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Jatropha curcas (Linn) leaf extract -a possible alternative for population control of *Rhipicephalus(Boophilus) annulatus*

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ABSTRACT

Objective: To study the effect of ethanolic extract of leaves of the plant Jatropha curcas as a step towards developing a safe and ecofriendly therapeutic agent to combat the problems of tick and tick-borne diseases. **Methods:** Pulverised leaves of *J. curcas* were subjected to soxhlet extraction using ethanol. The ethanolic extract of *J. curcas* at different dilutions such as, 50, 60, 70, 80, 90 and 100 mg / ml were tested against ticks using adult immersion test. The per cent adult mortality, inhibition of fecundity and hatching of laid ova were studied. **Results:** The extract caused significant blocking of hatching of the laid ova by the treated ticks. **Conclusions:** Eclosion blocking effect of *J. curcas* extract is a promising property of the plant that can be utilized for controlling the population of ticks.

1. Introduction

The control of ectoparasites of veterinary importance relies heavily on the use of chemicals and for the effective pest control around the world, it is necessary to have a range of compounds with different modes of action to enable the rotation of these chemicals and so help to manage existing problem of acaricidal resistance[1]. Tick control by use of chemical acaricides, is also fraught with various problems like residues, environmental pollution and high cost, clearly demanding the need for alternative approaches[2].

Traditional medicine using plant extracts continues to provide health coverage for over 80% of world population, especially in the developing world[3]. Even though many plants extracts with promising acaricidal effects were reported in literature, the feasibility of many of these extracts for the control of ticks infesting animals in the field conditions was not adequately studied[4].

Jetropha curcas Linn (*J. curcas*) belongs to the family Euphorbiaceae and are used in traditional folklore medicine to cure various aliments in Africa, Asia and Latin America[5]. The plant is native to North America but now thrives well in Africa and Asia. It is easy to establish as it grows relatively quickly with high yields[6]. It is one of the promising biodiesel plants. Traditionally, it is used to cure diseases like cancer, piles, snake bites, paralysis and dropsy[7]. The antimicrobial and larvicidal activities of the leaves of the plant[8, 9], stem bark[10] and the insecticidal property[11] of the *J. curcas* seed oil were already reported.

There were many previous reports on the acaricidal

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activity of essential oils from various plants against *R. (B.) annulatus*[12-15] and *R. (B.) microplus*[16-19]. Acaricidal activity of crude extracts from stem and leaves of different plants against the cattle ticks was also reported[20-25]. However, there are only meager reports on acaricidal properties of *J. curcas*. Therefore, an attempt has been made in this study to assess the effects of leaves of *J. curcas* against *R. (B.) annulatus*, the commonest tick species in southern region of Indian subcontinent[26-28].

2. Materials and methods

2.1. Plant material

The *J. curcas* L leaves were collected from Meenangady, Sulthan Bathery Taluk of Wayanad district, Kerala. The plant was identified and the voucher specimen was deposited in the herbarium of the National Botanical Research Institute, Lucknow.

2.2. Preparation of plant extract

The leaves were cleaned and dried in shade at room temperature. Dried plant leaves were finely pulverized using a grinder. The powdered plant material (100 g) was used for ethanolic extraction in a soxhlet extraction apparatus attached with rotary vacuum evaporator (Rotavac, Butchi, Switzerland). Solvent was completely removed by drying at room temperature. Required quantity of extract was weighed and dissolved in methanol for making six different dilutions at the rate of 50 mg/mL, 60 mg/mL, 70 mg/mL, 80 mg/mL, 90 mg/mL and 100 mg/mL.

2.3. Ticks collection

Fully engorged adult *R. (B.) annulatus* female ticks were collected from infested animals, washed with water and dried using tissue paper.

2.4. Adult immersion test

Various dilutions (50-100 mg/mL) of ethanolic extract of the plant leaves were tested using adult immersion test[4, 29]. A total of 168 numbers of ticks were used in the experiment. Four replicates of six ticks were used for each dilution of the extract. Group of six numbers of ticks were weighed prior to the experiment and they were immersed for 2 min in the respective dilution (10 mL) in a 50 mL beaker with gentle agitation and methanol used as control. Ticks were recovered from the solution, dried using tissue paper towels and placed in separate plastic specimen tube (25 X 50 mm). The tubes were incubated at 28℃ and 80% relative humidity in a BOD incubator.

2.5. Percentage of inhibition of fecundity and hatching

The eggs laid by the ticks of each tube were collected, weighed and observed at the same condition of incubation for the next 30 days for visual estimation of hatching. Ticks under different treatments were compared with that of controls.

The percentage inhibition of fecundity was calculated as follows:

Index of egg laying (IE) = weight of eggs laid (mg) / weight of females (mg)

Percentage inhibition of fecundity (IF) =[IE (control group) -IE (treated group)]×100 / IE (control group)[4].

2.6. Statistical analysis

Data were expressed as the mean ± SEM. Groups were compared using one-way ANOVA for repeated measurements using SPSS software. Duncan's test was used for post-hoc analysis. A value of $P<0.05$ was considered significant.

3. Results

Table 1
Effects of different concentration of ethanolic extract of *J. curcas* against *R. (B.) annulatus* (n=4). mean ± SEM.

Concerntration. (mg/mL)	Ticks weight per replicate (g)	Percentage adult mortality within 15 days (%)	Eggs mass per replicate (g)	Index of fecundity	Percentage inhibition of Fecundity (%)	Hatching percentage
Control	0.9425 ± 0.025^{ab}	0 ± 0^{a}	0.4255 ± 0.033^{a}	0.4501 ± 0.025^{a}	0	100
50	0.9903 ± 0.045^{b}	0 ± 0^{a}	0.4433 ± 0.046^{a}	0.4442 ± 0.030^{a}	1.31	10
60	0.8488 ± 0.007^{a}	0 ± 0^{a}	0.3682 ± 0.021^{a}	0.4333 ± 0.020^{a}	3.73	10
70	0.8865 ± 0.036^{ab}	0 ± 0^{a}	0.3798 ± 0.020^{a}	0.4284 ± 0.014^{a}	4.82	10
80	0.9265 ± 0.052^{ab}	0 ± 0^{a}	0.3885 ± 0.025^{a}	0.4189 ± 0.010^{a}	6.93	10
90	0.9490 ± 0.014^{ab}	0 ± 0^{a}	0.3893 ± 0.038^{a}	0.4090 ± 0.036^{a}	9.13	10
100	0.9745 ± 0.057^{ab}	0 ± 0^{a}	0.3980 ± 0.051^{a}	0.4045 ± 0.040^{a}	10.13	10

Values are Mean ± SEM, values in a column with different superscripts differ ($P<0.05$).

The results of adult immersion test using the ethanolic extract of *J. curcas* leaves are shown in table 1. The extract at all concentrations tested (50-100 mg / ml) considerably blocked the hatchability of eggs when compared to control. However, the extract of *J. curcas* did not produce mortality of adult engorged ticks. The extract also did not significantly reduce the mass of eggs laid by the treated ticks. A very low percentage of inhibition of egg laying was observed. Moreover, the eggs laid by the treated ticks were apparently glossy in their appearance.

4. Discussion

J. curcas has long been implicated in traditional medicine and also used as an insect repellant, a mollusicide and a rodenticide[30]. The insecticidal[11] and acaricidal[31] activities of J.curcas seed oil were previously reported. The oil of *J. curcas* was identified as efficacious in controlling sarcoptic mange in sheep when combined with ascorbic acid[31]. J curcas seeds showed high content of unsaponifiable matter and the insecticidal activity of seed oil was attributed to the presence of sterols and triterpene alcohols[32, 33]. The by-product after extraction of oil from the seeds of the plant also contained insecticides[34]. The antiovipositional and ovicidal effects of *J. curcas* against Callosobruchus maculatus Fab. were also reported. It is speculated that suffocation and / or lethal chemical poisoning due to Jatropha oil application prevented the adult emergence from the bruchid, *C. maculatus*[35].

The acaricidal properties of plant extracts against *R. (B.) microplus* were attributed to terpenoids[36-39] and tannins[40]. The presence of triterpenoids, volatile oils, alkaloids, flavonoids, saponins and tannins was previously confirmed in *J. curcas* leaves[41]. In the present study, the ethanolic extract from the leaves of the plant did not reveal any cidal effect on adult engorged female ticks even at a concentration of 100 mg/ml. Also, in another study 5 per cent aqueous leaf and bark extracts of *J.curcas* revealed less acaricidal effect against spotted spider mite *Tetranychus urticae* Koch[42]. The larvicidal effect of methanolic leaf extract of *J.curcas* against the first and fourth instar larvae of *Culex quinquefasciatus* Say[9] was reported. In another study, the acetone extract of *J. curcas* leaves extended the duration of the various larval instars and of pupation of *Aedes aegypti* even at very low concentration and showed toxicity at higher concentrations[43]. These effects could be due to very low content of triterpenoids and tannins in Jatropha leaves compared to seed oil.

J. curcas extract was highly effective in controlling hatching of eggs laid by the treated ticks. The eggs laid by the treated ticks were apparently glossy in their appearance. Thus, it is evident from the results of the present study that the oviposition was not at all inhibited but the eclosion was prevented. The results were consistent with the previous reports on the population limiting properties of various plant extracts. The ethanolic extracts of *Leucas aspera* induced a significant concentration dependent decrease in egg mass production and complete blocking of the hatching of the laid ova[25]. The hexane extract of aerial parts of the plant *Calea serrata* inhibited hatching of *B. microplus* eggs, when they were immersed in the dilution of the extract and the activity was attributed to chromenes present in the plant especially the precocene II[44]. Similarily, *Lysiloma latisilquum* extract also showed an inhibitory effect on egg hatching at concentration of 19, 200 μg /mL[40].

The molting hormones, ecdysteroids play an important role in the regulation of salivary gland function, production of pheromones, oogenesis and oviposition[45] in ticks. Ecdysone is metabolized by specific cytochrome P450 isozymes to the active form, 20 hydroxy ecdysone (20 HE). This is then transported through haemolymph to the target cells, where it binds to the ecdysone receptor to cause gene transcription[46-48].

Several phytochemicals, in particular, the flavones have the potential to interact with the vertebrate estrogen receptor as agonists or antagonists. Some of the flavones like luteolin, quercetin, apigenin and chrysin were reported to inhibit ecdysone mediated gene expression in an ecdysone responsive cell line, CL8+[49]. Apigenin inhibit both mammalian and insect cytochrome P450 isozyme expression and activity. The flavones, apigenins (apigenin 7-O-β-D-neohesperidoside, apigenin 7-O-β-D-galactoside), orientin, vitexin, vicenin II and the biflavone di-C-β-Dglucopyranoside-methylene-(8, 8′)-biapigenin were isolated from the leaves of *J. curcas*[50]. The efficacy of the extract in inhibiting the hatching of eggs laid by the treated ticks could thus be attributed to the presence of the flavone apigenin which can cause decrease in the level of active ecdysteroid by inhibiting the P450 enzyme. This could be attributed to the decreased levels of ecdysteroids leading to decreased incorporation of free ecdysteriods into the eggs or interference with the uptake of modified egg yolk protein, vitellin into the oocytes both being important for egg maturation and development.

The ethanolic extract of the leaves of *J. curcas* L at low concentrations can significantly inhibit the hatching of laid eggs and can be considered as a possible alternative for the control of ticks. The role of flavonoids and their mechanisms in modulating the tick reproduction need to

be explored further.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Graf JF, Gogolewski R, Leach-bing N, Sabatini GA, Molento MB, Bordin EL et al. Tick control: an industry point of view. *Parasitol* 2004; **129**: S427-S442.

[2] Ghosh S, Azhahianambi P, Yadav MP. Upcoming and future strategies of tick control: a review. *J Vector Borne Dis* 2007; **44**:79-89.

[3] WHO. Traditional medicine: growing needs and potential, WHO policy perspective on medicine. Geneva: World Health Organization; 2002, p.1-6.

[4] FAO. Guidelines for resistance management and integrated parasite control in ruminants. Rome: Food and Agriculture Organization of the United Nation; 2004.

[5] Burkill HM. The useful plants of West Tropical Africa (Families E-J). Kew:Royal Botanical Gardens; 1994, p. 90-94.

[6] Willis TE. Text book of Pharmacognosy. London: J and A Churchill Ltd; 1967, p. 513-524.

[7] Okujagu TF, Etatuvie SO, Eze I, Jimoh B, Nweke C, Mbaoji C editors. Medicinal Plants of Nigeria; South-west Nigeria. 1st Edited. Lagos: University of Lagos; 2006, p. 1-20.

[8] Kalimuthu K, Vijayakumar S, Senthilkumar R. Antimicrobial activity of the biodesel plant, *Jatropha curcas*. *Intern J Pharm Bio Sci* 2010; **1**(3): 1-5.

[9] Kalimuthu K, Kadarkaral M, Savariar V, Siva K. Larvicidal efficacy of *Jatropha curcas* and bacterial insecticide, *Bacillus thuringiensis*, against lymphatic filarial vector, *Culex quinquefasciatus* Say(Dipteria: Culicidae). *Parasitol Res* 2011;**109**(5):1251-1257

[10] Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *Afr J Pharm Pharacol* 2009; **3**(2): 58-62.

[11] Adebowale KO, Adedire CO. Chemical composition and insecticidal properties of the underutilized *Jatropha curcas* seed oil. *Afr J Biotechnol* 2006; **5**(10): 901-906.

[12] Pirali-Kheirabadi K, Razzaghi-Abyaneh M, Halajian A. Acaricidal effect of *Pelargonium roseum* and *Eucalyptus globulus* essential oils against adult stage of *Rhipicephalus (Boophilus) annulatus in vitro*. *Vet Parasitol* 2009; **162**:346-349.

[13] Pirali-Kheirabadi K, da Silva JAT. *Lavandula angustifolia* essential oil as a novel and promising natural candidate for tick (*Rhipicephalus (Boophilus) annulatus*) control. *Exp Parasitol* 2010; **126**: 184-186.

[14] Pirali-Kheirabadi K, da Silva JAT. In-Vitro Assessment of the Acaricidal Properties of *Artemisia annua* and *Zataria multiflora* essential oils to control cattle ticks. *Iranian J Parasitol* 2011; **6**: 58-65.

[15] Martinez-Velazquez M, Rosario-Cruz R, Castillo-Herrera G, Flores-Fernandez JM, Alvarez AH, Lugo-Cervantes E. Acaricidal effect of essential oils from *Lippia graveolens* (Lamiales: Lamiaceae) and *Allium sativum* (Liliales:Liliaceae) against *Rhipicephalus (Boophilus) annulatus* (Acari: Ixodidae). *J Med Entomol* 2010; **48**:822-827.

[16] de B F Ferraz A, Balbino JM, Zini CA, Ribeiro VL, Bordignon SA, von Poser G. Acaricidal activity and chemical composition of the essential oil from three Piper species. *Parasitol Res* 2010; **107**: 243-248.

[17] Ribeiro VLS, dos Santos JC, Bordignon SAL, Apel MA, Henriques AT, von Poser G. Acaricidal properties of the essential oil from *Hesperozygis ringens* (Lamiaceae) on the cattle tick *Boophylus microplus*. *Bioresource Technol* 2010; **101**:2506-2509.

[18] Gazim ZC, Demarchi IG, Lonardoni MVC, Amorim ACL, Hovell AMC, Rezende CM et al. Acaricidal activity of the essential oil from *Tetradenia riparia* (Lamiaceae) on the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari; Ixodidae). *Exp Parasitol* 2011; **129**(2):175-178.

[19] Ribeiro VLS, dos Santos JC, Martinsc JR, Schripsemad J, Siqueiraa IR, von Poser G et al. Acaricidal properties of the essential oil and precocene II obtained from *Calea serrata* (Asteraceae) on the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari:Ixodidae).*Vet Parasitol* 2011; **179**: 195-198.

[20] Kaaya GP, Mwangi EN, Malonza MM. Acaricidal activity of *Margaritaria discoidea* (Euphorbiaceae) plant extracts against the ticks *Rhipicephalus appendiculatus* and *Amblyomma variegatum* (Ixodidae). *Int J Acarol* 2009; **21**:123-129.

[21] Bagavan A, Kamaraj C, Elango G, Zahir AA, Rahuman AA. Adulticidal and larvicidal efficacy of some medicinal plant extracts against tick, fluke and mosquitoes. *Vet Parasitol* 2009; **166**: 286-292.

[22] Rosado-Aguilar JA, Aguilar-Caballero A, Rodriguez-Vivas RI, Borges-Argaez R, Garcia-Vazquez Z, Mendez-Gonzalez M. Acaricidal activity of extracts from *Petiveria alliacea* (Phytolaccaceae) against the cattle tick, *Rhipicephalus (Boophilus) microplus* (Acari: ixodidae). *Vet Parasitol* 2010; **168**: 299-303.

[23] Habeeb SM. Ethno-Veterinary and Medical Knowledge

of Crude Plant Extracts and its Methods of Application (Traditional and Modern) for Tick Control. *World Applied Sci J* 2010; **11**: 1047-1054.

[24] Madzimure SJ, Nyahangare ET, Hamudikuwanda H, Hove T, Stevenson PC, Belmain SR et al. Acaricidal efficacy against cattle ticks and acute oral toxicity of *Lippia javanica* (Burm F.) *Trop Anim Health Prod* 2011; **43**: 481-489.

[25] Ravindran R, Juliet S, Sunil AR, Kumar KGA, Nair SN, Amithamol KK et al. Eclosion blocking effect of ethanolic extract of *Leucas aspera* (Lamiaceae) on *Rhipicephalus (Boophilus) annulatus*. *Vet Parasitol* 2011;**179**: 287-290.

[26] Rajamohanan K. Identification of vector for babesiosis of cattle in Kerala. Proceedings of All India symposium of vectors and vectors and vector born diseases, Trivandrum, Kerala.1982, p.125-128.

[27] Koshy TJ, Rajavelu G, Lalitha CM. Ecology and bionomics of boophilids of Tamil Nadu. *Cheiron* 1982; **11**:25-30.

[28] Jagannath MS, Muraleedharan K, Hiregoudar LS. Prevalence of ixodid ticks of cattle at Bangalore. *Indian J Anim Sci* 1979; **49**: 890-894.

[29] Drummond RO, Ernst SE, Trevino JL, Gladney WJ, Graham OH. *Boophilus annulatus* and *Boophilus microplus* laboratory tests for insecticides. *J Econ Entomol* 1973; **66**:130-133.

[30] Duke JA. CRC Handbook of Medicinal herbs. CRC Press Inc Boca Raton, FL. 1985

[31] Dimri U, Sharma MC. Effects of sarcoptic mange and its control with oil of *Cedrus deodara, Pongamia glabra, Jatropha curcas* and benzyl benzoate, both with and without ascorbic acid on growing sheep: epidemiology;assessment of clinical, haematological, cell- mediated humoral immune responses and pathology. *J Vet Med A Physiol Pathol Clin Med* 2004; **55**:71-78

[32] Jazeen DH. How Southern cowpea weevil larvae (Bruchidae: *Collosoruchus maculatus* die on non-host seeds. *Ecology* 1977; **58**: 921-927.

[33] Heftmann E. Recent advances in photochemistry. New York: Appleton Century; 1970.

[34] Openshaw K. Concepts and Methods for collecting and compiling statistics in Biomas used as Energy. USA: UN statistical office New York; 1986.

[35] Jadhau KB, Jadhua LD. Use of vegetable oils, plant extracts and synthetic products as protectants from pulse beetle. *Callosobruchus maculatus* in stored grain. *Food Sci Technol* 1984; **21**: 110-113.

[36] Pereira JR, Famadas KM. The efficiency of extracts of *Dahlstedtia pentaphylla* (Leguminosae, Papilionoidae, Millettiedae) on *Boophylus microplus* (Canestrini, 1887) in artificially infested bovines. *Vet Parasitol* 2006; **142**:192-195.

[37] Fernandes FF, Freitas EPS. Acaricidal activity of oleoresinous extract from *Copaifera reticulate* (Legiminosae: Caesalpinioidae) against larvae of the southern cattle tick, *Rhipicephalus (Boophilus) microplus* (Acari:Ixodidae). *Vet Parasitol* 2007; **147**:150-154.

[38] Ribeiro VLS, Togio E, Bordignon SAL, Goncalves K, von Poser G. Acaricidal properties of extracts from the aerial parts of *Hipericum polyanthemum* on the cattle tick *Boophylus microplus*. *Vet Parasitol* 2007; **147**:199-203.

[39] Magadum S, Mondal DB, Ghosh S. Comparative efficacy of *Annona squamosa* and *Azadirachta indica* extracts against *Boophylus microplus* Izatnagar isolate. *Parasitol Res* 2009; **105**: 1085-1091.

[40] Fernandez-Salas A, Alonso-Diaz MA, Acosta-Rodriguez R, Torres-Acosta JFJ, Sandoval-Castro CA, Rodriguez-Vivas RI. *In vitro* acaricidal effect of tannin-rich plants against the cattle tick, *Rhipicephalus (Boophilus) microplus* (Acari:Ixodidae). *Vet Parasitol* 2011; **175**:113-118.

[41] Uche FI, Aprioku JS. The phytochemical constituents, analgesic and anti-inflammatory effects of methanol extract of *Jatropha curcas* leaves in mice and Wistar albino rats. *J Appl Sci Environ Manage* 2008; **12**:99-102.

[42] Reddy GVM, Girish R, Uma MS, Srinivas N. Acaricidal activity of aqueous extracts from leaves and bark of cinnamomum and jatropha against two spotted spider mite,*Tetranychus urticae* Koch. *Karnataka J Agric Sci* 2009; **22**: 693-695.

[43] Joish MM, Pathipati UR. Biological activity of certain botanical extracts as larvicides against the yellow fever mosquito, *Aedes aegypti*.L. *J bio pests* 2009; **2**(1): 72-76.

[44] Ribeiro VL, Avancini C, Goncalves K, Togio E, von Poser G Acaricidal activity of *Calea serrata* (Asteraceae) on *Boophilus microplus* and *Rhipicephalus sanguineus*. *Vet Parasitol* 2008; **151**:351-354

[45] Velayutham R, Sankaradoss N, Nazeer Ahamed KFH. Protective effect of tannins from *Ficus racemosa* in hypercholesterolemia and diabetes induced vascular tissue damage in rats. *Asian Pac J Trop Med* 2012; **5**(5): 367-373

[46] Osadebe PO, Okoye FBC, Uzor PF, Nnamani NR, Adiele IE, Obiano NC. Phytochemical analysis, hepatoprotective and antioxidant activity of *Alchornea cordifolia* methanol leaf extract on carbon tetrachloride-induced hepatic damage in rats. *Asian Pac J Trop Med* 2012: **5**(4): 289-293

[47] Kovendan K, Murugan K, Vincent S, Barnard DR. Mosquito larvicidal properties of Orthosiphon thymiflorus (Roth) Sleesen. (Family: Labiatae) against mosquito vectors, *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Asian Pac J Trop Med* 2012; **5**(4): 299-305

[48] Rees HH. Hormonal control of tick development and reproduction. *Parasitol* 2004; **129**: S127-S143.

[49] Oberdorster E, Clay MA, Cottam DM, Wilmot FA, McLachlan JA, Milner MJ. Common phytochemicals are ecdysteroid agonists and antagonist: a possible evolutionary link between vertebrate and invertebrate steroid hormones. *J Steroid Biochem Mol Biol* 2001; **77**: 229-238.

[50] Abd-Alla HI, Moharram FA, Gaara AH, El-Safly MM. Phytoconstituents of *Jatropha curcas* L. leaves and their immunomodulatory activity on humoral and cell mediated response in chicks. *Z Naturforsch C* 2009; **64** (7-8):495-501.