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Chemical composition of garlic wood (*Gallesia integrifolia*) (Phytolaccaceae) volatile compounds and their activity on cattle tick

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Abstract

The objective of this study was to evaluate the acaricidal and larvicidal activities of essential oil (EO) from fruits, leaves and flowers of garlic wood on cattle tick [*Rhipichephalus* (*Boophilus*) *microplus*]. The fruits were harvested from May to June (2015) and the leaves and flowers in December (2015). The EO was obtained by hydrodistillation (2h) and identified by GC/MS. Bioassays consisted of Adult Immersion Test and Larval Immersion Test. The results made the following major compounds evident: 2,3,5-trithiahexane (35.29%) in fruits, dimethyl sulfide (42.42%) in leaves and methanethiol (44.91%) in flowers. The EOs from fruits, leaves and flowers showed high activity on the tick larval cycle, presenting $LD_{99.9}$ of (0.23 ± 0.01 mg/mL), (2.15 ± 0.11 mg/mL) and (0.08 ± 0.00 mg/mL), respectively. However, when we compared different phases of cattle tick's live cycle, EO from fruits was more active on females' mortality, and EO from leaves was more efficient on the egg hatching inhibition, whereas EO from flowers presented better results on bovine tick larva. Thus, garlic wood (*Gallesia integrifolia*), a native plant of the Atlantic forest can be considered as a promising natural agent to control bovine tick.

Keywords: Garlic wood, 2,3,5-trithiahexane, dimethyl sulfide, methanethiol, *Rhipichephalus* (*Boophilus*) *microplus*, dynamic *headspace*.
Abbreviations: GC/MS_gas chromatographer coupled to mass spectrometer; LD_lethal dose; $LD_{99.9}$_lethal dose to eliminate 99.9% of larvae and ticks, OE_essential oil.

Introduction

Brazil has the greatest commercial cattle herd in the world with more than 200 million heads. However, the productivity could be greater if it were not affected by *Rhipichephalus* (*Boophilus*) *microplus*, which causes big losses to livestock (Cerri et al., 2015). Cattle is this parasite's main host and the principal economic losses are related to the low feed to gain ratio, chronic loss, reduction of milk and beef production, decrease in leather quality, skin lesions that favor the development of myiases, anemia and transmission of pathogens, leading to the animal's death (Alvaréz et al., 2008). One of the strategies to minimize these losses involves the reduction of tick populations to economically acceptable levels (Martins et al., 2002). Tick control is based on the utilization of chemical products such as pyrethroids, organophosphates and avermectins. However, the excessive use of these products has led to the development of ticks resistant to these acaricides (Kafle et al., 2012). Moreover, commercial acaricides are toxic to human and can contaminate the environment and leave residues in beef and milk once they are generally sprayed or injected in the animals (Furtado et al., 2013). In order to minimize these problems, an alternative to substitute these chemical products is the use of natural products such as extracts and essential oils (EO) from plants, which are becoming quite promising, contributing to intensify research studies and technologies that promote sustainable development (Souza et al., 2010). In this context, the Atlantic Forest is considered as one of the greatest biodiversity centers worldwide, which presents a great variety of aromatic and medicinal species, and among them garlic wood (pau d'alho in Portuguese). It is a native plant of South America that is found in Peru and Brazil, from Ceará to Paraná (Sambuichi, 2009) which stands out due to its broad application in popular medicine due to its alliaceous characteristic (Lorenzi, 2002).

Botanically, garlic wood is called *Gallesia integrifolia* (SPRENG.) Harms, because it was first described by Sprengel in 1821, and named *Thouinia integrifolia* Spreng.; however, this species presents scientific synonym's such as *Gallesia gorarema*, *Gallesia gorazema* (Vell.) Moq., *Crataeva gorazema* Vell. (Akisue et al., 1986). This large

species belongs to Phytolaccaceae family and when it is green, it characteristically exhales a strong alliaceous smell which is peculiar compared to all other plants (Duringan et al., 1997). The plant leaves are elliptical and shiny, flowers are bunchy and white which bloom from February to April. Its elongated samara-like fruits become brownish when ripening from June to October; and it is difficult to separate the seed from the fruit (Akisue et al., 1986; Duringan et al., 1997). *Gallesia integrifolia* also produces wood for several uses. It has characteristic smell of garlic when green and, therefore, it is popularly named garlic wood. The smell vanishes when the wood gets dry, and then the wood becomes durable and is used as a substitute of pine (Maia et al., 2013).

Thus, this study aimed to evaluate the acaricidal and larvicidal activity of garlic wood EO from fruits, leaves and flowers on *R.* (*B.*) *microplus* and to determine the chemical composition of *in natura* vegetal material by dynamic *headspace* technique.

Results

Physical and chemical indices

The results obtained for the refraction index and the relative density of garlic wood EO from fruits, leaves and flowers are shown in Table 1.

Chemical composition of garlic wood essential oil

The chemical identification was done by GC/MS using dynamic *headspace* technique. *In natura* fruits of garlic wood were utilized in chemical analysis and 44 compounds were eluted and 43 were identified (Table 2). Among them, the major compounds are: methanethiol (16.26%), dimethyl sulfone (9.08%), 2,3,5-trithiahexane (35.29%), 2,3,5,6-tetrathiaheptane (5.22%) and 3,6-dithiaoctan-1,8-diol (20.89%) (Fig 1).

In the chemical identification of *in natura* garlic wood leaves, 44 compounds were identified (Table 3), and the ones that stood out as major compounds were: dimethyl sulfide (42.42%), 3-methylbutanal (40.43%), ethanol, 2-(methylthio) (5.52%) and α-terpinolene (8.63%) (Fig 2).

The chemical identification was also done for *in natura* garlic wood flowers which represented 44 compounds (Table 4). The major compounds were: methanethiol (44.91%), dimethyl sulfide (43.72%) and 2-butanamine (4.70%) (Fig 3).

Acaricidal and larvicidal activity on *Rhipichephalus* (*Boophilus*) *microplus*

The results obtained for acaricidal activity of garlic wood EO from fruits, leaves and flowers on *R. (B) microplus* are shown on Tables 5, 6 and 7, respectively.

The inhibition of egg hatching is one of the relevant parameters to measure the acaricidal activity of Eos. This parameter allows acaricide control. Evaluation of garlic wood EOs from fruits, leaves and flowers verified that EO from leaves presented greater egg hatching inhibition (56.99%) at the concentration of 10.00 mg/mL (Table 6).

According to the Ministry of Agriculture (Brasil, 1990), for a product to be considered efficient, the value determined for the product efficiency (%) should be 95.00% minimum. Thus, garlic wood EO from fruits, leaves and flowers presented product efficiency (93.97, 93.97 and 98.67%), respectively, at the concentration of 100.00 mg/mL.

The results of lethal doses (LDs) of garlic wood EO from fruits, leaves and flowers needed to eliminate 50% (LD_{50}) and 99.9% ($LD_{99.9}$) of ingurgitated female ticks and larvae (Table 8).

Evaluation of garlic wood EO from fruits, leaves and flowers on female mortality verified that EO from fruits had greater action with $LD_{99.9}$ (251.59 mg/mL). However, the evaluation of the effect of garlic wood'd EO from fruits, leaves and flowers on tick larvae showed that EO from flowers provided greater larvicidal activity with $LD_{99.9}$ (0.08 mg/mL).

Discussion

Physical and chemical indices

Analysis of relative density of garlic wood EO from fruits, leaves and flowers verified that there was no significant difference among the results which presented values with greater density than water (d = 0.99 g/mL at the temperature of 20 °C) (Constantino et al., 2004)(Table 1). These results are in accordance with those observed during the hydrodistillation procedures of garlic wood EO from fruits, leaves and flowers when the EO was accumulated in the bottom end of the condenser, which meant it is denser than water. The refraction index is defined as the relation between the light speed in the vacuum and its speed in the substance, and is one of the physical and chemical parameters that characterizes EO of a specific species (Farmacopeia, 1988; Simões et al., 2010). In that parameter, the EO from fruits, leaves and flowers did not present significant difference as well (Table 1). The evaluation of physical and chemical indices of EO is considered fundamental for the industry since the parameters for the EO quality are considered. Moreover, the refraction index and the relative density allow the detection of EO adulterations (Gil, 2007).

Analysis of chemical compounds by dynamic headspace

The presence of natural sulfur products is a chemotaxonomic characteristic of the species of Phytolacacceae family. These compounds, volatile organophosphates also present in onions (Schünemann et al., 2006; De Souza et al., 2015) are related to the characteristic odor of garlic and are formed by the action of alinase enzymes (Block, 1992). The presence of these sulfur compounds was identified in the chemical composition obtained by dynamic *headspace* of fruits, leaves and fresh flowers of garlic wood (Tables 1, 2 and 3). It was also verified in garlic wood EO from fruits, leaves and flowers obtained by hydro distillation.

There are no studies that report the chemical composition obtained by dynamic *headspace* utilizing fruits, leaves and flowers of *in natura* garlic wood; however, some of the volatile sulfur compounds found in this study had already been identified.

The vegetal cell is able to biosynthesize a variety of metabolites that are important to the development, adaptation and protection of the plant. These metabolites are utilized in the pharmaceutical, food and biofuel industries. However, some secondary metabolites may have a sulfur atom in their molecule such as the amino acids methionine and cysteine, which provide the synthesis of organosulfur compounds through thermal or enzymatic processes (Kyung and Lee, 2001; Dewick, 2002); therefore, they are able to direct the synthesis of organosulfur compounds.

Therefore, methionine an amino acid found in Phytolaccaceae species (Dewick, 2002), degrades by thermal process and produces methional, which in turn degrades itself

Table 1. Physical and chemical indices of the essential oil of fruits, leaves and flowers of *Gallesia integrifolia*.

Essential Oil	Physical and Chemical Indices	
	Refraction Index	Relative Density (g/mL)
	n_D^{20}	d_{20}^{20}
Fruit	1.6205 [a]	1.48 [a]
Leaf	1.6075 [a]	1.45 [a]
Flower	1.6224 [a]	1.37 [a]

Means followed by the same letter do not differ from each other by the Scott-Knott test p (0.05).

Table 2. Chemical composition obtained from fruits *in natura* of *Gallesia integrifolia* by dynamic *headspace*.

Peak	[b]Compounds	Area (%)	Identification method
1	Methanethiol	16.26	a,b
2	Dimethyl sulfide	1.42	a,b
3	Dimethyl sulfoxide	0.36	a,b
4	3-methylbutanal	0.68	a,b
5	Dimethyl disulfide	1.06	a,b
6	Dimethyl sulfone	9.08	a,b
7	2-ethylfuran	0.45	a,b
8	Hexanal	0.23	a,b
9	Santene	t	a,b
10	2,4-dithiapentane	0.35	a,b
11	Artemesia triene	t	a,b
12	*α*-pinene	t	a,b
13	*α*-fenchene	t	a,b
14	Camphene	t	a,b
15	Sabinene	t	a,b
16	Isolimonene	t	a,b
17	Dimethyl thiosulfonate	3.49	a,b
18	Dimethyl trisulfide	0.22	a,b
19	Pentylfuran	0.33	a,b
20	*p*-mentha-1,8-diene	t	a,b
21	*α*-terpinene	t	a,b
22	*γ*-terpinene	t	a,b
23	*cis*-linalool oxide	t	a,b
24	*trans*-linalool oxide	t	a,b
25	Terpinolene	t	a,b
26	Linalool	0.33	a,b
27	2,3,5-trithiahexane	35.29	a,b
28	*trans*-*p*-menth-2-en-1,8-diol	t	a,b
29	*α*-terpineol	t	a,b
30	Trithiometoxymethane	0.39	a,b
31	2,8-dithianonane	t	a,b
32	3,5-dithiahexanol-5,5-dioxide	t	a,b
33	*γ*-terpineol	t	a,b
34	*α*-endo-fenchylacetate	t	a,b
35	2,3,5,6-tetrathiaheptane	5.22	a,b
36	Isobornyl acetate	0.64	a,b
37	L-methionine, ethyl ester	t	a,b
38	Thymol	t	a,b
39	*β*-ionone	t	a,b
40	3,6-dithiaoctan-1,8-diol	20.89	a,b
41	n.i.	t	a,b
42	N-ethyl-1,3-dithioisoindole	1.05	a,b
43	5-methyl-2-phenylindole	0.42	a,b
44	1,3-dimethyl-4-azaphenanthrene	1.82	a,b

[a]**MS** identification based on comparison of mass spectra using Wiley 275 libraries. [b]Compounds listed in order of elution in column HP-5MS. n.i. = not identified. t = traces. **Area** (%): percentage (%) of the area occupied by compounds within the chromatogram.

(Table 2) and flowers (Table 4) of garlic wood, which can be transformed into dimethyl disulfide in the presence of oxygen (Schutte and Teranishi, 1974). Through a transfer reaction of the methyl group, it can also form dimethyl sulfide (Fig 2 and 3) (Lomans et al., 2002), one of the major compounds in leaves (Table 3) and flowers (Table 4) of garlic wood (Fig 4). Sulfur compounds are found in food and beverages and can have an attractive or repulsive role (Landaud et al., 2009), as methanethiol, a very important compound for the aroma of some cheeses such as cheddar (Burbank and Qian, 2005). On the other hand, it is one of the compounds that contribute to halitosis produced by periodontal bacteria, *Porphyromonas gingivalis* (Yoshimura et al., 2000).

Methanethiol alongside with dimethyl sulfide are the main contributors of unpleasant odors that are developed by cruciferous vegetables such as broccolis, cabbage, radish and so on, which result in shorter shelf life to the food and decrease consumers acceptance (Engel et al., 2002).

Furthermore, dimethyl sulfide is released in significant amounts with other compounds as a volatile compound of paprika (*Capsicum annuum*) (Cremer and Eichner, 2000). It is also found in onion and garlic but it not important for the taste formation of these foods (Hattula and Granroth, 1974).

Table 3. Chemical composition obtained from leaves *in natura* of *Gallesia integrifolia* by dynamic *headspace*.

Peak	[b]Compounds	Area (%)	Identification method
1	Dimethyl sulfide	42.42	a,b
2	3-methylbutanal	40.43	a,b
3	Ethanol, 2-(methylthio)	5.52	a,b
4	Methyl disulfide	t	a,b
5	Dimethyl sulfone	t	a,b
6	2-ethylfuran	t	a,b
7	2,4-dithiapentane	t	a,b
8	Propanoic acid, 3-(methylthio)	t	a,b
9	2,2-thiodiethanol	t	a,b
10	Methyl (methylsulfinyl) methyl sulfide (FAMSO)	t	a,b
11	Santene	t	a,b
12	Tricyclene	t	a,b
13	Artemesia triene	t	a,b
14	Thujene	t	a,b
15	*α*-pinene	t	a,b
16	*α*-fenchene	t	a,b
17	Camphene	t	a,b
18	Sabinene	t	a,b
19	*β*-pinene	t	a,b
20	Myrcene	t	a,b
21	2-carene	t	a,b
22	*α*-phellandrene	t	a,b
23	3-carene	t	a,b
24	Terpinene	t	a,b
25	Limonene	t	a,b
26	*β*-phellandrene	t	a,b
27	*cis*-ocimene	t	a,b
28	*trans*-ocimene	t	a,b
29	*γ*-terpinene	t	a,b
30	*α*-terpinolene	8.63	a,b
31	Sabinene hydrate	t	a,b
32	Linalool	t	a,b
33	L-methionine	t	a,b
34	2,7-dithiaoctane	t	a,b
35	Trithiometoxymethane	t	a,b
36	Myrtenol	t	a,b
37	Damascenone	t	a,b
38	*trans*-farnesene	t	a,b
39	*cis*-farnesol	t	a,b
40	*trans*-farnesol	t	a,b
41	3,5-dithiahexanol-5,5-dioxide	t	a,b
42	Ethanol, 2-(octylthio)	t	a,b
43	1,3-dimethyl-4-azaphenanthrene	t	a,b
44	5-methyl-2-phenylindole	t	a,b

[a]MS identification based on comparison of mass spectra using Wiley 275 libraries. [b]Compounds listed in order of elution in column HP-5MS. t = traces. Area (%): percentage (%) of the area occupied by compounds within the chromatogram.

Dimethyl sulfide is the most abundant sulfur compound in beer, superior to the taste threshold of 30-45 µL. In levels less than 100.00 µL it contributes to the flavor characteristic of *lager* beer. However, in great amounts provides an unpleasant flavor of corn (Hansen, 1999). In Porto wine, aged samples developed aromas related to the presence of dimethyl sulfide (Silva Ferreira et al., 2003).

It is possible to obtain dimethyl sulfone from dimethyl sulfide (Fig 1), a major compound in *in natura* garlic wood fruits (Table 2). The process of dimethyl sulfone formation is possible because sulfides are easily oxidized into sulfones (Vollhardt and Schore, 2013) (Fig 5). Dimethyl sulfone is a compound that presents a simple structure and has already been isolated as a natural product in *Cladonia deformis* Hoffm liquen (Brunn and Sörensen, 1954). Moreover, dimethyl sulfone was also found in EO and extracts from garlic wood leaves (Barbosa et al., 1997).

Two aliphatic polysulfide were found as major ones in *in natura* garlic wood fruits (Table 1), 2,3,5-trithiahexane and 2,3,5,6-tetrathiaheptane (Fig 1), which have already been identified in EO from garlic oil leaves (Fraga et al., 2006). There are studies that reported the presence of 2,3,5-trithiahexane in cheeses, broccolis, cooked cabbage, cauliflower, *Lentinus edodes* Sing, Beaufort and Camembert and also in hop oil, which in latter was produced by the oxidation of another sulfur compound in the presence of methanethiol (Moir et al., 1990).

However, the EO of a plant has a great variety of compounds such as terpene hydro carbonates, aldehydes, amines among others (Lima et al., 2003; Simões et al., 2010). In *in natura* garlic wood leaves (Table 3), *α*-terpinolene was found (Fig 2), which is a compound belong to the terpene hydrocarbonate class. The flavor and aroma of fruits are related (Campbell-Platt, 2015) to this compound. Also, 3-methylbutanal was found (Fig 2), an aldehyde that is also

Table 4. Chemical composition obtained from flowers *in natura* of *Gallesia integrifolia* by dynamic *headspace*.

Peak	[b]Compounds	Area (%)	Identification method
1	Methanethiol	44.91	a,b
2	Dimethyl sulfide	43.72	a,b
3	2-butanamine	4.70	a,b
4	3-methylbutanal	t	a,b
5	Methyl disulfide	t	a,b
6	Propanenitrile, 3-(methylthio)	t	a,b
7	2,4-dithiapentane	t	a,b
8	Santene	t	a,b
9	Tricyclene	t	a,b
10	Artemesia triene	t	a,b
11	Thujene	t	a,b
12	Dimethyl trisulfide	t	a,b
13	*α*-pinene	t	a,b
14	*α*-fenchene	t	a,b
15	Camphene	t	a,b
16	Sabinene	t	a,b
17	*β*-pinene	t	a,b
18	*trans*-isolimonene	t	a,b
19	Myrcene	t	a,b
20	2-carene	t	a,b
21	3-carene	t	a,b
22	Limonene	t	a,b
23	*β*-phellandrene	t	a,b
24	*cis*-ocimene	t	a,b
25	*trans*-ocimene	t	a,b
26	*γ*-terpinene	2.25	a,b
27	*α*-terpinolene	t	a,b
28	Linalool	t	a,b
29	Sabinene hydrate	t	a,b
30	Myrtenol	t	a,b
31	Safranal	t	a,b
32	Sabinyl acetate	t	a,b
33	Neryl acetate	t	a,b
34	Elemene	t	a,b
35	*β*-caryophyllene	t	a,b
36	*α*-humulene	t	a,b
37	2,3,5-trithiahexane	t	a,b
38	Bisabolene	t	a,b
39	Caryophyllene oxide	t	a,b
40	*β*-sinensal	t	a,b
41	*cis-trans*-farnesol	t	a,b
42	*α*-sinensal	t	a,b
43	N-ethyl-1,3-dithioisoindole	3.69	a,b
44	3,4-dimetoxy-dl-phenylalamine	0.73	a,b

[a]**MS** identification based on comparison of mass spectra using Wiley 275 libraries. [b]Compounds listed in order of elution in column HP-5MS. t = traces. **Area** (%): percentage (%) of the area occupied by compounds within the chromatogram.

Table 5. Averages ± standard error of female mortality (%), egg mass (g), hatchability (%), reproductive efficiency (%) and product efficiency (%) in engorged *Rhipicephalus (Boophilus) microplus* females by Adult Immersion Test of essential oil (EO) of the fruits of *Gallesia integrifolia*.

Concentration (mg/mL)	Mortality females (%)	ofMass of females (g)	Egg mass (g)	Hatchability (%)	Product efficiency (%)
PC	100.00^{a}± 0.00	0.19^{a}± 0.02	0.00^{a}± 0.00	0.00^{a}± 0.00	100.00^{a} ± 0.00
300.00	100.00^{a} ± 0.00	0.19^{a} ± 0.05	0.00^{a} ± 0.00	0.00^{a} ± 0.00	100.00^{a} ± 0.00
200.00	96.67^{a} ± 3.33	0.17^{a} ± 0.04	0.0002^{a} ± 0.001	3.35^{a} ± 0.35	100.00^{a} ± 0.00
100.00	53.33^{b} ± 14.53	0.19^{a} ± 0.04	0.02^{b} ± 0.02	29.55^{b} ± 7.20	93.97^{a} ± 3.34
60.00	43.33^{c} ± 8.82	0.19^{a} ± 0.02	0.03^{b} ± 0.02	46.11^{b} ± 18.85	81.92^{b} ± 9.27
20.00	16.67^{d} ± 6.67	0.19^{a} ± 0.03	0.05^{c} ± 0.02	74.77^{c} ± 8.24	64.30^{b} ± 6.50
10.00	13.33^{d} ± 3.33	0.18^{a} ± 0.03	0.06^{c} ± 0.02	77.06^{c} ± 3.37	47.92^{c} ± 6.63
5.00	13.33^{d} ± 6.67	0.18^{a} ± 0.02	0.07^{c} ± 0.02	76.63^{c} ± 6.52	40.80^{c} ± 9.50
2.50	10.00^{d} ± 5.77	0.14^{a} ± 0.04	0.05^{c} ± 0.03	78.48^{c} ± 6.41	39.05^{c} ± 13.24
1.25	10.00^{d} ± 5.77	0.17^{a} ± 0.03	0.08^{d} ± 0.02	81.40^{c} ± 6.10	26.15^{d} ± 9.06
0.62	10.00^{d} ± 0.00	0.15^{a} ± 0.03	0.08^{d} ± 0.02	83.50^{c} ± 3.86	18.43^{d} ± 1.75
0.31	10.00^{d} ± 0.00	0.17^{a} ± 0.03	0.07^{d} ± 0.02	85.64^{c} ± 2.52	26.06^{d} ± 6.04
0.16	6.67^{d} ± 3.33	0.16^{a} ± 0.02	0.07^{d} ± 0.02	85.91^{c} ± 3.46	25.07^{d} ± 1.53
0.08	0.00^{d} ± 0.00	0.15^{a} ± 0.02	0.07^{d} ± 0.02	88.58^{c} ± 3.68	18.32^{d} ± 4.21
0.04	0.00^{d} ± 0.00	0.16^{a} ± 0.03	0.09^{d} ± 0.02	89.02^{c} ± 4.67	9.89^{d} ± 3.67
0.02	0.00^{d} ± 0.00	0.17^{a} ± 0.02	0.09^{d} ± 0.01	93.99^{c} ± 1.32	4.28^{d} ± 1.66
NC	0.00^{d} ± 0.00	0.20^{a}± 0.02	0.09^{d}± 0.01	100.00^{c}± 0.00	0.00^{d} ± 0.00

PC = positive control (0.125% commercial solution containing 15.00% cypermethrin, 25.00% chlorpyrifos and 1.00% citronellal); NC = negative control (polysorbate (80) at 2.00% solution). Means followed by the same letter do not differ from each other by the Scott-Knott test p (0.05).

Table 6. Averages ± standard error of female mortality (%), egg mass (g), hatchability (%), reproductive efficiency (%) and product efficiency (%) in engorged *Rhipicephalus (Boophilus) microplus* females by Adult Immersion Test of essential oil (EO) of the leaves of *Gallesia integrifolia*.

Concentration (mg/mL)	Mortality of females (%)	Mass of females (g)	Egg mass (g)	Hatchability (%)	Product efficiency (%)
PC	100.00^{a}± 0.00	0.19^{a}± 0.02	0.00^{a}± 0.00	0.00^{a}± 0.00	100.00^{a} ± 0.00
500.00	100.00^{a} ± 0.00	0.17^{a} ± 0.02	0.00^{a} ± 0.00	0.00^{a} ± 0.00	100.00^{a} ± 0.00
400.00	100.00^{a} ± 0.00	0.17^{a} ± 0.02	0.00^{a} ± 0.00	0.00^{a} ± 0.00	100.00^{a} ± 0.00
300.00	93.33^{a} ± 3.33	0.17^{a} ± 0.02	0.00^{a} ± 0.00	0.00^{a} ± 0.00	100.00^{a} ± 0.00
200.00	86.67^{a} ± 8.82	0.16^{a} ±0.03	0.01^{a} ± 0.01	5.49^{a} ± 0.00	100.00^{a} ± 0.30
100.00	63.33^{b} ± 6.67	0.16^{a} ± 0.03	0.03^{b} ± 0.02	18.86^{b} ± 2.65	93.97^{a} ± 2.00
60.00	40.00^{c} ± 20.00	0.16^{a} ±0.03	0.01^{a} ± 0.01	24.82^{b} ± 6.34	81.92^{b} ± 1.93
20.00	36.67^{c} ± 6.67	0.16^{a} ± 0.02	0.03^{b}± 0.02	31.23^{b} ± 4.60	64.30^{b} ± 2.74
10.00	30.00^{c} ± 0.00	0.16^{a} ± 0.02	0.05^{c} ± 0.01	43.01^{c} ± 7.63	47.92^{c} ± 5.82
5.00	26.67^{c} ± 17.64	0.16^{a} ± 0.03	0.05^{c} ±0.02	50.73^{d} ± 1.11	40.80^{c} ± 3.87
2.50	13.33^{d} ± 13.33	0.16^{a} ± 0.02	0.04^{c} ± 0.01	81.28^{e} ± 6.44	39.05^{c} ± 3.65
1.25	10.00^{d} ± 0.00	0.16^{a} ± 0.03	0.04^{c} ± 0.02	83.30^{e} ± 7.57	26.15^{d} ± 4.52
0.62	10.00^{d} ± 10.00	0.17^{a} ± 0.02	0.04^{c} ± 0.02	82.16^{e} ± 4.28	18.43^{d} ± 7.61
0.31	10.00^{d} ± 0.00	0.17^{a} ± 0.03	0.05^{c} ± 0.02	84.42^{e} ± 3.16	26.06^{d} ± 4.58
0.16	10.00^{d} ± 5.77	0.17^{a} ± 0.02	0.07^{c} ± 0.02	84.90^{e} ± 3.96	25.07^{d} ± 4.62
0.08	6.67^{d} ± 3.33	0.17^{a} ± 0.03	0.08^{d} ± 0.02	84.48^{e} ± 0.96	18.32^{d} ± 3.58
0.04	6.67^{d} ± 3.33	0.16^{a} ± 0.02	0.08^{d} ± 0.02	85.46^{e} ± 4.81	9.89^{d} ± 9.74
0.02	6.67^{d} ± 6.67	0.16^{a} ± 0.02	0.08^{d} ± 0.02	86.84^{e} ± 3.84	4.28^{d} ± 9.83
NC	0.00^{d} ± 0.00	0.20^{a}± 0.02	0.09^{d}± 0.01	100.00^{e}± 0.00	0.00^{d} ± 0.00

PC = positive control (0.125% commercial solution containing 15.00% cypermethrin, 25.00% chlorpyrifos and 1.00% citronellal); NC = negative control (polysorbate (80) at 2.00% solution). Means followed by the same letter do not differ from each other by the Scott-Knott test p (0.05).

Table 7. Averages ± standard error of female mortality (%), egg mass (g), hatchability (%), reproductive efficiency (%) and product efficiency (%) in engorged *Rhipicephalus (Boophilus) microplus* females by Adult Immersion Test of essential oil (EO) of the flowers of *Gallesia integrifolia*.

Concentration (mg/mL)	Mortality of females (%)	Mass of females (g)	Egg mass (g)	Hatchability (%)	Product efficiency (%)
PC	100.00^{a}± 0.00	0.19^{a}± 0.02	0.00^{a}± 0.00	0.00^{a}± 0.00	100.00^{a} ± 0.00
500.00	100.00^{a} ± 0.00	0.18^{a} ± 0.02	0.00^{a} ± 0.00	0.00^{a} ± 0.00	100.00^{a} ± 0.00
400.00	100.00^{a} ± 0.00	0.20^{a} ± 0.02	0.00^{a} ± 0.00	0.00^{a} ± 0.00	100.00^{a} ± 0.00
300.00	100.00^{a} ± 0.00	0.19^{a} ± 0.02	0.00^{a} ± 0.00	0.00^{a} ± 0.00	100.00^{a} ± 0.00
200.00	93.33^{a} ± 1.05	0.20^{a} ± 0.02	0.00^{a} ± 0.00	0.00^{a} ± 0.00	100.00^{a} ± 0.00
100.00	76.67^{b} ± 2.79	0.19^{a} ± 0.03	0.01^{a} ± 0.01	8.33^{a} ± 6.90	98.67^{a} ± 1.21
60.00	26.67^{c} ± 2.79	0.20^{a} ± 0.02	0.04^{b} ± 0.01	63.66^{b} ± 8.24	72.74^{b} ± 2.20
20.00	20.00^{c} ± 0.00	0.19^{a} ± 0.02	0.05^{b} ± 0.01	68.69^{b} ± 1.41	61.95^{c} ± 3.40
10.00	20.00^{c} ± 3.16	0.20^{a} ± 0.02	0.06^{c} ± 0.02	67.79^{b} ± 8.75	58.38^{c} ± 8.12
5.00	20.00^{c} ± 1.83	0.20^{a} ± 0.02	0.07^{c} ± 0.02	70.69^{b} ± 2.74	51.85^{c} ± 8.76
2.50	13.33^{c} ± 2.79	0.20^{a} ± 0.02	0.07^{c} ± 0.02	70.60^{b} ± 7.03	49.82^{c} ± 9.80
1.25	13.33^{c} ± 1.05	0.19^{a} ± 0.02	0.07^{c} ± 0.02	72.71^{b} ± 3.96	48.23^{c} ± 3.74
0.62	13.33^{c} ± 2.11	0.20^{a} ± 0.03	0.08^{d} ± 0.02	77.49^{b} ± 3.51	41.16^{c} ± 6.00
0.31	10.00^{c} ± 1.83	0.20^{a} ± 0.02	0.08^{d} ± 0.02	79.63^{c} ± 3.29	35.45^{d} ± 5.18
0.16	10.00^{c} ± 3.16	0.20^{a} ± 0.02	0.10^{d} ± 0.02	81.76^{c} ± 1.35	23.08^{d} ± 3.35
0.08	6.67^{c} ± 2.11	0.19^{a} ± 0.02	0.08^{d} ± 0.02	88.26^{c} ± 4.91	26.91^{d} ± 11.06
0.04	6.67^{c} ± 1.05	0.18^{a} ± 0.02	0.08^{d} ± 0.02	92.34^{c} ± 7.15	22.86^{d} ± 5.53
0.02	6.67^{c} ± 1.05	0.19^{a} ± 0.02	0.09^{d} ± 0.02	92.25^{c} ± 0.16	20.94^{d} ± 8.47
NC	0.00^{c} ± 0.00	0.20^{a}± 0.02	0.09^{d}± 0.02	100.00^{c}± 0.00	0.00^{d} ± 0.00

PC = positive control (0.125% commercial solution containing 15.00% cypermethrin, 25.00% chlorpyrifos and 1.00% citronellal); NC = negative control (polysorbate (80) at 2.00% solution). Means followed by the same letter do not differ from each other by the Scott-Knott test p (0.05).

Table 8. Averages ± standard error of Lethal Doses LD_{50} and $LD_{99.9}$ (mg/mL) and confidence interval (CI) of essential oil (EO) of fruits, leaves and flowers of *Gallesia integrifolia* on larvae and adults of the *Rhipicephalus (Boophilus) microplus* by Probitos analysis.

	EO of FRUITS		EO of LEAVES		EO of FLOWERS	
	LD_{50} (CI)	$LD_{99.9}$ (CI)	LD_{50} (CI)	$LD_{99.9}$ (CI)	LD_{50} (CI)	$LD_{99.9}$ (CI)
Mortality of Larvae	0.06^{a} ± 0.00 (0.06 – 0.07)	0.23^{A} ± 0.01 (0.22 – 0.24)	0.99^{b} ± 0.12 (0.75 – 1.12)	2.15^{B} ± 0.11 (1.95 – 2.31)	0.05^{a} ± 0.00 (0.04 – 0.05)	0.08^{A} ± 0.00 (0.08 – 0.09)
Mortality of Females	114.82^{a} ± 5.00 (104.91 – 120.88)	251.59^{A} ± 5.14 (243.00 – 260.76)	150.55^{b} ± 4.17 (144.29 – 158.45)	385.68^{B} ± 4.65 (376.49 – 391.44)	150.97^{b} ± 4.77 (141.45 – 156.08)	373.92^{B} ± 1.77 (371.15 – 377.21)

LD_{50}: Lethal Dose 50%; $LD_{99.9}$Lethal Dose 99.9%; CI: Confidence interval**.** Means followed by the same letter in the lines do not differ from each other by the Scott-Knott test p (0.05).

present in cauliflower (Van Langenhove et al., 1991). Furthermore, 2-butanamine was found (Fig 3) as one of the major compounds of *in natura* garlic word flowers (Table 4). Plants produce a lot of important amine compounds but they are known to have very unpleasant odors (Winter, 2011).

Acaricidal activity on Rhipichephalus* (*Boophilus*) *microplus

There are no studies reporting the acaricidal properties for the application of garlic wood EO from fruits, leaves and flowers. However, according to Reis et al. (2015), sulfur compounds can have acaricidal and fungicidal effects, which corroborate studies done on *Petiveria alliacea*, a plant from the same family as garlic wood and that also has sulfur compounds in its chemical composition such as dibenzyl trisulfide, which separately presented high acaricidal potential (Lyndon et al., 1997; Rosado-Aguillar et al., 2010; Kerudo et al., 2015). This effect was also confirmed in another study on *R.* (*B.*) *microplus* utilizing raw extract from *P. alliacea* stems at the concentration of 200.00 mg/mL, which showed a mortality rate of (86.60 ± 15.20%) on female ticks. Regarding the egg hatching inhibition, the raw extract from *P. alliacea* leaves provided an inhibition of (26.00 ± 5.20%) at the concentration of 100 mg/mL (Rosado-Aguillar et al, 2010).

Compared to the present study, the EO from garlic wood fruits presented a mortality rate of (96.67 ± 3.33%) on female ticks at the concentration of 200.00 mg/mL (Table 5) while the EO from leaves at the concentration of 100 mg/mL presented an inhibition of egg hatching of 81.14 ± 2.65% (Table 6), making evident that garlic wood EO was more active compared to *P. alliacea*.

Table 9 shows that EO of garlic wood provided greater activity on *R.* (*B.*) *microplus* larvae. This may have happened due to the fact that larvae presented greater vulnerability than adult. In this case, the EO could act by inhibiting chitin, the greatest compound in tick cuticle, which would prevent stage change, growth and larva hatching (Santos et al., 2015). The pest might also be killed by exhaustion, an effect caused by EO viscosity, and by asphyxiation when EO overlaps the larva body surface (Prates et al., 1993). On the other hand, in the adult stage, the EO action is more difficult because in this cycle stage the chitin layer is already completely formed, making the penetration of EO difficult.

Comparison of garlic wood EO from fruits, leaves and flowers shows that EO from fruits causes a better mortality rate on female ticks (Table 8), whereas the inhibition of egg hatching was observed utilizing EO of garlic wood leaves (Table 6).

Larvicidal activity on Rhipichephalus* (*Boophilus*) *microplus

Comparison of wood EO extracted from fruits leaves and flowers verified that EO from flowers can have better control since all larvae (100.00%) were killed at a concentration of 0.08 mg/mL (Table 8). For the larvicidal activity on *R. (B) microplus* there have not yet been studies to report this property of garlic wood EO from fruits, leaves and flowers. However, this activity was also done with raw extracts of *P. alliacea* (Phytolaccaceae) leaves and stems.

The larvicidal activity done with the raw extract of *P. alliacea* leaves showed ($LD_{99.9}$ of 12.29 mg/mL), while for the raw extract from stem ($LD_{99.9}$ of 16.52 mg/mL) (Rosado-Aguillar et al., 2010) was obtained. The results obtained by Rosado-Aguillar and collaborators (2010), compared the garlic wood EO from fruits, leaves and flowers (Table 8). It is verified that garlic wood EO (fruits, leaves and flowers) provided better results and consequently a better larvicidal activity.

Materials and Methods

Plant material

Garlic wood fruits, leaves and flowers were harvested in the city of Umuarama - PR, at the coordinates S23º46'16'' and WO53º19'38'', and 442 m of altitude. Fruits were harvested from May to June, 2015; leaves and flowers in December, 2015.

Extraction of garlic wood essential oil (EO)

Fruits, leaves and fresh flowers of garlic wood were separately utilized to extract the essential oil by hydrodistillation using a Clevenger-type apparatus for 2h. The oil was removed from the equipment with PA hexane using a Pasteur pipette and dried with anhydrous sodium sulfate (Na_2SO_4) (Simões et al., 2010), and then stored in amber flasks which were kept under refrigeration at (-4 ºC) (Omolo et al., 2004).

Physical and chemical indices of garlic wood essential oil

Relative density

The relative density of a substance is the ratio of its mass by its volume at 20 °C. It is determined according to the technique described in the Brazilian Pharmacopeia (Farmacopeia, 1988).

Refraction index

The refraction index was determined by an ABBE refractometer, model RL3, PZO Warszawa, at 20 °C (Farmacopeia, 1988).

Chemical characterization of garlic wood essential oil

Five grams of *in natura* garlic wood vegetal material (fruits, leaves and flowers) were stored in vials for dynamic headspace. The parameters for *headspace* performance were: incubation temperature (100 °C), incubation time (30 min), agitation (on for 1 min; off for 10 s), syringe temperature (150 °C) and agitation speed (500 rpm). The volatile compounds were analyzed by gas chromatography/mass spectrometry - GC/MS, using an Agilent chromatographer, model 19091S-433, coupled to an Agilent mass spectrometer, model 19091J-433. An HP-5MS 5% (30 m x 0.25 mm x 0.25 µm) analytical column was utilized with initial temperature of 60 °C and kept for 3 min. Then, the temperature raised to 300 °C with a ramp of 5 °C/min and remained in that temperature for 10 min, and finally reached 310 °C with a ramp of 10 °C/min for 10 min. The utilized carrier gas was helium with a linear velocity of 1 mL/min until 300 °C, and pressure release of 56 kPa. The injector temperature was 300 °C. The injection volume was 2 µL. The injection was done in split mode (20:1). The transfer line was kept at 285 °C and the ionization and quadrupole sources were at 230 °C and 150 °C, respectively. The detection system was EM in "Scan"' mode, in the mass/charge ratio (*m/z*) of 40-550 band, with "Solvent Delay" of 3 min. The compounds found in *in natura* vegetal material (fruits, leaves and flowers) of garlic

wood were identified by comparing its mass spectra with the mass spectra from WILEY 275 libraries (Adams, 2012).

Acaricidal and larvicidal activity of garlic wood essential oil on Rhipicephalus (Boophilus) microplus

The acaricidal activity of garlic wood essential oil from fruits, leaves and flowers were determined by *Adult Immersion Test* and *Larval Packet Test* as recommended by the Drummond et al. (1973) and Leite et al. (1995).

Harvesting of ingurgitated females

1550 *R. (B.) microplus* female ticks were randomly harvested from milk cattle with no spraying treatment against ticks for over 60 days, belonged to the Veterinary Hospital of Paranaense Univesity - UNIPAR, Umuarama - PR. The ticks were transported in a container with appropriate air circulation in the laboratory of Natural Products of UNIPAR.

Treatment groups

The ticks were selected considering the following aspects: normal appearance and motility, wholesome body and maximum ingurgitation. Then, they were cleaned with purified water (Leite et al., 1995) and were divided in groups of ten. Sixteen dilutions of EO from fruits, leaves and flowers were prepared in concentrations that varied from 500.00 to 0.02 mg/mL, utilizing polysorbate (80) at 2.00% (v/v) as emulsifier and purified water as solvent. As negative control, purified water and polysorbate (80) at 2.00% (v/v) were used, and as positive control, a commercial solution at 0.12% containing 15.00% of cipermetrine, 25.00% of chlorpyrifos and 1.00% of citronellal was utilized. The treatments were done in triplicate.

Sensitivity of ingurgitated females in adult immersion test (AIT)

According to the methodology described by Drummond and collaborators (1973), groups of 10 female ticks were weighed and immersed for 5 minutes in each treatment. They were dried in paper and distributed in a Petri dish, then were identified and labelled. The Petri dishes were stored in a styrofoam box containing moist cotton and kept for 14 days for egg laying.

After 14 days, the mass of eggs from each female tick was weighed and transferred to assay tubes appropriately (each female and its eggs). Tubes were placed in the styrofoam box containing moist cotton for 21 days to hatch.

After 21 days, the larvae were killed with sulfuric ether and counted using an entomologic loupe to verify the egg hatching rate. From data of female ticks' mass, egg mass and hatching percentage, the reproductive efficiency (RE) and product efficiency (PE) were determined by equations 1 and 2, respectively (Drummond et al., 1973).

$$RE = \frac{\text{Egg mass (g)} \times \text{Hatching rate (\%)}}{\text{Female tick mass}} \times 20.000 \quad \text{Eq.1}$$

$$PE = \frac{RE_{\text{negative control group}} \times RE_{\text{treated group}}}{RE_{\text{negative control group}}} \times 100 \quad \text{Eq.2}$$

Larvicidal activity of garlic wood essential oil on Rhipicephalus (Boophilus) microplus

The utilized technique was described by Leite and collaborators (Leite et al., 1995), in which 100 larvae were placed on 2.00 × 2.00 cm paper filter that had freshly been moistened with dilutions of EO from fruits, leaves and flowers of garlic wood, forming a sealed "sandwich", stored in a Petri dish and kept at room temperature. The reading was done after 24h when live and dead larvae were separated using an entomologic loupe.

Twenty dilutions of garlic wood EO from fruits, leaves and flowers were prepared in concentrations ranging from 10.00 to 0.0006 mg/mL, utilizing polysorbate (80) at 2.00% (v/v) as emulsifier and purified water as solvent. As negative control, purified water and polysorbate (80) at 2.00% (v/v) was utilized, whereas as positive control organophosphates from an acaricide (cipermetrine 15.00%; chlorpyrifos 25.00%; citronellal 1.00%).

The treatments were done in triplicate and the larval mortality (LM) (Equation 3) and the average larval mortality were determined.

$$LM\ (\%) = \frac{\text{Dead larvae}}{\text{Total of larvae}} \times 100 \quad \text{Eq.3}$$

Statistical analysis for the acaricidal and larvicidal activity

The tests were done in triplicate and the mortality percentage (%) of female ticks and larvae of *R. (B.) microplus* were obtained by calculating the average ± standard error utilizing the Microsoft Excel® program (Excel® Version 2010). The experimental design was completely randomized (CRD). The data were submitted to analysis of variance (ANOVA) and compared utilizing Sisvar 5.6 program by Scott-Knott's test ($p<0.05$).

Conclusion

The essential oil (EO) from garlic wood fruit, leaves and flowers showed sulfur compounds in its chemical analysis, which were active in different phases of the cattle tick's reproductive cycle. EO from garlic wood in three periods (fruit, leaves and flowers) presented greater activity in the phase that corresponds to the larval cycle. However, when compared to the other phases of the cattle tick's cycles, the EO from fruits was more active on the females' mortality and the EO from leaves was more efficient on the inhibition of egg hatching. Thus, the garlic wood EO can be considered a promising agent to combat cattle tick, once it causes losses to livestock.

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