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Toxicity evaluation and chemical composition of *Capsicum frutescens* for natural control of Asian blue tick, *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae)

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Abstract

Background: Indiscriminate use of synthetic acaricides in the management of veterinary parasites has led to environmental pollution, acaricidal resistance and their residues in the animal products. These problems are directly demanded an alternative acaricidal source for the ticks control and that should be cost-effective, eco-friendly and target specific. The current study demonstrated the acaricidal effects of *Capsicum frutescens* (fruits) against the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. In adult immersion test, the effects of the treatment on engorged females were assessed by measuring egg mass production, estimated reproductive factor, and % inhibition of reproduction.

Results: Methanol extract was observed the most effective against adults with LC_{50} 617.54 ppm and LC_{90} 1040.41 ppm. The other target extracts (petroleum ether and hexane) were less effective to the engorged females of *R. microplus*. Chemical analysis of the potent extract was elucidated by Gas Chromatography–Mass Spectrometry analysis and Cis-13-octadecenoic acid was observed as main compound (43.54%). The simultaneous evaluation of the qualitative chemical screening of the methanol extract showed the presence of tannins, saponins, carbohydrates, steroids, terpenoids, flavonoids, and alkaloids.

Conclusion: The study concludes that the methanol extract of *C. frutescens* fruits revealed the significant acaricidal properties and may be used as safe alternative for tick management.

Keywords: Acaricide, Botanical pesticides, GC–MS constituents, Phytochemical analysis, Bioassay

Background

Ticks act as the enervating parasites ranked fourth among the major infections of livestock and transmit an extent variety of the vector borne diseases to the livestock (Ghosh et al., 2007). The cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* is an economically important ectoparasite mainly threat to the domestic animals by direct effect on their production, quality of skin, and blood etc. and indirect effect related to the transmission of rocky mountain spotted fever, relapsing fever, meningoencephalitis, tularemia, colorado tick fever, crimeancongo hemorrhagic fever, babesiosis, etc. (Rosario-cruz et al., 2005). In addition, *R. microplus* causes high economic losses in terms of high mortality, morbidity rate and reduces production of milk, meat and also affect the quality of livestocks production. The economic loss due to deleterious effect of tick infestation in the host has been at dangerous levels. A single engorged female tick imposes daily 0.5–2.0 ml of blood, 8–9 ml of milk and one gram of body weight reduction (Minjauw & Mccleod, 2003). Various groups of synthetic chemical substances including

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arsenical compounds, carbamates, organophosphates, pyrethroids, ivermectin, etc., has been applied in the form of spray, dust, dipping and injection to the host to eliminate ectoparasites and plays an important role in tick control (Goncalves et al., 2007). However, the haphazard use of these chemical acaricides have some drawbacks viz. development of resistant, environment pollution and residues meat, milk, hide skin and natural toxicity (Graf et al., 2004; Lovis et al., 2013). These consistent problems are demanded alternative strategies to conventional control (Benelli et al., 2016; Fernandez-Salas et al., 2012).

Botanical pesticides are becoming a simple and sustainable method of vector control (Amer & Mehlhorn, 2006). These phytochemicals consist of mixture of substances derived from the different plant sources and can play an important role as larvicides, repellents, growth regulators and interrupt the transmission of diseases to their possible hosts (Bagavan et al., 2009; Mathew et al., 2009). *Capsicum frutescens* L., the green chilli (Fig. 1) is a small shrub of the family Solanaceae usually used for culinary purposes and exhibited traditional medicine activities against viruses and bacteria and is widely cultivated in tropical and sub-tropical countries (Ling et al., 2012). *C. frutescens* has seven alkaloid related compounds which are associated with burning, scathing or spicy characteristics (Reifschneider, 2000). Among these, capsaicinoids are usually found in seeds and responsible for 90% of the pungency. Capsaicin (8-methyl-N-vanillyl-6-nonemide), is a colorless, crystalline pungent alkaloid that is thermoliable, often soluble in oils and alcohols, and has major metabolite accounting for the fire in chilli peppers (Govindarajan & Sathyanarayana, 1991). It is imperative to note that *C. frutescens* also contains saponins, phenolics, flavonoids and diterpenoids, compounds having lethal, antifeedant, and parasite repellency effects (Iorizzi et al., 2000; Madhumathy et al., 2007). Some previous small-scale studies were identified the acaricidal activity, phytochemical and constituents of *C. frutescens* extracts. Therefore, taking into the deliberation of chemical acaricides as therapeutic agents, the present study was undertaken with aim to evaluate the potential of *C. frutescens* fruits for control of tick infestation against *Rhipicephalus microplus.*

Fig. 1 Fruits of *Capsicum frutesecens* L. (the green chilli)

Material and methods

Collection of plant material and preparation of extracts

Fruits of *Capsicum frutescens* were collected from the different localities (fields) of Agra (GPS coordinates of 27° 10′ 36.0120″ N and 78° 0′ 29.0592″ E), India. The separated fruits from the plants were washed in running tap water and whole fruit chopped by knife and left them for dried in shade at room temperature 27 °C. The completely dried chopped fruits were ground to make course powder in the mixer and kept it into air tight glass containers. The 500 g powdered plant material was packed into thimble of the Soxhlet's extractor and using same material for the extraction subjected to petroleum ether, hexane and methanol subsequently, up to 72 h or till the solvent in the siphon tube of an extractor becomes colorless for the complete extraction. The completion of the extraction was confirmed by taking the solvent from the thimble and evaporated to check the absence of residue. The extracts were taken out, filtered and distilled to concentrate to get the syrupy consistency in vacuum rotary evaporator. The extracts were kept in airtight glass containers in refrigerator at 4 °C to avoid the loss of volatile principle constituents. The percent yield of petroleum ether extract was 10.0 g, hexane extract was 8.00 g and methanol extract was 60.0 g.

Collection and rearing of ticks

The adult female ticks which dropped off from the body of infected host after engorgement and hidden in cow dung patches were collected from the farmer's animal houses (Gaushala's) of Agra and Mathura regions, Uttar Pradesh, India (Godara et al., 2018). After the collection, ticks were placed into plastic boxes having holes for aeration and transported to the laboratory, washed with water and dried by filter papers thoroughly, identified according to Walker et al., (2013). Adult immersion tests (AIT) were conducted on ticks weighing approximately 0.30–0.32 g. Some of these ticks were kept separately in 30-ml culture tubes with labeled for oviposition in desiccators having 10% KOH solution and then placed in BOD incubator at 27 ± 2 °C temperature.

Preparations of stock solutions and bioassay

The refrigerated crude extracts were used to prepare the stock solutions. The stock solutions of the extracts of desired concentrations (as shown in the Tables 2, 3, 4) were prepared by diluting the extracts in ethanol/distilled

water and kept into the stoppered conical flasks of borosilicate glass. Field collected engorged females of *R. microplus* were used for the adult immersion test and treated with six different concentrations of different extracts along with control in triplicates ($n=7\times3$). Adult ticks were immersed in 10 mL solution of the different concentrations for 3–5 min (FAO, 2004) whereas control group was immersed in ethanol/distilled water. After exposing, ticks were randomly transferred to the petridishes padded with Whatman No. 1 filter papers. All the petridishes were kept at 27 ± 2 °C temperature and 80 ± 5% relative humidity. The mortality of ticks was recorded after 24, 48, and 72 h and observed daily for 14 days. When the control ticks completed their egg laying afterward the inhibition of eggs laying was determined for all the treated groups by using the following formula Drummond et al. (1973).

$$\text{Inhibition of Oviposition (IO)} = \text{Average weight of eggs (mg)/initial weight of female (mg)}$$

$$\text{Estimated Reproductive Factor} = 20000 \times \text{eggs weight} \times \text{IO\%/ticks weight in grams}$$

where 20,000 = average no. of eggs per gram, Weight in grams of laid eggs, Estimated % hatchability of eggs (IO %).

Weight of experimental female in grams

$$\%\text{Inhibition of Reproduction} = \text{ERF (control)} - \text{ERF Treated/ERF control} \times 100$$

Preliminary phytochemical analysis

Qualitative analysis was conducted to know the phytochemicals present in the extract. Preliminary phytochemical analysis was carried by using a standard protocol (Harborne, 1998; Houghton & Raman, 1998; Raman, 2006) for the presence of active chemical groups such as tannins, alkaloids, saponins, carbohydrates, flavonoids, proteins, steroids, terpenoids, fixed oils, and fats determination.

GC–MS analysis

The methanol crude extract was subjected to analysis for the presence of active constituents by using gas chromatography–mass spectrometry (GC–MS) equipment (Milestone); supelcoequity 1 column with a film thickness of samples 0.20 micron was used. The total flow rate was 24 ml/min. Ultra high purity helium was used as the carrier gas with injector split ratio of 20:1. The ion source and interphase temperature were 200 °C. The solvent cut time of 4 min and detector gain was 0.70 kV. A Wiley-229 library was used to identify major peaks of the components of each sample. The relative percentage of each compound was determined by calculation of the area under the peak width at ½ heights (Houghton & Raman, 1998).

Statistical analysis

The mortality data observed in AIT were corrected by using Abbott's formula (Abbott, 1925) to remove the factors other than the extract tested. The corrected mortality data were subjected to Probit Analysis to calculate LC_{50} and LC_{90} values for concentration response by Finney (1971) along with other statistical parameters like 95 percent confidence level with upper and lower fiducial limits, Chi-square, and standard error by using the software developed by Reddy et al., (1992).

$$\text{Corrected\% mortality} = \frac{\%\text{Mortality in treatment} - \%\text{Mortality in control}}{100 - \%\text{Mortality in control}} \times 100$$

Results

Adulticidal bioefficacy shows that the methanol extract was the most effective among the tested extracts with LC_{50} value 617.54 ppm and LC_{90} value were 1040.41 ppm. Petroleum ether extract was least effective with LC_{50} and LC_{90} values were 2507.86 and 7493.0 ppm and hexane extract showed moderately effective exhibits with LC_{50} value 2194.93 ppm and LC_{90} value 5972.22 ppm (Table 1).

Table 1 Adulticidal bioefficacy of *C. frutescens* fruits against *R. microplus* after 24 h of treatment

Extraction solvent	χ^2	Regression equation	LC_{50} ± SE (UFL-LFL) (ppm)	Relative toxicity	LC_{90} ± SE (UFL-LFL) (ppm)	Relative toxicity
Petroleum ether	2.62	$Y=0.55X+1.20$	2507.86 ± 549.57 (3585.02–1430.70)	4.06	17,493.0 ± 8041.0 (33,255.0–7321.0)	16.8
Hexane	9.37	$Y=7.79X+2.94$	2194.93 ± 281.48 (3274.64–1643.21)	3.55	5972.92 ± 1110.74 (8149.98–3795.0)	5.74
Methanol	0.14	$Y=16.45X5.66$	617.54 ± 49.30 (714.89–520.91)	1.0	1040.41 ± 264.63 (1559.10–521.73)	1.0

Table 2 Adulticidal bioefficacy of petroleum ether extract of *C. frutescens* fruits against *R. microplus*

Concentration (ppm)	Total (15) wt. of ticks ratio (g)	% survival	Total wt. of egg laid survived ticks (g)	Egg index (egg wt./tick wt.)	Estimated reproductive factor (ERF)	%Inhibition of reproduction (IR)
600	2.10	86.67	0.52	0.25	396,190.48	62.18
1200	2.20	80.00	0.48	0.22	319,985.45	69.46
2400	2.09	73.33	0.41	0.20	265,499.52	74.66
4800	2.23	66.67	0.39	0.17	209,865.47	79.96
7200	2.24	46.67	0.24	0.11	85,714.29	91.82
9600	2.22	40.00	0.12	0.05	21,818.18	97.91
Control	2.10	100.0	1.10	0.52	1,047,619.05	–

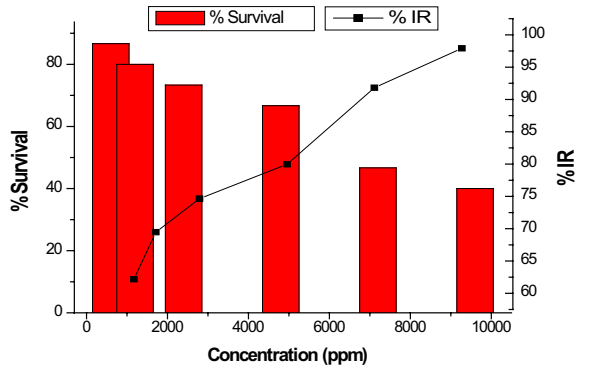

Fig. 2 Effect of petroleum ether extract on the survival and inhibition of reproduction of *R. microplus* engorged females

The petroleum ether extract of *C. frutescence* fruits against *R. microplus* at the concentrations of 600, 1200, 2400, 4800, 7200 and 9600 ppm and responded 86.67, 80.00, 73.33, 66.67, 46.67, and 40.00% survival of the target organism. The extract devastatingly affected the egg laid capacity of treated female ticks in concentration dependent manner and showed 62.18–97.9% inhibition of reproduction. In the case of control survival was 100% EI 0.52, estimated reproductive factor (ERF) 1,047,619.05 and no inhibition of reproduction (IR) was recorded. It is also mentioned in Table 2 that by increasing the test concentration survival, EI and ERF were decreased and IR increased gradually (Fig. 2).

The bioefficacy of hexane extract of *C. frutescence* fruits against *R. microplus* at 625, 1250, 2500, 3750, 5000, and 6250 ppm as the test concentrations mentioned in the Table 3. It responded by affecting % egg laying capacity with 0.16, 0.16, 0.12, 0.04, 0.04 and 0.00, respectively and 50.69–97.63% inhibition of reproduction. In the case of control no % IR was observed while survival was 100% and EI was 0.26%. The IR was increased but survival, EI and ERF were decreased by increasing the test concentrations of the extract (Fig. 3). Table 4 mentioned the effectiveness of methanol extract of *C. frutescence* fruits against *R. microplus* at 400, 450, 500, 550, 600, 650 ppm and observed 86.67, 80.00, 66.67, 60.00, 53.33, and 46.67% survival, 0.22, 0.20, 0.16, 0.14, 0.13, and 0.11 EI, and 36.00–84.35% IR, respectively. The control represented 100% of survival of the target organism, 0.27 EI and no %IR observed. On increasing the concentrations, the % IR was increased and survival, EI, ERF were decreased gradually (Fig. 4).

The physical characteristics of petroleum ether, hexane and methanolic crude extracts of *C. frutescens* fruits showed dark greenish, reddish, brownish-green color,

Table 3 Adulticidal bioefficacy of hexane extract of *C. frutescens* fruits against *R. microplus*

Concentration (ppm)	Total (15) wt. of ticks ratio (g)	%Survival	Total wt. of egg laid survived ticks (g)	Egg index (egg wt./tick wt.)	Estimated reproductive factor (ERF)	%Inhibition of reproduction (IR)
625	2.20	86.67	0.360	0.16	196,363.64	50.69
1250	2.20	80.00	0.358	0.16	195,272.73	50.96
2500	2.15	66.67	0.250	0.12	124,023.26	68.86
3750	2.20	20.00	0.098	0.04	17,818.18	95.52
5000	2.20	20.00	0.078	0.04	9452.18	97.63
6250	2.20	6.67	–	–	–	–
Control	2.30	100.0	0.606	0.26	398,260.87	–

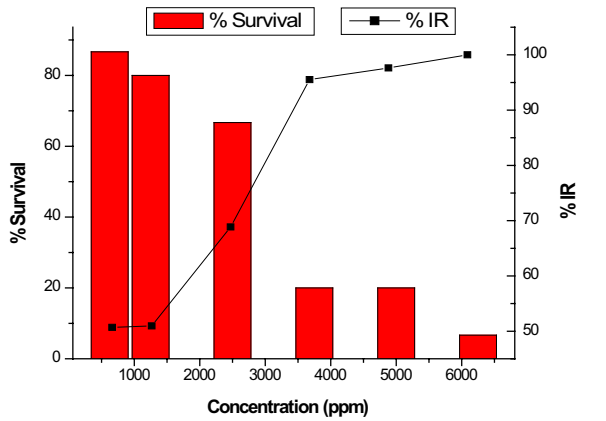

Fig. 3 Effect of hexane extract on the survival and inhibition of reproduction of *R. microplus engorged females*

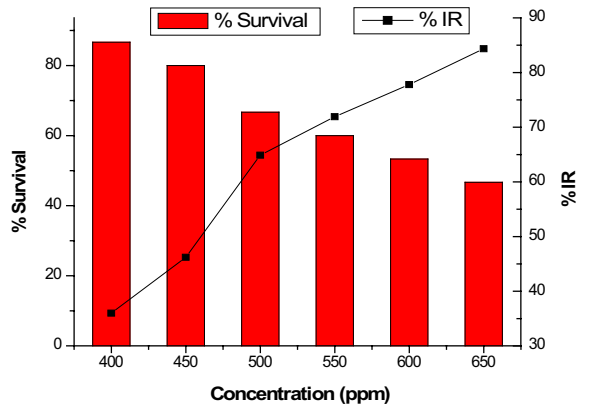

Fig. 4 Effect of methanol extract on the survival and inhibition of reproduction of *R. microplus* engorged females

sticky and non-sticky, solid semi-solid in consistency with non-agreeable odor. The crude methanol fruits extract of *C. frutescence* being the most efficient, was subjected for different qualitative tests to know the presence of the functional chemical groups in the extract. The tests showed the presence of the moieties of tannins, fixed oils, saponins, carbohydrates, terpenoids, steroids, flavonoids, and alkaloids in *C. frutescens* methanolic extract; proteins and amino acids tests responded negatively (Table 5).

The methanol crude extract was subjected for GC–MS for their spectral analysis. The spectral analysis assists to determine the presence of total number of peaks and their retention time, area % along with their peak report. The spectral analysis of methanol crude extract represented 6 peaks and out of them peak 6 (Cis-13-octadecenoic acid) was the dominant in area percent 43.45% with retention time 14.270 and followed by peaks 4 (n-Hexadecanoic acid), 3 (Pentadecanoid acid, 14-methylthyl ester), 1 ((1R,2S)-2-Acetyl-1-methylcyclobutanacetic acid)), 2 (Propanoic acid,2-methyl-,decylester), 5 (9-octadecenoic acid, methylester) with area percent 21.36, 12.32, 8.94, 8.48, 5.36%, respectively (Fig. 5; Table 6).

Table 5 Qualitative determination of the active constituents presents in the methanol crude extract of *C. frutescens* fruits

Name of the qualitative test conducted for the presence of	Status of the test
Alkaloids	+
Flavonoids	+
Saponins	+
Glycosides	+
Carbohydrates	–
Fixed oil	+
Tannins and phenols	+
Proteins	–
Amino acid	–

(+): present; (–): not present

Discussion

The present work was intended to evaluate the efficacy of crude petroleum ether, hexane and methanol extracts of *C. frutescens* (fruits) against common cattle ticks *R.*

Table 4 Adulticidal bioefficacy of methanol extract of *C. frutescence* fruits against *R. microplus*

Concentration (ppm)	Total (15) wt. of ticks ratio (g)	%Survival	Total wt. of egg laid survived ticks (g)	Egg index (egg wt./tick wt.)	Estimated reproductive factor (ERF)	%Inhibition of reproduction (IR)
400	2.20	86.67	0.480	0.22	349,090.90	36.00
450	2.20	80.00	0.440	0.20	293,320.00	46.22
500	2.20	66.67	0.351	0.16	191,454.55	64.89
550	2.23	60.00	0.320	0.14	153,054.70	71.93
600	2.15	53.33	0.279	0.13	121,124.93	77.79
650	2.25	46.67	0.240	0.11	85,333.33	84.35
Control	2.20	100.0	0.600	0.27	545,454.55	–

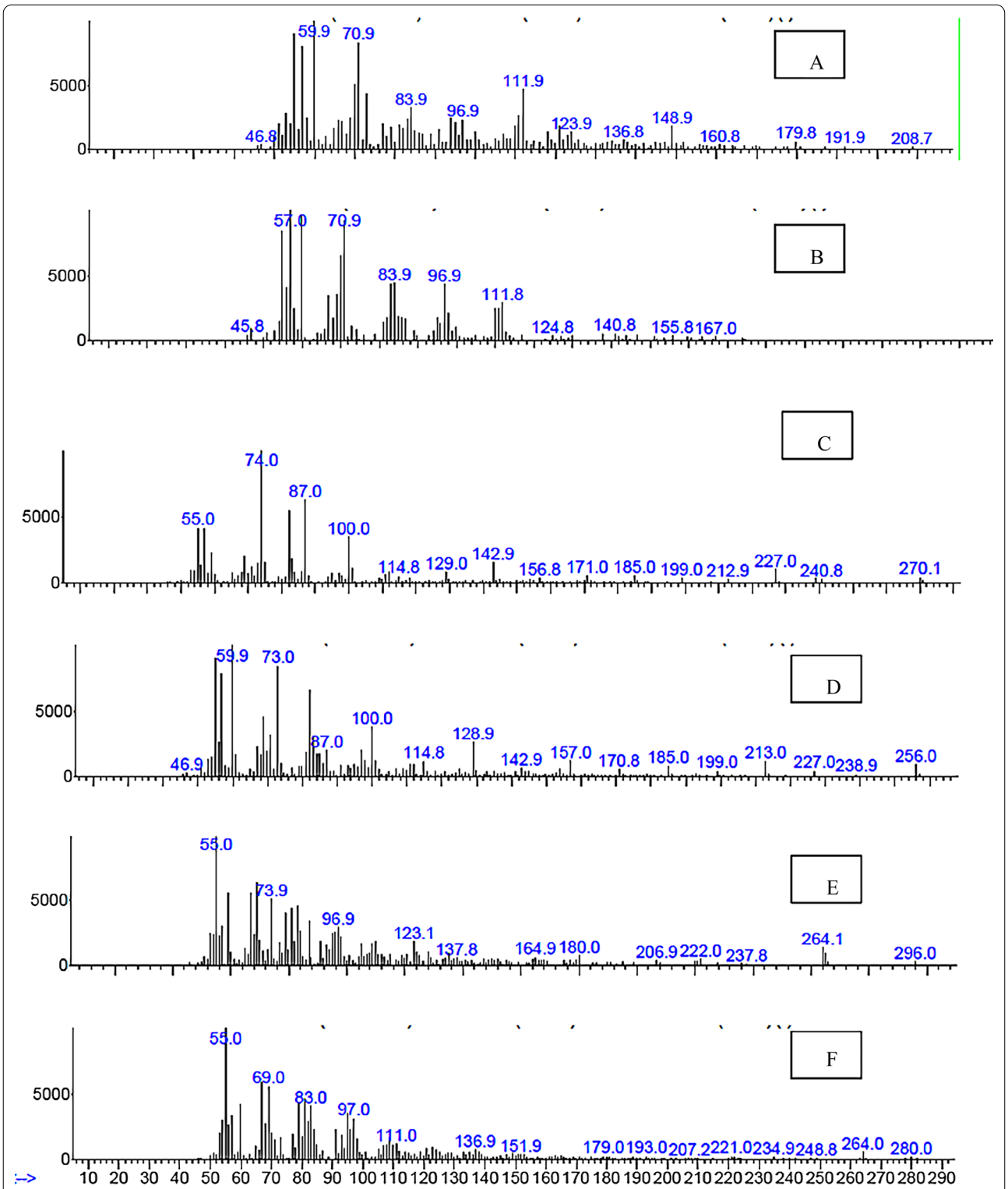

Fig. 5 GC–MS Chromatogram of methanolic extract of *C. frutescens* of major constituent compounds **A** (1R,2S)-2-Acetyl-1-methylcyclobutanacetic acid) **B** Propanoic acid, 2-methyl-,decylester **C** Pentadecanoid acid, 14-methylthyl ester **D** n-Hexadecanoic acid **E** 9-octadecenoic acid, methylester **F** Cis-13-octadecenoic acid

Table 6 Compounds identified by GC–MS in the methanolic extract

RT (min)	Compound	Area (%)	Properties
10.45	(1R,2S)-2-Acetyl-1-methylcyclobutanacetic acid	8.94	Not reported
10.74	Propanoic acid,2-methyl-,decylester	8.48	Not reported
12.11	Pentadecanoid acid, 14-methylthyl ester	12.32	Antioxidant (Adeyemi et al., 2017)
12.61	n-Hexadecanoic acid	21.36	Anti-inflammatory (Aparna et al., 2012), antioxidant, hypocholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5-alpha reductase Inhibitor (Kumar et al., 2010), potent mosquito larvicide (Rahuman et al., 2000)
13.76	9-octadecenoic acid, methylester	5.36	Anti-mosquito (Hemalatha et al., 2015), Anti-inflammatory, antiandrogenic cancer preventive, dermatitigenic hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge (Jegadeeswari et al., 2012)
14.27	Cis-13-octadecenoic acid	43.54	Therapeutic uses in medicine and surgery (Krishnamoorthy & Subramaniam, 2014)

(*B.*) *microplus* collected from naturally infested animals. The contemporary efforts, addressed a knowledge gap on the acaricidal properties of *C. frutescens* against *R. microplus* infecting livestock in India. As per our knowledge, no reports has been available on the preliminary phytochemical studies and compounds identification of selected plant from the northern area and semiarid region of the Indian subcontinent. Plants have a number of natural compounds that can disrupt the biological processes in arthropods and are considered as a vital implement in etho-veterinary practices (Zaman et al., 2012). In contrast to synthetic acaricides, botanicals can be less noxious to mammals, exhibit fewer residual effects, and have less possibility of acaricide resistance in tick populations (George et al., 2014; Isman, 2006; Wanzala, 2017). The results revealed that the extracts of *C. frutescens* exhibited significant acaricidal activity against *R.* (*B.*) *microplus* and methanol extract was found the most effective against the engorged females of the target species with LC_{50} 617.54 ppm and LC_{90} 1040.41 ppm, respectively. It was also noticed that generally no mortality was observed in the control during the study.

Juliet et al. (2012) evaluated ethanolic extract of *Jatropha curcas* leaves at different concentrations (50–100 mg/l) against *R. annulatus* adults and all the concentrations considerably blocked the hatchability of eggs, however, 100 mg/l concentration did not produce any mortality of adult engorged ticks. Rosado-Aguilar et al. (2010) evaluated the larvicidal and adulticidal efficacy of methanolic extracts of leaves, stem of *Petiveria alliacea R. (B.)microplus* and reported the great activity of both stem and leaves on the LIT with 100% mortality. On the other hand, AIT showed 26% (leaves) and 86% (stem) mortality at 20% concentration, respectively and hatchability inhibition 26% and 17%, respectively. Vasconcelos et al. (2014) found that ethanolic extract of this plant exhibits a potent acaricidal activity on adult females of *R. microplus* and reported significantly lowered the oviposition at all tested concentrations. Kishore et al. (2015) observed the effect of *Adhatoda vasica* roots against *Rhipicephalus microplus* engorged females and noted that petroleum ether extract strongly exhibits adulticidal activity (LC_{50} 271.49 ppm and LC_{90} 1148 ppm) and inhibition of reproduction was 97.30% at 600 ppm. Kishore et al. (2016) studied the adulticidal activity of *Adhatoda vasica* (roots) against multi-host tick, *Hyalomma anatolicum* and hexane extract was observed the most effective, showed 100% mortality at 2400 ppm and egg laying inhibition was 99.67% after 15 days of exposure. Dantas et al. (2016) studied acaricidal activites of crude ethanolic extract fractions of *Amburana cearensis* leaves on the cattle tick, *R. microplus* and reported among all fractions; hexane (2.5%) achieved the significant results in all parameters analyzed including 52.7% of inhibition of oviposition, 39% of hatching rate and 3271% index of reproductive efficiency. Rosado-Aguilar et al. (2017) showed the moderate % inhibition of oviposition activity of methanolic extract obtained from the leaf of *Havardia albicans* and *Caesalpinia gaumeri* i, e. 54.4 and 51.0% with 23.3 and 30% mortality rate against the adults of *R. microplus*. Similar to present study, Godara et al. (2018) observed the acaricidal and oviposition limiting properties of *Piper nigrum* and *P. longum* fruits metanolic extracts against engorged females of *R. microplus*. They reported minimum LC_{50} values of 0.48% (*P.nigrum*) and 2.3% (*P. longum*), respectively. Furthermore, the methanolic extracts adversely affected on oviposition inhibition showing, 28.1–96.9% by *P. nigrum* and 36.1–89.3% by *P. longum* in a dose dependent manner.

The phytochemical tests of methanolic extract showed the presence of the moieties of glycosides, alkaloids, flavonoids, saponins, fixed oils and fats, tannins, and phenolic compounds. This is consistent with the previous studies, which reported that the acaricidal properties of plant extracts against *R. (B.) microplus* were attributed to terpenoids (Fernandes & Freitas, 2007; Magadum et al.,

2009; Pereira & Famadas, 2006; Ribeiro et al., 2007) and tannins (Fernandez-Salas et al., 2011). Aronson (2008) further reported that the presence of flavonoids and terpenoids in the plants were assumed to be accountable for the many insecticidal properties and their findings are related to our present study. It was interesting to know that the absence of amines in crude extract of *C. frutescence* fruits which indicate toward the safety as existence of amines are poisonous in environment. It is implicit that alkaloids associated extracts may possibly have analogous mechanism of action to that of organophosphate compounds (Shyma et al., 2014). Fernández-Salas et al. (2011) observed that the extracts of *Acacia pennatula*, *Piscidia piscipula*, *Leucaena leucocephala* and *Lysiloma latisiliquum* were rich in tannins and had no significant effects on the oviposition index of *R. (B.) microplus*. Gurnani et al. (2016) assessed the antimicrobial activity of n-hexane and chloroform seeds extract of *C. frutescens* and reported that hexadecanoic acid (unsaturated fatty acids) was major constituent in both the extracts; this may be responsible for various pharmacological activites. Antonious et al. (2007) investigated the acaricidal (Spider mite, *Tetranychus urticae*) and insecticidal (Cabbage lopper, *Trichopulsia ni*) activity of hydro-methanolic crude extracts of *Capsicum Chinese*, *C. frutescens, C. baccatum*, *C. annum* with their different accessions numbers. They found 94% mortality of *C. annum* (PI-593566) against cabbage lopper while, *C. annum* (PI-310488) and *C. frutescens* (PI-241675) showed highest repellent activity against spider mite. GC–MS analysis of all accessions numbers were dominantly identified and detected pentadecanoic acid methylester, hexadecanoic acid methylester and octadecanoic acid were major compounds. Further, it reported that presence of significant amount of tannins in hot pepper fruits act as oviposition deterrent and toxin to insects. The prior findings of Frenandez-Salas are in contrary to this study, as an oviposition index and hatching percentage were significantly reduced after treatment of engorged females with tannin rich methanolic extract. It may be due the joint action of the different compounds present in the extract or plant produce enormous range of volatiles and tannins that play significant functions in plant protection (Aharoni et al., 2003) and behave as toxins which required to be studied further in details. The efficacy of phytochemicals may vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon geographical regions and an environmental factors.

Our current findings add to increasing list of plant-based materials that have an acaricidal and an anti-ovipositional activity against various tick populations that may serve to supplement or reinstate existing chemical methods for tick control (Abbas et al., 2014; Ghosh et al., 2011, 2013; Ravindran et al., 2012; Shyma et al., 2014). Further, studies must also take in hand for identification of active ingredients present in these plant extracts, particularly methanol extract that caused mortality of tick and decrease in egg production and inhibition of hatching of the eggs significantly. Results from the current study may provide useful information to help future studies in elucidating the modes of action of n-Hexadecanoic acid, 9-octadecenoic acid, methylester, Cis-13-octadecenoic acid against the *R. (B) microplus* (Costa-Júnior et al., 2016). Although, our study was not anticipated to determine the mode of action of extracts of the selected plant or their foremost chemical constituents. Further, in-vivo study of these extracts should be undertaken to assess the effect of the selected plant on animal's health. Different combinations of these extracts could also be studied to develop the antagonistic and synergistic effect of different active ingredients of the selected plant.

Conclusion

The present study concluded that the extracts of *C. frutescens* affected the reproductive ability of adult females of *R. microplus*. This is required for a realistic assessment of the opportunity to develop botanical treatment containing extracts of *C. frutescens*, and their components of emergent sustainable tactic for integrated tick management.

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Authors' contributions
LM and CNS designed the work, drafted and edited the manuscript. VK and NL performed all the adulticidal bioassay and analyzed the data. LM and NL given a major contribution in writing the manuscript. All authors read and approved the final manuscript.

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Declarations

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Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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