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Review article

Plant products and secondary metabolites with acaricide activity against ticks

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ABSTRACT

The present review documents the results of studies evaluating the acaricidal activity of different plant products and secondary metabolites against ticks that are resistant and susceptible to conventional acaricides. Studies published from 1998 to 2016 were included. The acaricidal activity of plant extracts, essential oils and secondary compounds from plants have been evaluated using bioassays with ticks in the larval and adult stages. There is variable effectiveness according to the species of plant and the concentrations used, with observed mortalities ranging from 5 to 100% against the *Rhipicephalus* (*Boophilus*), *Amblyomma, Dermacentor, Hyalomma,* and *Argas* genera. A number of plants have been reported to cause high mortalities and/or affect the reproductive capacity of ticks in the adult phase. In the majority of these trials, the main species of plants evaluated correspond to the families Lamiaceae, Fabaceae, Asteraceae, Piperaceae, Verbenaceae, and Poaceae. Different secondary metabolites such as thymol, carvacrol, 1,8-cineol and *n*-hexanal, have been found to be primarily responsible for the acaricidal activity of different essential oils against different species of ticks, while nicotine, dibenzyldisulfide and dibenzyltrisulfide have been evaluated for plant extracts. Only thymol, carvacrol and 1,8-cineol have been evaluated for acaricidal activity under *in vivo* conditions. The information in the present review allows the conclusion that the secondary metabolites contained in plant products could be used as an alternative for the control of ticks that are susceptible or resistant to commercial acaricides.

1. Introduction

Medicinal plants have proven to be an alternative method for the control of insects and mites by the secondary metabolites produced as a defense mechanism against conditions of biotic and abiotic stress (Gil and Carmona, 2006; Arceo-Medina et al., 2016). Increasing numbers of studies conducted since the end of the nineties have shown that the secondary metabolites of different plants can present different mechanisms of action against arthropods. Such secondary metabolites can inhibit feeding or the synthesis of chitin, can decrease growth, development, or reproduction, or could affect behavior without adverse effects on non-target species (Pamo et al., 2004; Invesco, 2005; Sardá et al., 2007, 2008; Silva et al., 2011). Additionally, some extracts of plants or specific phytochemicals have high effectiveness against arthropods that are resistant to insecticides and acaricides because they have different mechanisms of action (Soon et al., 2004).

Several techniques have been used to assess the acaricidal activity of plant products against ticks, including (a) the adult immersion test (AIT), (b) the larval immersion test (LIT) and (c) the larval packet test (LPT) (FAO, 2004), whereas for the identification of bioactive secondary metabolites, the most commonly used technique is gas chromatography coupled with mass spectrometry (GC/MS) (Prates et al., 1998; Sardá-Ribeiro et al., 2008; Apel et al., 2009; Ferraz et al., 2010; Martinez-Velázquez et al., 2011b; Garcia et al., 2012; De Oliveira-Cruz et al., 2013; Koc et al., 2013; Gomes et al., 2014).

Many species of plants have been evaluated for acaricidal activity, with the species studied mainly being members of the families Lamiaceae, Fabaceae, Asteraceae, Piperaceae, Verbenaceae and Poaceae (Ravindran et al., 2011; De Souza et al., 2012; Koc et al., 2013; Polished and Cruz, 2013; Godara et al., 2015; Muyobela et al., 2016; Dantas et al., 2016). Some studies have identified secondary metabolites (terpenes, stilbenes, coumarins, acids, alcohols, sulfurated compounds and aldehydes) of essential oils and plant extracts, associated with acaricidal effects against the genera *Rhipicephalus*, *Am-*

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blyomma, *Dermacentor*, *Hyalomma*, *Argas* and *Ixodes* (Pamo et al., 2005; Sardá-Ribeiro et al., 2008; Cetin et al., 2010). The large number of studies with different plant species, plant materials and species of ticks deserves to be systematized to be able to provide a clear view of what has been done so far in the evaluation of plant products against ticks, and what needs to be done in the future. Thus, the present review documents the results of studies evaluating the acaricidal activity of different plant products and secondary metabolites against ticks that are resistant and susceptible to conventional acaricides.

2. Techniques for evaluating acaricidal activity

2.1. Techniques for evaluating acaricidal activity against larval ticks

The acaricidal activity of plant products or their secondary metabolites are mainly assessed through two techniques that use larvae of the different tick genera: LPT and the LIT (FAO, 2004). These tests have been modified by several authors to improve their use and allow greater repeatability (Guerrero et al., 2014). It is recommended to use larvae that are 7–14 days in age in both tests. The LPT involves the exposure of larval ticks to filter paper that is 8.5 cm long by 7.5 cm wide and impregnated with a chemical compound or plant extract. The filter paper rectangles are folded in half to form an envelope, and the spaces on each side are closed with the help of a metal paper clip, leaving only the upper edge open to allow the packet to be filled with tick larvae. After the chemical compound or extract concentrations are prepared, the control group packet is impregnated first, followed by the treatment group. In trials with more levels of dilution or concentrations (for dose-response analyses), the lowest concentrations must be added first, followed by the higher concentrations. The impregnated packets are filled with approximately 100 larvae. Subsequently, the packets are placed on a clean tray and placed into an incubation chamber (27° ± 2 °C with a relative humidity (RH) of 85% to 90%) for 24 h. At the end of the incubation period, the number of dead larvae in the control packets must be counted, followed by those in the treatment group (Guerrero et al., 2014; Torres-Acosta et al., 2015).

In the larval immersion technique known as the sandwich or Shaw Test, the plant extract diluted with the solvent of choice is transferred to a Petri dish, where 300–500 larvae are placed between two Whatman No. 1 filter papers and are exposed to the extract for 10 min. Approximately 100 larvae are then transferred to envelopes of filter paper closed with metal paper clips using a No. 4 brush. The packages are placed in an incubator (27 ± 1.5 °C and 70–80% RH), and mortality is quantified after 48–72 h of incubation. Appropriate training is essential to achieve a high degree of confidence in this technique (Rosado-Aguilar et al., 2010a; Torres-Acosta et al., 2015).

There is a modification of the LIT in which the larvae are submerged in Eppendorf vials (1.5 ml) with defined plant extract dilutions, and after 10 min the larvae are removed and placed in filter paper envelopes, which are then incubated at 27 ± 1.5 °C and 70–80% RH. The results are recorded 24 h after the treatment. This test was designed to obtain percentages of mortality at different dilutions and to determine the degree of susceptibility or resistance according to the analyses of the results based on dose response (example: probit analysis) and to obtain the lethal concentrations (LC_{50} and LC_{99}) of the extracts evaluated with respect to the control and their respective 95% confidence intervals (CI95%) (Perez-Cogollo et al., 2010; Torres-Acosta et al., 2015).

In another LIT described and modified by Politi et al. (2012), 0.01 g of tick eggs are placed in Textile Non-Textile cloth bags (6.0 cm × 6.0 cm) and are incubated (27 + 1.5 °C and 70–80% RH) for three weeks. After the hatching period, approximately 200 viable larvae are transferred to new bags. Subsequently, the contents of the bags are cooled to −8 °C for one minute and are immersed for 5 min in 20 ml of the respective dilutions of the plant extract in Petri dishes. They are then incubated under the above conditions, and the mortality is quantified after 48 h.

2.2. Techniques for evaluating acaricidal activity against adult ticks

The most frequently used test to evaluate acaricidal activity against adult ticks is the adult immersion test (AIT), described by Drummond et al. (1967) and modified by FAO (1999). This bioassay is applied to engorged female ticks with a weight of approximately 200 mg. The size and weight of the tick has no influence on their ability to oviposit. The treated group of engorged female ticks is immersed for one minute in the diluted extract, and the control group is immersed in the solvent for one minute (Rosado-Aguilar et al., 2010a). The ticks are placed individually into plates of 24-well and are incubated for a period of 15 days (27 + 1.5 °C and 70–80% RH). In this bioassay, the mortality rate, oviposition rate and inhibition of larval hatching are measured. The first response variable indicates the effectiveness of the extract on treated engorged females. The oviposition rate highlights the effect of the extract on egg production in treated engorged females. Finally, the inhibition of hatching is evidence of the effect of the extract on the eggs of engorged females (Rosado-Aguilar et al., 2010a).

3. Families of plants evaluated against different species of ticks

The acaricidal activity of several species of plants has been evaluated against different kinds of ticks, and in some cases, the secondary metabolites responsible for the acaricidal activity occurring in different species of plants or even in different parts of some species of plant (leaves, stems, rind, roots, etc.) have been identified. Species of the families Lamiaceae, Fabaceae, Asteraceae, Piperaceae, Verbenaceae and Poaceae have been the most studied to determine the effectiveness of their essential oils, extracts or their respective secondary metabolites on different species of ticks (Tables 1 and 2). Plant products that have been evaluated to a lesser extent are those produced by members of the families Caesalpiniaceae, Ericaceae, Winteraceae, Solanaceae, Phytolaccaceae, Apiaceae, Myrtaceae, Meliaceae, Rutaceae, Amaryllidaceae, Euphorbiaceae and Bromeliaceae (Ravindran et al., 2011; De Souza et al., 2012; Koc et al., 2013; Pulido and Cruz, 2013; Godara et al., 2015; Muyobela et al., 2016; Dantas et al., 2016).

4. Evaluation of the acaricidal activity of essential oils against different kinds of ticks

The acaricidal activity of essential oils from different plant species has been tested on different genera of ticks, with the most evaluated being the genus *Rhipicephalus*. Here, studies evaluating essential oils are described, and Table 1 presents a summary of the data obtained from these studies along with the techniques used to evaluate the acaricidal activity and identify secondary metabolites active against ticks. Few studies have evaluated the active compounds of essential oils responsible for the acaricidal activity (Prates et al., 1998; Daemon et al., 2009; De Oliveira-Cruz et al., 2013; Koc et al., 2013).

4.1. Rhipicephalus genus

Lamiaceae is the family of plants that has been the most studied in terms of the activity of essential oils against members of the *Rhipicephalus* genus (included *Boophilus* subgenus (Murrell et al., 2000; Beati and Keirans, 2001)). Secondary metabolites produced by different species of this genus have been identified; however, only one study has evaluated the acaricidal activity of a principal compound on ticks (Koc et al., 2013). Below, studies on different species of plants belonging to family Lamiaceae are presented.

In Brazil, the chemical composition of the essential oils produced in the aerial parts of *Cunila angustifolia, C. incana, C. spicata, C. microcephala, C. incisa* and their toxicity against the *Rhipicephalus microplus* (formerly *Boophilus microplus)* tick have been evaluated. The principal

Table 1
Evaluation of the acaricidal activity of essential oils against different genera and stages of ticks and secondary metabolites present.

Family	Essential oils	Tick	ST	T	E (%)	SMIT	SM	Author
Lamiaceae	*Cunila angustifolia*	*Rhipicephalus microplus*	Larvae	LIT	100	GC and GC/MS	Sabinene	Apel et al. (2009)
	Cunila incana						α-pinene and β-pinene	
	Cunila spicata						Menthofuran	
	Cunila incisa				18.0		1,8-cineol	
	Cunila microcephala				5.0		Menthofuran	
	Ocimum basilicum			LPT	0.0	GC	Linalool and Estragole	Martinez-Velázquez et al. (2011a)
	Rosmarinus officinalis			LPT	> 85.0	GC/MS	α-pinene, Verbenone, and 1,8-cineol	Martinez-Velázquez et al. (2011b)
	Origanum bilgeri	*Rhipicephalus turanicus*	Adults	AIT	–	GC/MS	Carvacrol	Koc et al. (2013)
Piperaceae	*Piper amalago*	*Rhipicephalus microplus*	Larvae	LIT	–-	GC and GC/MS	Monoterpenes and Sesquiterpene hydrocarbons	Ferraz et al. (2010)
	Piper mikanianum						Phenylpropanoids	
	Piper xylosteoides						Phenylpropanoids	
Verbenaceae	*Lippia graveolens*	*Rhipicephalus microplus*	Larvae	LPT	100	GC/MS	Thymol, Carvacrol, p-cymene, and γ-terpinene	Martinez-Velázquez et al. (2011b)
	Lippia gracilis	*Rhipicephalus microplus*	Larvae and Adults	LIT AIT	> 90.0	GC/MS	Monoterpenes, Sesquiterpenes, Thymol, and Carvacrol	De Oliveira-Cruz et al. (2013)
	Lippia sidoides	*Rhipicephalus sanguineus*	Larvae Larvae	LPT LPT	99.0	GC/MS GC/MS	Thymol and Oxygenated monoterpene Hydrocarbonated sesquiterpene and Oxygenated sesquiterpene	Gomes et al. (2014)
		Amblyomma cajennense			100			
Poaceae	*Melinis minutiflora*	*Rhipicephalus microplus*	Larvae	LIT	100	GC/MS	Propionic acid, Butyric acid, Phenyl ethyl alcohol, Hexanal, 1,8-cineol, and 9-E-eicosane	Prates et al. (1998)
	Cymbopogon martinii	*Rhipicephalus microplus*	Adults	AIT	75.8	GC	Geraniol	De Souza et al., 2012
	Cymbopogon schoenanthus				39.2		Geraniol	
Meliaceae	*Azadirachta indica*	*Hyalomma anatolicum excavatum*	Eggs, Larvae and Adults	–	60 100	–	–	Abdel-Shafy and Zayed (2002)
	Carapa guianensis	*Rhipicephalus microplus*	Adults	AIT	55.4	GC	Oleic acid	De Souza et al. (2012)
Winteraceae	*Drimys brasiliensis*	*Rhipicephalus microplus* *Rhipicephalus sanguineus*	Larvae	LIT	100	GC/MS	Sesquiterpenoids, Cyclocolorenone, Bicyclogermacrene, and Alpha-gurjunene	Sardá-Ribeiro et al. (2008)
Apiaceae	*Cuminum cyminum*	*Rhipicephalus microplus*	Larvae	LPT	100	GC	Cuminaldehyde, γ-terpinene, 2-caren-10-al, o-cimene, and β-pinene	Martinez-Velázquez et al. (2011a)
Myrtaceae	*Pimienta dioica*	*Rhipicephalus microplus*	Larvae	LPT	100	GC	Methyl eugenol, Eugenol	Martinez-Velázquez et al. (2011a)
Amaryllidaceae	*Allium sativum*	*Rhipicephalus microplus*	Larvae	LPT	100	GC/MS	Diallyl trisulfide, Diallyl disulfide, and Methyl allyl trisulfide	Martinez-Velázquez et al. (2011b)
Asteraceae	*Tagetes minuta*	*Rhipicephalus microplus* *Rhipicephalus sanguineus* *Amblyomma cajennense* *Argas miniatus*	Larvae and adults	LPT and AIT	> 95.0	GC/MS SA	Limonene, β-ocimene, Dihydrotagetone, and Tagetone	Garcia et al. (2012)
Rutaceae	*Zanthoxylum caribaeum*	*Rhipicephalus microplus*	Adults and eggs	AIT	100	GC/MS	Monoterpenes oxygenated Sesquiterpenes oxygenated Aldehydes Alcohols	Nogueira et al. (2014)

ST: Stage of the tick. **T**: Technique. **E**: Efficacy. **SMIT**: Secondary metabolite identification technique. **SM**: Secondary metabolite. **LIT**: Larval immersion test. **AIT**: Adult immersion test. **LPT**: Larval packet test. **GC/MS**: Gas chromatography with mass spectrometry. **SA**: Spectroscopic analysis.

Table 2
Evaluation of the acaricidal activity of plant extracts against different genera and stages of ticks and secondary metabolites present.

Family	Plant extract	Tick	EG	T	E (%)	TIMS	MS	Author
Fabaceae	*Acacia pennatula*	*Rhipicephalus microplus*	Larvae	LIT	54.8	–	–	Fernández-Salas et al. (2011)
	Piscidia piscipula				88.1			
	Leucaena leucocephala				66.7			
	Lysiloma latisiliquum				56.0			
	Amburana cearensis	*Rhipicephalus microplus*	Adults	AIT	67.0	CLAR	Benzoic acid, Cinnamic acid, Flavonoids, Procatechuic acid, Epicatequin, p-coumaric acid, Gallic acid, and Kaempferol	Dantas et al. (2016)
	Bobgunnia madagascariensis	*Amblyomma variegatum*	Adults	FC	100	–	–	Muyobela et al. (2016)
Asteraceae	*Calea serrata*	*Rhipicephalus microplus* *Rhipicephalus sanguineus*	Larvae	LIT	100	–	–	Sardá-Ribeiro et al., 2008
	Tagetes patula	*Rhipicephalus sanguineus*		LIT	99.8	MS	Glycosylated flavonoids	Politi et al., 2012
	Calendula officinalis	*Rhipicephalus microplus*	Adults and larvae	AIT	60.0	–	–	Godara et al., 2015
				LPT	100			
Lamiaceae	*Leucas aspera*	*Rhipicephalus annulatus*	Adults	AIT	54.1	–	Nicotine	Ravindran et al., (2011)
	Leucas indica			AIT	66.6	HPTLC	Nicotine	Divya et al. (2014)
Solanaceae	*Nicotiana tabacum*	*Boophilus* ssp.	Larvae	Baths	70.0	–	–	Neira et al. (2009)
			Adults		64.9			
Phytolaccaceae	*Petiveria alliacea*	*Rhipicephalus microplus*	Larvae	LIT	LC_{50} 3.8	GC/MS	Dibenzyltrisulfide and Dibenzyldisulfide	Rosado-Aguilar et al. (2010b)
			Adults	AIT	LC_{99}16.5	–		
			Larvae	LIT	86.0 Synergism		Dibenzyltrisulfide and Dibenzyldisulfide	Arceo-Medina et al. (2016)
Rutaceae	*Ruta graveolens*	*Rhipicephalus microplus*	Adults	AIT	63.0	ST WT LR SR	Alkaloids, Coumarins, and Saponins	Pulido and Cruz (2013)
Verbenaceae	*Verbena officinalis*	*Rhipicephalus microplus*	Adults	AIT	66.6	ST WT LR SR	Flavonoids, Alkaloids, and Saponins	Pulido and Cruz (2013)
Euphorbiaceae	*Croton sphaerogynus*	*Rhipicephalus microplus*	Larvae	LPT	99.3	GC/MS	Abietans, Podocarpens and Clerodane-type diterpene furans	Righi et al. (2013)
Bromeliaceae	*Neoglaziovia variegata*	*Rhipicephalus microplus*	Adults	AIT	61.9	HRLC	Isoquercetin, p-coumaric acid, Vanillic acid, Caffeic acid, and Kaempferol-3-O- rhamnoside	Dantas et al. (2015)
Ericaceae	*Rhododendron tomentosum*				95.0		Myrcene and Palustrol	
Hypericaceae	*Hypericum polyanthemum*	*Rhipicephalus microplus*	Larvae and Adults	LIT AIT	52.7–100 –	–	–	Sardá et al. (2007)
Piperaceae	*Piper tuberculatum*	*Rhipicephalus microplus*	Adults	AIT	91.6	–	–	De Souza et al. (2012)

ST: Stage of the tick. **T**: Technique. **E**: Efficacy. **SMIT**: Secondary metabolite identification technique. **LIT**: Larval immersion test. **AIT**: Adult immersion test. **LPT**: Larval packet test. **FC**: Free contact. **GC/MS**: Gas chromatography with mass spectrometry. **ST**: Shinoda test. **WT**: Wagner Test. **LR**: Legal reaction for coumarin identification. **SR**: Saponin reaction. **HPTLC**: High-performance thin-layer chromatography. **HRLC**: High-resolution liquid chromatography.

secondary metabolites were identified as sabinene in *C. angustifolia*, α-pinene and β-pinene in *C. incana*, menthofuran in *C. spicata* and *C. microcephala* and 1–8 cineole in *C. incisa* using gas chromatography (GC) and mass spectrometry (MS). The evaluation of acaricidal activity was performed by LIT, and the essential oils were diluted in ethanol to obtain concentrations of 10, 5 and 2.5 µl/ml. *Cunila angustifolia, C. incana* and *C. spicata* showed 100% mortality of the larvae at concentrations of 10 and 5 µl/ml. *Cunila incisa* showed 18% mortality at 10 µl/ml, and *C. microcephala* showed the lowest efficacy on larvae, with 0–5% mortality at all concentrations tested (Apel et al., 2009).

Martinez-Velázquez et al. (2011a) evaluated the essential oil of the leaves of *Ocimum basilicum* using the LPT. The oil did not show acaricidal activity against *R. microplus* ticks; however, the analysis identified linalool and estragole as secondary metabolites of the plant. Martinez-Velázquez et al. (2011b) studied the acaricidal effects of the essential oil of the leaves of *Rosmarinus officinalis* using the LIT. The essential oil was dissolved in trichlorethylene and olive oil (2:1) to obtain serial concentrations of 20–1.25% and presented efficacies

of > 85% at concentrations of 10 and 20% against *R. microplus* larvae. The principal oil compounds were identified as α-pinene (31.0%), verbenone (15.2%) and 1,8-cineol (14.2%) using GC/MS. In addition, Koc et al. (2013) identified carvacrol (93.02%) as the principal compound of *Origanum bilgeri* and evaluated the acaricidal activity of the essential oil obtained from the aerial parts of the plant and its principal component on *R. turanicus* ticks using the AIT. The concentrations of 0.2, 0.4 and 0.8% were evaluated for the essential oil of *O. bilgeri*, and concentrations of 0.4 and 0.8% were evaluated for carvacrol, which were diluted with Tween 80 (1%). A mortality of > 83% was obtained for the 0.8% concentration of the *O. bilgeri* essential oil, and mortality > 63% was obtained for the 0.4% concentration of carvacrol. Several species of plants belonging to the family Piperaceae have been evaluated against *Rhipicephalus* ticks. The main compounds in the essential oils have also been identified; however, the components that are active against ticks have not been evaluated. Ferraz et al. (2010) evaluated the acaricidal activity of essential oils from the aerial parts of three species of *Piper* (*Piper amalago, P. mikanianum* and *P. xylosteoides*) on *R. microplus* ticks. They showed that *P. xylosteoides* and *P. mikanianum* contain phenylpropanoids (67.89% and 48.53%, respectively), while *P. amalago* principally contains monoterpene and sesquiterpene hydrocarbons (84.95%). The evaluation of the acaricidal activity was performed using the LIT by evaluating serial concentrations (10–0.65 μl/ml) dissolved in ethanol, in which *P. mikanianum* had an LC_{50} = 2.33 μl/ml and *P. xylosteoides* had an LC_{50} = 6.15 μl/ml. However, *P. amalago* showed no acaricidal activity. It is important to note that this is the first study to report the LC_{50} of these essential oils.

Thymol has been identified as the principal compound of the different essential oils tested in the Verbenaceae family. This compound has demonstrated up to 100% acaricidal activity against *R. microplus* ticks (Daemon et al., 2009). Carvacrol is another principal compound that has been found in some species of plants belonging to this family and has been evaluated, showing high acaricidal activity (De Oliveira-Cruz et al., 2013). Below, five studies conducted on plant species belonging to this family are presented.

A total of 16 compounds were identified in the essential oil extracted from the leaves of *Lippia sidoides*, representing 99.97% of the oil samples. Thymol was the most abundant compound (67.6% of the sample), whereas the other less abundant compounds were an aliphatic unsaturated alcohol, two hydrocarbonated monoterpenes, eight oxygenated monoterpenes, a hydrocarbonated sesquiterpene, an oxygenated sesquiterpene, and two monoglycerides. Oil samples from this plant were tested on *R. microplus* ticks. They were previously diluted with Tween 80 (2%) to obtain concentrations of 2.5, 5.0, 10.0, 15.0 and 20.0 μl/ml for the LPT and 10.0, 20.0, 40.0, 60.0 and 80.0 μl/ml for the AIT. The mortality of *R. microplus* larvae was greater than 95% starting at the concentrations of 10.0 and 20.0 μl/ml. In addition, significant reductions ($p < 0.05$) in the weight of egg masses and in the egg production index were observed for engorged females starting at 40.0 μl/ml. Egg viability was affected by all treatment concentrations, with egg hatching rates being significantly lower ($p < 0.05$) in the treatment groups relative to the control group in all cases (Gomes et al., 2012).

Gomes et al. (2014) previously reported that thymol was the most abundant compound (69.9%) in samples of essential oil from the leaves of *L. sidoides*. This compound in combination with 22 other compounds represented 98.5% of the samples studied. These less abundant compounds included nine hydrocarbonated monoterpenes (21.0%), eight oxygenated monoterpenes (72.2%), four hydrocarbonated sesquiterpenes (4.7%), and one oxygenated sesquiterpene (0.5%). The acaricidal activity of this essential oil was evaluated by means of the LPT on *R. sanguineus* at concentrations of 2.35-18.80 mg/ml, resulting in 20.6-99% larval mortality. The LC_{90} value was 11.56 mg/ml for the larvae of *R. sanguineus*. In addition, Daemon et al. (2009) found that thymol had a negative effect on the larvae of *R. sanguineus*, particularly on engorged larvae, for which a concentration of 2% resulted in 100% mortality.

Up to 20 compounds were identified in samples of essential oil from the leaves of *Lippia gracilis*, among which 92% were terpenes (mono and sesquiterpenes). Thymol was the most abundant compound found in the genotype LGRA-106, whereas carvacrol was the most abundant compound found in the other genotypes (LGRA-108, LGRA-109 and LGRA-201). Following from this, the acaricidal effects of carvacrol and thymol were tested on larvae and engorged females of *R. microplus* by means of the LIT and AIT. Carvacrol showed LC_{50} values of 0.22 and 4.46 mg/ml for larvae and engorged females, respectively, whereas thymol, presented LC_{50} values of 3.86 and 5.50 mg/ml for larvae and engorged females, respectively. The LC_{50} values obtained for the essential oil samples isolated from the genotypes LGRA-201 (1.31 mg/ml for larvae) and LGRA-106 (4.66 mg/ml for females) corroborated the acaricidal activity of *L. gracilis* (De Oliveira-Cruz et al., 2013).

Martinez-Velázquez et al. (2011b) evaluated the acaricidal effect of the essential oil extracted from the leaves of *Lippia graveolens* by means of the LIT. The essential oil was diluted in trichloroethylene and olive oil (2:1) to obtain serial concentrations of 20–1.25%, showing effectiveness of 90–100% against larvae of *R. microplus* for all the concentrations used.

Several secondary metabolites have been identified in the essential oils from species of Poaceae. However, only one study has evaluated the effects of the active compounds in this family (1,8-cineol and *n*-hexanal) against ticks, demonstrating up to 100% acaricidal activity. Specifically, the acaricidal effects of the essential oil extracted from the leaves and stems of molasses grass (*Melinis minutiflora*) were tested against *R. microplus* using the LIT and showed a lethal effect of up to 100% in larvae exposed to the oil at a concentration of 80 mg/ml. Six main compounds were identified from extracts by means of GC/MS, namely propionic acid (43%), butyric acid (3.3%), phenylethyl alcohol (4.5%), *n*-hexanal (5.0%), 1,8-cineol (10.6%), and 9-E-eicosane (8.0%). 1,8-cineol and *n*-hexanal individually caused 100% mortality in tick larvae by means of the LIT after five minutes of exposure (Prates et al., 1998). On the other hand, De Souza et al. (2012) performed an *in vitro* evaluation of the effectiveness of essential oils from the leaves of *Cymbopogon martinii* and *C. schoenanthus* against engorged females and larvae of *R. microplus* by means of the AIT at concentrations ranging from 0.03% to 10% and by means of the LPT at concentrations ranging from 0.02% to 10%. Oil extracts from *C. martinii* (5%) had 75.8% effectiveness, whereas oil extracts from *C. schoenanthus* (5%) had an effectiveness of 39.2%, in both cases against engorged females. In addition, the oil extracts from *C. martinii* had the highest effectiveness against adult females, with LC_{50} values of 2.93% and LC_{90} values of 6.66%. Using the LIT, the LC_{50} and LC_{90} values were 0.47% and 0.63%, respectively, for *C. martinii* and 0.57% and 0.96%, respectively, for *C. schoenanthus*. The presence of large amounts of geraniol in samples from *C. martinii* explains the higher acaricidal activity observed for this species compared with *C. schoenanthus*. Chemical analyses of oil extracts were performed by means of GC and indicated that geraniol was the main compound in *C. martinii* (81.4%) and *C. schoenanthus* (62.5%).

For Meliaceae, the only available studies tested the effectiveness of oil extracts from the seeds of *Carapa guianensis* against engorged females of *R. microplus* using the AIT at concentrations from 0.03% to 10%. Oil extracts from this species exhibited an effectiveness of 55.4% against adult females at a concentration of 5%. Chemical analyses of oil extracts were conducted by means of GC, which indicated that oleic acid was the most abundant compound (46.8%), followed by palmitic acid (39%) and stearic acid (1.7%) (De Souza et al., 2012).

Winteraceae is another poorly studied family, for which the only available study evaluated the effects of essential oil extracts from leaf and stem bark samples from *Drimys brasiliensis* by means of the LIT against larvae of *R. microplus* and *R. sanguineus*. The results showed 100% mortality using samples diluted in ethanol to obtain concentra-

tions of 25, 12.5 and 6.25 µl/ml, and the lowest dosage (3.12 µl/ml) caused 95–98% larval mortality. GC/MS revealed that the oil extracts from this plant are primarily composed of sesquiterpenes (66%), among which cyclocolorenone (30.4%) was the most abundant, followed by biciclogermacrene (11.8%) and alpha-gurjunene (6%) (Sardá-Ribeiro et al., 2008).

For Apiaceae and Myrtaceae, previous work has examined the essential oils from the seeds of *Cuminum cyminum* and the berries of *Pimenta dioica*, respectively. Both species had 100% acaricidal effectiveness against *R. microplus* using the LIT and diluting samples with trichloroethylene and olive oil (2:1). The acaricidal activity of the essential oil of *C. cyminum* was attributed to high levels of cuminaldehyde (22.03%), γ-terpinene (15.69%) and 2-caren-10-al (12.89%). However, the authors suggested that minor components such as o-cimene and β-pinene could also contribute to an increase in activity. The components presumably responsible for the acaricidal activity of the essential oil from *P. dioica* are methyl eugenol (62.7%) and eugenol (8.3%) (Martinez-Velázquez et al., 2011a).

Martinez-Velázquez et al. (2011b) evaluated the acaricidal effect of essential oil extracts from the bulbs of *Allium sativum* (Amaryllidaceae) by means of the LIT and diluted the samples in trichloroethylene and olive oil (2:1) to obtain serial concentrations of 20–1.25%. The authors reported an effectiveness of 90–100% against larvae of *R. microplus*. By means of GC/MS, they found that diallyl trisulfide (33.5%), diallyl disulfide (30.9%), and allyl methyl trisulfide (11.2%) were the most abundant compounds in the oil samples from *A. sativum*.

For Asteraceae, Garcia et al. (2012) evaluated the acaricidal activity of essential oils from the leaves and stems of *Tagetes minuta* against *R. microplus* and *R. sanguineus* using the LIT and AIT at concentrations ranging from 2.5 to 40.0%. The oil extracts were diluted in Tween 20 at 2% and exhibited an effectiveness > 95% against the larvae and adults of both tick species at a concentration of 20%. The chemical composition of the essential oils of this species was determined by GC/MS and spectroscopy analysis, based on which four main compounds were identified that together represented 70% of the oil samples, namely limonene (6.96%), β-ocimene (5.11%), dihydrotagetone (54.21%), and tagetone (6.73%).

On the other hand, Andreotti et al. (2013) performed an *in vivo* evaluation of the effectiveness of *T. minuta* essential oil against engorged females and larvae of *R. microplus* in a cattle pen trial. The solution applied to the animals was prepared as a pour-on formulation (T. minutaessential oil 20%, isopropanol 72%, DMSO 8%, v/v). Treatment with the *T. minuta* essential oil prepared in this study promoted significant effects on all biological indicators analyzed. Based on the biological indicators, the essential oil showed 99.98% efficacy compared to the control group when used at a 20% concentration.

Nogueira et al. (2014) used *R. microplus* to evaluate the acaricidal properties of the essential oil from the leaves of *Zanthoxylum caribaeum* (Rutaceae). They tested serial concentrations (5.0, 2.5 and 1.25%) in 1% dimethylsulfoxide. Using the AIT at a 5% concentration, the mortality rates of adult ticks were 65% on the first day of treatment, 85% on the second day and 100% on the fifth day. Decreasing the concentration to 2.5 and 1.25% caused 55 and 19% mortality, respectively, on the last day of the experiment (day 14). A 5% concentration of essential oil inhibited the oviposition capability by 100%, while concentrations of 2.5 and 1.25% inhibited oviposition by 80.9 and 2.5%, respectively. The chemical components of *Z. caribaeum* were also identified, with a predominant complex mixture of monoterpenes (47.3%) and sesquiterpenes (41.2%).

4.2. Amblyomma genus

The only published work concerning the acaricidal activity of essential oils against ticks of the *Amblyomma* genus was conducted by Garcia et al. (2012). These authors assessed the acaricidal activity of essential oils extracted from the leaves and stems of *Tagetes minuta* (Asteraceae) against the larvae of *Amblyomma cajennense* using the LIT at concentrations from 2.5 to 40%, with an efficiency of 95% at a concentration of 20%. Similar results were obtained using the AIT technique. The chemical composition of the essential oil was determined by GC/MS and spectroscopy analysis, in which four main components were identified comprising more than 70% of the essential oil, including limonene (6.96%), β-ocimene (5.11%), dihydrotagetone (54.21%) and tagetone (6.73%).

4.3. Dermacentor genus

Few essential oils have been tested for the control of *Dermacentor* spp. Gomes et al. (2012) assessed the essential oil extracted from the leaves of *Lippia sidoides* (Verbenaceae) and identified thymol (67.60%) as the main compound. The oil was analyzed by GC/MS, identifying another 15 compounds mentioned above that corresponded to 99.97% of the essential oil. This oil was evaluated against *Dermacentor nitens* ticks at concentrations of 2.5, 5, 10, 15, and 20 µl/ml for the LPT and 10, 20, 40, 60 and 80 µl/ml for the AIT. The larval mortality of *D. nitens* was over 95% starting from the 10 and 20 µl/ml concentrations. In the case of engorged females, a significant reduction ($p < 0.05$) in the egg mass weight and the rate of egg production was observed starting from the 40 µl/ml concentration. The egg viability was affected in all the treated groups, with significantly lower ($p < 0.05$) hatching rates when compared to the control groups (Gomes et al., 2012).

4.4. Hyalomma and argas genera

The plant family Meliaceae has been investigated due to the action of its essential oil on the ticks of the genus *Hyalomma*. The Neem tree (*Azadirachta indica*) belongs to this botanical family. The oil extracted from the seeds (at 12.8% concentration) has demonstrated a 60% inhibition of egg hatching in *Hyalomma anatolicum excavatum* at 15 days post-treatment. Additionally, 100% efficiency in mortality has been reported in recently hatched larvae as well as in unfed larvae and adults 3 and 15 days post-treatment (Abdel-Shafy and Zayed, 2002).

Another tick species that has been used for the evaluation of essential oils is *Argas miniatus*. Garcia et al. (2012) evaluated the acaricidal activity of essential oils extracted from the leaves and stems of *Tagetes minuta* (Asteraceae), reporting efficiency above 95% against the larvae of *A. miniatus* at concentrations from 2.5 to 40% and against adult ticks at a 2.5% concentration. The chemical composition of the essential oil was determined by GC/MS and spectroscopy, in which four main components that compose more than 70% of the essential oil were identified, including limonene (6.96%), β-ocimene (5.11%), dihydrotagetone (54.21%) and tagetone (6.73%).

5. Evaluation of the acaricidal activity of plant extracts against different tick genera

The acaricidal activity of different plant extracts against various tick genera has been previously evaluated, with the species of *Rhipicephalus* having been the most studied. The studies that have evaluated plant extracts are described in the next paragraphs, and a summary of the data obtained in such studies is provided in Table 2, including the techniques used to evaluate acaricidal activity and to identify the secondary metabolites of plant extracts that are effective against ticks. It is important to mention that there are few studies in which the identified compounds in the extracts have been evaluated to prove their acaricidal activity (Neira et al., 2009; Divya et al., 2014; Arceo-Medina et al., 2016).

5.1. Rhipicephalus genus

Plant extracts from the Fabaceae family have been the most widely

evaluated against the *Rhipicephalus* genus. Only one study has identified the main compounds contained in extracts; however, the acaricidal activity of these compounds has not yet been evaluated. Fernandez-Salas et al. (2011) evaluated the acaricidal activity of acetone-water extracts (70:30) from the fresh leaves from four tannin-rich plants (*Acacia pennatula, Piscidia piscipula, Leucaena leucocephala and Lysiloma latisiliquum*) against the larvae and adult ticks of *R. microplus*. These four plants were evaluated using the LPT, and the following mortality rates were obtained: 54.8% for *A. pennatula*, 88.14% for *P. piscipula*, 66.79% for *L. leucocephala* and 56.0% for *L. latisiliquum*. When using the AIT, none of the tested plants displayed acaricidal activity against adult ticks. The extracts from *L. latisiliquum* and *P. piscipula* showed greater dose-responses against the larvae of *R. microplus*. The plant extract LC_{50} values were 6.40 and 2.47 μg/ml, respectively. Recently, Dantas et al. (2016) evaluated the partitions obtained with organic solvents (hexane, chloroform and ethyl acetate) of the ethanol extract of *Amburana cearensis* leaves against a plethora of *R. microplus* females using the AIT technique. The three fractions were evaluated at concentrations of 5, 10 and 25 mg/ml. Among the three evaluated fractions, the hexanic partition (25 mg/ml) obtained the best results in all the analyzed parameters: 52.7% oviposition inhibition, 39% in hatching inhibition, 3.27% reduction in the reproductive efficiency rate and 67.0% in mortality. Using high-performance liquid chromatography (HPLC), the profile of the phenolic compounds was analyzed, identifying benzoic acid, cinnamic acid and flavonoids in the ethanol extract. Procatechuic acid, epicatequin, p-coumaric acid, gallic acid and kaempferol were also identified as major constituents. In the extract partitions with hexane, chloroform and ethyl acetate, flavonoid compound derivatives were identified as the major components.

Three plant species belonging to the Asteraceae family have been evaluated; however, only one study has identified the secondary metabolites of the plants, which were not evaluated to demonstrate their acaricidal activity. Sardá-Ribeiro et al. (2008) assessed the hexane extract from the aerial parts of *Calea serrata* against the larvae and adults of *R. microplus* and *R. sanguineus* using the AIT and LPT techniques, showing 100% mortality in the larvae of both species and a reduction in oviposition by 11–14% at concentrations from 50 to 6.25 mg/ml. Conversely, the ethanol extract from the aerial part of *Tagetes patula* was evaluated against the larvae and adult females of *R. sanguineus* using the AIT and LPT techniques. The extract did not demonstrate mortality in adult ticks of *R. sanguineus* at any of the evaluated concentrations (12.5-100.0 mg/ml). However, the concentration of 50.0 mg/ml showed a reduction of 21.5% in the oviposition rate of adult females and 99.78% mortality in the larvae. Identification of the compounds using MS showed the presence of 12 *O*-glycosylated flavonoids as secondary metabolites was determined (Politi et al., 2012). Recently, Godara et al. (2015) evaluated the efficacy of ethanolic and aqueous *Calendula officinalis* flower extracts in concentrations of 1.25, 2.5, 5 and 10% against larval and adult *R. microplus* ticks that were already resistant to synthetic pyrethroids using the LPT and AIT. They obtained 60% and 20% mortalities in adult ticks for the ethanolic and aqueous (10%) extracts, respectively, and LC_{50} values of 9.9 and 12.9. A significant reduction in the weight of eggs from treated females compared with the control group was observed, and the inhibition of eclosion was 10% for the ethanolic extract. In the larvae, 100% mortality was observed after 24 h at a concentration of 10%. The LC_{50} values for the ethanolic and aqueous extracts were 2.6 and 3.2%, respectively. The authors concluded that the ethanolic extract of *C. officinalis* presents better acaricide properties against the larvae and adults of *R. microplus* ticks in comparison with the aqueous extract.

Three plant species from the family Lamiaceae have been evaluated against *Riphicephalus* ticks. Ravindran et al. (2011) reported that ethanolic extract from the aerial parts of *Leucas aspera* in concentrations from 1.56 to 100 mg/ml induced mortalities of 4.16–54.15% in adult *R. annulatus* ticks using the AIT and demonstrated that the extract induced a reduction in egg production and inhibited tick egg eclosion by 23.21–69.36%. In another study by Divya et al. (2014), an efficacy of 66.67% in the mortality of adult ticks and 55.16% in the inhibition of fecundity were obtained at a concentration of 50 mg/ml when the acaricidal activity against engorged *R. (Boophilus) annulatus* ticks was evaluated in the alkaloid and non-alkaloid fractions obtained through ethanolic extraction of *L. indica* using the AIT. Nicotine was identified as one of the components of the alkaloid fraction, but it did not show acaricidal activity when evaluated *in vitro* at concentrations from 65.2 from 1000 μg/ml, eliminating the possibility of nicotine being responsible for the acaricidal activity of *L. indica*. However, Neira et al. (2009) obtained efficacies of 70% for larvae and 64.9% for adult ticks when the effect of a tincture of tobacco (*Nicotiana tabacum)* from the *Solanaceae* family was evaluated for the control of *Rhipicephalus* spp. ticks in dogs through bathing. The tincture is the result of an extraction method using ethylic alcohol or ethanol. These results were evaluated using a concentration of 0.0117% nicotine and compared with a classic treatment using amitraz at 12.5% in water, which had an efficacy of 92% in the two live stages of the tick (larvae and adults).

Rosado-Aguilar et al. (2010b) evaluated the efficacy of 45 methanolic plant extracts on acaricide-resistant *R. microplus* larvae, reporting mortalities of 5% to 99%. In another study, Rosado-Aguilar et al. (2010a) reported the efficacy of an extract of the stem of *Petiveria alliacea* (Phytolaccaceae) on the larvae of *R. microplus*, finding an LC_{50} of 4.38 and an LC_{99} of 16.5%. In adult ticks, a mortality of 86% and oviposition inhibition of 91% were reported. In the stem of *P. alliacea*, dibenzyltrisulfide and dibenzyldisulfide were identified as the principal compounds in the active fraction using GC/MS. This work is so far the most complete regarding the acaricidal activity against larvae and adult ticks and the identification of the possible compounds responsible for such activity. To confirm the acaricidal activity of these compounds, Arceo-Medina et al. (2016) tested commercially available compounds (Sigma®) individually and in combination, finding a synergistic effect against *R. microplus* larvae and adult resistant to commercial acaricides.

Pulido and Cruz (2013) evaluated 70% aqueous and ethanol extracts (1:3) of *Verbena officinalis* (Verbenaceae) and *Ruta graveolens* (Rutaceae) on adult *R. microplus* ticks using the AIT. These authors carried out four tests to determine if the secondary metabolites responsible for the ixodicidal effects were obtained after maceration. In the Shinoda test for the identification of flavonoids, the *V. officinalis* extract showed a change in color, turning green-purple, while in the Wagner colorimetric evaluation, both extracts were positive for alkaloids. In the Legaĺs reaction test for the identification of coumarins, the *R. graveolens* extract was positive. Finally, the saponin reaction test was carried out, and more was found in the *V. officinalis* extract than in that of *R. graveolens*. The latter extract showed a 63.3% efficacy in small and medium ticks using a pure extract, while *V. officinalis* had 66.6% efficacy.

In a dichloromethane extract of *Croton sphaerogynus* (Euphorbiaceae) leaves, the main compounds identified by GC/MS were abietanes, podocarpens and clerodane-type furan diterpenes, particularly abieta-8,11-diene-3-one (20%), abieta-8,11,13-triene-12-ol (11%) and podocarp-7-ene, 13-methyl-13-vynil-3-one (12%). The extract was evaluated using the LPT against *R. microplus* ticks using serial concentrations of 0.625% to 20%; mortalities from 2.25 to 99.32% were obtained (Righi et al., 2013).

Dantas et al. (2015) evaluated the acaricidal activity of the leaves and aerial parts of *Neoglaziovia variegata* (Bromeliaceae) on engorged *R. microplus* females using the AIT. Ethanol, hexane, chloroform and ethyl acetate extracts from the leaves and aerial parts of the plant in concentrations of 5, 10, and 25 mg/ml were used. As a result, all concentrations of ethanol and hexane extracts from *N. variegata* leaves showed a significant effect on adult *R. microplus* ticks at the highest concentration (25 mg/ml). The result for the aerial parts using the ethanol extract was 57.25% and for the hexane extract was 61.92%. The hexane extract from *N. variegata* leaves showed a 94.1% inhibition of oviposition, a 0.33% reduction in egg eclosion, and a 99.8% efficacy

in terms of the mortality of adult *R. microplus* ticks. The phenolic components of the plant extract were identified using liquid chromatography, showing the presence of isoquercetin (flavonoid) in the ethanolic extracts of the leaves and aerial parts. Furthermore, p-coumaric acid was found in the chloroform extract from the leaves, and vanillic acid was found in the aerial parts of the plant. The vanillic, p-coumaric and protocatechuic acids were identified in the ethyl acetate extract from the *N. variegata* leaves and aerial parts. In the aerial parts, caffeic and kaempferol-3-*O*-ramnosid acids were also found.

Sardá et al. (2007) evaluated hexanic and methanolic extracts from the aerial parts of *Hypericum polyanthemum* (Hypericaceae) on *R. microplus* adult and larval ticks. Using the AIT, the hexanic extract showed a 19.2% reduction in egg production for the 50 mg/ml concentration, while the methanolic extract did not show any activity against engorged females. In the LIT, the hexanic extract showed 100% efficacy at all evaluated concentrations after 48 h of exposure to the extract. The methanolic extract caused mortalities of 100, 96.7, 84.7 and 52.7% in *R. microplus* larvae at the concentrations of 50, 25, 12.5 and 6.25 mg/ml, respectively, after 48 h of exposure. Despite the high acaricidal activity of the extract against larvae, the secondary metabolites in the extract were not identified.

De Souza et al. (2012) evaluated *in vitro* the efficacy of the raw extract of *Piper tuberculatum* (Piperaceae) leaves against *R. microplus* ticks using the AIT at concentrations of 0.03% to 10% as well as the LPT with concentrations of 0.02% to 10%. The *P. tuberculatum* extract (10%) showed an efficacy of 91.6% against adult females and LC_{50} and LC_{99} values of 3.76 and 25.03%, respectively. In the LIT, LC_{50} and LC_{99} values of 0.41% and 0.79%, respectively, were obtained. However, no tests were carried out to identify the main compounds in the extract.

5.2. Amblyomma genus

Makeri et al. (2007) evaluated the acaricidal activity of an extract of *Azadirachta indica* seeds on goats infested with *Amblyomma variegatum*, showing 100% mortality of ticks after 24 h of exposure to concentrations of 5.0, 2.5 and 1.0%.

Recently, Muyobela et al. (2016) determined the acaricidal properties of leaf, bark and fruit extracts of *Tephrosia vogelii* and *Bobgunnia madagascariensis* against adult ticks of *Amblyomma variegatum*. The extracts were prepared with methanol, acetone and chloroform as extraction solvents and were evaluated by free contact at doses of 0.1, 0.2 and 0.3 g/ml. Only the methanol extracts of the bark and leaves of *T. vogelii* and the methanol extract of the fruit of *B. madagascariensis* at 0.3 g/ml showed 100% mortality of ticks after 24 h. The extracts from leaves of *T. vogelii* and the fruit of *B. madagascariensis* were selected for topical application, obtaining a LC_{50} of 0.03 g/ml for the *B. madagascariensis* extract, which was more effective than the extract of *T. vogelii* (LC_{50} 0.55 g/ml).

6. *In vivo* studies on efficacy and toxicity of the plant secondary compounds responsible for the acaricidal activity

As it has been revised above, the majority of tests searching for acaricidal activity of different plant products have been performed under laboratory conditions. It is logical that the results obtained under laboratory condition cannot necessarily be extrapolated to field conditions due to: alteration of the chemicals in the field by environmental factors, habituation of arthropods, use of artificial substrates in the laboratory experiments, arthropods movement away from treated plants and composition of the plant community. In fact, there is no specific procedural design devised for field evaluation of acaricidal compounds from plants. However, there is a need to evaluate these compounds in the field to determine its practical application potential (Koul, 2005).

In spite of the difficulties mentioned above, there have been *in vivo* studies testing the acaricidal or insecticidal activity of different plant products (essential oils and plant extracts) as reviewed by different authors (Habeeb, 2010; Ferreira-Borges et al., 2011; Parte et al., 2014; Gosh et al., 2015). However, those studies cited in those review papers fail to provide conclusive data on the plant compounds associated with the antiparasitic activity. Only a few studies have identified the plant secondary compounds associated with the insecticidal or acaricidal activity under *in vitro* conditions, including thymol, carvacrol, 1,8-cineol, *n*-hexanal, nicotine, dibenzyldisulfide and dibenzyltrisulfide (Prates et al., 1998; Daemon et al., 2009; Neira et al., 2009; De Oliveira-Cruz et al., 2013; Koc et al., 2013; Divya et al., 2014; Arceo-Medina et al., 2016). Amongst the latter, only thymol, carvacrol and 1,8-cineol have been confirmed either with insecticidal or acaricidal activity under *in vivo* conditions. All the other compounds have not been tested for the antiparasitic activity against insects or acari under *in vivo* conditions.

6.1. Thymol and carvacrol

The study by Ranjbar-Bahadori et al. (2014) evaluated two plant preparations (garlic & thyme) to show efficacy against to *Dermanyssus gallinae* by spraying. The extract was prepared from thyme plant based on 4.4–7.0 mg of thymol. During spraying the hens remained in the cages. There were significant differences between the mean total number of trapped mites on days 1 and 7 after the spraying with the thyme essential oil compared to untreated control group and mean trapped mite before treatment ($p < 0.05$) (89.4% day 1 and 95.4% on day 7).

Shang et al. (2016) evaluated the clinical acaricidal efficacy of oregano oil (OR) and its major components thymol and carvacrol against *Psoroptes cuniculi* on rabbits. Four groups were evaluated: group A (5% OR), group B (1% OR), group C (Ivermectin) and group D (negative control). Before treatment, no differences were observed between the four selected groups (day 0). After two treatments with OR (Oregano oil) or ivermectin, the numbers of scabs and mites on the right ears were substantially decreased. At day 8, there were significant differences between treatment groups and the untreated control group. After three treatments (day 15), the right ears of the rabbits that were treated with 5% OR or ivermectin were free of scabs and/or mites, while those treated with 1% OR exhibited only small scabs or minimal secretions in the ear canals and no mites. The untreated control group remained infested, and their statuses become poor, and they exhibited marasmus. At the end of the test (day 20), the rabbits in all of the treatment groups exhibited favorable mental and physical statuses, and no mites or scabs were found in the right ears of these rabbits. These results indicated that oregano oil could be widely applied as a potential acaricidal agent in the treatment of animal acariasis in the future.

A battery of toxicity studies has been performed for thymol. On acute oral administration, thymol is harmful whereas it is practically non-toxic following acute dermal application (LD50 rat oral 980 mg/kg body weight; LD50 mouse oral 640–1800 mg/kg body weight; LD50 rat dermal > 2000 mg/kg body weight). In the rabbit, thymol can be corrosive to the skin and eye. Rats subjected to subchronic administration in the feed for a period of 19 weeks tolerate thymol at 10000 ppm. Thymol did not increase the incidence of spontaneous lung tumors in mice (HSDB Thymol, 2017). Andersen (2006) reported that the oral LD50 of thymol in males mice was 1200 mg/kg and 1050 mg/kg body weight in females. Acute toxic signs were hypoactivity and ataxic gait and there were some small intestinal congestions observed. Also adult rats were exposed by intubation with 20% thymol in propylene glycol. The thymol LD50 in rats was 980 mg/kg (with 95% confidence limits of 817–1180 mg/kg). Rats died within 4 h to 5 days after dosing. Depression and ataxia were noted in most dose groups and coma at greater doses.

In embryonic chickens, thymol causes multiple malformations on injection into the air bubble or the yolk sac. Oral administration of

thymol does not induce micronuclei in mice even in the toxic dose range. In the Salmonella/microsome assay, thymol exhibits no mutagenic effect. However, it has been reported to give positive results in the Unscheduled DNA Synthesis test (liquid scintillation) and in the Sister chromatid exchanges test with embryonic cells of the Syrian hamster. The findings are statistically significant, though there is no strict dose-response relationship. The various other actions of thymol include cytotoxic, antineoplastic, antibacterial, fungicidal, anti-inflammatory, spasmolytic and other pharmacodynamic effects (HSDB Thymol, 2017).

Essential oils (including thymol) are used by beekeepers to control the Varroa mites infesting honeybee colonies. The effects of thymol on olfactory memory and gene expression in the brain of the honeybee were explored in bees previously exposed to thymol, and the specificity of the bee response to the conditioned stimulus was lost 24 h after learning. The results also indicated that the genes coding for the cellular targets of thymol could be rapidly regulated after exposure to this molecule (HSDB Thymol, 2017).

Andre et al. (2016), evaluated the toxicity of carvacrol on mice. In the acute toxicity test, the LD10 and LD50 of carvacrol were 546.8 mg/kg (266.3–718) and 919 mg/kg (693.3–1,251.9), respectively. No changes were observed in the behavior of mice during the acute toxicity test. Clarke and Clarke (1977) report that substances with a LD50 value above 1000 mg/kg via oral route are safe or of low toxicity. However, carvacrol applied full strength to intact or abraded rabbit skin for 24 h under occlusion was severely irritating (HSDB Carvacrol, 2017).

6.2. 1,8-cineol

Perrucci et al. (2001) tested the *in vivo* efficacy of 20% aqueous extract and 5% essential oil of *A. verlotorum* (1,8 cineol 18.8% in the chemical composition) on rabbits naturally infested with *Psoroptes cuniculi*. Five rabbits were treated with the oil diluted at 5%. All the compounds were applied directly to the infected ears at a dose rate of 2–5 ml. The compounds were administered daily during two periods of three consecutive days, seven days apart. After each treatment, the rabbits were kept under observation in order to detect the toxicity of the tested compounds. The treatment with 5% essential oil resulted in a clinical and parasitological recovery in all the five treated rabbits (100% effectiveness). Neither clinical symptoms nor mites in ear cerumen were found in these rabbits seven days after the treatment protocol. The long period in which they remained free of mites (about one month) demonstrated the complete efficacy of the treatment with 5% essential oil.

A variety of toxicity studies have been performed for the 1,8 cineol. The ocular irritancy potential of eucalyptol (1,8 cineol as the main compound) to the isolated bovine cornea showed that eucalyptol is not an ocular corrosive or severe irritant. Another study tested the 1,8 cineol administered by gavage to three groups of five male and five female rats, for twenty-eight consecutive days, at dose levels of 30, 300 and 600 mg/kg BW/day. For both sexes at 300 and 600 mg/kg BW/day, examination of the liver revealed a dosage dependent incidence of centrilobular hypertrophy of the hepatocytes. No other indicators of liver damage were apparent. Following the two week recovery period, hypertrophy of hepatocytes was no longer present at 600 mg/kg BW/day for either sex. 1,8-cineol induced accumulation of protein droplets in proximal tubular epithelial cells in male rats. The renal changes were specific to the male rat and of no toxicological relevance to man (HSDB Cineole, 2017). Koul et al. (2008), reported the toxicity of 1–8-cineol on rats by acute oral toxicity test obtaining LD50 of 2480 mg/kg. In another study, Eucalyptol was tested as a constituent of toothpaste in an oral long-term study with mice. Groups of 52 male mice were given 0, 8 and 32 mg/kg BW/day eucalyptol in toothpaste base by gavage for 80 weeks followed by an observation period between 16 and 24 weeks. No treatment-related effects on body weight, food consumption, survival, weight of adrenals, kidneys, liver, lungs or spleen, or on the microscopic appearance of brain, lungs, liver and kidneys or on tumor incidence was observed (HSDB Cineole, 2017).

6.3. n-hexanal, nicotine, dibenzyl disulfide and dibenzyl trisulfide

To the best of our knowledge, there are no *in vivo* studies where these compounds have been tested against any ectoparasite of veterinary importance under *in vivo* conditions. However, dibenzyl disulfide and dibenzyl trisulfide will be tested soon after the interesting results obtained under *in vitro* conditions as mentioned above (Arceo-Medina et al., 2016). The following are studies on the toxicity of *n*-hexanal, nicotine, dibenzyl disulfide and dibenzyl trisulfide.

Acute exposures to the concentrated vapor of *n*-hexanal for 1 h or to 2000 ppm for 4 h result in mortality to rats and is cytotoxic to rat hepatocytes. Rats receiving diets containing hexyl aldehyde for 3 weeks showed a decrease in serum cholesterol and triglyceride. *n*-hexanal stimulates dopamine release but does not inhibit dopamine uptake in the brain striatum of rats. *n*-hexanal influenced the length of time virgin female mice engage in the maternal crouching behavior. *n*-hexanal was mutagenic in mammalian cells. *n*-hexanal produced DNA single-strand breaks, or lesions which were converted to breaks in alkali. The compound was tested externally on the eyes of rabbits, and, according to the degree of injury observed after 24 h, rated on a scale of 1–10. The most severely injurious substances have been rated 10. *n*-hexanal rated 5 on rabbit eyes (NCBI Hexanal, 2017). Lewis (2004), reported for *n*-hexanal an oral LD50 of 4890 mg/kg on rats.

Nicotine is acutely toxic (Category I) by all routes of exposure (oral, dermal, and inhalation). The LD50 of nicotine is 50 mg/kg for rats and 3 mg/kg for mice. A dose of 40–60 mg can be a lethal dosage for adult human beings and doses as low as 1–4 mg can be associated with toxic effects in some individuals. Nicotine is a ganglionic (nicotinic) cholinergic-receptor agonist. The pharmacologic actions of nicotine are complex and include a variety of effects mediated by stereospecific binding to receptors in autonomic ganglia, the adrenal medulla, the neuromuscular junction, and the brain. This product is designated a restricted use pesticide due to very high acute inhalation, oral, dermal, and eye toxicity to humans. Production and usage are now quite limited (Okamoto et al., 1994; Werley et al., 2014; US-EPA, 2008; NLM-HSDB-TOXNET, 2017).

Munday and Mans (1985) performed an *in vivo* evaluation of the toxicity of dibenzyl disulfide (DD) on eight-week-old male littermate rats. The DD was administered, as suspensions in 0.1% Tween 80, to 10 rats by oral intubation on seven consecutive days. The daily dose was made equal on a molar basis and was administered at 500 pmol kg/BW/day. Toxicity was determinated by analysis of blood packed cell volume and hemoglobin. Weight and iron content of the spleen of each rat was assessed at post-mortem. No evidence of hemolysis was recorded and no significant difference in the other parameters was detected between control rats and those receiving DD. On the other hand, The MAK Collection for Occupational Health and Safety (2012), reported that the oral LD50 of DD for rats is 3780 mg/kg. After oral administration of 123 mg/kg body weight to rats, daily on 7 consecutive days, there were no significant changes in erythrocyte count or hemoglobin level in blood. No Heinz bodies were found. Spleen weights and iron content were unchanged and the spleen tissue was not darkened. However in rabbits, 24 h after application, 500 mg/kg dibenzyl disulfide was seen to be moderately irritating to the skin and eyes. The high oral LD50 indicates that DD is likely to have a relatively low acute toxic potential. However, the possibility of irritation of skin and eyes during its handling, cannot be excluded.

The acute toxicity was established in BALB/c mice for *Petiveria alliacea* fraction containing Dibenzyl trisulfide administered via I.P. Different doses of the fraction were inoculated in 4 groups of mice (n = 5) and after 72 h of treatment the deceased animals were counted and the data analyzed with Probit test (Minitab software). The LD50 calculated was 1545 mg/ kg and according to Hodge and Sterner

toxicity scale the fraction was classified as slightly toxic (Hernández et al., 2014). Dibenzyl trisulphide at 10 mM did not have any effect on the sensitive process of protein biosynthesis in Starfish (*Asternia pectinifera*) embryos. Concentrations of up to 34 mg/kg body weight did not cause mortality to mice (Williams et al., 2007).

7. Potential use of plant products and secondary compounds on ticks resistant to ixodicides

This review showed that the different parts of plants that have been assessed so far contain complex mixtures of secondary compounds. In most cases, it is unknown which of these secondary compounds are associated with the acaricidal activity (Tables 1 and 2). Of the few studies that have identified secondary compounds associated with the acaricidal activity, the presence of more than one secondary compound has been found to be required (Arceo-Medina et al., 2016). This means that associations should be studied in terms of additive effects, synergistic effects and possible antagonistic effects. The possible existence of additive and synergistic effects could mean that the development of parasite resistance to these compounds would be more difficult than normally occurs with conventional acaricides. According to Katoch et al. (2006), the efficacy of a single ectoparasiticidal plant can be improved in combination with another plant or in combination with an active ingredient with adjuvant properties. Plant products or their secondary compounds could act in one or more of the following ways: neutralization of growth-regulating hormones, anti-feeding effects, inhibition of the development of eggs, disruption of mating and sexual communication, and inhibition of the formation of chitin (De Souza et al., 2012).

It is evident that *in vivo* trials with ruminants are needed, but will be more complex to perform due to the quantity of the material required to treat large animals. However, it is evident that thymol, carvacrol or 1,8-cineol could be good candidates for testing against some ruminant ectoparasites as it could be applied by spraying or in the form of a topic oil.

The *in vivo* evaluation of plant secondary metabolites different to essential oils will be a great challenge as it will require finding the right vehicle for the safe and effective administration to the animals. Thus, in the search for an *in vivo* use of materials such as dibenzyl disulfide and dibenzyl trisulfide there is also a need for methods of application including vehicles that have no antagonistic effect that could limit their antiparasitic effect.

8. Conclusions

This review shows that plant products containing bioactive metabolites represent a promising alternative for the control of ticks that are susceptible and resistant to conventional acaricides. Studies of the effects of essential oils and plant extracts against different classes of ticks showed efficacies of 5–100%, with the Lamiaceae family and the genus *Rhipicephalus* being the most studied. In tests of the inhibition of egg hatching, an efficacy of 60–100% was observed. In tests against larvae and adults, mortalities of 5–100% and 60–100%, respectively, were observed, and the larval stage was the most studied. Only a few secondary metabolites have confirmed acaricidal effect. For the essential oils, the plant compounds with confirmed activity were thymol, carvacrol, 1,8-cineol and *n*-hexanal. Meanwhile, for the plant extracts the active secondary metabolites were nicotine, dibenzyldisulfide and dibenzyltrisulfide. Only thymol, carvacrol, 1,8-cineol have been evaluated for acaricidal activity under *in vivo* conditions. Work *in vivo* conditions is required to evaluate these secondary compounds responsible for the acaricidal activity against different types of ticks..

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