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Acaricidal Bioefficacy of *Calotropisprocera* (Asclepiadaceae) of Middle Gangetic Region against Cattle Tick, *Rhipicephalusmicroplus* and Their Gc-Ms Analysis

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ABSTRACT

Pharmaceutic plants are used by classical and veterinarians as an alternate to the traditional drug to cure an extensive variety of irregularity along parasitic illnesses. A few compounds from these plants exhibit acaricidal action and also resist arthropods. The livestock tick Rhipicephalus microplus is one of the most disturbing pests to cattle breeding in tropical and subtropical regions of the world. The ability to obtain herbal acaricides to regulate R. microplus is crucial in retaining cattle flock fertility, decreasing financial damages, and reducing the misuse of artificial chemical acaricides. Extracts derived from Calotropis procera were calculated for acaricidal activity across R. microplus. The botanicals are considered an important source of promising bioactive compounds and the target plant is known to have biochemical constituents with potential medicinal properties. Thus, the present study was aimed to calculate the phytochemical properties of raw methanolic distillation and their fractions of C. procera. Hexane, ethyl acetate, chloroform, and aqueous fractions of the plant possess alkaloids, flavonoids, and saponins which were analyzed using Gas chromatography-Mass spectrometry (GC-MS) and exhibit remarkable acaricidal activity.

Keywords: Calotropis procera, Rhipicephalus microplus, Tick control, Acaricides.

INTRODUCTION

Plant centered natural goods, minerals animal developed and natural product have been the establishment of cure of diverse human illnesses.1 A lot of people across the globe, specifically in emerging nations are progressively trusting on plant derivative conventional drugs. W.H.O describes a therapeutic plant that produces biologically active compounds in one or more body parts that can be used for therapeutic purposes.[2] Calotropis procera (Figure 1) is a plant in the Asclepiadaceae family. It is an Ayurvedic and acaricidal plant with important therapeutic functions. It occurs in several parts across the globe with a hot weather in dry, dirty and limy soils.[3] Dry and semi-dry areas are the foremost parts where C. procera grow significantly with no necessity of insect killer, chemical composts and irrigation.[4] Various portions C. procera were working for medical reasons in the conventional drugs and the various scientist were highlighted their therapeutic features.[5]

Rhipicephalus microplus is considered to be the utmost inexpensively leading tick classes disturbing cattle in hot and subtropical area of globe. Beyond the 904 tick types currently recognized, this classes cause an projected the annual worldwide loss of around $ 30 billion.[6,7] R. microplus permeates a wide-ranging variety of host animals and is a known transmitter of microbial infectious agent producing illnesses alike Anaplasmosis and Babesisosis, that can cause substantial death in cattle, herd.[8,9,10] An effectively implemented joined tick

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management (ITM) programme to handling ticks essential to consist of dissimilar approaches with varied means of act to maximize efficiency, At the same time, it reduces the risk of target pests becoming resistant to treatment with designated chemical acaricides.[11,12] Intense cures with artificial acaricides like pyrethroids, organophosphates, fluazuron, amitraz, organachlorines, carbamets, fipronits, and tri based Ivermectin. Doramectin chooses for the progresses of acaricides resistance in pests species like R. microplus. These compounds can damage the environment and cause fatal harm to humans, domestic animals and wildlife.[13]

Reports of anti-acaricide tick insects are becoming more and more common around the world for a variety of reasons, all of which emphasize the failure of existing control technologies. There is an urgent need for a new sustainable system to address the tick and tick-borne diseases and to replace the existing chemotherapy, which requires research and development and ultimately implementation in the region.[14,15,16]

Study on new control technologies include investigation plants to recognized any anti-tick properties they may possess. At that time, before integrating plant acaricides into the ITM program, additional tests had to be performed to identify different bioactive metabolites.[17]Compared with artificial acaricides, plant-derived compounds are generally easier to degrade and less lethal, but before large-scale development, the harmfulness to humans and other mammals should be carefully evaluated.[18,19,20] Various reviews of R. microplus only found acaricidal properties from extracts from different plant categories around the world, which are naturally present in specific topographical locations. Various researches advise that botanicals can be used alone or in combination to improve present chemical control approaches.[21] Leaf extracts from Baccharis trimna (Asteraccae) inborn to various areas of South America, reported significant egg hatching of R. microplus in Brazil,[22] Aegle marmolas (Rutaceae), Andographis lineata (Acanthaceae), Spilanthes acmella (Asteraceae), displayed the acaricidal action against R. microplus.

The current investigation was focused to assess the extracts of C. procera for acaricidal and their chemical composition and generative inhibition action against R. microplus. The adult immersion test (AIT) was done to determine the capacity of the plant extracts and their separation fraction with chloroform, hexane, ethyl acetate, and aqueous to inhibit hatchability and oviposition. C. procera (aerial parts) exhibited different compound characterized by GC-MS data.

The results of this study indicate that more research is necessary to establish a comparative relationship between the different active compounds from the aerial parts of C. procera in their acaricidal effects.

MATERIALS AND METHODS

(a) Plant collection and extraction

The plant, *Calotropis procera* was identified and authorized by CSIR-National Botanical Research Institute, Lucknowwith Accession No. 106922 and its family Asclepiadaceae. Fresh aerial parts of the plant were collected from Mathura, Uttar Pradesh,Middle gangetic plains region and washed, shade dried at room temperature, after that grinded by steel grinder as coarse powder. 327 g coarse powder was extracted in methanol and solvent was recovered in rotary evaporator and 79.80 g extract was obtained with and 24.40 % yield.

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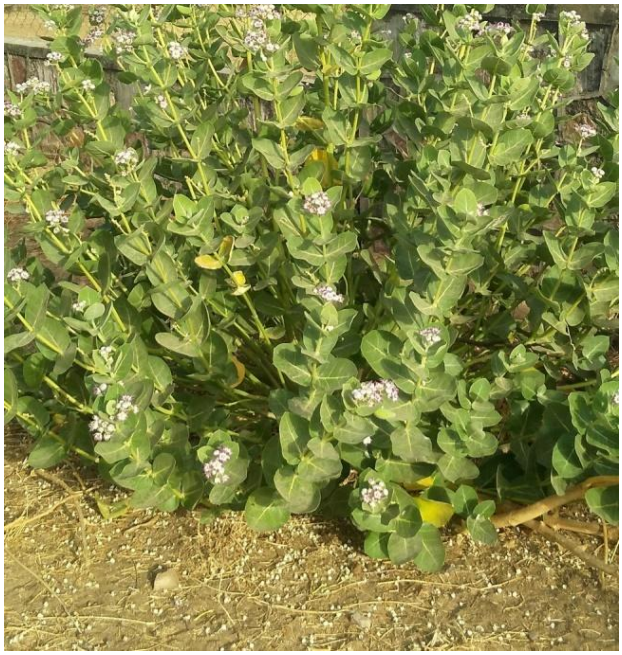

Figure 1: Aerial parts of *Calotropis procera* plant

(b) Collection of target organism

Adult stage of *Rhipicephalus* (*Boophilus*) *microplus* was collected. The ticks dropped after taking full blood meal from the animal's body during nightwere collected early in the morning from farmer's house in plastic bottle (like container) from Mathura, Uttar Pradesh, India and covered with cotton cloth for proper oxygen supply. Ticks were washed in running tap water to remove dust and other impurity on the body surface and dried by filter paper after thenkept into refrigeratorat 4^0C for further studies.

(c) Fractionation of methanolic extract

Fractionation was done in three different solvents (hexane, ethyl acetate and chloroform). 2 g crude ~~of~~ methanolic extract was dissolved in 25 ml distilled water and 15 ml hexane mixed and shaked well. The mixure was allowed to settle for 30 minutes for separation of solvents. Hexane part was collected (top layer) in 50 ml beaker. Rest amount of aqueous part similarly were processed with ethyl acetate and chloroform successively. Solvents (hexane, ethyl acetate, chloroform) and aqueous parts were dried on water bath and yields were calculated (table 1).

(d) Adult Immersion Test (AIT)

Ticks were taken in petri dishes of 9 cm in diameter containing bed of filter paper (Whatman No.1). 105 ticks were divided into seven groups and six groups treated one kept as control. 15 ticks in each group were weight approximately 2 to 3 g and treated group was immersed into the respective test fraction concentrations (hexane, ethyl acetate, chloroform and aqueous parts) and the control group was immersed in distilled water for approximately 2-3 minutes. Treated ticks were placed on petri dishes and incubated into BOD incubator at

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28±2.0ºC temperature and 70-80 % relative humidity. Mortality data were observed after 24 hours and died ticks removed from petri dishes and observed in the same way up to 15 days until complete oviposition[23].

(e) Preparation of GC-MS sample

10 mg extract of fractions dissolved in 2 ml of GC-MS grade methanolwas used for injection in the machine using GC-MS Model -TQ8030 of Japan with a program for real time analysis. GCMS data of (aerial parts) *Calotropis procera*of different Agro-climatic regions accordingly peak area shown in GC-MS and data is presented in figure 2 to figure 6.

RESULTS AND DISSCUSSION

The *Calotropis procera* (aerial part) has been biological managed effect on live beings. In table 1 shows yield of fractions of *Calotropis procera*hexane yield 0.332 g, ethyl acetate yields 0.382 g, chloroform yield 0.046 g and aqueous yield 1.24 g.

Table 1: % yield of *Calotropis procera* (aerial part) fractionations using different solvents

Solvents	Mass extracted (g)	Yield weight (g)
Hexane	2	0.332
Ethyl acetate	2	0.382
Chloroform	2	0.046
Aqueous	2	1.24

The acaricidal bioefficacy of hexane, ethyl acetate and chloroform fractions of *Calotropis procera*(aerial parts) against *Rhipicephalus (Boophilus) microplus* at test concentrations 20, 40, 60, 80, 100, 120 mg/ml responded % mortality of hexane fraction 6.67, 26.67, 60.00, 66.67, 80.00, 86.67, ethyl acetate fraction 13.33, 33.33, 53.33, 66.67, 80.00, 86.67 and chloroform fraction % mortality 20.00, 40.00, 66.67, 73.33, 80.00, 93.33. The test concentrations were 60, 120, 180, 240, 300 and 360 of aqueous fraction, % mortality 13.33, 20.00, 40.00, 60.00, 80.00, 93.33 (table 2).

Table 2 Adulticidal bioefficacy of fractions of methanolic extract of *Calotropis procera* against *Rhipicephalus (Boophilus) microplus*

Fractions	Concentration (mg/ml)	Total 15 ticks, average body weight (g)	Number of died ticks after 24 hrs.	Mortality % after 24 hrs.
Hexane fraction	20	2.20	01	6.67
	40	2.20	04	26.67
	60	2.21	09	60.00
	80	2.21	10	66.67
	100	2.20	12	80.00
	120	2.22	13	86.67
	Control	2.21	00	00.00

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Ethyl acetate fraction	20	2.20	02	13.33
	40	2.20	05	33.33
	60	2.22	08	53.33
	80	2.21	10	66.67
	100	2.22	12	80.00
	120	2.21	13	86.67
	Control	2.22	00	00.00
Chloroform Fraction	20	2.20	03	20.00
	40	2.21	06	40.00
	60	2.20	10	66.67
	80	2.22	11	73.33
	100	2.20	12	80.00
	120	2.23	14	93.33
	Control	2.24	00	00.00
Aqueous Fraction	60	2.21	02	13.33
	120	2.20	03	20.00
	180	2.20	06	40.00
	240	2.22	09	60.00
	300	2.23	12	80.00
	360	2.21	14	93.33
	Control	2.21	00	00.00

LC_{50} value of hexane fraction 56.50 mg/ml along with 68.23 and 44.78mg/ml Upper Fiducial Limit (UFL) and Lower Fiducial Limit (LFL) and 135.41mg/ml LC_{90} value with UFL and LFL 182.89 and 87.93 mg/ml, in ethyl acetate fractionLC_{50} value 53.51 mg/ml with UFL and LFL 66.41 and 40.61 mg/ml, LC_{90} value 150.70 mg/ml with UFL and LFL 216.71 and 84.69 mg/ml. Chloroform fraction LC_{50} value 44.41 mg/ml with UFL and LFL 56.39 and 32.43 mg/ml, LC_{90} value 130.51 mg/ml with 184.80 and 76.22 mg/ml UFL and LFL values. In aqueous fraction LC_{50} value 179.87 mg/ml with 218.29 and 141.45 mg/ml UFL and LFL, LC_{90} value 449.12 mg/ml with UFL 628.23 and LFL 270.01 mg/ml (table 3).

Table 3 Relative acaricidal bioefficacy of the fractions of methanolic extracts of *Calotropis procera*against *Rhipicephalus (Boophilus) microplus*

Target Species	Target Stage	Fraction Solvent	X^2	Regression Equation	LC_{50}+SE UFL-LFL	RT	LC_{90}±SE UFL-LFL	RT
Rhipicephalus (Boophilus) Microplus	Adult	Hexane	0.43	Y=4.29X+3.38	56.50±5.98 (68.23-44.78)	1.27	135.41±24.23 (182.89-87.93)	1.04
		Ethyl acetate	0.29	Y=2.78X+2.85	53.51±6.58 (66.41-40.61)	1.20	150.70±33.68 (216.71-84.69)	1.15

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		Chloroform	0.79	Y=2.25X+2.74	44.41±6.11 (56.39-32.43)	1	130.51±27.70 (184.80-76.22)	1
		Aqueous	4.34	Y=5.49X+3.22	179.87±19.60 (218.29-141.45)	4.05	449.12±91.38 (628.23-270.01)	3.44

In Table 04 shows active compound in all fractions. In hexane 3-Isopropoxy-1,1,1,7,7,7-hex, Naphthalene, 1,6-dimethyl-4- compound present, ethyl acetate Cyclohexane, isocyanato-, 3-Isopropoxy-1,1,1,7,7,7-hex, 2-(2',4',6',8',8'-Hept, Phenol, 2-(1-phenylethyl)-, active compound present, chloroform 3-Isopropoxy-1,1,1,7,7,7-hex, 2-(2',4',6',8',8'-Hept active compound and aqueous fraction 2-(2',4',4',6',6',8',8'-Hept active compound.

Table 4 Retention time of active compound and peak area

Extract	Peak	Retention time (min.)	Active compound	Amount
Hexane fraction	1	6.883	Cyclohexane, isocyanato-	0.358
	2	6.918	Cyclohexane, isocyanato-	0.795
	3	10.259	4-(2-Acetylamino-1-	0.084
	4	10.909	(trimethy	0.484
	5	11.735	1-[2-Pyridyl]-2,2-	0.142
	7	12.970	dimethyl-2	0.030
	9	14.233	Benzothiazole	0.099
	10	**16.195**	2-Methylnaphthalene	**1.815**
	11	16.741	Anisaldehyde dimethyl	0.328
	12	16.896	acetal	0.003
	14	18.644	**3-Isopropoxy-1,1,1,7,7,7-**	0.883
	15	18.990	**hex**	0.059
	17	19.615	N,N-Dimethyl-1-(4-[3-(1-	4.774
	18	20.301	pipe	0.028
	20	**20.732**	2(4H)-Benzofuranone,	**1.229**
	23	21.430	5,6,7,7	0.074
	24	22.049	2-(2',4',6',8',8'-Hept	0.423
			1,1'-Biphenyl, 2,2',5,5'-tet	
			Phenol, 2-(1-phenylethyl)-	
			Benzene, 1,1'-(3-methyl-1-pr	
			Naphthalene, 1,6-	

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			dimethyl-4- Di-N-butylphthalate Pentadecanoic acid, 14-methy	
Ethyl acetate fraction	**3** 5 6 7 9 10 11 **13** 14 16 **17** 18 **19** 21 22 23 25	**6.851** 10.233 10.883 11.714 12.884 12.949 13.316 **16.180** 16.521 18.513 **18.635** 19.345 **19.594** 20.295 20.494 20.721 20.944	**Cyclohexane, isocyanato-** 4-(2-Acetylamino-1-(trimethy 1-[2-Pyridyl]-2,2-dimethyl-2 Benzothiazole 1-Proline, N-methoxycarbonyl 2-Methylnaphthalene 2-Methoxy-4-Vinylphenol **3-Isopropoxy-1,1,1,7,7,7-hex** Phenol, 2,4-bis(1,1-dimethyl 2-Cyclohexan-1-one, 4-(3-hyd **2-(2',4',6',8',8'-Hept** 2-Octyl benzoate **Phenol, 2-(1-phenylethyl)-** Benzene, 1,1'-(3-methyl-1-pr 2-Cyclohexan-1-one, 4-hydrox Naphthalene, 1,6-dimethyl-4- 3,7,11,15-Tetramethyl-2-hexa	**1.030** 0.103 0.605 0.156 0.114 0.050 0.085 **2.410** 0.085 0.034 **1.077** 0.072 **1.988** 0.051 0.107 0.509 0.062
Chloroform Fraction	1 2 6 7 8 10 11 13	6.722 6.891 10.913 11.663 11.735 12.973 13.337 14.444	Phosphonic acid, (p-hydroxy p Cyclohexane, isocyanato- 4a, 8a-(Methaniminomethano)na Benzaldehyde, 3-methyl- Benzothiazole 2-Methylnaphthalene	0.052 0.690 0.635 0.048 0.117 0.048 0.135 0.133

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	14	**16.199**	2-Methoxy-4-Vinylphenol	**2.347**
	15	16.545	7-Chloro-1,3,4,10-	0.128
	17	**18.647**	tetrahydro	**1.253**
	18	18.884	**3-Isopropoxy-1,1,1,7,7,7-**	0.118
	19	19.355	**hex**	0.058
	20	19.606	Phenol, 2,4-bis(1,1-	2.093
	21	19.823	dimethyl	0.138
	23	20.301	**2-(2',4',6',8',8'-Hept**	0.032
	24	20.512	2(3H)-Benzothiazolone	0.275
	25	20.730	2-Octyl benzoate	0.552
			Phenol, 2-(1-phenylethyl)-	
			4-((1E)-3-Hydroxy-1-	
			propenyl	
			Benzene, 1,1'-(3-methyl-	
			1-pr	
			5,5,8a-Trimethyl-	
			3,5,6,7,8,8	
			Naphthalene, 1,6-	
			dimethyl-4-	
Aqueous	3	10.230	4-(2-Acetylamino-1-	0.204
fraction	5	10.888	(trimethy	0.704
	6	11.644	4a,8a-	0.037
	7	12.950	Methaniminomethano)na	0.046
	10	14.422	Benzaldehyde, 3-methyl-	0.013
	11	14.484	2-Methylnaphthalene	0.089
	13	15.489	Fumaric acid, monoamide,	0.089
	15	**16.180**	N-m	**2.659**
	16	16.515	7-Chloro-1,3,4,10-	0.104
	19	18.148	tetrahydro	0.098
	20	18.179	Quinoline, 1,2-dihydro-	0.109
	21	18.476	2,2,4	0.028
	22	18.546	**3-Isopropoxy-1,1,1,7,7,7-**	0.035
	23	**18.636**	**hex**	**1.278**
	24	18.724	Phenol, 2,5-bis(1,1-	0.162
	25	18.811	dimethyl	0.025
	26	18.847	N,N, 3-Trimethyl-5-oxo-	0.031
			1-phen	
			Diphenylamine	
			Phenol, 4-(1-	
			methylpropyl)-	
			Hexestrol, O-	

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			trifluoroacetyl **2-(2',4',4',6',6',8',8'-Hept** Carbamic acid, N-[1,1-bis(tr Hexestrol, O-trofluoroacetyl Phenol, 4-(1-methylpropyl)-	

The four fractions were subjected to GCMS-MS analysis assists us to determine the presence of total numbers of peaks and their retention time, peak amount along their peak report. The spectral analysis of (**Figure 2**) hexane fraction was observed the highest peak amount (1.815) with retention time 16.195 min. and the peak mentioned the compound 3-Isopropoxy-1,1,1,7,7,7-hex. Spectral analysis of (**Figure 3**) ethyl acetate fraction observed as sharp peak amount with 2.410 and retention time 16.180 min. and the peak mentioned the compound 3-Isopropoxy-1,1,1,7,7,7-hex. The analysis of (**Figure 4**) chloroform fraction of *Calotropis procera* of peak amount was highest 2.347 and retention time 16.199 min. and the peak mentioned the compound 3-Isopropoxy-1,1,1,7,7,7-hex. In (**Figure 5**) aqueous fraction observed peak amount2.659 was and showed retention time 16.180 min., as per the peak was named as 3-Isopropoxy-1,1,1,7,7,7-hex.

In the GCMS-MS spectral data of all the fractions of methanolic extract of *Calotropis procera* that the compound 3-Isopropoxy-1,1,1,7,7,7-hex was highest peak amount which showed in all the fractions and it was responsible to acaricidal activity.

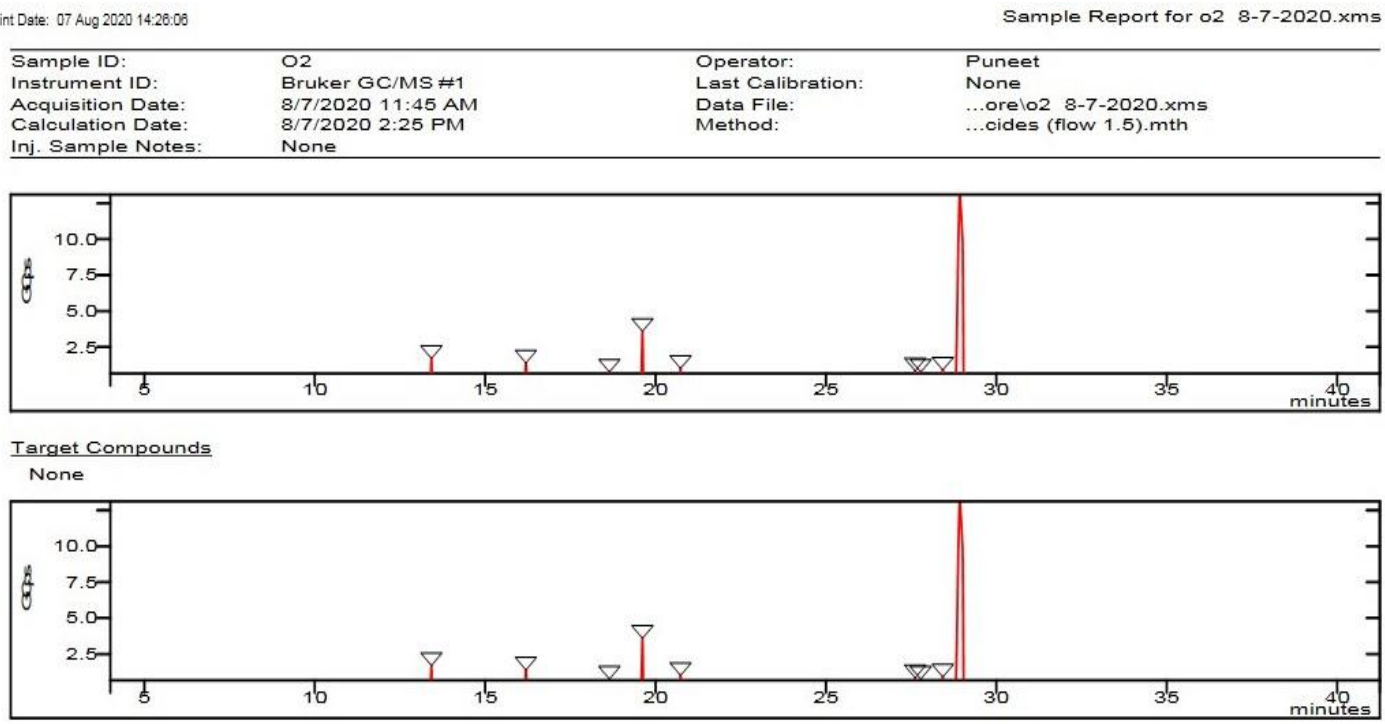

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Figure 2: GCMS-MS spectral analysis of Hexane fraction.

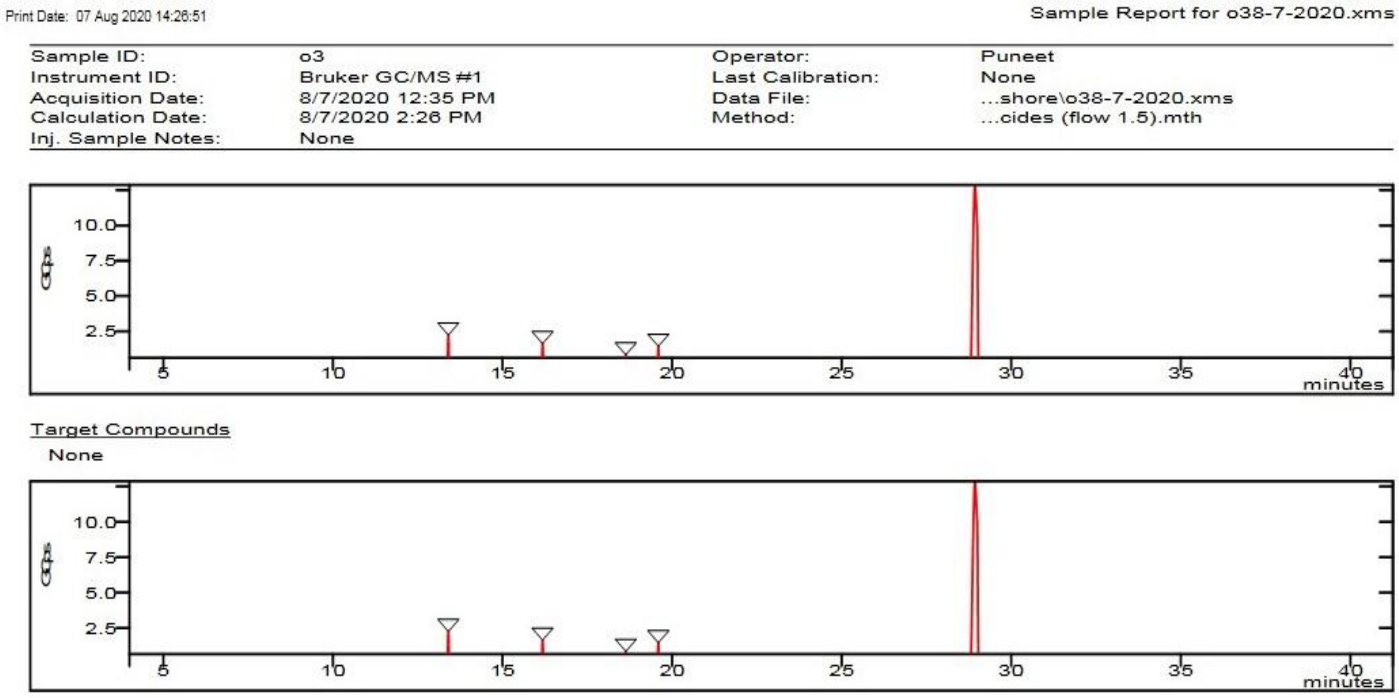

Figure 3: GCMS-MS spectral analysis of Ethyl acetate fraction.

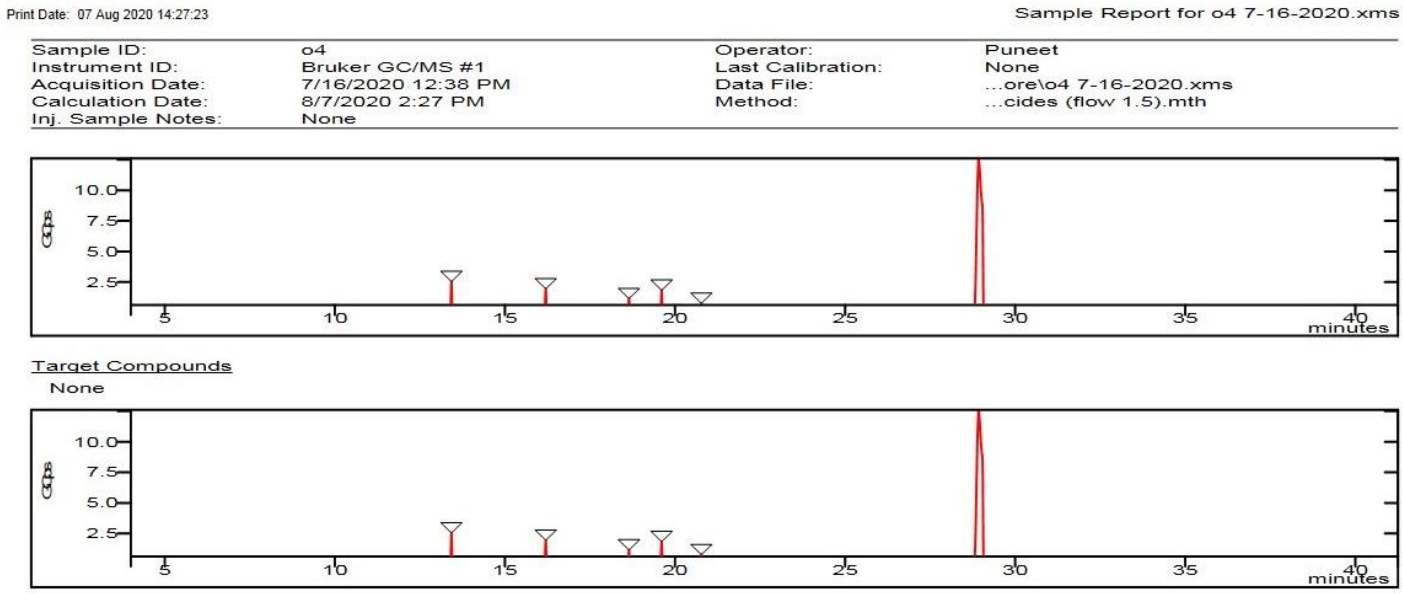

Figure 4: GCMS-MS spectral analysis of chloroform fraction.

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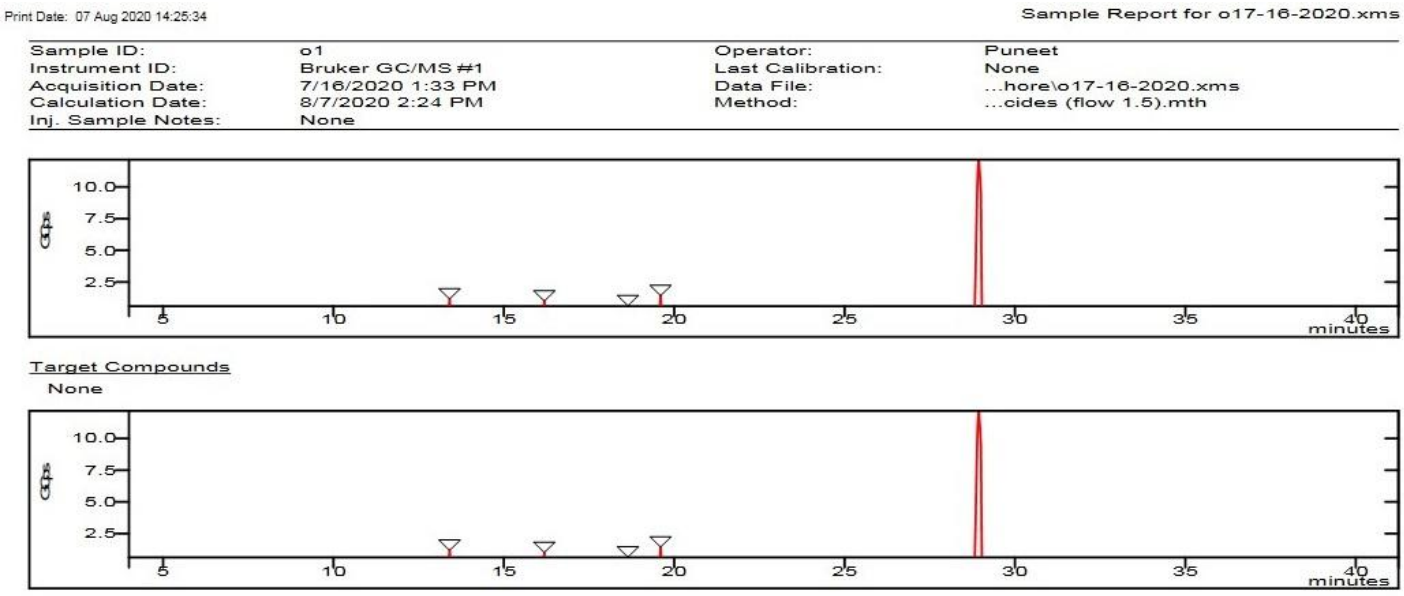

Figure 5: GCMS-MS spectral analysis of aqueous fraction.

DISCUSSION

Present investigation identified the acaricidal compounds of crude and fractional extracts from *Calotropis procera* against field farm population of *Rhipicephalus microplus* in India. Acaricidal activity of *Calotropis procera* plant extracts against some species of tick like *Hyalommaanatolicum* and *Rhipicephalus microplus* was reported in Arbia[24] and Pakistan[20] respectively. *Calotropis procera* is popular for tis ectoparasiticidal[25], insect repllent[26] and insecticidal properties[27].

It is evident from different reports that *Rhipicephalus microplus*is is becoming resistant day by day from several commercially acaricides available in the market [20]. In certain cases, it has been seen that it even resistant to commercially available multi-acaricides application[12]. In order to figure out this situation there are several alternative strategies is under process. Use of plant based acaricide i.e. phyto-acaricides is one of them and could be a helpful alternative to manage the problem[28].

Although the acaricidal properties of Indian Calotropis procera were investigated, we investigated the acaricidal properties of Indian Calotropis procera on livestock, dairy farms and cattle farms in the Mathura district of Uttar Pradesh, India. In ethno-veterinary practices, extract from beneficiary natural compounds is considered as a powerful tool against pest as it can disturb the biological mechanism of arthropod [29,20]. It has been seen that natural plant derived acaricides (botanical) is less harmful for human and animals compared to synthetic acaricides. On the other hand, use of natural derived acaricides can be a potent strategy to escape resistance proerty in tick population [30,31,32]. *Calotropis procera* is used by physician to treat ailment like cough, swelling, diarrhoea and inflammation both in animal and human[33].

Here the results from *Calotropis procera is* indicating that *it* could be a better solution against *Rhipicephalus microplus.* Using fully engorged adult *Rhipicephalus microplus*female collected from Mathura, Uttar Pradesh, India to run the bioassays in the *in-vitro* testing measure the current sensitivity of *Rhipicephalus microplus* infesting livestock in district Mathura to methanol extract from *Calotropis procera*and their four fractions in

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hexane, ethyl acetate, chloroform and aqueous at 44.41 mg/ml concentration shows 50% mortality and 130.51 mg/ml concentration 90% population of ticks. Effect of *Calotropis procera* extracts to other arthropod pests was also checked by violent excitation and aggressive anal biting behavior of fourth instar *Aedes aegypti* in some studies and noticed that it acts on nerve where neuro-muscular synchronization is disrupted and on the other hand some deformation of structure was also noticed in the papillae [34.] However, to confirm its toxicity on the nervous system of *Rhipicephalus microplus there is a need of* further studies are recommended.

Female *Rhipicephalus microplus i*s susceptible to this extract in terms of reproduction where the egg production is reduced. This is benificial because using this plant extracts from Melia azedarach to treat animals ectoparasites with engorged female tick would help manage populations of *Rhipicephalus microplus*in infested livestock farms[35]. However, in a study they [36]showed that popular natural acaricdie neem oil is failed to inhibit oviposition in female adult tick despite the 90% mortality against ticks.

Our finding on plant-based material that produce acaricidal activity may help to reduce toxicity in human and animal and could be a better replacement of synthetic chemical compound against various tick populations [37,35,38,39,40,41].

In addition, it was also determined that whether individual compound or a mixture of several compounds is responsible for the primary cause of the acaricidal activity[42]. Targeted toxicity test was also checked to confirm about the nontoxic nature on non target animal and humans. But till now the major drawback is the cost as it has been seen that the cost of additional extraction, purification and formulation methods is significantly higher than traditional chemical control [43]. Although micro and nano encapsulation methods are in progress to overcome the issue [44].

At the end it can be said that the plant derived material should be implemented to overcome acaricides resistance. There is some controversy persists regarding the belief of overcoming resistance property by plant derived materials but there is at least once instance of cross resistance in tick between DDT and Natural Pyrethroids that has already presented in nature[45]. *Calotropis procera* extracts and their fractions compounds are required to be studied *in-vivo* for their efficacy in controlling *Rhipicephalus microplus* infestation. It is therefore necessary for real assessment to develop natural plant extracts of *Calotropis procera* or their components against *Rhipicephalus microplus* affecting livestock in India.

CONCLUSION

In this study, the acaricidal properties of Calotropis bioassays that are common in tropical and subtropical India are presented. The extract of Calotropis procera controls the tiny adult females of Rhipicephalus and affects their ability to reproduce. Our findings provide the basis for determining whether phytotherapy of livestock can be obtained from these plants. These acaricide compounds need to address livestock infestation and the ectoparasite problem of cerebellar fluke populations that are resistant to synthetic acaricides.

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