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Short Communication

DETERMINATION OF *IN VITRO* EFFICACY OF AQUEOUS AND CHLOROFORM EXTRACTS OF *ADHATODA VASICA* AGAINST *RHIPICEPHALUS MICROPLUS* TICKS

Nitin D Jadhav[1], S. R. Rajurkar[1], M. Vijay[2], B. W. Narladkar[3], S. G. Jadhao[3], M. S. Vaidya[4], C. S. Mamde[5], Gajanan M. Chigure[3]*

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ABSTRACT: The aqueous and chloroform extracts obtained from leaves of *Adhatoda vasica* were evaluated for acaricidal activity against engorged females of *Rhipicephalus microplus* and their larvae. No significant mortality of adult ticks was observed in the tested concentration of aqueous extract except at 80%. Whereas, chloroform extract showed a dose-dependent increase in adult mortality, and LC50 and LC90 values were determined as 10.47% and 11.74%, respectively. A significant difference in egg hatching of laid eggs was observed at all concentrations tested and 98.55% inhibition of hatching was observed at 12.5% concentration. Dose-dependent larval mortality was observed in larval immersion test with various concentrations of chloroform extract with LC 50 and LC 90 values of 0.51%, and 1.09%, respectively. The results indicate that the chloroform extract of *Adhatoda vasica* could have very good *in vitro* acaricidal activities on adult and larval stages of *Rhipicephalus microplus* ticks.

Key words: *Rhipicephalus microplus,* Acaricidal activity, *Adhatoda vasica.*

Ticks and tick-borne diseases pose a serious threat to livestock health in many parts of the world including India. Among different species of ticks, *Rhipicephalus microplus* is widely prevalent and considered as the most economically important tick infesting dairy animal (Ghosh *et al*. 2007). Besides the involvement in transmitting diseases, *R. microplus* inflicts harm to animals through blood loss, general stress and irritation, depression of immune function, damages to hides and skins (Ghosh *et al*. 2007). According to the food and Agriculture Organization (FAO 2004), more than 80% of the world cattle population is exposed to tick infestations and global losses due to diseases transmitted by ticks and the costs of tick management was estimated to be US$ 13.9 -18.7 billion annually (de Castro 1997). Additionally, the tick infestation is also responsible for the annual loss of US$ 500,000 through hiding and skin downgrading (Bekele 2002) and US$57.2 million from tick worry (Minjauw and McLeod 2003). The control of ticks is mainly dependent on the use of acaricides of different chemical groups, but its indiscriminate and extended use has resulted in the development of resistance. Furthermore, *R. microplus* stands sixth among the most resistant arthropods globally (Villar *et al*. 2020) and the spread of acaricide resistance in the population of *R. microplus* of different regions has been revealed in many studies (Kumar *et al*. 2016, Chigure *et al*. 2018, Nandi *et al*. 2018). In this scenario, screening of natural products has received attention as an alternative tick control measure (Kebede *et al*. 2010) and may provide potential alternatives to chemical control agents as they constitute a rich source of bioactive chemicals (Qin *et al.* 2010). Accordingly, the efficacy of many plant extracts and essential oils produced from different plant species needs to be tested against economically important ticks.

[1]Department of Veterinary Pharmacology & Toxicology, [3]Department of Veterinary Parasitology, [4]Department of Animal Genetics & Breeding, [5]Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Parbhani, Maharashtra, India.
[2]National Institute of Animal Biotechnology, Hyderabad, Telangana, India.
**Corresponding author. e-mail: gajananchigure@gmail.com*

The study

Collection, transportation and maintenance of ticks

Fully engorged female ticks of *R. microplus* were collected manually from the cattle and buffaloes and the cracks, crevices of the shed. Ticks were collected in separate vials, closed with a muslin cloth to allow air and moisture exchange, and brought to the laboratory. The collected ticks were pooled, washed thoroughly in tap water, and dried using absorbent paper. The female ticks which were not used for the adult immersion test were kept in desiccators maintained at 85±5% relative humidity (RH) for oviposition. The laid eggs were allowed to hatch and the larvae of 12-14 days old were used for larval immersion.

Collection of plant material

The matured leaves of *Adhatoda vasica* were collected from the botanical garden of Vasantrao Naik, Marathwada Agriculture University and nearby areas of the Parbhani region and were authenticated from the botanist.

Preparation of aqueous extracts

The collected leaves were shade dried at the departmental laboratory for 15 to 20 days and ground to a powder using an electrical grinder. After preparation of powder, 100 gm was added in distilled water, and volume was adjusted to 1 liter. An aqueous mixture of the plant material was macerated at room temperature. Then the aqueous mixture was kept in the refrigerator for 48 hours with intermittent shaking using an electric shaker. The obtained macerate was strained off using a muslin cloth and then filtered through the filter paper. The obtained filtrate was transferred into evaporating Petri plates and kept at room temperature. The final extract was stored in screw-capped vials and kept at 4°C till its use.

Preparation of chloroform extract

The dried leaves powder (100g) was subjected to soxhlation unit with 600 ml chloroform in round bottom flask at 70^0C for 48 hours. Then the extract was removed from the flask and concentrated by using Rota evaporator at 40^0C (water bath). After complete evoration of solvent, the final extract was collected from the flask and stored at 4^0C.

The extractability of plant was calculated as

Extraction rate (%) = [Weight of extracts (g)/ Weight of the plant material (g) before extraction] X100.

Bioassays

Adult immersion test (AIT)

Acaricidal activity of extracts was evaluated using an adult immersion test (AIT) as described by Ghosh *et al.* (2013). Initially, a series of different concentrations of the extracts *viz*., 2.5, 5.0, 7.5, 10, and 12.5% were prepared. 20% acetone was used as a solvent. The adult female ticks were immersed for 5 minutes in different concentrations of the extracts while the control ticks were immersed in their respective solvents. After 5 min, the immersed ticks were transferred to the Petri dishes dried with a paper towel, weighed, and kept in a BOD incubator maintaining at 28 °C and 85 ± 5% RH. The data on biological parameters were monitored up to 14 days and the ticks which did not oviposit were considered as dead.

The inhibition of oviposition was determined as per the method of Stendel (1980):

Table 1. Dose response data of aqueous extract of *Adhatoda vasica* on *Rhipicephalus microplus* ticks by AIT.

Conc. (Dose) (%)	Mean tick weight (mg)/replicate (Mean ± SE) [N=5]	Mortality (%) (Mean± SE)	Mean egg mass (mg) (Mean ± SE)	Reproductive index (RI) (Mean ± SE)	Inhibition of Oviposition (IO%) (Mean ± SE)
10	546.00±18.81	0.00±0.00	218.25±9.35	0.41±0.003	1.89±1.06
20	503.50±11.06	0.00±0.00	194.50±11.35	0.41±0.003	5.26±4.25
30	505.50±13.64	0.00±0.00	195.00±10.42	0.41±0.007	5.37±3.43
40	503.50±36.29	0.00±0.00	189.50±27.49	0.40±0.005	9.03±8.29
50	497.50±13.30	0.00±0.00	183.25±13.12	0.40±0.005	9.84±3.75
60	493.00±6.24	0.00±0.00	178.50±8.66	0.40±0.005	11.24±2.63
80	497.25±10.71	45.00±5.00	123.75±9.49	0.177±0.01	38.88±4.39
Control	546.00±18.81	0.00±0.00	218.25±9.35	0.41±0.003	0.00±0.00

Table 2. Dose response data of chloroform extract of *Adhatoda vasica* on adult *Rhipicephalus microplus* ticks by AIT.

Conc. (Dose) (%)	Mean tick weight (mg)/replicate (Mean ± SE) [N=5]	Mortality (%) (Mean± SE)	Mean egg mass (mg) (Mean ± SE)	Reproductive index (RI) (Mean ± SE)	Inhibition of Oviposition (IO%) (Mean ± SE)
2.5	462.75±22.74	0.00±0.00	230.00±12.40	0.49±0.006	19.70±2.58
5	539.00±20.26	0.00±0.00	264.50±2.59	0.49±0.01	20.41±3.45
7.5	596.00±47.80	0.00±0.00	273.00±11.32	0.46±0.01	25.10±4.56
10	657.75±14.14	45.00±15.00	88.25±22.61	0.13±0.03	78.04±6.12
12.5	638.50±18.79	95.00±5.00	5.25±5.25	0.008±0.00	98.55±1.44
Control	623.00±18.59	0.00±0.00	385.50±3.12	0.62±0.01	0.00±0.00

*LC_{50}=10.47%; LC_{90}=11.74%; R2=0.964; Slope=30.35.

Reproductive Index (RI) = Egg masses/ Engorged tick weight.

Percentage inhibition of oviposition (% IO) = [RI (Control) - RI (Treatment)/ RI (control)] X 100.

Larval immersion test (LIT)

For LIT, approximately 100 larvae were transferred to 1.5 ml centrifuge tubes containing 300 µL of extracts and shaken vigorously to get it immersed. After 10 min, the solution was pipetted out and the entire tube was dried by absorbing the solution with a filter paper strip, and then the tubes were covered with cotton cloths tied with rubber bands. The control group was immersed in respective solvents. For each working concentration of the extract, three replications were maintained, and treated larvae were placed in desiccators placed in BOD incubator maintained at 28 °C and 85 ± 5% RH. After 24 hrs, the tubes were opened. The dead and live larvae were counted to calculate the mortality.

Statistical Analysis

The dose-response data were subjected to probit analysis (Finney1962). The LC50 (lethal concentration of extract producing 50% mortality in ticks) and LC90 (lethal concentration of extract producing 90% mortality) values were obtained from the regression equation and compared at the 95% confidence limit.

Results and discussion

The acaricidal efficacy of crude aqueous and chloroform extracts of *Adhatoda vasica* was assessed against *R. microplus* female ticks. The entomological parameters like adult mortality percentage, reproductive index, inhibition of oviposition, hatching rate and larval mortality were obtained. The aqueous extract of *Adhatoda vasica* caused no mortality of adult ticks in concentration up to 60% while only 45% adult mortality was noted in 80% (Table 1).

The chloroform extract showed strong acaricidal activity and a dose-dependent increase in adult mortality was observed in the range from 45.00±15.00 to 95.00±5.00 (Table 2). The LC50 and LC90 values were determined as 10.47% and 11.74%, respectively. The significant difference in the hatching of eggs passed by treated females was observed at all tested concentrations and 98.55% inhibition of hatching was observed at 12.5% concentration.

The chloroform extract also exhibited good larvicidal activity against larvae and dose-dependent increase in mortality was observed (Table 3). Significant larval mortality ($p<0.001$) at 24 hrs was observed in all the doses starting from 0.312% in comparison to the control. The LC50 and LC90 were 0.51% and 1.09%, respectively.

Table 3. Dose response data of chloroform extract of *Adhatoda vasica* on larvae of *Rhipicephalus microplus* by LIT.

Concentration (%)	Mortality (%) (Mean±SE)
0.312	22.94±1.20
0.625	54.83±1.54
1.25	83.36±2.04
2.5	100±0.00
5	100±0.00
Control	00.00

*LC_{50} = 0.51%; LC_{90}=1.09%, R^2 = 0.904, Slope = 3.797.

Adhatoda vasica is being used in an indigenous system of medicine for more than 2000 years and possesses numerous biological activities. The leaves of *Adhatoda vasica* have proven insect repellent properties (Saxena *et al*.1986) and also used as insecticide particularly, to control insect pests in oil seeds (Srivastava *et al*. 1965). In the present study, the selection of the *Adhatoda vasica* for acaricidal activity was based on the published literature about the high safety profile (Yadav and Yadav 2018). Moreover, World Health Organization (1990) has also considered *A. vasica* as a medicinal plant with therapeutic utility and non-toxic nature, as recorded in the WHO manual.

The results obtained in the present study using chloroform extract showed very strong anti-adult activity and effect on oviposition of treated female ticks. Similar to our findings, Kishore *et al*. (2016) reported the acaricidal efficacy of root extracts of *Adhatoda vasica* against the same species of tick and observed LC50 271.49, 388.80, and 863.61 ppm for petroleum ether, methanol, and hexane extracts respectively. Suggesting strong insecticidal activity of extracts of *Adhatoda vasica*, Gamble (1922) reported efficacy against hematophagous mosquitoes and flies.

Among the chemical constituents (vasicinone, vasicinol, adhatodine, adhatonine, adhavasinone, anisotine and peganine) present in leaves of *Adhatoda vasica*, vasicine and vasicinone are the two major alkaloids (Chihara 1997). The antifertility effect of vasicine by causing blockage of the oviduct has been reported against several insect species. In the present study, a significant difference in inhibition of oviposition and reduction in the hatching of eggs was also observed in female ticks treated with different concentrations of chloroform extract. Previously, Borges *et al*. (2003) obtained similar results using 0.25% *M. azedarach* (Meliaceae).

Besides adult tick mortality, dose-dependent larvicidal activity was also recorded in larvae treated with chloroform extracts of *A. vasica*. Similar larvicidal efficacy was reported by Sadek (2003) using crude methanolic extract of *A. vasica* against larvae of *Spodoptera littoralis* larvae and by Al-shaibani *et al.* (2008) against gastrointestinal nematodes of sheep.

From the results obtained in the present study, it can be concluded that the chloroform extract of *Adhatoda vasica* has shown to have very good *in vitro* acaricidal activities on adult and larval stages of ticks.

However, *in vivo* studies regarding the direct effect of this extract on animals needs to be undertaken. The present study will further help to develop a sustainable strategy for integrated tick management at least in the shed as chloroform extract exhibited the effect on oviposition and larvae.

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REFERENCES

Al-Shaibani IRM, Phulan MS, Arijo A, Qureshi TA (2008) Ovicidal and larvicidal properties of *Adhatoda vasica* (L.) extracts against gastrointestinal nematodes of sheep *in vitro*. Pak Vet J 28(2): 79-83.

Bekele T (2002) Studies on seasonal dynamics of ticks of Ogaden cattle and individual variation in resistance to ticks in eastern Ethiopia. J Vet Med 49: 285-288.

Borges, LMFPH, Ferri WJ, Silva WC, Silva JG (2003) *In vitro* efficacy of extracts of *Melia azedarach* against the tick *Boophilus microplus*. Med Vet Entomol 17(2): 228-231.

Chigure GM, Sharma AK, Kumar S, Fular A, Sagar SV *et al.* (2018) Role of metabolic enzymes in conferring resistance to synthetic pyrethroids, organophosphates, and phenylpyrazole compounds in *Rhipicephalus microplus*. Int J Acarol 44(1): 28-34.

Chihara J, Yasuba H, Tsuda A, Urayama O, Saito N *et al.* (1997) Elevation of the plasma level of rantes during asthma attacks. J Allergy Clin Immunol 100(6): S52-S55.

de Castro JJ (1997) Sustainable tick and tickborne disease control in livestock improvement in developing countries. Vet Parasitol 71(2-3): 77-97.

FAO (2004) Resistance management and integrated parasite control in ruminants. Guidelines, Animal production and health division, Rome, FAO. 25-77.

Finney DJ (1962) Probit analysis: A statistical treatment of the sigmoid response curve. 2 nd. edn. Cambridge University Press.

Gamble JS (1922) A manual of Indian timbers: an account of the growth, distribution, and uses of the trees and shrubs of India and Ceylon, with descriptions of their wood-structure. Sampson low, London. 205.

Ghosh S, Bansal GC, Gupta SC, Ray DD, Khan MQ *et al.* (2007) Status of tick distribution in Bangladesh, India and Pakistan. Parasitol Res 101(2): 207-216.

Ghosh S, Tiwari SS, Srivastava S, Sharma AK, Kumar S *et al*. (2013) Acaricidal properties of *Ricinus communis* leaf extract against organophosphate and pyrethroids resistant *Rhipicehalus (Boophilus) microplus*. Vet Parasitol 192 : 259-267.

Kebede Y, Gebre-Michael T, Balkew M (2010) Laboratory and field evaluation of neem (*Azadirachta indica* A. Juss) and Chinaberry (*Melia azedarach* L.) oils as repellents against *Phlebotomus orientalis* and *P. bergeroti* (Diptera: Psychodidae) in Ethiopia. Acta Trop 113(2): 145-150.

Kishore V, Kumar A (2016) Toxic potentiality of *Adhatoda vasica* root extracts against *Hyalomma anatolicum* (Acari: Ixodidae). J Entomol Res 40(2): 149-155.

Kumar S, Sharma AK, Nagar G, Rawat SS, Tiwari SS *et al.* (2016) Characterization of acaricide resistance in tick isolates collected from Rajasthan, India. Indian J Anim Sci 86: 14-23.

Minjauw B, McLeod A (2003) Tick-borne diseases and poverty: the impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa, DFID animal health programme, centre for tropical veterinary medicine, 124.

Nandi A, Sagar SV, Chigure GM, Fular A, Sharma AK *et al.* (2018) Determination and validation of discriminating concentration of ivermectin against *Rhipicephalus microplus*. Vet Parasitol 250: 30-34.

Qin S, Xing K, Jiang JH, Xu LH, Li WJ (2011) Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl Microbiol Biotechnol 89(3): 457-473.

Sadek MM (2003) Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Lep., Noctuidae). J Appl Entomol 127(7): 396-404.

Saxena BP, Tikku K, Atal CK, Koul O (1986) Insect antifertility and antifeedant allele-chemics in *Adhatoda vasica*. Int J Trop Insect Sci 7(4): 489-493.

Srivastava AS, Saxena HP, Singh DR (1965) *Adhatoda vasica*, a promising insecticide against pests of storage. Lab Dev 3(2): 138.

Stendel W (1980) The relevance of different test methods for the evaluation of tick controlling substances. J S Afr Vet Assoc 51(3): 147-152.

Villar D, Klafke GM, Rodríguez-Durán A, Bossio F, Miller R *et al.* (2020) Resistance profile and molecular characterization of pyrethroid resistance in a *Rhipicephalus microplus* strain from Colombia. Med Vet Entomol 34(1): 105-115.

Yadav S, Yadav VK (2018) Ethnomedicinal value and pharmacognosy of the member of Acanthaceae: *Adhatoda vasica* (Linn.). Asian Pac J Health Sci 5(2): 40-43.

WHO (1990) The use of traditional medicine in primary health care. A manual for health workers in South-East Asia, SEARO Regional Health Papers, No. 19. New Delhi, 1-2.

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