

## Acaricidal effects of seven Brazilian plant extracts

Efecto acaricida de siete extractos de plantas brasileñas

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**Abstract:** This investigation was carried out to evaluate the effects of the plant extracts of *Rheedia longifolia* (Clusiaceae), *Garcinia xanthochymus* (Clusiaceae), *Plumbago scandens* (Plumbaginaceae), *Hovenia dulcis* (Rhamnaceae), *Malpighia glabra* (Malpighiaceae), *Euphorbia tirucalli* (Euphorbiaceae) and *Nerium adelfa* (Apocynaceae) on eggs of *Dermacentor (Anocentor) nitens* and *Rhipicephalus sanguineus* (Acari: Ixodidae) at concentrations of 1,000 ppm. The extracts had a significant effect on the hatching rate of eggs. The extracts of *R. longifolia*, *H. dulcis*, *M. glabra* and *E. tirucalli* delayed the hatching of *R. sanguineus* eggs by three days; however, the group treated with the extracts of *G. xanthochymus* and *P. scandens* did not hatch until day fourteen. For the tick *D. (A.) nitens*, extracts of *H. dulcis* and *N. oleander* delayed egg hatching by three days; however, the action of extracts from *R. longifolia*, *G. xanthochymus*, *P. scandens* and *E. tirucalli* caused a delay of seven days. For the control groups of both species, eggs hatched in one day. *H. dulcis* and *M. glabra* were effective *in vitro* on eggs of *R. sanguineus* and *D. (A.) nitens* (89 and 100 %, respectively), and can be considered to be potential candidates for the biocontrol of those ticks.

**Key words:** Botanical acaricide, control, arthropod, tick.

**Resumen:** Esta investigación se llevó a cabo para evaluar los efectos de los extractos de *Rheedia longifolia* (Clusiaceae), *Garcinia xanthochymus* (Clusiaceae), *Plumbago scandens* (Plumbaginaceae), *Hovenia dulcis* (Rhamnaceae), *Malpighia glabra* (Malpighiaceae), *Euphorbia tirucalli* (Euphorbiaceae) y *Nerium adelfa* (Apocynaceae) sobre la tasa incubación de los huevos de *Dermacentor (Anocentor) nitens* y *Rhipicephalus sanguineus* (Acari: Ixodidae) en concentraciones de 1.000 ppm. Los extractos de *R. longifolia*, *H. dulcis*, *M. glabra* y *E. tirucalli* retrasaron significativamente la incubación de los huevos de *R. sanguineus* en tres días, pero los grupos tratados con los extractos de *G. xanthochymus* y *P. scandens* eclosionaron después de 14 días. Para las garrapatas *D. (A.) nitens*, los extractos de *H. dulcis* y *N. adelfa* retrasaron la eclosión de los huevos durante tres días, sin embargo, la acción de los extractos de *R. longifolia*, *G. xanthochymus*, *P. scandens* y *E. tirucalli* causó un retraso mayor de siete días. Para los dos tipos de garrapatas, los huevos del grupo control tardaron un día para eclosionar. Los extractos *H. dulcis* y *M. glabra* fueron eficaces *in vitro* en huevos de *R. sanguineus* (89 %) y *D. (A.) nitens* (100 %) y se pueden considerar como candidatos potenciales para el control biológico de estas garrapatas.

**Palabras clave:** Acaricida botánico, control, artrópodo, garrapata.

### Introduction

*Dermacentor (Anocentor) nitens* (Neumann, 1897) is a tick species that causes several serious problems as a drop-in productivity and can lead to loss of the pinna by secondary bacterial invasion (Pfeifer-Barbosa 1993; Suzuki *et al.* 2003). The brown tick *Rhipicephalus sanguineus* (Latreille, 1806) is a natural ectoparasite of domestic dog and can parasitize different hosts, including man (Venzal *et al.* 2003; Louly *et al.* 2006; Ribeiro *et al.* 2006).

Controlling these ticks is difficult because of the increase of some strains of resistance to acaricides such as pyrethroids and the inefficacy of other compounds (Fernandes and Freitas 2001; Miller *et al.* 2001), so new agents and alternative strategies are necessary. According to Gionetto and Chávez (2000), metabolites of plant origin reduce the persistence and accumulation of pesticides in the environment, being biodegradable and showing no side effects.

The use of secondary phytochemical metabolites has been able to interfere with the physiology of arthropod such as neuroendocrine systems, feeding, metamorphosis, which are vulnerable points for population control based on the life cycle of arthropods (Garcia and Azambuja 2004).

Some studies revealed satisfactory results from the use of several plants substance for insect and tick management such as brow dog tick *R. sanguineus* (Acari: Ixodidae) (Denardi *et al.* 2010; Pinto *et al.* 2011), cow tick *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887) (Acari: Ixodidae) (Borges *et al.* 2011); mites of the honeybees *Varroa destructor* Anderson and Trueman (2000) (Acari: Varroidae) (Ghasemi *et al.* 2011); the maize weevil adults, *Sitophilus zeamais* Motschulky, 1885 (Coleoptera: Curculionidae) (Fazolin *et al.* 2007); housefly and blowflies (Insecta: Diptera) (Carriço *et al.* 2014; Pinto *et al.* 2015a, b) and *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Liviidae) (Mendoza-García *et al.* 2015).

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Searching for alternative anti-tick products led to this study which had the aim to test the acaricidal effects of *Rhedia longifolia* Planch and Triana (Clusiaceae), commonly known as “Bacupari”, *Garcinia xanthochymus* Hook f (Clusiaceae), commonly known as “Falso Mangostão”, *Plumbago scandens* L. (Plumbaginaceae), commonly known as “Bela Emilia”, *Hovenia dulcis* Thunb ( Rhamnaceae), commonly known as “Uva do Japão”, *Malpighia glabra* L. (Malpighiaceae), commonly known as “Acerola”, *Euphorbia tirucalli* L. (Euphorbiaceae), commonly known as “Aveloz” and *Nerium oleander* L. (Apocynaceae), commonly known as “Espirradeira” on the eggs of *D. (A.) nitens* and *R. sanguineus* (Acari: Ixodidae). Some of these plants have been reported possess insecticidal properties, but their acaricidal properties have not been investigated until now.

### Materials and methods

Fresh leaves of the plants *R. longifolia*, *G. xanthochymus*, *P. scandens*, *H. dulcis*, *M. glabra*, *E. tirucalli* and *N. oleander*, were dried at 40 °C in an oven with air circulation and reduced to small fragments. The dried plant materials were submitted to a static extraction with methanol for about seven days and dried under reduced pressure. The crude extracts were prepared at the Instituto de Tecnologia em Fármacos/FIOCRUZ, by researchers from the Laboratório de Produtos Naturais. The extracts were diluted in distilled water, in order to obtain the concentration of 1,000 ppm.

Gravid females of *R. sanguineus* and *D. (A.) nitens* were collected from naturally infested dogs and horses, respectively, in Rio de Janeiro-RJ. The females were placed in BOD incubators acclimatized to 27 ± 1 °C, 80 % RH and a 12:12 h light/dark photoperiod. In order to obtain eggs of the same age cohort, eggs from *R. sanguineus* and *D. (A.) nitens* were counted under a dissecting scope and divided into four replicates per extracts, totalizing 48 eggs per group. The eggs were placed in filter paper envelopes (4.0 x 5.5 cm) and immersed for three seconds in 200 mL of the extracts in a Becker (Bicalho *et al.* 2001); distilled water was used as control. Subsequently, the eggs were transferred to an apparatus similar to the one proposed by Fernandes (1997). This apparatus was constructed with a Petri dish (9.4 x 1.5 cm) with filter paper adhered to the top part. The eggs were incubated at 27 ± 1 °C, 80 % RH and with a 12:12 h (L:D) hr cycle and daily examined until the end of the observation period. Hatched eggs were counted after 24 hours of exposure

and the observation lasted four weeks. The experimental design was completely randomized, with four replicates; the treatments were constituted by the control (water distilled), as well as solutions with 200 mL of the extracts.

The significance of the data was evaluated by ANOVA and Tukey’s test ( $P < 0.05$ ). The difference between the percentages of hatching eggs of *R. sanguineus* and *D. (A.) nitens* were evaluated through the chi-square test (Sokal and Rohlf 1979). The hatching percentage was calculated concerning the number of hatched larvae divided by the total number of incubated eggs.

### Results and discussion

The efficacy of the extracts against eggs of *R. sanguineus* and *D. (A.) nitens* were assessed by measuring hatchability of eggs. There was a significant difference in the percentage of hatched eggs among treatments for the species *R. sanguineus* ( $\chi^2 = 568.496$ , DF = 7,  $P < 0.01$ ) (Table 1) and for the species *D. (A.) nitens* ( $\chi^2 = 919.337$ , DF = 6,  $P < 0.01$ ) (Table 2).

The extracts of *R. longifolia*, *H. dulcis*, *M. glabra* and *E. tirucalli* delayed hatching of eggs of *R. sanguineus* by three days, but the group treated with the extracts of *G. xanthochymus* and *P. scandens* just hatched on day 14. For the tick *D. (A.) nitens*, the extracts of *H. dulcis* and *N. oleander* delayed by three days the eggs hatching, however, the action of extracts *R. longifolia*, *G. xanthochymus*, *P. scandens* and *E. tirucalli* caused a delay of seven days. For the two control groups, the eggs hatched in one day. Pinto *et al.* (2011) evaluated the acaricidal activity of the latex from *Euphorbia splendens* var. *hislopii* (Euphorbiaceae) and verified that eggs of *R. sanguineus* treated with 25 µL/L began hatching 24 h after treatment, while for the groups treated with other concentrations (50, 100, 125, 250 and 500 µL/L), the hatchings began 72 h after exposure.

Shafy and Zayed (2002) using a different methodology, showed that when eggs of *Hyalomma (Anatolicum) excavatum* (Acari: Ixodidae) are immersed in neem extracts *in vitro*, the hatching accelerates, and the mortality of newly hatched larvae increases. Extracts of *M. azedarach* inhibit egg production of immersed *Rhipicephalus (B.) microplus* ticks (Borges *et al.* 2003). According to Silva *et al.* (2009) Hexanic, ethyl acetate and ethanolic extracts from leaves of *Piper aduncum* (Piperaceae) were tested against engorged females of *Rhipicephalus (B.) microplus*. For all extracts, even at the highest concentration, reproductive control was

**Table 1.** Average of eggs hatched of *Rhipicephalus sanguineus* (n = 192), per days after treatment, mean, standard deviation and eclosion (%), exposed to different aqueous extracts of plants and control group, under laboratory conditions.

Extracts	Days after treatment						Eggs hatched X ± S. D.
	1	3	7	14	21	28	
Control	7	0.75	10	12.7	8.3	0	4.2 ± 2.9 <sup>a</sup>
<i>Reedia longifolia</i>	0	0.5	0.25	4.25	2.25	0.75	2.7 ± 6.5 <sup>ab</sup>
<i>Garcinia xanthochymus</i>	0	0	0	1.25	5.75	0	2.7 ± 6.8 <sup>ab</sup>
<i>Plumbago scandens</i>	0	0	0	2.75	0	0	1.5 ± 4.9 <sup>b***</sup>
<i>Hovenia dulcis</i>	0	0.25	1	3.25	1	0	1.4 ± 4.3 <sup>b***</sup>
<i>Malpighia glabra</i>	0	3.25	3.5	4	0.5	0	0.8 ± 2.3 <sup>bc***</sup>
<i>Euphorbia tirucalli</i>	0	2	1.75	6.25	2	0.25	3.4 ± 6.6 <sup>a</sup>
<i>Nerium oleander</i>	0.25	0.5	1.75	3.5	2.25	0	1.7 ± 4.9 <sup>b***</sup>

Numbers followed by the same letter did not differ among themselves and those followed by different letters have a significant difference (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\* $P < 0.001$ ) when the Tukey test was used.

**Table 2.** Average of eggs hatched of *Anocentor nitens* (n = 192), per days after treatment, mean, standard deviation and eclosion (%), exposed to different aqueous extracts of plants and control group, under laboratory conditions.

Extracts	Days after treatment						Eggs hatched X ± S. D.
	1	3	7	14	21	28	
Control	12.25	15.5	18.75	1	0	0	4.2 ± 2.9 <sup>a</sup>
<i>Reedia longifolia</i>	0	0	1.25	0	0	0	0.1 ± 1.1 <sup>b***</sup>
<i>Garcinia xanthochymus</i>	0	0	1.5	0.5	0	0	0.3 ± 1.8 <sup>bc***</sup>
<i>Plumbago scandens</i>	0	0	1.25	1.25	0.75	0	0.8 ± 3.5 <sup>c***</sup>
<i>Hoveni dulcis</i>	0	0.25	0.25	0	0	0	0.03 ± 0.5 <sup>b***</sup>
<i>Malpighia glabra</i>	0	0	0	0	0	0	0.0 ± 0.0 <sup>b***</sup>
<i>Euphorbia tirucalli</i>	0	0	0.5	0	0.25	0	0.1 ± 1.6 <sup>b***</sup>
<i>Nerium oleander</i>	0	3.75	2.25	0	0.5	0	0.7 ± 2.6 <sup>bc***</sup>

Numbers followed by the same letter did not differ among themselves and those followed by different letters have a significant difference (\* P < 0.05, \*\* P < 0.01, \*\*\*P < 0.001) when the Tukey test was used.

no higher than 62 %. Hexanic extracts caused larvae mortality of 70.42 % and, its hydrodistillation produced 6.8 % essential oil, 94.84 % consisting on the sesquiterpene dill apiol, which caused 100 % larval mortality.

Some experiments have demonstrated that plants extracts interfere with tick oviposition, by acting directly on growth control and development (Borges *et al.* 2003; Silva *et al.* 2009; Ribeiro *et al.* 2010; Silva *et al.* 2011). The *H. dulcis* extracts inhibited 89 % of the *R. sanguineus* eggs to hatch. Among secondary metabolites found in *H. dulcis* were triterpene saponins, glycosides, triterpenes and dihydroflavonoids (Castro *et al.* 2005). Recent studies of chemistry and pharmacology activity of *H. dulcis* have shown promising potential as a bioactive species, especially due to its antineoplastic activity (Martínez *et al.* 1997; Popoca *et al.* 1998). They also reported a 95 % trypanocidal inhibition by the aqueous extracts and 100 % inhibition for the methanolic extracts from the leaves of plants.

The extracts from the plants *G. xanthochymus*, *P. scandens*, *N. oleander* and *R. longifolia* reduced hatchability of *R. sanguineus* eggs by 85, 85, 84 and 83 %, respectively, but the newly hatched larvae died just after eclosion. Extracts from *M. glabra* and *E. tirucalli* showed 76 % ovicidal activity. The data showed that the extracts act directly on the biology of the tick *R. sanguineus*.

Eggs of the tick *D. (A.) nitens* treated with aqueous extracts from *M. glabra*, presented 100 % of mortality, which suggests that it produces a substance that affects embryo development. It was observed that extracts *H. dulcis*, *E. tirucalli*, *R. longifolia*, *G. xanthochymus* and *P. scandens* prevented hatching of *D. (A.) nitens* eggs in 99, 98, 97, 96 and 93 %, respectively. *N. oleander* extracts was less active and caused only an 85 % ovicidal effects. For the control group, the egg hatching rate was of approximately 99 % for the two species of tick studied.

The results obtained by Pinto *et al.* (2011), using latex of *E. splendens* var. *hislopii* on eggs of *R. sanguineus* showed that the lowest number of eclosions was observed at higher concentrations 125, 250 and 550 mL (1 % for these concentrations), but the newly hatched larvae died just after eclosion. For the other concentrations tested, the egg hatching rates were 45 % at 25 µL, 30 % at 100 µL. This was confirmed by those extracts of *Artocarpus alitilis* (Moraceae) and *Azadirachta indica* (Meliaceae) on *R. (B.) microplus* eggs, and we observed unfeasible hatching in 65 and 80 %, respectively.

Results found in this research are in agreement with other authors who have studied the effects of Neem Azal F (Trifolio-M GmbH, Germany) seed extracts against the postembryonic development and adults of *H. (A.) excavatum* (acari: Ixodidae). They found that neem at concentrations of 1.6 and 3.2 % significantly affects this species of tick (Shafy and Zayed 2002).

Our data suggest that the bioassays conducted offered excellent results for the potential activity of the samples, particularly in relation to the extracts of *H. dulcis* and *M. glabra* that produced ovicidal action, preventing hatching of the eggs of *R. sanguineus* (89 %) and *D. (A.) nitens* (100 %), respectively. As a result, the unhatched eggs were pale and showed rough surfaces morphologically altered and the embryos become opaque within the shell.

These extracts could play an important role in the future to control the ticks. Anyway, the isolation and characterization of the active compounds of each extract and the minimum dosage necessary to achieve the desired effects still depends on further studies to be properly defined.

The results indicated a significant effect of extracts from *R. longifolia*, *G. xanthochymus*, *P. scandens*, *H. dulcis*, *M. glabra*, *E. tirucalli* and *N. oleander* on embryonated eggs from *R. sanguineus* and *D. (A.) nitens*.

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