GAU Research Journal 48 (4) : 341-350 (October 2023)

Preliminary in-vitro acaricidal screening of selected Indian medicinal plants against *Rhipicephalus microplus*

Dharmik A. Shah, Prachi K. Nariyani, Punit R. Bhatt*, Kishorkumar R. Sorathia
Bhanubhai N. Suhagia

Faculty of Pharmacy, Dharmsinh Desai University, Nadiad
*Email: puns003@gmail.com

ABSTRACT

Rhipicephalus microplus is also known as Asian blue tick or cattle tick. This tick is prominent in all parts of India including Gujarat. In this study, in-vitro acaricidal activity was evaluated by the Adult Immersion Test against *Rhipicephalus (Boophilus) Microplus* also known as cattle ticks. Aqueous extracts of various medicinal plants were evaluated for their acaricidal activity. In the assay, test solutions were prepared to consist of 100, 500 and 1000 µg/ml of extracts and 10 to 50% v/v solution of oils in 50% v/v ethanol. Ticks were immersed in the test solutions for 2 minutes and mortality of the ticks was recorded after 24 hours. Except for the test solution of aqueous extract of *A. marmelos* leaves (100 µg/ml), ticks died (100% mortality) in all the test solutions containing extracts and oils. All the tested plants could be used against *R. microplus* as a potential alternative to chemical-based drugs.

Keywords : Acaricidal, Tick, Herbal, Medicinal plants extract, *Rhipicephalus (Boophilus) microplus*

INTRODUCTION

Ticks are harmful hematophagous arthropods that parasitize domestic and wild animals and result in significant financial damages, thereby contributing to food scarcity with an approximate global expense of prevention and efficiency losses of 7 billion USD annually. Their impacts are varied, such as diminished growth and lactation, paresis/poisoning, and dissemination of tick-borne diseases (TBDs) that impair production or induce fatality (Nchu *et al.*, 2012).

Globally, tick control relies primarily on the recurrent application of acaricides, which have caused issues related to environmental degradation, milk and meat adulteration, and the emergence of resistance leading to increased cost of control. In India, for the last several years, ticks are chiefly controlled by using various synthetic acaricides. Nevertheless, ticks have acquired resistance against commercial acaricides in India with the extensive, under- or over-diluted, and regular use of these substances (Obaid *et al.*, 2022). Therefore, there is a need for new tick control methods to overcome the disadvantage associated with the use of synthetic chemicals. One alternative control method could be phytotherapy, a vital component of ethnoveterinary medicine (Madzimure *et al.*, 2011).

The use of natural products, predominantly acaricides from the plant origin used for the management of ticks, has been the centre of research in many countries, mainly to counteract the evident increasing prevalence of acaricide-resistant tick populations (Dzemo *et al.*, 2022). The application of botanicals for the management of ticks is an indigenous practice in India, where most low-income farmers use plant substances to treat ectoparasites and endoparasites of livestock (Wanzala, 2017). Acaricidal activity of crude extracts from various medicinal plants

against ticks has been well documented. The phytoextracts exhibit acaricidal properties through different mechanisms: eliminating adult ticks, diminishing tick feeding, and impeding egg hatching, moulting, fertility, and viability of eggs, *etc.* (Kemal *et al.*, 2020).

Thus, it is quite obvious that acaricidal effectiveness of natural remedies can considerably contribute to manage tick infestation in a more economical and sustainable manner (Rajput *et al.*, 2006). Furthermore, there is a possibility to develop sources of acaricides from the existing medicinal plants which can be easily produced by livestock producers themselves or processed by small-scale industries and used as inexpensive and effective natural acaricide for tick management. Implementing a successful tick management strategy appropriate to a specific livestock production system is a dual benefit, managing ticks and also the TBDs (Quadros *et al.*, 2020). Therefore, it is essential to conduct an acaricidal effectiveness evaluation of botanicals which can be used in-future for the development of effective acaricidal herbal formulations. In the present work, attempts have been made to evaluate an acaricidal activity of locally available medicinal plants at various concentrations or percentage.

MATERIALS AND METHODS

Collection, identification and processing of plant materials

The plants were collected from the local surrounding areas of Nadiad and Anand (22°40'27.6"N 72°53'02.6"E). Some plants like *Cassia absus, Enicostema littorale*, *etc.*, were bought directly from the market as their powder form. Whereas some plants like *Datura stramonium, Datura metel*, *etc.* were collected from the local areas like the Faculty of Pharmacy, Dharmsinh Desai University campus garden (Table 1). The plants were identified by one of the authors (PRB) and voucher specimens were submitted to the department for future reference. All the collected plant materials were dried in shed at room temperature for up to 10 days. After drying, powder was made by mixer grinder and stored in an air-tight plastic container for further use.

Table 1 : List of medicinal plant used for in-vitro acaricidal activity

Sr. No.	Botanical Name	Family	Vernacular name	Part used
1.	*Aegle marmelos*	Rutaceae	*Billi*	Leaves
2.	*Annona sqamosa*	Annonaceae	*Sitaphal*	Seed oil
3.	*Azadirachta indica*	Meliaceae	*Limdo*	Fruit oil
4.	*Calotropis procera*	Apocynaceae	*Ankado*	Flowers
5.	*Cassia absus*	Fabaceae	*Chimed*	Seed
6.	*Centratherum anthelminticum*	Asteraceae	*Kalijiri*	Seed
7.	*Datura metel*	Solanaceae	*Dhaturo*	Leaves
8.	*Datura stramonium*	Solanaceae	*Dhaturo*	Leaves
9.	*Embelia ribes*	Primulaceae	*Vaavading*	Seed
10.	*Enicostema littorale*	Gentianaceae	*Mamejavo*	Leaves
11.	*Justicia adhatoda*	Acanthaceae	*Ardusi*	Leaves
12.	*Lantana camara*	Verbenaceae	*Gandhari*	Leaves & Flower
13.	*Millettia pinnata*	Fabaceae	*Karanj*	Leaves
14.	*Moringa oleifera*	Moringaceae	*Saragavo*	Leaves
15.	*Tagetes erecta*	Asteraceae	*Galgota*	Flower
16.	*Trigonellia foenum graceus*	Fabaceae	*Methi*	Seed
17.	*Vitex negundo*	Verbenaceae	*Nagod*	Leaves

Preparation of extracts

Except oils, the extracts were prepared by soaking the powder in the suitable solvent in the ratio of 1:10 (for each 1 g powder 10 ml extracting solvent was added) in a conical flask for at least 5 to 7 days. Then, the content was filtered; filtrate was collected, dried below 40°C in petri dish. The dried extracts were scraped and stored in an air-tight plastic container (Table 2).

Table 2 : List of medicinal plant, its part and type of extract used

Sr. No.	Vernacular Name of plant	Part of plant	Extract used
1.	*Aakdo*	flower	water extract
2.	*Billi*	leaves	water extract
3.	*Chimed*	seed	water extract
4.	*Chimed*	seed	methanol extract
5.	*Black datura*	leaves	methanol extract
6.	*White datura*	leaves	methanol extract
7.	*Galgota*	flower	water extract
8.	*Gandhari*	flower	water extract
9.	*Gandhari*	leaves	water extract
10.	*Kalijiri*	seed	water extract
11.	*Karanj*	leaves	water extract
12.	*Mamejavo*	leaves	water extract
13.	*Mamejvo*	leaves	methanol extract
14.	*Methi*	seed	methanol extract
15.	*Methi*	seed	water extract
16.	*Nagod*	leaves	water extract
17.	*Nagod*	leaves	methanol extract
18.	*Sitaphal*	seed	water extract
19.	*Vasaka*	leaves	water extract
20.	*Vasaka*	leaves	methanol extract
21.	*Vavding*	seed	water extract

Collection of ticks for in-vitro testing

The ticks were collected from various livestock owners of Nadiad and surrounding regions. The ticks were collected mainly from the ground surface near the cattle and cow feeding regions or their staying places. The ticks were then brought to the lab by keeping them in containers that allowed some oxygen and air to pass. Once brought into the labs, the ticks were then cleaned from the dirt and the foreign matter present in them and then stored in the container until the in-vitro test was to be performed (Figure 1).

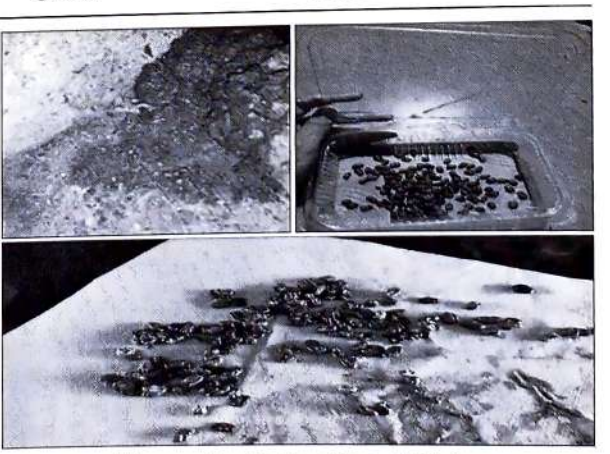

Figure 1 : Collection of ticks

Preparation of different concentrations of the test samples

Preparation of stock solution (1000 microgram per ml) - 0.1 g of the prepared extract was taken and then it was diluted with 10 ml of water. The stock solution was then diluted to 100 micrograms per ml and 500 micrograms per ml. All 3 concentrations of the extracts were then labelled and kept for testing the acaricidal activity. All the oils were separately mixed with a sufficient volume of 50% v/v ethanol in water to get the desired concentrations. All these concentrations were then labelled and tested for their acaricidal activity.

In-vitro testing of the acaricidal activity

The prepared solutions of different concentrations were tested for their acaricidal activity with ticks by performing Adult Immersion Test. The solutions of test extract or oils were taken in a suitable apparatus. Ticks were dipped in the solution of extract/oil for 2 min. After two minutes, ticks were put in a petri dish containing moistened filter paper. Petri dishes were put in an incubator for 24 hours. The ticks were then observed in the daylight after 24 hours if they were alive or dead. If the ticks were found alive, they were then kept for observation for their egg-laying capacity.

RESULTS AND DISCUSSION

All the plant materials were subjected to cold extraction i.e. maceration process (Figure 2). The per cent for each selected extracts were calculated as % w/w per 100 g dry plant material (Table 3).

Water extract of *chimed seed, galgota* flower, *mamejavo* leaves, *methi* seed, *sitaphal* seed, *gandhari flower*, *gandhari* leaves, *kalijiri* seed and *vavding* seed showed 100% mortality. Methanol extract of *methi* seed, *nagod* leaves also showed 100% mortality of ticks in all the concentrations. However, methanol extract of chimed seed and white datura leaves and karanj leaves water extract did not show any mortality. In this assay, 100% water and methanol were used as control as all the extracts were prepared either in water or methanol. Control did not show any mortality (Table 4). In case of neem seed and *karanj* seed oil which were prepared in ethanol, 100% mortality was observed from 10 to 50% oil. Control sample (50% ethanol) showed no mortality (Table 5). All the samples were assayed at constant incubation temperature.

A. Preparation of Extract

B. Drying in a hot air oven

Figure 2 : Extraction and drying of the plant extract.

Table 3 : Per cent yield of different extracts of various medicinal plants

Name of extract	Percentage yield (%w/w)	Name of extract	Percentage yield (%w/w)
Aakdo flowers water extract	8.3	*Karanj* leaves water extract	9.8
Billi leaves water extract	10.3	*Mamejavo* leaves water extract	9.9
Chimed seed methanol extract	13.0	*Mamejvo* methanol extract	11.1
Chimed seed water extract	10.3	*Methi* seed methanol extract	19.9
Black datura methanol extract	6.3	*Methi* seed Water extract	21.4
White datura methanol extract	9.7	*Nagod* leaves methanol extract	9.8
Galgota flower water extract	2.8	*Nagod* leaves water extract	9.1
Gandhari flower water extract	6.5	*Sitaphal* seed water extract	16.8
Gandhari leaves water extract	3.4	*Vasaka* leaves methanol extract	10.7
Kalijiri seed water extract	6.8	*Vasaka* leaves water extract	7.9
		Vavding seed water extract	1.5

Table 4 : Acaricidal activity of extracts

Name of the medicinal plant extract	No. of ticks included in assay	Total No. of dead ticks/percentage mortality		
		The concentration of the extract (µg/ml)		
		100	500	1000
Control (Distilled water)			0 (n=5)	
Aakdo flowers water extract	9	1 (n=3), 33%	1 (n=3), 33%	2 (n=3), 66%
Billi leaves water extract	15	0 (n=5), 0%	5 (n=5), 100%	5 (n=5), 100%
Chimed seed methanol extract	9	0 (n=3), 0%	0 (n=3), 0%	0 (n=3), 0%
Chimed seed water extract	15	5 (n=5), 100%	5 (n=5), 100%	5 (n=5), 100%
Black datura methanol extract	9	2 (n=3), 67%	1 (n=3), 33%	1 (n=3), 33%
White datura methanol extract	9	0 (n=3), 0%	0 (n=3), 0%	0 (n=3), 0%
Galgota flower water extract	6	2 (n=2), 100%	2 (n=2), 100%	2 (n=2), 100%
Gandhari flower water extract	6	2 (n=2), 100%	2 (n=2), 100%	2 (n=2), 100%

Name of the medicinal plant extract	No. of ticks included in assay	Total No. of dead ticks/percentage mortality		
		The concentration of the extract (µg/ml)		
		100	500	1000
Gandhari leaves water extract	6	2 (n=2), 100%	2 (n=2), 100%	2 (n=2), 100%
Kalijiri seed water extract	9	3 (n=3), 100%	3 (n=3), 100%	3 (n=3), 100%
Karanj leaves water extract	9	0 (n=3), 0%	0 (n=3), 0%	0 (n=3), 0%
Mamejavo leaves water extract	9	3 (n=3), 100%	3 (n=3), 100%	3 (n=3), 100%
Mamejvo methanol extract	9	1 (n=3), 33%	0 (n=3), 0%	0 (n=3), 0%
Methi seed methanol extract	9	3 (n=3), 100%	3 (n=3), 100%	3 (n=3), 100%
Methi seed Water extract	9	3 (n=3), 100%	3 (n=3), 100%	3 (n=3), 100%
Nagod leaves methanol extract	9	3 (n=3), 100%	3 (n=3), 100%	3 (n=3), 100%
Nagod leaves water extract	9	0 (n=3), 0%	0 (n=3), 0%	3 (n=3), 100%
Sitaphal seed water extract	6	2 (n=2), 100%	2 (n=2), 100%	2 (n=2), 100%
Vasaka leaves methanol extract	9	2 (n=3), 67%	1 (n=3) , 33%	2 (n=3), 67%
Vasaka leaves water extract	9	2 (n=3), 67%	1 (n=3) , 33%	3 (n=3), 67%
Vavding seed water extract	6	2 (n=2), 100%	2 (n=2), 100%	2 (n=2), 100%
Control (methanol/ water)	3		0 (n=3)	

"n" here shows the total number of ticks taken for the assay and the number outside the bracket shows a total number of ticks died. The number of ticks may vary due to their availability or the number of ticks collected.

Table 5 : Acaricidal activity of various oils with different concentrations

Concentration of oil	Number of ticks evaluated	Number of ticks dead	% mortality
	Neem oil		
10 %	4	4	100
20 %	4	4	100
30 %	4	4	100
40 %	4	4	100
50 %	4	4	100
Control	4	0	0
	Karanj oil		
10 %	4	4	100
20 %	4	4	100
30 %	4	4	100
40 %	4	4	100
50 %	4	4	100
Control	4	0	0

Nyahangare *et al.* (2019) reported that *C. absus* seed possesses acaricidal action. The activity might be due to the quaternary alkaloids like chaksine and isochaksine present in the seed. These alkaloids are also reported for its antibacterial and antifungal action (Ahmad *et al.*, 1935). Ghotge and Ramdas (2020) cited *Enicostemma littorale* as deworming plant as per the field trials carried out on animals as a part of ethnoveterinary practices. Swertiamarin is a glycoside compound present in *E. littorale* which might be responsible for the action. Worku (2018) reported the use of *Embelia ribes*for its anti-parasitic and anti-nematode activity. The plant have been widely used in India and Africa under the ethnoveterinary practices. Elango and Rahuman (2011) studied and reported acaricidal activity of *Aegle marmelos* against *Boophilus microplus* and other species of the ticks. Presence of various phytochemicals like aegelin, marmenol, marmin, marmelin, marmelosin, marmelide, psoralen, limonene, anhydromarmelin, betulinic acid, α-phellandrene, *etc.* Oliveira De Sousa *et al.* (2020) showed the acaricidal activity of *Lantana camara* essential oil. GC/MS analysis showed the presence of various molecules like Bicyclogermacrene, isocaryophyllene, valencene and germacrene-D. Results showed that leaves are also possessing acaricidal action. Adenubi *et al.* (2016) reviewed various plant extract for tick management and ethnoveterinary use. *Tagete erecta* exhibited its action due to the presence of phytochemicals like Thiophenes, flavonoids, carotenoids, triterpenoids. Elkertati *et al.* (2013) studied the acaricidal activity of *Trigonella foenum graecum*.The results showed that *T. foenum graecum* extract exhibited strong acaricidal action against *Tetranychus urticae*. In the present results, 100% mortality was observed in case of *methi* seed extract. Garg *et al.* (2019) reported an ethnoveterinary practices in Rajasthan. It has been observed that most

farmers use *C. anthelminticum* with other plants for tick infestation. In the present research, *kalijiri* showed very good acaricidal action against *B. microplus* tick. Jadhav *et al.* (2021) reported the acaricidal activity of chloroform extract of *A. vasica* against *R. microplus* ticks. The acaricidal action of *A. vasica* might be due to alkaloid present in the leaves like vasicine and vasicinone, are the two major alkaloids. Sharma *et al.* (2022) developed the phytoformulation contains *Annona squamosa* for the management of tick infestation. The formulation showed more than 70% of efficacy in the field trial. In our research, seeds of *A. squamosa* showed good action against tick. Denardi *et al.* (2011) reported the vitallogensis action of neem leaves against *Rhipicephalus sanguineus*. In the present research, neem oil showed very good action against *R. microplus* ticks. It may be concluded that azadirachtin present in the plant might be responsible for the acaricidal action. Raghav *et al.* (2019) reviewed the use of *P. pinnata* seed oil for its various applications like pesticidal, insecticidal and acaricidal activities. Karanjin from seed oil is an active constituent of the oil which might be responsible for the acaricidal activity in the present research.

FUNDING SOURCE

The research work was financed by Dharmsinh Desai University, Nadiad Student Startup Innovation Policy cell (DDU SSIP cell).

ACKNOWLEDGEMENT

Authors are thankful to all the animal owners of Nadiad city to provide ticks from their cowsheds.

PERIOD OF EXPERIMENT

The total duration of experiment was 6 months.

REFERENCES

Adenubi, O., Fasina, F., McGaw, L., Eloff, J., & Naidoo, V. (2016, July). Plant extracts to control ticks of veterinary and medical importance: A review. *South African Journal of Botany*, *105*, 178–193. https://doi.org/10.1016/j.sajb.2016.03.010

Ahmad, S., Hassan, A., Abbasi, W. M., Rehman, T., 2017. Phytochemistry and pharmacological potential of *Cassia absus* – a review. *Journal of Pharmacy and Pharmacology 70*(1): 27–41. https://doi.org/10.1111/jphp.12816

De Sousa, E. O., Lima, A. D. S., Lopes, S. G., Costa-Junior, L. M., & Da Costa, J. G. M. (2020, April 28). Chemical composition and acaricidal activity of Lantana camara L. and Lantana montevidensis Briq. essential oils on the tick Rhipicephalus microplus. *Journal of Essential Oil Research*, *32*(4), 316–322. https://doi.org/10.1080/10412905.2020.1752320

Denardi, S. E., Bechara, G. H., de Oliveira, P. R., & Camargo Mathias, M. I. (2011). Inhibitory action of neem aqueous extract (Azadirachta indica A. Juss) on the vitellogenesis of Rhipicephalus sanguineus (Latreille, 1806) (Acari: Ixodidae) ticks. *Microscopy research and technique*, *74*(10), 889–899. https://doi.org/10.1002/jemt.20973

Dzemo, W. D., Thekisoe, O., Vudriko, P., 2022. Development of acaricide resistance in tick populations of cattle: A systematic review and meta-analysis *Heliyon*, *8*(1): e08718. https://doi.org/10.1016/j.heliyon.2022.e08718

Elango, G., & Rahuman, A. A. (2011). Evaluation of medicinal plant extracts

against ticks and fluke. *Parasitology research*, *108*(3), 513–519. https://doi.org/10.1007/s00436-010-2090-9

Elkertati, M., Blenzar, A., Jotei, A. B., Belkoura, I. & Tazi, T. (2013). Acaricide effect of some extracts and fractions on Tetranychus urticae Koch (Acari: Tetranychidae). *African Journal of Agricultural Research, 8*(23), 2970-2976. https://doi.org/10.5897/AJAR2013.8765

Garg, S. L., Sharma, N. K., Rajput, D. S. & Singh, V. (2019). Ethno-veterinary Practices of Parasitic Infestation with Their Extent of Use Followed by Livestock Owners in Western Zone of Rajasthan. *International Journal of Livestock Research, 9*(6), 220-225. http://dx.doi.org/10.5455/ijlr.20190222095854

Ghotge, N. S., & Ramdas, S. R. (2008). *Plants Used in Animal Care*. Anthra, Pune. Retrieved January 28, 2024, from https://www.anthra.org/wp-content/uploads/2016/11/Plants-Used-in-Animal-Care-Anthra.pdf

Jadhav, N. D., Rajurkar, S. R., Vijay, M., Narladkar, B. W., Jadhao, S. G., Vaidya, M. S., Mamde, C. S., & Chigure, G. M. (2021, December 31). Determination of in vitro efficacy of aqueous and chloroform extracts of Adhatoda vasica against Rhipicephalus microplus ticks. *Exploratory Animal and Medical Research*, *11*(2), 255. https://doi.org/10.52635/eamr/11.2.255-259

Kemal, J., Zerihun, T., Alemu, S., Sali, K., Nasir, M., Abraha, A., Feyera, T., 2020. *In-vitro* Acaricidal Activity of Selected Medicinal Plants Traditionally Used against Ticks in Eastern Ethiopia. *Journal of parasitology research 2020*:7834026. https://doi.org/10.1155/2020/7834026

Madzimure, J., Nyahangare, E. T., Hamudikuwanda, H., *et al.*, 2011. Acaricidal efficacy against cattle ticks and acute oral toxicity of *Lippia javanica* (Burm F.) spreng. *Tropical Animal Health and Production* 43(2):481–489. doi: 10.1007/s11250-010-9720-1.

Nchu, F., Magano, S. R., Eloff, J. N., 2012. *In-vitro* anti-tick properties of the essential oil of *Tagetes minuta* L. (Asteraceae) on *Hyalomma rufipes* (Acari: Ixodidae) *Onderstepoort Journal of Veterinary Research* 79(1):E1-E5. doi: 10.4102/ojvr.v79i1.358.

Nyahangare, E. T. (2019). *Acaricidal characteristics of ethnoveterinary plants used for tick control in Southern Africa* [Doctoral thesis, University of Pretoria]. University of Pretoria institutional repositories. https://repository.up.ac.za/handle/2263/76751

Obaid, M. K., Islam, N., Alouffi, A., Khan, A. Z., da Silva Vaz, I., Jr, Tanaka, T., & Ali, A., 2022. Acaricides Resistance in Ticks: Selection, Diagnosis, Mechanisms, and Mitigation. *Frontiers in cellular and infection microbiology*, *12*, 941831. https://doi.org/10.3389/fcimb.2022.941831

Quadros, D. G., Johnson, T. L., Whitney, T. R., Oliver, J. D., Oliva Chávez, A. S., 2020. Plant-Derived Natural Compounds for Tick Pest Control in Livestock and Wildlife: Pragmatism or Utopia?. *Insects 11*(8):490. https://doi.org/10.3390/insects11080490

Raghav, D., Mahanty, S., & Rathinasamy, K. (2019). Biochemical and toxicological investigation of karanjin, a bio-pesticide isolated from Pongamia seed oil. *Pesticide biochemistry and physiology*, *157*, 108–121. https://doi.org/10.1016/j.pestbp.2019.03.011

Rajput, Z. I., Hu, S. H., Chen, W. J., Arijo, A. G., Xiao, C. W., 2006. Importance of ticks and their chemical and immunological control in livestock. *Journal of Zhejiang University Science-B* 7(11):912–921. https://doi.org/10.1631/jzus.2006.B0912

Sharma, A. K., Tiwari, S. S., Kumar, S., Rawat, A. K. S., Srivastava, S., Ray, D., Singh, N. K., Rawat, S. S., Sangwan, A. K., & Ghosh, S. (2022). Establishment of antitick efficacy of a phytoformulation prepared from Annona squamosa leaf extracts for the management of acaricide resistant tick infestations on cattle. *Acta tropica*, *233*, 106463. https://doi.org/10.1016/j.actatropica.2022.106463

Wanzala, W., 2017. Potential of Traditional Knowledge of Plants in the Management of Arthropods in Livestock Industry with Focus on (Acari) Ticks. *Evidence-based complementary and alternative medicine: eCAM 2017*:8647919. https://doi.org/10.1155/2017/8647919

Worku, T. (2018). Review on Importance of Ethnoveterinary Practices in Pastoral Areas Of Ethiopia. *International Journal of Research Studies in Biosciences*, 6(9), 16–27.