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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(11): 281-284

www.thepharmajournal.com
Received: 04-09-2020
Accepted: 09-10-2020

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Comparative study of different solvents seed extracts of *Azadirachta indica* (*neem*) and its *in-vitro* acaricidal properties against *Rhipicephalus* (B.) *microplus*

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DOI: https://doi.org/10.22271/tpi.2020.v9.i11e.5347

Abstract
The seeds of Azadirachta indica **(**Neem) were analyzed for gradient yields in different solvents and its acaricidal properties. The solvents used were aqueous (distilled water), ethanol, hydroethanol and methanol in Soxhlet apparatus for the extraction of extracts. The acaricidal properties of A. indica seed extract was assessed by using the solution of 8% aqueous, ethanolic, hydro-ethanolic and methanolic extracts. The highest yield obtained was methanolic extract about 14.84%, followed by ethanolic, hydroethanolic and aqueous extract 13.24%, 9.76% and 8.12% respectively. Variable amounts of yields might be possible due to the method, solvents, time and temperature applied for the extraction. During the assessment of acaricidal properties of A. indica seed extract, aqueous extract was found more effective as compared to ethanolic extract, hydroethanolic extract and methanolic extract and lowest efficacy was recorded in methanolic extract, might be happened by the presence of sufficient biomolecules in aqueous extract.

Keywords: Neem extract, Herbal Acaricide, Azadirachta indica, Rhipicephalus (B.) microplus and Ectoparasite

Introduction
Ticks are important blood sucking parasites, transmitting many infectious diseases to human, animals and birds. The tick infestation has direct impact on leather industry, because tick pierces inside the leather and damage the hide (Biswas, 2003) [5]. *Rhipicephalus microplus* is blood sucking parasite can transmit haemoprotozoan diseases. *Rhipicephalus (B.) microplus* directly and indirectly affects the cattle lead to great economic losses due to decrease in body weight and milk production, anaemia, tick paralysis and mortality through spreading protozoal, ricketsial, bacterial and viral diseases (Ghosh *et al.,* 2006; Kirthi *et al.,* 2011 and Grisi *et al.,* 2014) [8, 14, 11].

The acaricides mostly used to control the ticks are amitraz, fipronil, pyrethroids (deltamethrin, cypermethrin) and carbaryl and Indian tick population has also developed resistance to the acaricides commercially available (Alcaino *et al*., 1995; Franc and Cadiergues, 1999; Jernigan *et al*., 2000; Otranto *et al*., 2005 and WHO, 2006) [2, 7, 13, 17, 24]. Control and eradication of ticks are difficult due to indiscriminate and irregular application of acaricides, mismanagement in tick control programme, multiple natural hosts and the use of synthetic chemical mediated acaricides against which resistance has been developed.

Ticks resistance against synthetic acaricides shifted the investigation towards alternative methods to control the ticks (Ghosh *et al.,* 2007 and Kiss *et al.,* 2012) [9, 15]. The herbal acaricides are not only eco-friendly in nature but it also slowing the development of resistance, because it has multiple active acaricidal components. Single-point mutations in ticks against the herbal acaricides are not effective for resistance, whereas, majority of the chemical acaricides become ineffective after single-point mutation against the targeted molecules (WHO, 1992; Benavides *et al.,* 2000) [23, 3].

Azadirachta indica (Neem) has potential medicinal value and known as the solution of many problems because it posses the properties of insecticidal, nematicidal, larvicidal including many other activities (Sharma *et al.,* 2011) [18]. Neem derived compounds are effective for controlling the tick populations.

Neem seed extracts having azadirachtin and some other limonoids, showing deleterious effects on reproductive performance of female *Rhipicephalus microplus* (Giglioti *et al.,* 2011) [10]. Therefore, the present investigation was planned to assess the yield of different solvent extract of *Azadirachta indica* (Neem) seeds extracts and its comparative acaricidal properties against *Rhipicephalus (B.) microplus.*

Materials and Methods

Collection and identification and preparation of plant materials

The seeds of *Azadirachta indica* **(**Neem) were collected from the medicinal plant garden, IVRI, Izatnagar, Bareilly campus and washed with distilled water for three times to eliminate the impurities and completely air dried.

The neem seed was classified and identified at the Department of Botany, Bareilly College, Bareilly.

Extraction of extracts

The dried *Azadirachta indica* seeds were crushed in mixer grinder. The crushed coarse neem seed powder about 50 gm was transferred to the columnar tube of Soxhlet apparatus. About 250 ml each of distilled water (aqueous), ethanol, hydroethanol (1:1) and methanol was used as solvents for extraction of extract.

After assembling the Soxhlet apparatus, ceramic heater was started under round flask. The temperature was maintained to the ceramic heater of Soxhlet apparatus as per the solvent used for the boiling and vaporization of the content and cooling the vapour with running tap water.

The procedure of extraction was continued up to 12 to 15 cycles which was completed between 5 to 6 hours. The condensed liquid of the last cycle collected in columnar tube of the soxhlet apparatus was colourless and clear. The liquid form of different neem seed extract was collected and after filtration, dried below 40°C. The dried extract of different types of solvents were weighed and preserved.

Assessment of acaricidal properties of extract

Adult Tick Immersion test (AIT) was performed as per the protocol mentioned by Drummond (1983) [6] with slight modification. The solution of 8% aqueous, ethanolic, hydroethanolic and methanolic extracts were prepared to assess the acaricidal efficacy of extract.

Whatman filter paper no. 1 was placed in 90 mm Petridish. Total four petridish for each extract and one petridish for control was prepared. Total 6 ticks for each petridish were dipped in 1ml 8% concentration of different solvent extract for one minute and placed in petridish. The different solvent extracts used for dipping the ticks were soaked on Whatman filter paper in petridish.

The control ticks and filter paper was treated with distilled water. All petridish were placed in an incubator maintaining the temperature 28-30°C and RH 80% for 24 hrs. At distinct time interval of 3, 6, 12 and 24 hrs, the mortality of ticks was recorded. The selection of best extract was based on the mortality of ticks.

Results and Discussion

As per the results of gradient solvent extraction of *A. indica* (neem) seed extracts, the highest yields obtained was methanolic extract (14.84%) followed by ethanolic (13.24%), hydroethanolic (9.76%) and aqueous extract (8.12%). Bendigeri *et al.* (2019) [4] also described about the extracted yields of methanolic extract and aqueous extract of neem seeds about 11.95% and 6.14% respectively, whereas Srivastava *et al.* (2008) [20] obtained 11.82% yields of ethanolic neem (*A. indica*) seed extract during extraction. Similarly, Ibrahim and Kiranmai, (2012) [12] obtained 15.25% hydro-alcoholic neem (*A. indica*) seed extract. Alcohols are best solvent for oil and widely used for the extraction of oils of the seeds. Hence the highest yield in the alcoholic extracts might be possible due to availabilities of the oily substance in the extracts.

In present investigation, minor difference was noted between the yields of methanolic and ethanolic extract and similarly between hydroethanolic and aqueous extracts. On account of Tesfaye and Tefera, (2017) [21] solvent type, size of the particle, invested time and temperature are possible factors, which affect the quality and quantity of the neem oil during extraction.

Differences in the extracts yield might be possible by the methods applied for the extraction, type of the solvent used, solubility of the phytochemicals, impact of the time and temperature maintained for the extraction of the extracts, because different phytochemical constituents are not able to dissolve at certain time and temperature. Variable specific times and temperature are required to dissolve the certain phytochemical.

In Adult tick immersion test, the mortality of ticks was recognized according to the absence of physical signs and inactivity of the dead ticks.

The dead ticks had no movement and laid on their dorsal surface with facing ventral surface upward. No mortality of ticks was recorded in control group.

In this experiment aqueous extract was found highly potent as compared to the other extract because in aqueous extract, ticks mortality started on 6th hrs with 33.33% and reached up to 66.66% and 83.33% on 12th hrs and 24th hrs respectively. In ethanolic extract ticks mortality started on 12th hrs with 50% and 83.33% achieved by 24 hrs. In hydroethanolic extract ticks mortality started on 6th hrs with 16.66% and the mortality reached up to 50% and 83.33% on 12th and 24th hrs respectively. Only 33.33% and 66.66% mortality was recorded in methanolic extract on 12th and 24th hrs respectively.

According to Srivastava *et al*. (2008) [20] ethanolic neem seeds extracts exhibited higher efficacy against *Boophilus microplus* up to 80% mortality after 5 hrs treatments. Similarly, Magadum *et al*. (2009) [16] also found ethanolic neem (*A. indica*) seed extract more efficacious against *Boophilus microplus*. Abdel-Shafy and Zayed (2002) [1], postulated that neem Azal F induces significant mortality up to 100% in new larvae, unfed larvae and adults of *H. anatolicum excavatum*. But, Shyma *et al*. (2014) [19] revealed about the lowest acaricidal effects of methanolic neem (*A. indica*) seed extract and recorded only 33.33% mortality rates of *R. (B.) microplus*, similar to the findings of the present study. Zaman *et al.* (2012) [25] evaluated anti-tick property of aqueous extract of leaves of *A. indica* with other extracts of plants and observed effective in reduction of ticks *Rhipicephalus (B.) microplus* infection intensity in infested calves. Similarly, Varadharajan and Gnanasekar (2019) [22] postulated that the weekly spray of aqueous neem (*A. indica*) seed extracts were able to decrease the ticks number due to high dose of *Azadirachtin*. Thus, as per the above mentioned facts and mortality of the ticks, aqueous extracts of neem seed was found best for the acaricidal property in comparison to the

other extracts might be happened by the presence of sufficient biomolecules in aqueous extract because the water is the best solvent for the phytoconstituents of neem seed.

Table 1: The yields of different solvent extract of A. *indica* (neem) seeds

Sl. No.	Plant Materials (50 g)	Solvent (250 ml)	Yield (%)
1.	*A. indica* seeds	Methanol	14.84
2.	*A. indica* seeds	Ethanol	13.24
3.	*A. indica* seeds	Hydroethanol	9.76
4.	*A. indica* seeds	Aqueous	8.12

Table 2: Evaluation of acaricidal properties of 8% seed extracts of *A. indica* against *Rhipicephalus (B.) microplus*

Groups	Total Ticks	Percent (%) Mortality			
		3 hrs	6 hrs	12 hrs	24 hrs
I. Control Group	6	-	-	-	-
II. 8% Aqueous Extact	6	-	33.33	66.66	83.33
III. 8% Ethanolic Extact	6	-	-	50	83.33
IV. 8% Hydroethanolic Extract	6	-	16.66	50	83.33
V. 8% Methanolic Extract	6	-	-	33.33	66.66

Fig 1: Extraction of extracts in Soxhlet apparatus

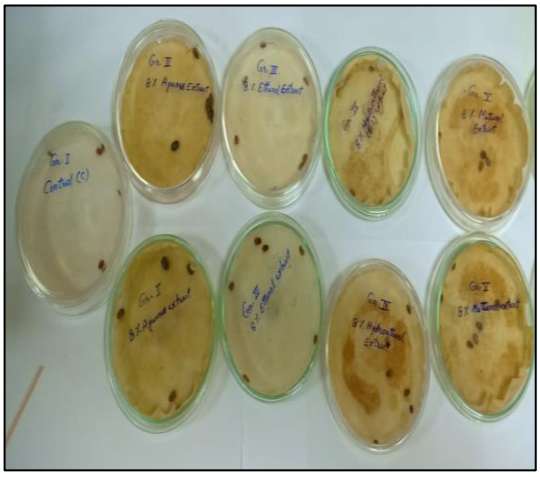

Fig 2: Adult tick immersion test in different Neem seed extract

Conclusion

Tick Rhipicephalus microplus is a blood sucking parasite causing huge economic loos in dairy industry. Gradient solvent extraction of *A. indica* (neem) seeds revealed the highest yield of methanolic extract followed by ethanolic, hydro-ethanolic and aqueous extract due to the presence of oily substance in alcoholic extracts, methods applied, types of solvent used, time and temperature maintained. Highest mortality of ticks was recorded in aqueous extract and lowest in methanolic extract during 24 hrs *in-vitro* screening. Thus, aqueous extracts of *A. indica* (neem) seed was found best for the acaricidal property because of sufficient biomolecules present in aqueous extract.

References

1. Abdel-Shafy S, Zayed AA. *In vitro* acaricidal effect of plant extract of neem seed oil (*Azadirachta indica*) on egg, immature, and adult stages of *Hyalomma anatolicum excavatum* (Ixodoidea Ixodidae). Vet. Parasitol 2002;106:89-96.
2. Alcaino H, Gorman T, Acosta P, Fredes F. Evaluacio´n de cinco esquemas de control con cipermetrina del *Rhipicephalus sanguineus* en la Regio´n Metropolitana de Chile. Arch. Med. Vet 1995;27:45-51.
3. Benavides E, Rodriguez JL, Romero A. Isolation and partial characterization of the Montecitos strain of *Boophilus microplus* (Canestrini, 1877) multi-resistant to different acaricides. Ann. N.Y. Acad. Sci 2000;916:668-671.
4. Bendigeri S, Das G, Shrman K, Kumar S, Khare RK, Sachan S, Saiyam R, *et al*. Phytochemical analysis of Saraca asoca bark and Azadirachta indica seeds. *Int. Jour*. Chem. Stud 2019;7(4):126-131.
5. Biswas S. Role of veterinarians in the care and management during harvest of skin in livestock species. In: Proc. National Seminar on Leather Industry in Today's Perspective, Kolkata, India 2003, pp. 62-64.
6. Drummond RO. Tick borne livestock diseases and their vectors. Chemical control of ticks. Wimld An. Rev. (FAO) 1983;36:28-33.
7. Franc M, Cadiergues MC. Activity of a deltamethrin shampoo against *Ctenocephalides felis* and *Rhipicephalus sanguineus* in dogs. Vet. Parasitol 1999;81:341-346.
8. Ghosh S, Azhahianambi P, De la Fuente J. Control of ticks of ruminants with special emphasis on livestock farming system in India- present and future possibilities for integrated control: a review. Expl. Appl. Acarol 2006;40:49-66.
9. Ghosh S, Azhahianambia P, Yadavb MP. Upcoming and future strategies of tick control: a review. J. Vect. Borne Dis 2007;44:79-89.
10. Giglioti R, Forim MR, Oliveira HND, Chagas ACS, Ferrezini J, Brito LG, *et al*. *In vitro* acaricidal activity of neem (Azadirachta indica) seed extracts with known azadirachtin concentrations against Rhipicephalus microplus. Vet. Parasitol 2011;181(2-4):309-315.
11. Grisi L, Leite RC, Martins JRDS, Barros ATMD, Andreotti R, Cançado PHD, *et al*. Reassessment of the potential economic impact of cattle parasites in Brazil. Rev. Bras. Parasitol. Vet 2014;23(2):150-156.
12. Ibrahim M, Kiranmai M. Successive solvent extraction and free radical scavenging activity of Azadirachta indica A. Juss., Int. J. Green Pharm 2012;6:237-240.
13. Jernigan AD, Mctier TL, Chieffo C, Thomas CA, Krautmann MJ, Hair JA, *et al*. Efficacy of selamectin against experimentally induced tick (*Rhipicephalus sanguineus* and *Dermacentor variabilis*) infestations on dogs. Vet. Parasitol 2000;91:359-375.
14. Kirthi AV, Rahuman AA, Rajakumar G, Marimuthu S, Santhoshkumar T, Jayaseelan C, *et al*. Biosynthesis of

titanium dioxide nanoparticles using bacterium *Bacillus subtilis*. Mater Lett 2011;65(17-180):2745-2747.
15. Kiss T, Cadar D, Spinu M. Tick prevention at a crossroad: New and renewed solutions. Vet Parasitol 2012;187(3-4):357-66.
16. Magadum S, Mondal DB, Ghosh S. Comparative efficacy of *Annona squamosa* and *Azadirachta indica* extracts against *B. microplus Izatnagar* isolates. Parasitol. Res 2009;105:1085-1091.
17. Otranto D, Lia RP, Cantacessi C, Galli G, Paradies P, Mallia E, *et al*. Efficacy of a combination of imidacloprid 10% permethrin 50% versus fipronil 10%/ (S) methoprene 12% against ticks in naturally infected dogs. Vet. Parasitol 2005;130:293-304.
18. Sharma P, Lokeshwar T, Mukesh B, Vishnu B. Review on neem (Azadirachta indica): thousand problems one solution. Inter. Res. J. Pharma 2011;2(12):97-102.
19. Shyma KP, Gupta JP, Ghosh S, Patel KK, Singh V. Acaricidal effect of herbal extracts against cattle tick Rhipicephalus (Boophilus) microplus using *in vitro* studies. Parasitol Res 2014. DOI: 10.1007/s00436-014-3839-3
20. Srivastava R, Ghosh S, Mandal DB, Azhahianambi PS, Singaghal PS, Pandey NN, *et al*. Efficacy of Azadirachta indica extracts against Boophilus microplus. Parasitology Research 2008;104:149-153.
21. Tesfaye B, Tefera T. Extraction of essential oil from neem seed by using soxhlet extraction methods. Int. J. Adv. Eng. Manag. and Sci 2017;3(6):239870.
22. Varadharajan A, Gnanasekar R. Acaricidal activity of herbal extracts against cattle tick (Rhipicephalus microplus), The Phar. Inno. J 2019;8(1):609-611.
23. WHO. Vector resistance to pesticide. Fifteenth Report of the WHO Expert Committee on Vector Biology and Control, Geneva. Technical Report Service 1992;818:58.
24. WHO. Pesticides and their Application for the Control of Vectors and Pests of Public Health Importance, 6th ed. Geneva 2006, pp. 114.
25. Zaman MA, Iqbal Z, Abbas RZ, Khan MN, Muhhammad G, Muhhammad Y, *et al*. *In vitro* and *in vivo* acaricidal activity of a herbal extract. Vet. Parasitol 2012;186:431-436.