
E. globulus	60-85
E. lecoxylon	65-75
E. oleosa	45-52
E. polybractea	60-93
E. radiate subspecie radiate var. cineole	65-75
E. sideroxylon	60-75
E. smithii	70-80

Table 6. Eucalyptus species typically used to produce medicinal oils.

In several cases according to the source, after extraction certain crude oils has to be rectified to increase the percentage of 1,8-cineole required for medicinal purposes.

The oils intended to perfumery are rich in citronellal, citronellol and geranyl acetate. One important source of perfumery *Eucalyptus* EOs is *E. citriodora* in which the major component is citronellal and its content should be in the range 65%-85%. This oil is used in whole form for fragrance purposes, usually in the lower-cost soaps, perfumes and disinfectants, but its main use is as a source of citronellal for the chemical industry [44,228,231].

The term industrial oil is commonly used to describe the employment of the oil as raw material for isolation of chemical constituents. The industrial oils are characterized for the high level of phellandrene and piperitone, mainly obtained from *E. dives* species [228].

As described by Coppen [232] "any attempt to accurately quantify and analyse production and consumption trends for Eucalyptus oil is fraught with difficulties. Unlike some other commodities, or some other essential oils such as the citrus oils, quantitative information is not always available or accessible".

5. Concluding remarks

Nature is a formidable source of a myriad of compounds that are useful for several purposes. The demand for natural extracts from manufactures of foods, cosmetics, perfumes, pharmaceuticals and agrochemicals is continuously increasing. Due to such, demand research on essential oils is of fundamental importance. In this review, it was demonstrated the tremendous chemical variability that exists among EOs from several species of *Eucalyptus*. In addition, it was described the usefulness of those EOs in terms of their antimicrobial, insecticidal, acaricide and phytotoxic activity. In some cases, the observed biological activity of the EOs is superior to products available in the market. These facts demonstrate the importance of conducting studies on Eucalyptus EOs. Considering that and also taking into consideration that there are several species of Eucalyptus unexplored in terms of EOs content and composition, it is projected that investigations in this field will continue active in the future. Certainly, new activities will be reported for *Eucalyptus* EOs. In this regard, more efforts should be devoted not only in terms of the chemical composition but also in the possibility of linking chemical contents with particular functional properties.

Supplementary Materials: Table S1. Major chemical components for Eucalyptus spp. essential oils.

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