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Acaricide activity of leaves extracts of *Sambucus australis* Schltdl. (Caprifoliaceae) at 2% on engorged females of *Rhipicephalus (Boophilus) microplus*

Atividade acaricida de extratos de folhas de *Sambucus australis* Schltdl (Caprifoliaceae) a 2% sobre teleóginas de *Rhipicephalus (Boophilus) microplus*

Felipe da Silva Krawczak[I*] Andréia Buzatti[I] Felipe Lamberti Pivoto[I] Luís Antônio Sangioni[I] Fernanda Silveira Flores Vogel[I] Sônia de Avila Botton[I] Gilberto Dolejal Zanetti[II] Melânia Palermo Manfron[II]

ABSTRACT

*Parasite infections caused by **Rhipicephalus (Boophilus) microplus** are responsible for the most of economic losses in producing-cattle countries in tropical and subtropical areas. Indiscriminate uses of chemical acaricides have contributed with the appearance of tick resistance to many drugs available in the marketplace, and it is a serious problem in the tick control. Flowers of **Sambucus australis** (South America), called "sabugueiro-from-Brazil", are used on infusions or decoctions forms in the folk medicine with diuretic, antipyretic, anti-inflammatory, and laxative purpose, also employed for treating respiratory diseases in human. The main goal of this study was to evaluate in vitro potential of **S. australis** leaves extracts as an acaricide agent. Ethanol extract at 70% has been dehydrated and a fraction was suspended in 70% ethanol or in distillated water at final concentration of 2% ($0.2mg^{-1}$). Using the immersion test of engorged females the efficiency results were obtained in 34% and 66% with the leaves extract diluted in water and 70% ethanol, respectively. This study is the first report on acaricidal activity of **S. australis** against cattle tick. Further studies to determine the active metabolites in different stages of **S. australis** could aid to identify suitable extracts to be tested in the **R. (B.) microplus** control.*

Key words: *Acaricide, crude extract, tick, **R. (B.) microplus**, **Sambucus australis**.*

RESUMO

*A infestação por **Rhipicephalus (Boophilus) microplus** é responsável por grandes perdas econômicas em países produtores de bovinos nas áreas tropicais e subtropicais. Usos indiscriminados de acaricidas químicos têm contribuído para o aparecimento da resistência dos ixodídeos a múltiplas drogas, representando um sério problema no controle de carrapatos. As flores da planta **Sambucus australis,** conhecidas pelo nome popular de sabugueiro do Brasil, são usadas, na forma de infusão ou emplastros, na medicina popular como diurético, antipirético, antiinflamatório e laxativo; também empregado no tratamento de doenças respiratórias em humanos. O objetivo principal deste trabalho foi avaliar o potencial in vitro de extratos de folhas de **S. australis**, como agente carrapaticida. O extrato etanólico a 70% foi dessecado e retomado parte em etanol a 70% ou em água destilada, numa concentração final de 2% ($0,2mg^{-1}$). No biocarrapaticidograma obteve-se resultados de eficácia entre 34% e 66% para o extrato de folhas diluído em água e etanol a 70%, respectivamente. Esse estudo é o primeiro relato de ação carrapaticida de **S. australis**. Estudos adicionais para determinar os metabólitos ativos, em diferentes estágios de **S. australis,** fornecerão subsídios para identificar os extratos apropriados a serem testados no controle do **R. (B.) microplus**.*

Palavras-chave: *Acaricida, extrato bruto, carrapato, **R. (B.) microplus**, **Sambucus australis**.*

INTRODUCTION

Rhipicephalus (Boophilus) microplus is the most important haematophagous ectoparasite of cattle (FORTES, 1993); it has larger geographic distribution and economic importance for producing-cattle countries in tropical and subtropical areas in the world. The economic losses caused by tick cattle infestation in Brazil overcome 2 billion dollars a year

[I]Departamento de Medicina Veterinária Preventiva (DMVP), Centro de Ciências Rurais (CCR), Universidade Federal de Santa Maria (UFSM), Av. Roraima, 1000, Prédio 44, Sala 5149, Bairro Camobi, 97105-900, Santa Maria, RS, Brasil. E-mail: fkrawczak@yahoo.com.br. *Autor para correspondência.
[II]Departamento Farmacognosia (DF), Centro de Ciências da Saúde (CCS), UFSM, Santa Maria, RS, Brasil.

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(GRISI et al., 2002). In addition, there are direct damages as well as decreases in the production of milk and meat (SUTHERST et al., 1983), a lot of injuries on the leather due to inflammatory reactions in the spots where the ticks were fixed on the animal skin (SEIFERT et al., 1968); besides this mite is an important carrier of infectious agents to farm animals such as ***Anaplasma*** spp. and ***Babesia*** spp., responsible for Tick Fever (GRISI et al., 2002). ***R. (B.) microplus*** is found in all Brazilian territory, being the south region the most affected by the occurrence of ticks on cattle mainly during the warmer seasons. Two factors seem to contribute significantly for this: the predominance of animals of European origin and the large population density of cattle (HORN, 1983).

In Brazil, regular acaricide usage constitutes the main control method for this tick (FARIAS, 1999; VARGAS et al., 2003). However, historically, after some time of use of the majority of the pesticides available in the marketplace, the development of a population of resistant ticks was detected (FURLONG et al., 2002; GRAF et al., 2004). The foremost issues in the selection of resistant individuals involve defects in conservation, dilution and application of the pesticide, as well as mistaken interval and application methods that lead to the use of the products in non-lethal concentration for the ticks. All of these aspects contribute directly to the emergence of ***R. (B) microplus*** resistant strains (VARGAS et al., 2003). Due to those problems, the alternative use of plants with medical potential emerges as an important choice in the parasite control. Advantages for using this control include lower prices than industrialized chemical products, it does not leaving toxic residues in the animal-food products (AVANCINI, 1994; HEIMERDINGER et al., 2006) and it is biodegradable, avoiding severe damages to the environment (ROEL, 2001). Unfortunately, researches aiming the use of plant extracts in the control of parasites are still rare. Additionally, there is a lack of further information concerning to various aspects as well as variety of plant will be usefulness, the adequate conditions for plants cultivate, the correct period of harvest, the quantity needed to produce extracts with potential therapeutic use, and the specific parts of the plants will be employed to elaborate the plant extracts for medicinal purposes (HEIMERDINGER et al., 2006).

Sambucus australis Schltdl. (Caprifoliaceae) is a native plant in Brazil and its occurrence is reported in the Northeast, Southeast and South Regions (TORRES et al., 2005). ***S. australis*** is a many-branched shrub or a small tree, up to 4.0m high, rarely more, irregular crown leaves are petiolate, opposite, composed of seven (rarely five) to 13 leaflets, oval-lanceolate form, asymmetric, membranous, 4.0 to 7.5cm long, serrated margin, acuminate at the apex, and glabrous. The flowers present white to yellowish-white color, they are small, arranged in large terminal corymbs summits morphologically monoclinic or tetramer pentamerous, and actinomorphic (NUNES et al., 2007). The flowers have been employed in popular medicine, in the form of infusion or decoction, as a diuretic, antipyretic, anti-inflammatory, laxative, diaphoretic, demulcent; as well as in the treatment of respiratory diseases, analgesic and anti-rheumatic agent (SIMÕES et al., 1988; JORGE et al., 1999; SCOPEL, 2005; NUNES et al., 2007).

The central goal of this study was to evaluate the potential acaricidal activity *in vitro* of extracts of ***S. australis*** against ***R. (B). microplus***.

MATERIAL AND METHODS

Leaves of **S. *australis*** Schltdl. were collected in Santa Maria city, Rio Grande do Sul state in Brazil. The exsiccates were deposited in the Herbarium of the Botanic Department at Federal University of Santa Maria (UFSM) as HDFI 136 code (NUNES et al., 2007). The leaves were submitted to dehydration and stabilization in an incubator at 40°C, grounded in a knife mill and stocked in an amber bottle. This plant material was macerated in ethanol at 70% to total depletion and filtered to obtain the crude extract. The leaves extract was lead to dry crude extract (DCE_L) by evaporation of the solvent into a rotary evaporator with temperature around 30ºC overnight. Later on, in order to carry the *in vitro* tests out, the DCE_L was suspended in two fractions, one in ethanol at 70% and another one in distilled water. All tests were performed with both solutions adjusted to the final concentration of 2% (0.2 mg^{-1}).

Engorged females ticks of ***R. (B). microplus*** measuring 4.5 to 7.5mm in length were manually collected of crossbred European cattle naturally infested from a rural property situated in Cacequi town, in Rio Grande do Sul state, in Brazil, during December

of 2009. The cattle received acaricidal treatment with commercial products during the summer season. The engorged females were placed in a strip cup, washed in distilled water, dried on absorbent wipes and separated in ten groups containing ten engorged females in each group. All of the ticks were selected based on the mobility and their noticeable features such as intact body and maximum of engorgement (LEITE et al., 1995). All of the engorged females were weighed in order to maintain the uniformity along with each tested group.

The groups individually were submitted to the immersion test and maintaining the liquid (20ml of solution test) in constant agitation for five minutes. The excess of solution was removed from the engorged females with absorbent wipes, and the ticks from each group were fixed on a Petri dish on a double-sided adhesive tape and kept in the incubator at 27°C and humidity of 80% per 40 days (DRUMMOND et al., 1973).

Fourteen days later the weighing of the egg mass from each group was proceeded and 0.3g was transferred from each group into test tubes. These tubes were closed with cotton and incubated in the same conditions previously described. After 26 days the percentage of hatchability was evaluated by using a stereoscope and the visual verification was applied as parameter for this test (DRUMMOND et al., 1973). Aqueous and ethanol at 70% solutions were used in the tests, using the DCE_L of the leaves of ***S. australis*** adjusted to a final concentration of 2%. As positive control (acaricide solution), aqueous solution containing Cypermethrin at 15% ($150mg^{-1}$), Chlorpyrifos at 25% ($250mg^{-1}$) and Citronella at 1% ($10mg^{-1}$) diluted at 1:1000 was employed. As control groups, distilled water and 70% ethanol solution were used. The method developed to analyze the effectiveness of the solutions used the respective control for every tested diluent.

The effectiveness tests of the plant were performed in triplicate, calculating the average of the results. The calculi of the effectiveness of the products (EP) were executed applying the formula described by DRUMMOND et al. (1973).

The statistical analysis of the observed results was carried out by the Chi-square test (SAS, 1997), in 5% level of probability ($P<0.05$).

RESULTS AND DISCUSSION

The results obtained *in vitro***,** referring to the efficacy of the treatments are represented in table 1. The effectiveness of ***S. australis*** DCE_L in aqueous solution demonstrated results ranging from 22 to 50%, with an average rate of 34% and the standard error (SE) for its value ±8.3%. The percentage of effectiveness of ***S. australis*** DCE_L at 70% ethanol solution ranged 50 to 83%, with the average of 66%, where the SE established was of ±9.5% (Table 1).The positive control used in this research presented 100% of efficacy (Table 1), indicating that this pesticide association is efficient facing the ticks of the rural property where they were collected, according to BRASIL (1990).

CLEMENTE et al. (2007) tested, in similar conditions, the *in vitro* effectiveness of the crude extract of ***Sapindus saponaria*** diluted in distilled water, in four different concentrations, and no significant values for any acaricide action on engorged females or

Table 1 - *In vitro* results of the average effectiveness ± Standard Error (SE) of the dry crude extract (DCE_L) of ***Sambucus australis*** at 2% in distilled water or ethanol at 70%, on engorged females of ***Rhipicephalus (B) microplus*** and laying average of the engorged female facing the treatments.

Treatment	Laying average (g)	Average effectiveness ± SE (%)
Aqueous solution of the DCE_L of ***S. australis*** at 2% ($0.2mg^{-1}$)	0.66	$34^b \pm 8.3$
Ethanol solution at 70% of DCE_L of ***S. australis*** at 2% ($0.2mg^{-1}$)	0.50	$66^a \pm 9.5$
Control 1: distilled water	0.85	-
Control 2: ethanol at 70%	0.87	-
Positive control: solution of Cypermethrin at 15%, Clorpyrifos at 25% and Citronella at 1% in the dilution of 1:1000.	0.11	100±0

a, b: Average followed by different letters in the column, differ by the Chi-square test ($P<0.0163$).

on larvae were observed. On the other hand, in the present study it was possible to verify any acaricidal effect of the ***S. australis*** leaves extract on the ticks, although it displayed low rates of effectiveness. HEIMERDINGER et al. (2006) obtained an effectiveness of 46.56% on the control of ***R. (B.) microplus*** in Holstein dairy cattle, through aspersion, using alcoholic extracts of ***Cymbopogon citratus*** (lemongrass). However, in that study, the concentration used it was superior (2.72%) than employed in our study (2%). In addition, both results do not demonstrate high effectiveness on ***R. (B.) microplus*** control. Conversely, MANSINGH & WILLIAMS (1998), applying *in vitro* ethanol extract of leaves of ***Simarouba glauca*** on engorged females of ***R. (B.) microplus***, achieved 100% of effectiveness. Differences in efficacy in the results could be justified by varying the concentration of active substances in the different plants that hold distinct origin and diverse extraction methods used in the extract preparation. In relation to the Chi-square test, there was a significant difference ($P<0.0163$) among the treatments employed in this study. It was possible to observe that the plant extract of ***S. australis*** diluted in ethanol at 70% was higher (66%) than the extract diluted in distilled water (34%) if they are compared to the control groups (distillated water and 70% ethanol). Probably the ***S. australis*** DCE_L effect was potentialized by the ethanol used during the extraction procedures that might enhance the concentration of active acaricidal ingredients in the plant extract.

In this study, an average hatchability obtained with ***S. australis*** DCE_L recovered in distilled water and 70% ethanol was 84% and 28% respectively (Table 2). It was possible that the effectiveness produced by the ***S. australis*** DCE_L at 70% ethanol does not apply only to the action of the plant, but also the ethanol action. SILVA et al. (2008) observed similar results studying ***Cymbopogon citratus***, indicating that the ethanol could potentiate the effect by presenting a degree of acaricidal efficacy and/or facilitating the release of the active substances of the plant extract. CHAGAS et al. (2003) evaluated the susceptibility of larvae and engorged females of ***R. (B.) microplus*** to several solvents and observed that only the ethanol at 100% led to boost of mortality rates. According to those authors, the ethanol demonstrated low toxicity; in contrast, in the present study, it was found that ethanol interfered in the hatchability of the eggs (Control 2) (Table 2). Thus, the results suggested that ethanol could potentiate the effectiveness of the treatments which it was used as solvent.

The results obtained in this study demonstrated that ***S. australis*** DCE_L presented any acaricidal effect *in vitro*. However, other studies testing different solvents and extraction procedures, several concentrations, other parts of the plant, as well as further *in vivo* experiments are required. Additionally, phytochemical studies are needed for evaluating the chemical composition effective this plant on the tick.

CONCLUSION

The obtained results suggest that plant extracts of ***S. australis*** presented acaricide potential, although it displayed low rates of effectiveness. This study was the first report that demonstrated any acaricidal activity of this plant against ***R. (B.) microplus***. To incorporate high effectiveness on acaricidal effect of ***S. australis***, there is the need to perform additional studies. Analysis to identify the active metabolites in different physiological stages of ***S. australis*** will provide important data in order to optimize and apply the extracts of this plant in the control of ***R. (B.) microplus***.

Table 2 - Average hatchability ± Standard Error (SE) and reproductive effectiveness obtained *in vitro* facing the exposure of the ***Rhipicephalus (B) microplus*** to the dry crude extract (DCE_L) of ***Sambucus australis*** at 2% recovered in distilled water or ethanol at 70% compared to the control groups.

Treatment	Average hatchability ± SE(%)	Reproductive average effectiveness
Aqueous solution of DCE_L of ***S. australis*** at 2% ($0.2mg^{-1}$)	84^a ±4.7	613883.40
Ethanol solution at 70% of DCE_L of ***S. australis*** at 2% ($0.2mg^{-1}$)	28^b ±7.3	144864.51
Control 1: distilled water	98 ±0	941242.94
Control 2: ethanol at 70%	50 ±0	430693.07
Positive control: solution of Cypermethrin at 15%, Chlorpyrifos at 25% and Citronella at 1% in the dilution 1:1000	0 ±0	0

a, b: Average followed by different letters in the column, differ by the Chi-square test ($P<0.0001$).

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