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Chemical composition and acaricidal activity of *Eucalyptus globulus* essential oil against the vector of tropical bovine piroplasmosis, *Rhipicephalus* (*Boophilus*) *annulatus*

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Abstract

Ticks are of great economic importance to humans and animals due to their role in disease transmission. The application of synthetic, chemical acaricides on the animal and/or the environment (the most used tick control method globally) has led to the selection of tick populations that are resistant. Their adverse effects on ecology and human and animal health cannot be overemphasised. As a result, the search for alternatives that are natural and can overcome these adverse effects are strongly indicated. Using gas chromatography-mass spectrometry (GC–MS) and adult immersion test (AIT), this study evaluated the chemical composition and acaricidal activity, respectively, of *Eucalyptus globulus* essential oil (EO) on *Rhipicephalus* (*Boophilus*) *annulatus* ticks. This is a major tick species implicated for the transmission of bovine piroplasmosis in Nigeria. The acaricidal activity was evaluated using different concentrations (0.625, 1.25, 2.5, 5 and 10%) of *E. globulus* EO. Amitraz (1 and 2%) and cypermethrin (2%) served as the positive control and 2% dimethylsulfoxide in distilled water was the negative control. Three replicates of 10 engorged female ticks each were immersed in the test samples for 2 min and the experiment was done twice. The GC–MS analysis identified the major constituents of *E. globulus* EO as eucalyptol (1,8-cineole) (78%), menthol (20%) and menthone (3%). *Eucalyptus globulus* EO caused 97% acaricidal mortality at 10% concentration. The lower concentrations reduced tick fecundity up to 90% in a dose-dependent manner. This study provides support for plant EOs as alternative tick control strategy for humans and animals.

Keywords Acaricidal · Eucalyptol · Essential oil · Ticks · Fecundity · *Eucalyptus*

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Introduction

Ticks are obligate hematophagous arthropods next in importance to mosquitoes as vectors of bacterial, viral, rickettsial and protozoal disease agents (Opara and Maxwell 2012). Tick infestation and associated tick-borne diseases (TBDs) such as babesiosis, theileriosis and anaplasmosis affect the health and productivity of companion animals, livestock, wildlife and human populations around the world (Greay et al. 2016). Ticks are responsible for 22.8% of emerging infectious diseases and 28.8% of these diseases have emerged over the past decade (Failloux and Moutailler 2015). This resurgence has coincided with climate change, as vectors are sensitive to environmental conditions such as rainfall and temperature (Ogden and Lindsay 2016).

Globally, the direct losses caused by tick infestation and TBDs as well as indirect losses incurred for their treatment and control has been calculated at USD 13.9–18.7 billion per year (Bentacourt-Craioto et al. 2017). The Food and Agriculture Organization (FAO) estimated the average financial loss per animal per year at USD 7.3 (Hurtado and Giraldo-Rios 2018).

Current strategies to control TBDs are largely based on measures to avoid exposure and/or control the vector species. Chemical control of ticks using synthetic acaricides applied on the animal and/or the environment is the most used method in Africa as in many other parts of the world. However, the indiscriminate use of chemical acaricides exerts a strong selection pressure for the evolution of biological processes in ticks, which results in populations that are resistant to these chemicals (Klafke et al. 2017). The intense use of chemical acaricides has also raised public health concerns (presence of chemical residues in meat and milk products) and environmental contamination (De Meneghi et al. 2016).

Nigeria, Africa's most populous country and largest economy, has a livestock population of approximately 34.5 million goats, 22.1 million sheep and 13.9 million cattle (about 1.4% of the global livestock populations) (Lawal-Adebowale 2012). However, livestock population has not kept pace with human population growth due to many constraints, one of which is the infestation with the tick *Rhipicephalus* (*Boophilus*) *annulatus*, the vector of tropical bovine piroplasmosis. This species has been reported to be very prevalent in Nigeria (Lorusso et al. 2013). In recent years, extremely high tick populations and chemoresistance of marketed acaricides have been observed by many livestock farmers and reported in Nigeria (Akande et al. 2020). This has made the use of synthetic chemicals for tick control no longer sustainable. As such, the search for alternative control measures such as the use of medicinal plants with acaricidal activity is strongly advocated.

Medicinal plants synthesize a large variety of secondary metabolites (phytochemicals) that form part of adaptation and defense mechanisms against the pathogens and predators in their environment. These metabolites can act synergistically, in different ways, making the development of resistant tick populations more difficult than would occur with conventional chemical acaricides. Thus, they constitute a desirable alternative for pest control (Álvarez et al. 2017). Research in recent decades has supplied much information on the extraordinary diversity of chemicals and several biological activities of plant-derived products against many pathogens (Li and Lou 2018; Mawalagadera et al. 2019). According to the World Health Organisation (WHO), the present demand for medicinal plants is approximately USD 14 billion annually and is expected to reach USD 5 trillion by the year 2050 (Bhat et al. 2018).

Many medicinal plants produce volatile, odorous compounds known as essential oils. Essential oils (EOs) play important roles in nature and have been utilized for pharmaceuticals, agrochemicals, aromatherapy, perfumes and as food flavorants (Djebir et al. 2019). In a recent review by Camillo et al. (2017), the Myrtaceae family was the second highest reported on EOs with acaricidal activities. The *Eucalyptus* genus is one of the largest genera (about 900 species) in the family, with antibacterial, antifungal, anti-inflammatory and insecticidal activities (Rossi and Palacios 2015). *Eucalyptus globulus* Labill., commonly called 'Southern blue gum' in English, 'Nku-ishi' in Igbo and 'Kafur' in Hausa, is one of the approximately 300 EOs in commercial use (Barbosa et al. 2016). Though the species originated from Australia, it has become well acclimated in Nigeria, where the weather is favorable for its development. In African traditional medicine, the plant is used for the treatment of common infections such as cough, asthma, cold, fever, joint pains, loss of appetite, wounds, fungal infections and ectoparasites (Bipin et al. 2015; Ekhuemelo et al. 2017).

Despite the use of *E. globulus* as a biopesticide traditionally in Africa, its potential acaricidal properties have not been fully explored. This study therefore seeks to evaluate the chemical composition and acaricidal property of *E. globulus* in vitro as a first step in a series of proposed studies to find leads for the development of new products which are more efficient and will overcome the problem of increasing tick resistance to treatments.

Materials and methods

Collection of plant material

Fresh *E. globulus* leaves were collected in March, 2018, from Itokin, Epe area, Lagos State, Nigeria (6.656°N, 3.795°E). The plant species was identified and authenticated at the Nigeria Natural Medicine Development Agency (NNMDA), Lagos State, where a voucher specimen number (2019/01298) was given.

Extraction of essential oil and analysis

Essential oil from fresh leaves of *E. globulus* was obtained by hydrodistillation using an all glass Clavenger apparatus (Elyemni et al. 2019). Approximately 500 g of the sample leaves were packed into a 10-l round bottom flask containing 4 l of distilled water. The flask was then fitted with a Clavenger apparatus and heated. Water at 0 °C flowed counter currently through the condenser to condense the ensuring steam. The EO that was extracted from the leaf mixed with the water vapour at 100 °C and both passed through the condenser and condensed into liquid. Cooling was done using an ice block and volatilization of the EO was avoided. The condensate was collected in a 500-ml beaker and then poured into a separating funnel. This formed two layers of oil and water. The EO was separated and immediately collected into a 100-ml stoppered bottle and tightly closed. The volume of EO obtained was weighed. The EO was thereafter collected in an airtight container, dried over anhydrous sodium sulphate to remove any entrapped water and stored at 4 °C for further use.

The gas chromatography-mass spectrometry (GC–MS) analysis of *E. globulus* EO was performed on a Hewlett-Packard ITP5890A GC, interfaced with a VG analytical 70-250S double focusing mass spectrometer as described by Akolade et al. (2012). Helium was the carrier gas at 1.2 ml/min. The MS operating conditions were: ionization voltage 70 eV, ion source 2300C. The GC was fitted with a 25 m × 0.25 mm, fused silica capillary column coated with CP-sil 5. The film thickness was 0.15 µm. The GC operating conditions were identical with those of GC analysis. The percentage compositions of the constituents of the EO were computed in each case from GC peak areas. Identification of components in the EO was accomplished by comparison of retention indices (determined relative to the retention time of series of *n*-alkanes) and mass spectra with those of authentic samples (NIST 05.L database/chemstation data system) and with data from literature. The analysis was done in duplicate.

Collection and housing of ticks

Engorged female *R.* (*B.*) *annulatus* ticks were carefully handpicked from their predilection sites on herds of cattle at the Directorate of the Federal University of Agriculture, Abeokuta Farms (DUFARMS) and the lairage of Lafenwa abbatoir, Abeokuta. The cattle had no history of acaricide treatment in the last 2 months. The ticks were kept in labeled and perforated, plastic bowls to allow air and moisture exchange. The bowls were then sealed with rubber rings to prevent the ticks from escaping. The ticks were transported to the Parasitology laboratory, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, where they were identified to the species level using both taxonomic descriptions and morphological keys (Walker 2003). Each batch of ticks collected were tested on the same day of collection.

Experimental procedure

The adult immersion test (AIT) described by Pirali-Kheirabadi et al. (2009) was employed. Engorged females which have been thoroughly cleaned and dried, were weighed, marked and kept in plastic bowls padded with absorbent paper. *Eucalyptus globulus* EO concentrations (10, 5, 2.5, 1.25 and 0.625% wt/vol) were prepared in series diluted in 2% dimethylsulfoxide (DMSO).

The ticks were divided into groups of 10 ticks each and immersed in the various concentrations of the test sample for 2 min with gentle agitation. Each tick was examined immediately post immersion (PI) to determine their photo response under a USB digital microscope (Unimake 2mp, China). The negative control was 2% DMSO in distilled water, whereas 2% cypermethrin (Cyperpharma®) and 1 and 2% amitraz (Amitraz 20EC®) were used as positive controls. The ticks were then dried on absorbent paper before transfer into clean padded plastic bowls (one tick per bowl). Treated ticks were thereafter kept in a rearing chamber and maintained at 25 ± 1 °C, 70–80% relative humidity and L14:D10 photocycle to ensure their survival (Thorsell et al. 2006). Each concentration was tested in triplicates and 10 engorged females were used per replication. The experiment was performed twice.

The bowls were examined on an hourly basis for the first 6 h after treatment. The tarsal reflex was also checked by mechanical stimulation of the ipsilateral front leg tarsus with a probe and by light (see below). Thereafter, they were examined every 24 h (in the morning) to check for mortality up to 14 days to allow for oviposition (Pamo et al. 2005). Engorged females that oviposit were considered as live and females that did not oviposit were considered dead. Eggs of females that oviposited were weighed and transferred to bowls that were covered with Tissue-Non-Tissue (TNT) fabric and fastened by elastic bands. The reproductive index (RI) was calculated as [egg mass (mg)]/[weight of engorged female (mg)], and the inhibition of oviposition (IO, %) was calculated as $[(RI_{control} - RI_{treated})/RI_{control}] \times 100$.

Tarsal reflex scoring

The tarsal response to light and touch was observed, recorded and graded on a scale of 0–5 based on the scoring system described by Akande et al. (2020). Score 0 signifies death of the tick, whereas 1–5 indicates that the ticks are alive and display a range of responses (see Table 2). The touch and light stimuli were delivered using a probe and light source, respectively. This was done immediately after treatment to check for possible death through drowning and/or immediate paralysis.

Data analysis

Data were recorded in Microsoft Excel. Dose response data were analyzed by probit method (Lieberman 1983) using GraphPad Prism v.8.3.0 (San Diego, CA, USA). The LC_{50} and LC_{90} were determined by applying regression equation analysis to the probit transformed data of mortality. One-way ANOVA was used to analyze the effect of the EO concentrations on tick mortality, followed by Tukey's posthoc test to compare group means ($\alpha = 0.05$).

Results

Yield of essential oil

The EO extracted from the *E. globulus* leaves was straw colored with a pleasant, menthol-like odour. The volume of EO obtained from 500 g of *E. globulus* leaves was 8.0 ml, giving an extraction yield of 1.6%.

GC–MS analysis

Three compounds were identified in the *E. globulus* EO namely eucalyptol, menthone [cyclohexanone (5-methyl-2-1-methylethyl)] and menthol (Table 1, Fig. 1).

Effect of essential oil on tarsal reflex

None of the ticks got drowned in the test sample or control solutions used in this study (Table 2). The response rates to each test sample varied, but overall ticks seemed to move their limbs less with increasing EO concentration (Table 2).

Table 1 Retention time and peak area of identified compounds in *Eucalyptus globulus* leaves

Compound	Retention time (min)	Peak area (%)
Eucalyptol	4.219	77.62
Menthone [cyclohexanone(5-methyl-2–1-methylethyl)]	5.842	2.50
Menthol	6.082	19.88

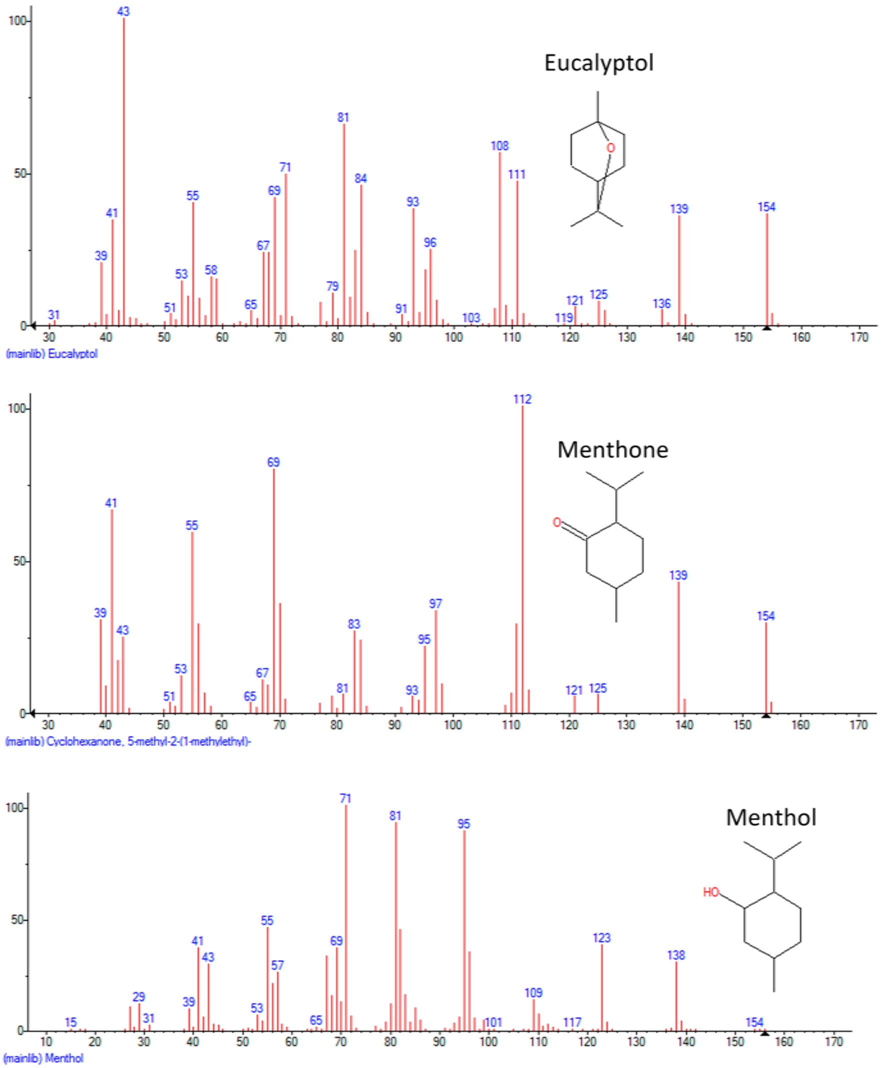

Fig. 1 Mass spectra of the three compounds identified in *Eucalyptus globulus* essential oil: eucalyptol, menthone [cyclohexanone (5-methyl-2–1-methylethyl)] and menthol

Effect of essential oil on mortality

The AIT of engorged female *R.* (*B.*) *annulatus* ticks displayed a dose-dependent response (Fig. 2). There was 97% mortality at 10% concentration. Mortality reached the peak on day 3 PI and at this point 90–100% of the ticks were dead or moribund. For ticks immersed in 5% concentration, 60–80% mortality occurred between day 5 and 7 and 90% mortality was recorded on day 12 PI. At 2.5% concentration approximately 40–50% mortality was achieved on day 3 PI and up to 70% mortality on day 12. The 1.25% concentration of *E. globulus* EO caused much lower mortality of 20–30% on day

Table 2 Tarsal reflex score of groups of 10 engorged female *Rhipicephalus* (*Boophilus*) *annulatus* ticks treated with *Eucalyptus globulus* oil (0.625–10%), positive controls (PC; i.e., 2% cypermethrin, 1 and 2% amitraz), and a negative control (NC; i.e., 2% dimethylsulfoxide in distilled water)

Response	*E. globulus* oil (%)					PC	NC
	0.625	1.25	2.5	5	10		
(0) No response	0	0	0	0	0	0	0
(1) Moving 1–2 limbs slowly	0	2	2	3	4	0	0
(2) Moving 1–2 limbs at a fast rate	0	0	1	3	1	0	0
(3) Moving more than 2 limbs but slowly	0	1	0	1	3	0	0
(4) Moving ˃2 but ˂8 limbs but slowly	0	2	2	0	2	1	0
(5) Moving all limbs	10	5	5	3	0	9	10

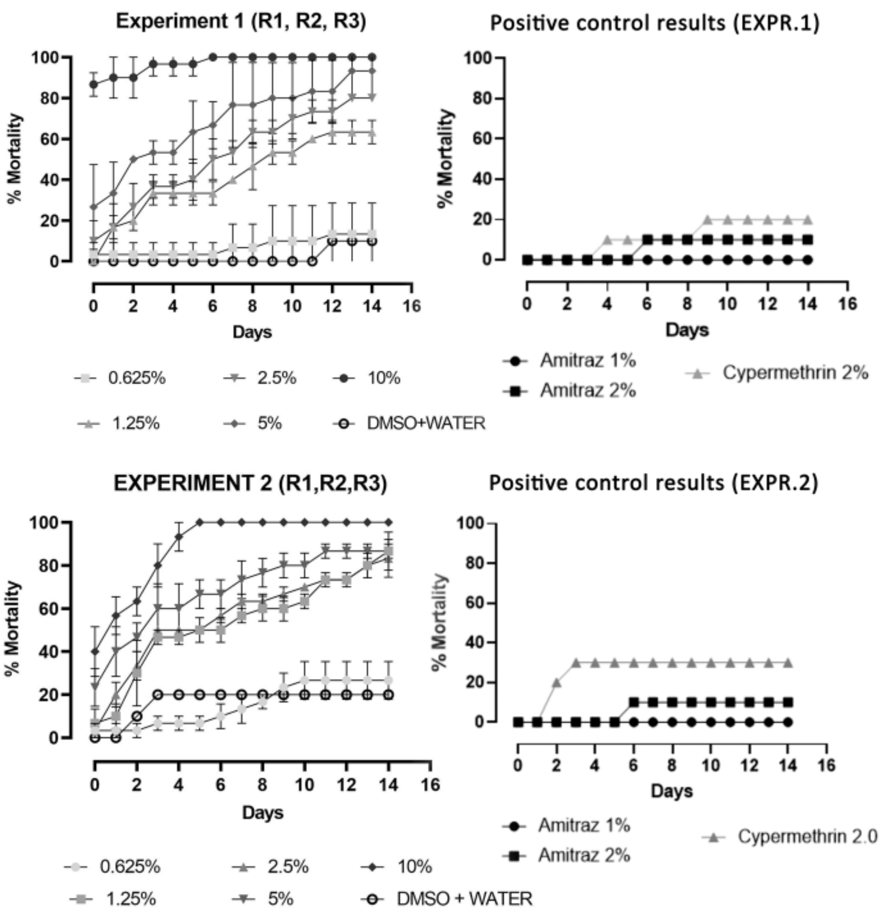

Fig. 2 Mean (± SE) mortality (%) of engorged female *Rhipicephalus* (*Boophilus*) *annulatus* ticks exposed to various concentrations of *Eucalyptus globulus* essential oil and 1 or 2% amitraz and 2% cypermethrin (as positive controls), in two replicate experiments

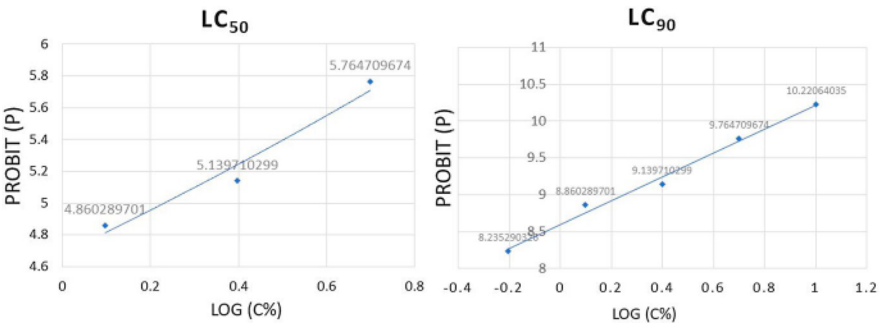

Fig. 3 Lethal concentration causing 50% (LC_{50}) and 90% (LC_{90}) mortality of engorged female *Rhipicephalus* (*Boophilus*) *annulatus* ticks treated with various concentrations of *Eucalyptus globulus* essential oil

Table 3 Mean (±SEM) adult mortality within 14 days (MAM, %), reproductive index (RI), inhibition of oviposition (IO, %) and larvae hatching (LH) of engorged female *Rhipicephalus* (*Boophilus*) *annulatus* ticks treated with various concentrations of *Eucalyptus globulus* essential oil, two positive controls or a negative control (2% DMSO in distilled water)

Test sample	Conc. (%)	MAM ± SEM (%)	Egg mass (mg)	RI ± SEM	IO (%)	LH
Eucalyptus globulus essential oil	10	90.0 ± 1.6a	0.00	0.0 ± 0.0	100	Absent
	5	67.5 ± 7.9b	50.00	0.05 ± 0.0	60	Present
	2.5	51.6 ± 3.9c	83.33	0.1 ± 0.1	45	Present
	1.25	40.8 ± 2.6d	51.25	0.3 ± 0.1	35	Present
	0.625	20.7 ± 2.4e	73.33	0.7 ± 0.1	25	Present
Amitraz (pos. control)	2	12 ± 0.0e	71.43	0.3 ± 0.0	0	Present
Cypermethrin (pos. control)	2	27 ± 5.2e	74.70	0.2 ± 0.1	10	Present
Negative control		10 ± 0.0	100	0.3 ± 0.0	0	Present

Means within a column followed by different letters are statistically significant ($P < 0.05$)

3 and 50% on day 14 PI. At 0.625% concentration the lowest mortality was recorded, of less than 20% on day 14 PI.

All the concentrations of *E. globulus* EO caused higher mortality than the positive controls (Fig. 2). Amitraz (1%) produced no mortality as all the ticks were alive at day 14 PI, whereas 2% amitraz caused 10% mortality on day 6 PI. Cypermethrin (2%) presented a higher mortality achieving 30% mortality on day 3 PI. The LC_{50} and LC_{90} of *E. globulus* EO determined by applying regression equation analysis to the probit transformed data of mortality was 5% (0.7) and 10% (1.0), respectively (Fig. 3).

Effect of essential oil on oviposition and hatching

The results of the effect of *E. globulus* EO on oviposition and egg hatching of engorged female *R.* (*B.*) *annulatus* ticks are presented in Table 3. The 10% concentration of *E. globulus* EO caused 100% inhibition of oviposition (IO) as most of the ticks had died before day 3 PI and did not lay any eggs before dying. The 5% concentration caused 57% IO, with the few surviving females laying only a few eggs with a much reduced weight. At 2.5%, treated ticks exhibited a 46.4% drop in quantity and weight of eggs. The 1.25% concentration of

E. globulus achieved transient reduction in oviposition (on average 36.5%), whereas the 0.625% concentration achieved good oviposition (Table 3).

Discussion

The EO from the fresh *E. globulus* leaves in Nigeria was straw colored with a pleasant, menthol-like odour. This is similar to the EO from the fresh *E. globulus* leaves in Algeria which was reported to be yellow in color with a camphor-like, pleasant odor (Atmani-Merabet et al. 2018). The extraction yield of the EO obtained in our study was 1.6%. This was higher than the yield of *E. globulus* reported in Morocco (1.21%), North central Nigeria (0.96%) and Algeria (0.93%) (Derwich et al. 2009; Akolade et al. 2012; Atmani-Merabet et al. 2018). However, it was lower than the yield of *E. globulus* reported in Brazil (3.1%) (Mossi et al. 2011). The disparity of yields of the same plant species grown in different countries can be linked to genetic factors, environmental factors, time/season of collection and extraction method.

Essential oils are complex mixtures of volatile and lipophilic liquid compounds which consist of terpenes, mainly monoterpenes and sesquiterpenes, and phenylpropanoids (Camilo et al. 2017). In this study, the main compounds identified in the *E. globulus* EO grown in Southwest Nigeria are eucalyptol (1,8-cineole) (78%), menthol (20%) and menthone (3%). 1,8-Cineole was also isolated as the major compound in *E. globulus* grown in Algeria and has been identified as a major acaricidal agent around the world (Djebir et al. 2019). Also *E. globulus* EO samples from Brazil and Australia showed a high amount of 1,8-cineole, with an average of 85 and 90%, respectively (Chagas et al. 2002; Yang et al. 2004). In contrast, analysis of *E. globulus* EO samples from Argentina displayed a moderate percentage (60%) of 1,8-cineole (Lucia et al. 2009), whereas those of Morocco, Kenya and North central Nigeria gave low percentages of 22.4, 17.2 and 2.5% of 1,8-cineole, respectively (Derwich et al. 2009; Akolade et al. 2012; Karemu et al. 2013). This variation denotes the existence of several chemotypes of the plant species as previously reported by Barbosa et al. (2016).

In this study, the acaricidal and inhibition of oviposition activity of *E. globulus* EO was studied against engorged female *R.* (*B.*) *annulatus* ticks. The acaricidal activity of *E. globulus* was highest at 10% concentration, which caused 100% mortality. This agrees with the study by Pirali-Kheirabadi et al. (2009) in Iran, who reported a dose-dependent ovicidal, larvicidal and adulticidal activity of *E. globulus* EO against *R.* (*B.*) *annulatus*. Their mortality rates were much lower as 5% concentration caused mortality of 16.7 and 37.6% on days 1 and 6 PI, respectively. Several other studies have reported the acaricidal and insecticidal activities of *E. globulus* EO against various species of mites, ticks and mosquitoes (Prates et al. 1998; Roh et al. 2013; Hussein et al. 2013; Dehghani-Samani et al. 2015; Immediato et al. 2016; Madreseh-Ghahfarokhi et al. 2019). The high percentage of 1,8-cineole present in *E. globulus* in this study may be responsible for the acaricidal activity seen. It may act by inhibiting cytochrome P450 enzymes or interfering with biochemical and physiological functions such as inhibition of acetylcholinesterase, antagonism with the receptors of the octopamine neurotransmitter and the closure of the GABA chloride channels of the parasite (Isman and Machial 2006; Badawy et al. 2010; Marcic 2012; Regnault-Roger et al. 2012).

According to FAO (2004), a potential acaricide tested using AIT should have a delineating effect on the egg production efficiency (oviposition capacity) of treated engorged female ticks. Two acaricides of different classes (pyrethroid and formamidine) and mechanisms of action (sodium ion blockage and octopamine receptor agonist, respectively), were used as positive controls for this study. The low efficacy of the commercial acaricides in this study may be associated with the development of resistance to these molecules in our local populations of *R.* (*B.*) *annulatus* in Nigeria. Possibly, the misuse of acaricides such as application of sub-lethal doses, sequential use of same chemical group for long periods and short intervals between treatments, has favored the development of resistance (Akande et al. 2020).

Conclusion and recommendations

This study further confirms the efficacy of *E. globulus* EO in reducing oviposition and hatchability in engorged female *R.* (*B.*) *annulatus*, a widely distributed tick species with an important role in zoonosis. As the EO of *Eucalyptus* species has been placed under GRAS (generally regarded as safe) category by the U.S. Food and Drug Administration (FDA) (Vuong et al. 2015), it is a good candidate for further studies. It is necessary to determine the structure–activity relationship of compounds associated with acaricidal activity, where many compounds that act on the same target or mechanism of action can be synergistically combined and improved. In addition, the need for chemical standardization and quality control of the plant species to overcome some challenges like long-term stability, storage and transportation cannot be overemphasized. New advances in the formulation of acaricides, such as microencapsulation and nanoformulation, could provide clues to solving these problems.

Author contributions OTA designed the experiment and wrote the manuscript, AOA and TS carried out the experiment and data analysis, FAA contributed to data analysis and finalizing the manuscript and HL did the EO and GC–MS analysis.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Consent for publication All authors gave their consent for publication.

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