Journal MVZ Cordoba
2020; 25(1):e1951.
https://doi.org/10.21897/rmvz.1951

Original

Acaricidal effect of *Momordica charantia*, *Megaskepasma erythrochlamys* and *Gliricidia sepium* on the *Rhipicephalus microplus*

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Received: February 2019; Accepted: August 2019; Published: December 2019.

ABSTRACT

Objective. The acaricidal activity of *Momordica charantia (Mc)*, *Megaskepasma erythrochlamys (Me)* and *Gliricidia sepium (Gs)* on *Rhipicephalus microplus* (Rm) was evaluated. **Materials and methods.** Preliminary phytochemical analysis of leaves of the methanolic extract of *Mc* (EMc), the ethanolic extract of *Me* (EMe) and the acetone extract of *Gs* (EGs) were carried out through the technique of colorimetry and thin layer chromatography (CCD). The acaricidal activity was performed through *in-vitro* tests using the larval immersion test (LIT) and the adult immersion test (AIT). For *in-situ* tests, grazing cattle naturally infested with ticks were used, using the LC_{50} obtained from the *in-vitro* AIT tests; subsequently engorged females were taken to incubation to assess their reproductive capacity **Results.** The presence of several groups of secondary metabolites of acaricidal interest was determined. The acaricidal effect of plants extracts on engorged females was demonstrated; although only EGs showed larvicidal activity. Extracts at 160 mg/mL affected the life cycle of Rm by inhibiting oviposition in 46.9%, 66.1% and 84.03% ($p<0.05$) for EGs, EMc and EMe, respectively. On the other hand, the i*n-situ* testing, significant difference ($p<0.05$) was observed between EMc and EMe regarding control groups. **Conclusions.** The results obtained are promising to strengthen the possibility of linking the extracts of these plants within integrated plans to control ticks in cattle production systems.

Keywords: Acari, cattle diseases, ethnopharmacology (Source: *DeSC*).

RESUMEN

Objetivo. Se evaluó la actividad acaricida de *Momordica charantia (Mc)*, *Megaskepasma erythrochlamys (Me)* y *Gliricidia sepium (Gs)* sobre *Rhipicephalus microplus* (Rm). **Materiales y métodos**. Se realizó la marcha fitoquímica preliminar de hojas del extracto metanólico de *Mc* (EMc), del extracto etanólico de *Me* (EMe) y del extracto acetónico de *Gs* (EGs) a través de la técnica de colorimetría y cromatografía en capa delgada (CCD). La actividad acaricida se realizó a través de pruebas *in-vitro* utilizando la prueba de inmersión de larvas (LIT) y la prueba de inmersión de adultos (AIT). Para las pruebas *in-situ* se usaron bovinos en pastoreo infestados naturalmente con

How to cite (Vancouver).
Jaramillo HD, González RA, Pedraza CN, Sierra AG, García MG, Jara AR. Acaricidal effect of *Momordica charantia*, *Megaskepasma erythrochlamys* and *Gliricidia sepium* on the *Rhipicephalus microplus*. Rev MVZ Cordoba. 2020; 25(1):e1951. DOI: https://doi.org/10.21897/rmvz.1951

ISSNe: 1909-0544

garrapatas, utilizando las CL_{50} obtenidas en las pruebas *in-vitro* AIT; posteriormente las teleoginas se llevaron a incubación para evaluar su capacidad reproductiva. **Resultados.** Se determinó la presencia de varios grupos de metabolitos secundarios de interés acaricida. Se demostró el efecto acaricida de los extractos de las plantas sobre teleoginas; aunque sólo EGs mostró actividad larvicida. Los extractos a 160 mg/mL afectaron el ciclo de vida de Rm inhibiendo la ovoposición en un 46.9%, 66.1% y 84.03% ($p<0.05$) para EGs, EMc y EMe, respectivamente. Por otro lado, en las pruebas *in situ* se observó diferencia significativa ($p<0.05$) entre el tratamiento de EMc y EMe respecto a los grupos controles. **Conclusiones.** Los resultados obtenidos son prometedores para fortalecer la posibilidad de vinculación de los extractos de estas plantas dentro de planes integrados de control de garrapatas en sistemas de producción de bovinos.

Palabras clave: ácaros y garrapatas, enfermedades de los bovinos, etnofarmacología (*Fuente: DeSC*).

INTRODUCTION

A cattle ranching is an outstanding activity in the national economic context in general 3.5% of the nationals GDP and 56% of Livestock GDP (1). The ectoparasites, specifically the tick Rm has been associated with large economic losses for this productive screed by declining reproductive indices and weight gain associated with the transmission of pathogenic agents (2).

The use of acaricides has had a limited efficacy in reducing infestations and is accompanied by problems of resistant tick strains, environmental pollution and of meat or dairy products (3,4); this reinforces the need for alternative approaches to control tick infestations, where plant extracts are an option within the incursion of the integral management of external parasitic diseases (5,6).

Farmers who through traditional knowledge in the flora have sought and found solutions for the control of ectoparasites; species such as "matarratón" (*Gs*) and "Melón amargo" (*Mc*) were reported with antiparasitic effect in Colombia (7).

The objective of this research work was to validate the ethnopharmacological information on the use of these forage plants, in addition of "manto rojo" (Me), as an antiparasitic for the control of Rm, both in *in vitro* and *in situ* models.

MATERIALS Y METHODS

Collection of plants and elaboration of extracts. Leaves of *Mc* L. N°COL 580477, *Me* Lindau N°COL 575462 y *Gs* (Jacq.) Kunth ex Walp N°COL576461; were collected between the months of April and July, on the farm of Universidad de los Llanos, located on rural area Barcelona, Villavicencio (Meta). 4° 04'32,91" North latitude , 73° 35'02,27 West Latitude, altitude 386 MASL, 3500 mm/year precipitation, 87% HR and average ambient temperature 27°C.

The plant material was treated according to the methodology of the phytochemicals analysis proposed by Sanabria (8), thus: it was dried in a circulating air furnace at 40°C for 72 H and subsequently ground to pulverization for the elaboration of the extracts were weighed 150 g of dry plant material ground of *Mc, Me* y *Gs*; subsequently exposed to 600 mL of Methanol 99.8%, Ethanol 99.8% y Acetone 70% (Sigma-Aldrich, St. Louis, MO), respectively; each in a glass decanting funnel, for 72 hours, carrying out the continuous percolation technique to exhaustion.

The percolation liquid was filtered and concentrated at 40°C in a rotary evaporator IKA® RV10 control, at reduced pressure, eventually taking to dryness in water bath (bain-marie). The extracts were transferred to amber glass vials and stored at 4°C until the time of testing, the average yield of the extraction process was 5%, subsequently, preliminary phytochemicals analyses of the extracts were performed to determine the presence of secondary metabolites through the colorimetric and CCD technique.

Colony of Ticks. The collection of engorged females was developed in the bovine herd (21 animals naturally parasitized) of the fam of Universidad de los Llanos; engorged females were washed with a 2.5% hypochlorite solution (6); groups of 10 engorged females were placed on Kraft paper in Petri dishes that were then placed in plastic boxes for the generation of microenvironments and incubated using a

refrigerated incubator MEMMERT® IPP110 a 27–28ºC y HR 85-95%.

Fourteen days after incubation, the eggs of the female engorged groups are heavy, identified and placed in an individual glass vial; the vials are covered with a gauze plug and then placed in the plastic boxes for incubation; once hatched, the larvae were used in the acaricide tests *in vitro*.

***In vitro* test**. The AIT test proposed by Drummond et al (9) was applied, with adaptations. The engorged females were selected according to their morphology, motility and maximum engorgement; then weighed and separated in groups of 10 with homogeneous weights. Six repetitions for each concentration including the negative control group (water-Tween 80 2.5%) and positive control Amitraz 0.025% were developed, where engorged females were exposed to extracts at declining concentrations of 320, 160, 80, 40, 20 y 10 mg/mL conducted in water-Tween 80 2.5%; submerging them in 10 mL of these solutions for 10 minutes, then dried and placed in plastic Petri dishes, then incubated in the same conditions described above.

By stereoscopy, mortality was determined at 24 hours, 7 days and 14 days post-treatment; on day 14 post immersion the eggs were collected and weighed, the index of oviposition (IO), the percentage of inhibition of oviposition (IO%), reproductive control (CR%) and reproductive efficiency (ER) was determined for each treatment and controls through the following formulas:

$$IO = \frac{Weight\ of\ eggs\ (g|)}{Initial\ weight\ of\ females\ (g)}$$

$$IO\% = \frac{(IO\ negative\ control - IO\ extract)}{IO\ negative\ control}\ x\ 100$$

Subsequently, 100 eggs of each experimental unit were placed in glass vials covered with a gauze plug, taking to incubation for 21 days until hatching; determined the percentage of hatching larvae (E%).

ER was calculated with IO and E%, Like this:. The ER of each group of treated ticks was compared with the ER of the groups positive control and negative control; then CR% was calculated as well:

$$CR\% = \frac{(ER\ negative\ control - ER\ treated)}{ER\ negative\ control}\ x\ 100$$

For the LIT test, 7-14 day-old larvae were used; exposing them at different concentrations of extracts 20, 40, 80, 160 mg/mL, negative and positive controls equal to those described above; developing three repetitions per treatment. For the bioassay the vials with larvae were placed in the center of a petri dish that was subsequently filled with soap and water; methodology described by Klafke et al (10) was used, where 1 mL of each final immersion solution was distributed in three 1.5 mL microcentrifuge plastic tubes; with the use of a n°4 nylon brush, approximately 300 larvae were transferred to each tube, which was closed and vigorously agitated to ensure the immersion by 10 minutes of the larvae; subsequently 100 larvae are removed and moved to a Whatman filter paper #1 which was folded and closed, these assemblies were placed in plastic boxes for incubation for 24 hours.

Dead larvae were considered: those that did not move those that presented ataxia or appendicular movements only to the stereoscopic exam. To calculate mortality the formula used was:

$$Mortality = \frac{Number\ of\ dead\ larvae}{Total\ larvae}\ x\ 100$$

***In situ* testing.** The Acaricide activity of Eme y EMc was evaluated in Barcelona´s dairy herds, located in Km 12 runway Puerto López in Villavicencio, Meta; The evaluation of the EGs was carried out in the Agroindustry Center of the Meta "Hachón" (SENA), located in Km 17 runway Villavicencio - Puerto López. The use of animals was with prior written informed consent of the owners and approval by the Ethics Committee of the Universidad de los Llanos.

For each bioassay, 9 mixed bovine animals were used with high to medium infestations of Rm (approximately 20 engorged females per animal), with average ages of 18 months and average live weight of 300 Kg; the bovines did not receive any kind of acaricide topical or systemic medication for a period of two months before the experiment; the animals grazed a grass meadow and had water at will; which were distributed in three experimental groups, where 3 animals underwent a spray bath with the solution of the extracts to the CL_{50}; 3 animals were the negative control (water-Tween 80 2.5%); and finally 3 animals as positive control sprinkled with Amitraz 0.025%, for each experiment. The treatments in the bovines

were carried out with manual back pump with pressure, each animal received treatment for ten minutes with 1 liter of solution for every 100 Kg of live weight.

For the evaluation of the effect of extracts in cattle, the methodology described by Bianchi et al (3) was carried out, in which engorged females (ticks >4.5 y <8 mm body length) and larvae (ticks <4.5 mm body length) were counted on the right side of the bovine body, specifically in 4 areas of 10 cm^2: ventral lateral and dorsum lateral cervical aspect, tail base and perineum (Figure 1).

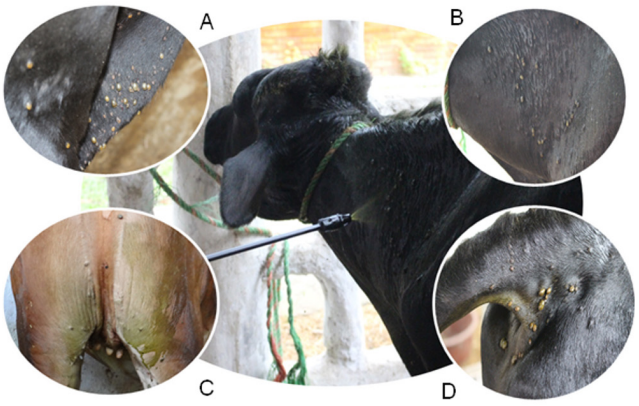

Figure 1. Evaluation of the infestation of ticks and counting of larval stages and engorged females of Rm. A. ventral lateral cervical area, B. dorsum lateral cervical area, C. Perineum area, D. Tail base area.

Tick count was done before treatment (day 0), days 1 (D1), Day 2 (D2), and Day 3 post-treatment (D3). Daily tick counts were always at the same time (07:00-8 AM) and by the same researcher. At the end of the D3 the engorged females were collected moving them to *in vitro* conditions, in this way, groups of 10 engorged females were organized for the different treatments, which were taken to incubation according to the AIT methodology for evaluating reproductive capacity after receiving the spray baths.

Statistical analysis. Sample size of the experiments *in-situ* was calculated from a power 80, confidence interval of 95%, maximum error 5%, average 12 and SD ±2 engorged females, through the OPenEpi program, version 3.01. The results of mortality of larvae and engorged females were subjected to variance homogeneity test, Bartlett's Test, and Kolmogorov-Smirnov test for normality, subsequent parametric analysis through the Probit method for the calculation of lethal doses 50 and 90; in addition to Chi-square to demonstrate differences between treatments.

Other results obtained from the *in-vitro* tests (%IO, ER, CR%); in addition to the results obtained in the tests *in-situ* were subjected to the tests of homogeneity of variance and normality described previously, to be processed through the analysis of one way variance and multiple comparison of averages with the Tukey-Kramer test, With a reliability range of 95% ($p<0.05$). The data were organized and analyzed in the statistical program OpenStat 4.0, version 7.0.

RESULTS

EMc's preliminary phytochemistry march determined the presence of alkaloids, saponins and sterols; of EMe was obtained alkaloids and saponins; and EGs flavonoids, terpenoids, coumarins, cardiac glycosides, saponins and tannins were found.

***In vitro* tests.** EMc and EMe showed no larvicide activity; while EGs showed a CL_{50} 78 mg/mL (IC 71–83 mg/mL) y CL_{90} 146 mg/mL (IC 128–182 mg/mL) in LIT. The results of the AIT test with the extracts evaluated were found to be a CL_{50} 125 mg/mL (IC 69–266 mg/mL) and a CL_{90} 930 mg/mL (IC 377–2085 mg/mL) for EMc; for EMe CL_{50} 69 mg/mL (IC 20–176 mg/mL) and CL_{90} 846 mg/mL (IC 268–94906 mg/mL); and for EGs CL_{50} 155 mg/mL (IC 110–241 mg/mL) and a CL_{90} 562 mg/mL (IC 324–2601 mg/mL). In addition, it was established that at the concentration of 160 mg/mL evaluated on engorged females, there are significant deleterious effects ($p<0.05$) on the life cycle of this parasite (Table 1).

Table 1. Effectiveness of the three plant extracts *in-vitro* by means of the AIT test in the inhibition of oviposition and reduction of the emergence of Rm.

160mg/mL extract	IO	IO%	ER	CR%
Gs	0.22	46.9	16.46	62.35
Mc	0.164	66.1	16.40	66.15
Me	0.06	84.03	4.03	91.3

***In situ* testing.** The results shown in averages and standard deviation of the engorged females counts on the right side of the animals, are evidenced in tables 2, 3 and 4.

Table 2. Mc effect on the averages of engorged females counts on the right side of bovine animals.

Treatments	Day 0	Day 1	Day 2	Day 3	Average
Mc	9.33 ±7.57^{A}	7.33 ±5.51^{A}	6.67 ±5.69^{A}	3.67 ±4.04	5.89 ±1.95^{B}
Negative control	13.33 ±9.02^{A}	9.33 ±5.69 ^{A}	11.67 ±7.51^{A}	NR	10.50 ±1.64^{A}
Positive Control	3.67 ±2.31^{B}	2.67 ±1.53^{B}	6.67 ±4.93^{A}	NR	4.67 ±2.82^{B}

Different letters in the same column indicate significant differences p<0.05, ANOVA posthoc Tukey–Kramer.

Table 3. Affect Me on the averages of engorged females counts on the right side of bovines.

Treatments	Day 0	Day 1	Day 2	Day 3	Average
Me	21.3 ±2.3^{A}	9 ±4^{A}	8.3 ±2.5^{A}	9.6 ±3.2^{A}	9 ±0.6^{A}
Negative control	4.6 ±2.0^{B}	3.6 ±1.5^{AB}	18.6 ±8^{A}	32.6 ±13.5^{B}	18.3 ±14.5^{B}
Positive Control	6.6 ±1.5^{B}	1.6 ±1.1^{B}	1.3 ±0.5^{B}	0.6 ±1.1^{C}	1.2 ±0.5^{C}

Different letters in the same column indicate significant differences p<0.05, ANOVA posthoc Tukey–Kramer.

Table 4. Effect of Gs on the averages of engorged females counts on the right side of bovine animals.

Treatments	Day 0	Day 1	Day 2	Day 3	Average
Gs	25 ±9.54^{A}	27 ±9.85^{A}	36 ±20.52^{A}	32.3 ±18.8^{A}	31.7 ±4.5^{A}
Negative control	23 ±14.18^{A}	24 ±18.08^{A}	33 ±22.87^{AB}	24.3 ±19.14^{AB}	27.11± 5.10^{A}
Positive Control	27.33± 20.03^{A}	9 ±6.08^{A}	8.33 ±4.73^{B}	5.33 ±3.21^{B}	7.56 ±1.95^{B}

Different letters in the same column indicate significant differences p<0.05, ANOVA posthoc Tukey–Kramer.

About the averages and standard deviations of the larval count are presented in tables 5, 6 and 7.

Table 5. Results *in-situ* test for Mc and its effects on larval stages.

Treatments	Day 0	Day 1	Day 2	Day 3	Average
Mc	6.67 ±7.33^{A}	2.75 ±1.76^{A}	1.67 ±2.35^{A}	1.17 ±0.94	1.86 ±0.80^{B}
Negative control	4.42 ±4.38^{A}	3.92 ±3.68^{A}	2.83 ±2.55^{A}	NR	3.38 ±0.76^{A}
Positive Control	12.08 ±11.56^{A}	4 ±5.13^{A}	4 ±5.10^{A}	NR	4 ±0.1^{A}

Different letters in the same column indicate significant differences p<0.05, ANOVA posthoc Tukey–Kramer.

Table 6. Results *in-situ* test for Me and its effects on larval stages.

Treatments	Day 0	Day 1	Day 2	Day 3	Average
Me	23.7 ±30.6^{A}	15 ±13.8^{A}	19.5 ±17.5^{A}	18 ±10.6^{A}	17.5 ±2.2^{A}
Negative control	39.1 ±28^{A}	20.6 ±19.8^{A}	22.7 ±21.9^{A}	28.2 ±19.9^{A}	23.8 ±3.9^{B}
Positive Control	17 ±20.8^{A}	11.2 ±8.8^{A}	7.3 ±4.5^{A}	7.3 ±4.8^{A}	8.6 ±2.2^{C}

Different letters in the same column indicate significant differences p<0.05, ANOVA posthoc Tukey–Kramer.

Table 7. Results *in-situ* test for Gs and its effects on larval stages.

Treatments	Day 0	Day 1	Day 2	Day 3	Average
Gs	15.67 ±18.6^{A}	11.67 ±11.70^{A}	11.25 ±14.83^{A}	14.17 ±9.84^{A}	12.36 ±1.57^{A}
Negative control	11 ±13.31^{A}	12,33 ±15.29^{A}	10.33 ±13.28^{A}	14 ±15.96^{A}	12.22 ±1.83^{AB}
Positive Control	21.92 ±20.16^{A}	10.67 ±9.63^{A}	8.42 ±7.55^{A}	8.42 ±8.15^{A}	9.16 ±1.29^{B}

Different letters in the same column indicate significant differences p<0.05, ANOVA posthoc Tukey–Kramer.

Likewise, in the evaluation of the effects of the vegetal extracts on the reproductive capacity of the engorged females collected on day D3, the results are presented in tables 8, 9 and 10.

Table 8. Effect on inhibition of ovoposition and reduction of the hatching of Mc on engorged females collected D3.

Treatments	IO	IO%	ER	CR%
Tween-80 2.5%	0.650^{A}	NA	66.25^{A}	NA
Amitraz 0.025%	0.434^{B}	33.2^{A}	47.90^{B}	33.21^{A}
AIT CL_{50} 125 mg/mL	0.378^{B}	41.8^{A}	37.80^{B}	41.83^{A}

Different letters in the same column indicate significant differences p<0.05, ANOVA posthoc Tukey-Kramer. NA, does not apply.

Table 9. Effect on inhibition of ovoposition and reduction of the hatching of Me on engorged females collected D3.

Treatments	IO	IO%	ER	CR%
Tween-80 2.5%	0.523^{A}	NA	52.29^{A}	NA
Amitraz 0,025%	0.243^{B}	53.5^{A}	7.29^{B}	79.80^{A}
AIT CL_{50} 69 mg/mL	0.361^{B}	30.9^{B}	36.11^{C}	30.95^{B}

Different letters in the same column indicate significant differences p<0.05, ANOVA posthoc Tukey-Kramer. NA, does not apply.

Table 10. Effect on inhibition of ovoposition and reduction of the hatching of Gs on engorged females collected D3.

Treatments	IO	IO%	ER	CR%
Tween-80 2.5%	0.417^{A}	NA	41.67^{A}	NA
Amitraz 0,025%	0.414^{A}	0.6^{A}	20.71^{B}	33.91^{A}
Extract 100 mg/mL	0.313^{A}	24.8^{B}	31.34^{B}	24.78^{A}

Different letters in the same column indicate significant differences $p<0.05$, ANOVA posthoc Tukey-Kramer. NA, does not apply.

DISCUSSION

No Prior studies have been conducted on the acaricide properties of EMc, EMe, and EGs on Rm ticks, this being the first report. The application of plants or their extracts to control microorganisms has been used by practitioners of traditional medicine; for the last decades, acaricide and insecticide properties of plant extracts have been widely used as biological pest control (11).

The Mc plant has been widely used as a medicinal plant, its medicinal properties include its antidiabetic potential, anti-inflammatory, antipyretic, antimicrobial activity, antiviral, antiparasitic, anthelmintic, antimalarial and antifungal (12). Gs is a tree legume mainly used as a forage species in cattle, its leaves have a high nutritional value with crude protein 23% (13); this plant is used as an antihistamine, antipyretic, expectorant, diuretic, antioxidant, antimicrobial, antimalarial, anthelmintic, trypanocidal, fly killer and acaricide effect (14,15).

On the other hand Me is a flowering shrub very colorful and commonly used as ornamentation plant (16), unlike other plants this does not report information about other possible uses.

Several studies have demonstrated the ability of groups of secondary metabolites type coumarins, condensed tannins, flavonoids, triterpenes, rotenoides, among others; to alter the biology of mites and insects (15,17,18,19): Several of these secondary metabolite groups were found in the phytochemistry march of EGs, EMc, and EMe collected under the Piedemonte of Meta conditions. Based on the determination of these promising chemical groups from the external and internal antiparasitic perspective, several studies have been developed; for example in tests performed *in vitro* with extracts of Gs was observed its effect anthelmintic reducing the motility of larvae stage L3 of *Haemonchus contortus*. This effect was related to secondary metabolites such as tannins/polyphenolic compounds (20), also terpenoids, flavonoids and lectins (21); it has also been shown that plants rich in these same groups of secondary metabolites have shown acaricide activity on Rm (22-25).

On the other hand, in the group of secondary metabolites type coumarins, group determined in the EGs, its antiparasitic capacity has been associated with mites through the inhibition of digestive enzymes belonging to the family of endopeptidases type serine, cysteine, aspartate; in addition metalloproteases, trypsin, chymotrypsin and cathepsin (26), enzymes with a proteolytic capacity of Rm who are responsible for the digestion of hemoglobin and act as reserve proteins during embryogenesis (27). Possibly this mechanism could explain the results obtained in the powerful inhibition of the life cycle of Rm (%IO, CR%, ER)(Tables 1) (8,9,10) by affecting the larval and adult stages used in the study; and the significant differences on mortality in larval stages and engorged females (Tables 2-7); being these enzymes vital in the survival of this mite.

Some studies have shown Gs *in vitro* acaricide activity on Rm, with results similar to those found in this research (Table 1), for example Rodríguez et al (28) evaluated the *in vitro* efficacy of *Morus Alba* extract and Gs in Rm control and its oviposition; in which the pure Gs extract manifested a mortality of 53.33%; they also obtained an OI of 58%.

Although *in vitro* acaricide activity on engorged females of Rm exposed to the plant extracts of this study show promising results of the incursion of these plants within the integrated control of external parasites in bovine production systems of the Piedemonte of the Meta department (Table 1-7).

The interest of this type of phitoterapeutic rests on the infeasibility of the eggs of this parasite; finding for this study significant results by obtaining 62.35%, 66.15% and 91.3% of CR% and ER% of 16.46, 16.40% and 4.03%, for Gs, Mc and Me; within the life cycle of the Rm to the concentration of 16% of the extract (Table 1), that is, the extracts under this model of experimentation generate a phenomenon of inhibition in the process of hatching (CR%) above 50% of the eggs ovoposited by the engorged females exposed to these herbal preparation.

Similar data were reported by Alvarez et al (15) where the alcoholic extract of Gs showed a significant effect ($p<0.05$) on IO% in Rm of 94%. Also about its acaricide capacity has been published that the 5% ethanolic extract of leaves of Gs reduced a 57% the ovoposition of the *Tetranychus Cinnabarinus* mite, obtained from black bean plants; in addition, concentrations of 5, 10, 15, and 20% of the ethanolic extract of the plant caused 41.7, 80.6, 88.9 and 100% of mortality, respectively (14).

For these reasons the results obtained from the *in-vitro* and *in-situ* study developed validate the ethnopharmacologic reports of the use of the Gs and Mc plants (29,30) as acaricides; in addition to linking as a new candidate to the plant Me as phytotherapeutic promising in animal production systems. On the other hand, in the bovine herds of the tropics under the parasitic load of the population is established in a 92% associated with the agroecosystem (preparasitic or free-life phases) and only 8% or less is in its obligatory parasitic stage; emphasizing the importance of linking within the integrated control of parasites the opportunity to control the preparasitic phases of the parasitic agent to treat; opportunity to be supported in sustainable processes and procedures from the environmental perspective (11).

At that time of the parasitic control the vegetal extracts play an important role, by being able to be used in the reduction of the free parasitic load with minimal environmental impact (30); in addition to its possible use on bovine, action to be validated through future *in-vivo* and *in situ* studies to compare the results obtained in this study. The above information is relevant and can be used to consolidate the cultivation of Gs, Mc and Me in the bovine production systems of the Piedemonte of the Meta department, in order to be used as an alternative method within the integrated control of the common livestock tick, strengthening the silvopastoral development of Colombian stockbreeding in the context of the economic opening of the primary or extractive productive sector (1).

Conflict of interests.

The author (s) declared no potential conflicts of interest with respect to the research, authorship and/ou publication of this article.

Acknowledgements.

To the National Funding Fund for Science, Technology and innovation, Francisco José de Caldas and the Universidad de los Llanos; for funding the research proposal within the macro research project: "Alternativa terapéutica para el control de Rhipicephalus microplus en bovinos con base en plantas forrajeras del piedemonte del Meta" Code 1122-569-35063; and to professor Carlos Enrique Hoyos Diez, from Human Sciences and Education College, for the translation of the manuscript.

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