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ORIGINAL ARTICLE

Acaricidal Bioefficacy and phytochemical Analysis of *Citrus limetta* fruit peels against common cattle tick, *Rhipicephalus microplus* (Acari: Ixodidae)

Shweta Gupta, Neha Loach, Shivani Sharma, Mahima Chaudhary, Arun Pratap Sikarwar, Lalit Mohan*
Applied Entomology & Vector Control Laboratory
Department of Zoology, Faculty of Science
Dayalbagh Educational Institute (Deemed to be University), Agra – 282005 (INDIA)
*E-mail: lalitmohan_dei@rediffmail.com

ABSTRACT
Present investigation revealed the acaricidal potential of Citrus limetta fruit peels extracts with different solvents (petroleum ether, hexane and methanol) against cattle tick, Rhipicephalus (Boophilus) microplus. Petroleum ether extract with LC_{50} value 9.35 and 6.15 mg/ml mg/ml was found most effective in adult and larval stages respectively while hexane extract with LC_{50} value 7.21 mg/ml was found most effective in eggs of target organism. Further phytochemical analysis of all the extracts shows the presence of alkaloids, coumarins, tannins, terpenes, volatile oils, glycosides, flavanoids and saponins. GC-MS analysis of potent extract shows the presence of n-Hexadecanoic acid ($C_{16}H_{32}O_2$) in highest concentration 28.90% area. Presence of alkaloids, flavanoids, tannins and terpenes may be responsible for acaricidal activity.
Keywords: *Citrus limetta, Rhipicephalus microplus,* Phytochemical analysis, GC-MS analysis, Acaricidal, Extract.

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INTRODUCTION

Animals, birds, and reptiles are all hosts for blood-feeding ectoparasites called ticks [1]. A large number of infections that affect both domestic animals and people are transmitted by around 10% of the 867 tick species that are currently recognized [2]. *Rhipicephalus (Boophilus) microplus*, one host tick in India, is the carrier of a wide variety of crippling parasites that damage cattle and their products both directly and indirectly [3]. Ticks convey important pathogens (such as *Anaplasma marginale, Arbovirus, Babesia bovis,* and *B. bigemina*) to the veterinary and human health, acting as a vector for a wide range of illnesses [4]. Various synthetic pesticides, such as organophosphates, pyrethroids, amidines, and macrocyclic lactones, have been used to manage this group of parasites in India in an effort to control *R. microplus* with varying degrees of effectiveness [5]. Moreover, the random use of these chemical acaricides has certain disadvantages, including the emergence of resistant strains, environmental contamination, traces in meat, milk, and skin from animals, and inherent toxicity [6]. Hence, Researchers have thus been looking for potentially efficient and secure alternatives to traditional synthetic medications.

From ancient times to the present, herbal remedies have consistently been viewed as promising treatments [7]. Every year, millions of tonnes of byproducts are produced by a variety of businesses, mostly the food industry [8]. As a strong source of antioxidants, the plant family Rutaceae is frequently referred to as the "Citrus Fruit Family". Citrus peels have a significant amount of phenolic chemicals, including numerous flavonoid compounds. Citrus peel extracts are known to display a variety of biological actions, including antibacterial, antioxidant, and prospective sources for anticancer drug screening [9]. Various phytochemicals included in the waste of *C. limetta* can be effectively utilised in the production of medicinal medications and skin care products [10]. Peel oil of *Citrus limetta* shows antifungal activity against

Aspergillus flavus, Aspergillus niger and Penicill ium griseofulvum [11], antibacterial potential against *Propioni bacterium acnes* [7] and insecticidal activity against *Anopheles stephensi* and *Culex quinquefasciatus* [12].

Aqueous extracts of *Citrus limetta* peels shows some antimicrobial activity against *Staphylococcus aureus, S. epidermidis, Enterococcus faecalis, Streptococcus pneumoniae, S. agalactiae, S. pyogenes, Pseudomonas aeruginosa, Enterobacter aerogenes, Klebsiella pneumoniae, Escherichia coli, Salmonella Typhi, Proteus spp., Moraxella catarrhalis, Acinetobacter spp*. and *Candida albicans* [13]. Juice and methanolic extract of *Citrus limetta* seed shows some antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* [14]. Ethanolic extracts of *Citrus limetta* leaves possesses antihelminthic activity against *Pheretima posthuma* [15], insecticidal activities of methanol extract of *Citrus limetta* leaves against *Aedes albopictus, Anopheles maculatus* and *Culex mimulus* [16]. Hexane extract of *Citrus limetta* seeds shows acaricidal potential against *Rhipicephalus microplus* [10].

It is important to keep in mind that *Citrus limetta* also includes alkaloids, phenolics, flavonoids, steroids, and terpenoids, substances with deadly, insecticidal, and parasite repellent effects. Because of the link between consuming flavonoids and illness prevention, *C. limetta* peels are gaining greater focus from the scientific community [9].

In light of these facts, the current experiment is intended to determine the effectiveness of an extract of *Citrus limetta* fruit peels in petroleum ether, hexane, and methanol against the different life stages of common cow tick, *Rhipicephalus (Boophilus) microplus.* Additional phytochemical research was carried out to determine the types of compounds contained in various extracts.

MATERIAL AND METHODS

Collection of plant material and their extraction

Collection of *Citrus limetta* peels from the local market of Dayalbagh, Agra. Collected Plant parts were air dried in the shade. A coarse powder was first made, and then it was extracted using Soxhlet's equipment in petroleum ether, hexane, and methanol as the solvent. Vacuum rotary evaporators were used to evaporate the extracted solvent at a temperature of 37 °C (Heidolph, Germany). Crude extracts of the plant were transferred to a glass jars and kept into the refrigerator at 4 °C.

Phytochemical analysis of the extracts

Test for alkaloids

Mayer's test was performed for the test of alkaloids. In a test tube, place 2 mL of the extract and then add 2N-diluted HCl. Filtering the generated aqueous layer allowed 1 mL of Mayer's reagent to be added to the filtrate. The formation of yellowish-cream coloured precipitate or turbidity indicates the presence of alkaloids.

Test for amino acids

Ninhydrin test were performed for the test of amino acids. In a test tube, 1 mL of extract was taken and add 4-5 drops of Ninhydrin reagent. The formation of purple colour indicates the presence of amino acids.

Test for corbohydrates

Fehling's test were performed for carbohydrates. Fehling's solutions "A" and "B" were combined in a solution of 5 mL using 2 mL of extract, and the mixture was then heated for 2-3 minutes. The formation of brick-red coloured precipitate indicates the presence of carbohydrates

Test for coumarins

In a test tube, 2 ml of extracts combine with 1 ml of 10% NaOH. The appearance of yellow colour indicates the presence of coumarins.

Test for flavonoids

Alkaline reagent's test were performed for flavanoids. In 2 mL of extract 1 mL of NaOH was added. The appearance of yellow colour that becomes colourless after adding the diluted HCL, it indicates the presence of flavonoids.

Test for glycosides

Keller-Kiliani's test were performed for glycosides. In a test tube, 2 mL of extract mixed with 1 mL glacial acetic acid and 3–4 drops of ferric chloride then added 1–2 drops of conc.
sulphuric acid in solution. The appearance of reddish-brown colour at the junction of two liquid layers and upper layer was appeared bluish-green, it indicates the presence of glycosides.

Test for saponins

Froth test were performed for saponins. In a test tube, 2 mL of extract mixed with 5 mL of distilled water. Vigorously shake the mixture. The presence of saponins is indicated by the generation of frothy lather or steady frothing that lasts for at least 3-5 min.

Test for tannins

Ferric chloride test were performed for tannins. In a test tube, 2 mL of extract mixed with 3-5 drops of ferric chloride (10%) solution. The appearance of bluish-green colour indicates the presence of tannins.

Test for terpenes

Salkowski test were performed for terpenes. 1 mL of chloroform and 1 mL of saturated H_2SO_4 gently added to 2 mL of essential oil. The layer of chloroform appeared as reddish-brown in colour; it indicates the presence of terpenes.

Test for volatile oils

In a test tube, 2 ml of extract mixed with 0.1 ml NaOH then added 2-3 drops of dil HCl. Formation of white coloured precipitate indicates the presence of volatile oils.

Gas Chromatography Mass Spectrometry (GC-MS) analysis

The extract was subjected to determine the presence of the different phytocompounds by using GC-MS (BRUKER SCION SQ). A single column was employed, with a sample film thickness of 0.25 micron. The helium gas was used as a carrier at 1 ml/min flow rate. Initially, the oven temperature was programmed at 80°C for 4-5 minutes and then it was increased to 280°C at the rate of 8°C/min and finally held isothermal for 30 minutes. The injector temperature was adjusted at 270°C and 1.0 µl of each sample was injected into the system for the detection of compounds and the running time for each sample was 45-50 minutes. The 70 eV ionization energy was used. To determine the components of extracts, a Wiley 229 and NIST–Mass spectral library investigation was done. The retention time and area percentage of each compound was determined by the generated data [12].

Ticks collection

In communities in the Agra district of India, engorged female *Rhipicephalus (Boophilus) microplus* ticks were collected from naturally infected cattle grazing on farmer's flocks. The collected ticks were cleaned under running water from the faucet and dried on paper towels. A species-specific taxonomy book allowed for the identification of the female as *Rhipicephalus (Boophilus) microplus.*

Egg immersion test (EIT)

The engorged females of *R. microplus* were gathered from diverse flocks of animals at distinct Agra locales. The ticks were collected, cleaned with water, and then patted dry using paper towels. The engorged females were put in 30 ml flat-bottom glass culture tubes with muslin fabric covering them and a rubber band to secure them. A single engorged female was present in each culture tube for oviposition. The culture tubes were set within a dessicator that contained 10% KOH solution. Prior to the eggs being deposited, the dessicator was maintained in the BOD incubator for two weeks at 27.2°C and 75±5% relative humidity. For the egg immersion test, these eggs were utilized. 100 to 150 eggs were subjected to the necessary test concentrations in triplicate, along with the control, in 3 ml flat-bottom glass tubes covered with muslin fabric and rubber-banded with a cap. For a period of three weeks, all of the glass tubes were stored in the BOD incubator. The number of hatched and unhatched eggs was counted, the observations for egg hatching were made, and the information was then utilised to determine the fatal concentration [6].

Larval immersion test (LIT)

For the experiments, laboratory-raised R. microplus larvae between 7 and 14 days old were employed. Selected hatching vials with the greatest larval eclosion rate (90–100%) were put in the centre of a petri dish, which was then filled with water and soap to prevent the escape of the larvaee. The extracts were prepared independently in triplicate together with a control sample at each required test concentration. 100 to 150 larvae were exposed for two to three minutes to the required concentration in 3 ml flat-bottom glass tubes covered with muslin fabric and secured with a rubber band. For 24 hours, the glass tubes containing the treated larvaee were kept in the BOD incubator at a temperature of 28°C and a relative humidity of 75±5%. After exposure for 24 hours, mortality results were obtained. larvae were deemed dead when they were unable to walk or move (FAO, 2004).

Adult Immersion Test (AIT)

Weighted and placed five per petri dish, 105 engorged females of *Rhipicephalus* (*Boophilus*) *microplus* were used. Six of the seven groups received each extract at a varied concentration, while the other two received distilled water as a control. The experiment was carried out in triplicate. The treated group was submerged for two to three minutes in diluted extracts and distilled water, respectively, in 15 ml tubes, whereas the control group did not receive any treatment. The ticks were put into a BOD incubator at a temperature of 28°C and a relative humidity of 75±5%. Following a 24-hour treatment period, the dead ticks were removed from petri plates to assess tick mortality using a stereo-microscope. Dead ticks might be distinguished by their cuticular blackness and absence of skin ulcers. The number of females depositing eggs was counted after 15 days of incubation, and the reproduction factor was determined using [17,6].

Data analysis

Estimated Reproductive Factor (ERF) = 20000 × Egg Index × % of hatched eggs
20000 = average number of eggs per gm
Egg weight = egg produced in gram
% Inhibition of Reproduction (% IR) = ERF control – ERF treated / ERF control ×100

RESULTS AND DISCUSSION

Bioassay

Acaricidal activity of different extracts of *Citrus limetta* peels were reported against different stages of *Rhipicephalus (Boophilus) microplus.*

Table 1 and Figure 1 represent the ovicidal bio-efficacy of petroleum ether, hexane, and methanol extracts of *Citrus limetta* peels against *Rhipicephalus microplus* eggs and revealed that p. ether extract was the most successful with the LC_{50} value 7.21 mg/ml along with upper fiducial limit 7.86 mg/ml and lower fiducial limit 6.56 mg/ml followed by hexane with the LC_{50} value 49.65 mg/ml along with upper fiducial limit 50.70 mg/ml and lower fiducial limit 48.61 mg/ml and methanol with the LC_{50} value 236.95 mg/ml along with upper fiducial limit 252.45 mg/ml and lower fiducial limit 221.46 mg/ml. Peels and leaves oil of *Citrus aurantifolia* showed more ovicidal activity than larvicidal activity against *Aedes aegypti* insect eggs and larvae respectively [18]. However, hydro-alcoholic extracts of *Melia azedarach* L. have some ovicidal and larvicidal activity against *Haemonchus contortus*, gastro-intestinak nematodes [19].

Table 1: Relative acaricidal bio-efficacy of different extracts of *Citrus limetta* peels against different stages of *R. microplus.*

Target stage	Extraction solvent	χ^2	Regression equation	LC_{50} ± SE (mg/ml) (UFL-LFL)	LC_{90} ± SE (mg/ml) (UFL-LFL)
Adult	Petroleum ether	0.063	y=2.04x+2.55	9.35 ± 2.50 (14.26-4.44)	100.95 ± 59.14 (216.88-14.97)
	Hexane	3.032	y=2.71x-2.64	43.14 ± 5.23 (53.40-32.89)	119.27 ± 28.49 (175.12-63.42)
	Methanol	1.457	y=4.06x-16.25	878.56 ± 83.47 (1042.18-714.95)	1518.94 ± 143.58 (1800.37-1237.52)
Egg	Petroleum ether	38.82	y=1.81x+2.12	7.21 ± 0.331 (7.86-6.56)	48.63 ± 6.29 (60.96-36.30)
	Hexane	2.49	y=2.71x-13.17	49.65 ± 0.531 (50.70-48.61)	76.93 ± 2.16 (81.17-72.69)
	Methanol	37.74	y=3.49x-1.64	236.95 ± 7.905 (252.45-221.46)	1061.70 ± 76.012 (1210.68-912.71)
Larva	Petroleum ether	19.10	y= 2.03x+1.84	6.15 ± 0.33 (6.80-5.50)	32.91 ± 2.48 (37.77-28.04)
	Hexane	93.45	y= 2.15x+0.99	9.34 ± 0.33 (9.99-8.69)	39.93 ± 2.27 (44.39-35.47)
	Methanol	9.86	y= 3.09x-10.74	110.92 ± 1.37 (113.61-108.23)	196.28 ± 4.25 (204.63-187.93)

χ^2 Chisquare value; LC_{50} Lethal Concentration at 50% mortality; LC_{90} Lethal Concentration at 90% mortality; UFL Upper Fiducial Limits; LFL Lower Fiducial Limits; RT Relative Toxicity.

Larvicidal bioefficacy of petroleum ether, hexane, and methanol extracts of *Citrus limetta* peels (Table 1 and Figure 2) against *Rhipicephalus microplus* larvae and show that petroleum ether extract was the most successful with the LC_{50} value 6.15 mg/ml along with upper fiducial limit 6.80 mg/ml and lower fiducial limit 5.50 mg/ml followed by hexane with the LC_{50} value 9.34 mg/ml along with upper fiducial limit 9.99 mg/ml and lower fiducial limit 8.69 mg/ml and methanol with the LC_{50} value 110.92 mg/ml along with upper fiducial limit 113.61 mg/ml and lower fiducial limit 108.23 mg/ml. In contrast, hexane extract of *Citrus limetta* fruit peels shows 84.23% mortality at 50 mg/ml against larvae of *R. microplus* [10]. In contrast, methanol extract of *Citrullus colocynthis* fruits was found most efficient against *R. microplus* larvae (LC_{50} value 19.84 ppm) in comparison to hexane and petroleum ether [4]. According to Shezryna *et al.*, 2020 larvicidal activity of essential oil of *Citrus hystrix* shows 44.44% mortality at 12.50% concentration but the combination of *C. hystrix* and *Cymbopogon citratus* essential oil shows 100% mortality even at 5% concentration against larvae of cattle tick, *Rhipicephalus (Boophilus) microplus* [20].

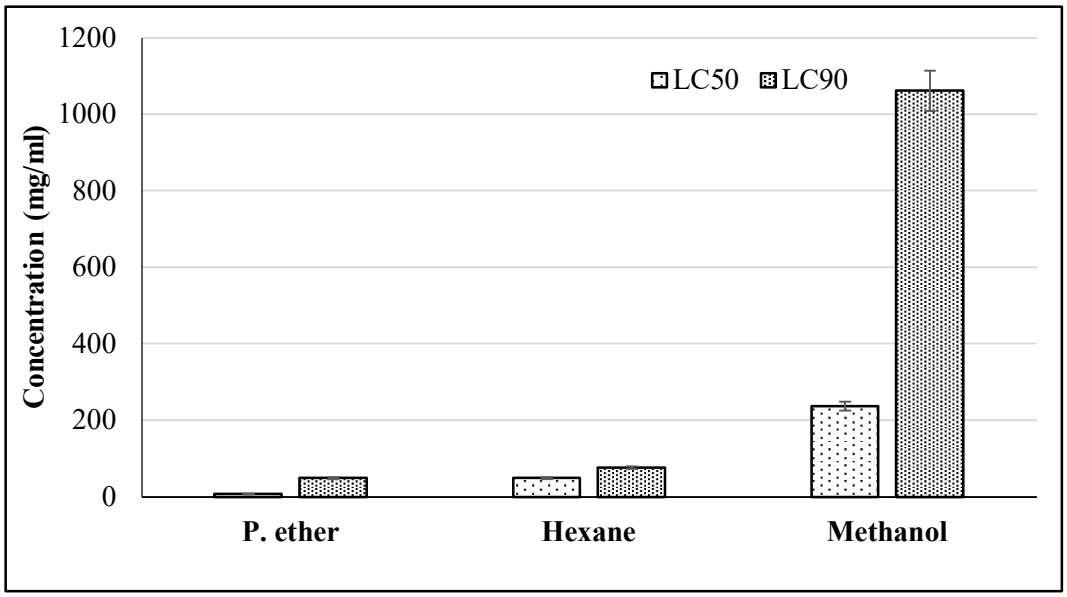

Figure 1: Relative acaricidal bio-efficacy of different extracts of *Citrus limetta* peels against eggs of *R. microplus.*

Adulticidal bioefficacy of petroleum ether, hexane, and methanol extracts of Citrus limetta peels (Table 1 and Figure 3) against Rhipicephalus microplus adults and show that p. ether extract was the most successful with the LC50 value 9.35 mg/ml along with upper fiducial limit 14.26 mg/ml and lower fiducial limit 4.44 mg/ml followed by hexane with the LC50 value 43.14 mg/ml along with upper fiducial limit 53.40 mg/ml and lower fiducial limit 32.89 mg/ml and methanol with the LC50 value 878.56 mg/ml along with upper fiducial limit 1042.18 mg/ml and lower fiducial limit 714.94 mg/ml. Similarly, petroleum ether extract found to be most effective among three different solvents (P. ether, hexane and methanol) extract of *C. colocynthis* roots against adults of *R. microplus* at 400 ppm concentration 54% mortality were observed **[5].** Extract of *Citrus limetta* leaves shows insecticidal activity against larvae of *Aedes albopictus, Anopheles maculatus* and *Culex mimulus* with 50% lethal concentration at 88.21, 86.49 and 79.72 µg/mL respectively [16].

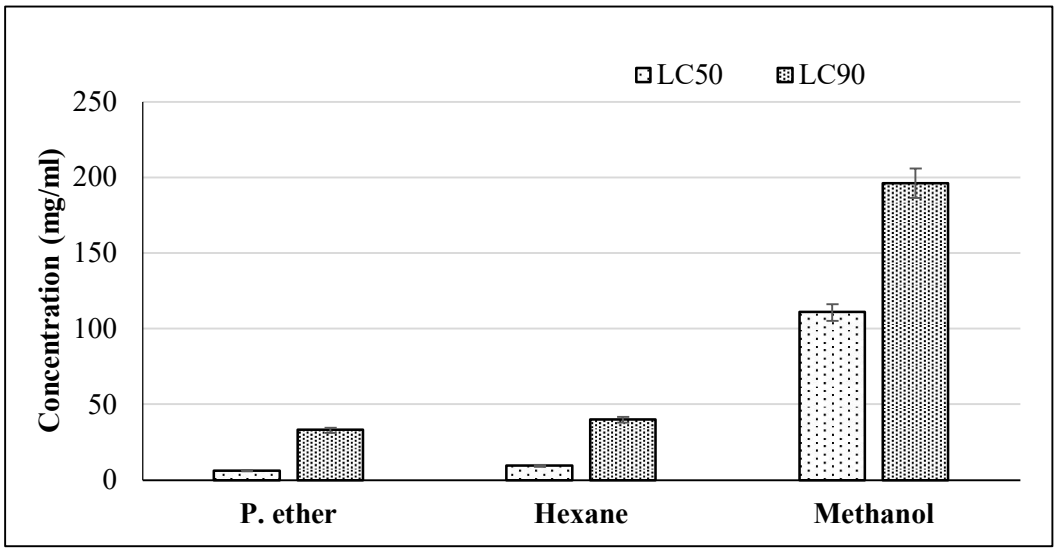

Figure 2: Relative acaricidal bio-efficacy of different extracts of *Citrus limetta* peels against larvae of *R. microplus.*

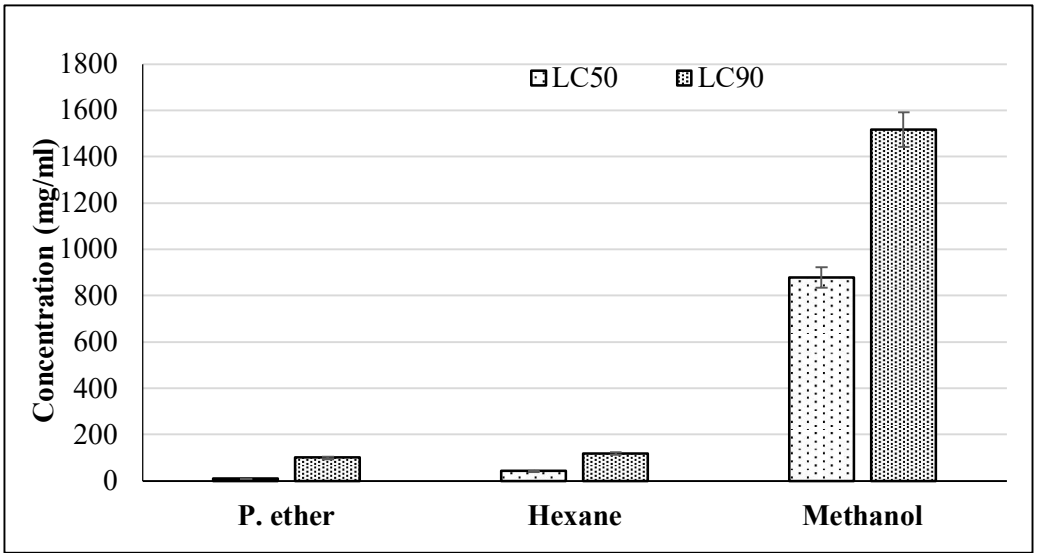

Figure 3: Relative acaricidal bio-efficacy of different extracts of *Citrus limetta* peels against adults of *R. microplus.*

However, methanol extract of *Lantana camara* was discovered to be the most efficient against *R. microplus* adults among various organic solvents (chloroform, ethyl acetate, and methanol), with a 50% fatal concentration at 1314.40 ppm [21]. In contrast, *R. microplus* adults exposed to hexane extract of C*itrus limetta* fruit peels, die at a rate of 70% when the concentration is 50 mg/ml [10]. On the other hand Kishore *et al.,* 2021 investigated that acaricidal potential of extracts of *Capsicum frutescens* fruits against *Rhipicephalus microplus* and found that adults of target organism was more sensitive to methanol extracts (LC_{50} value 617.54 ppm) than hexane and petroleum ether extracts [6]. Essential oil of C*itrus limetta* fruit peels exhibits insecticidal activity against *Anopheles stephensi* and *Culex quinquefasciatus* with 50% fatal concentration at 16.31 and 29.20 ppm respectively while essential oil of C*itrus limetta* leaves were relatively less effective than peels against *Anopheles stephensi* and *Culex quinquefasciatus* with 50% fatal concentration at 23.77 and 35.12 ppm respectively [12].

Table 2: Adulticidal bio-efficacy of petroleum ether extract of *Citrus limetta* peels.

Concentration (mg/ml)	Total (15) wt. of ticks (mg)	% Survival	Total wt. of egg laid survived ticks (mg)	Egg Index	ERF	% IR
2	710	80	200	0.28	340144	68.34
4	720	66.66	170	0.24	162720	84.85
8	750	53.33	130	0.17	60690	94.35
16	750	40	120	0.16	30688	97.14
32	920	26.66	110	0.12	5616	99.47
64	1140	13.33	80	0.07	74.2	99.99
Control	1380	93.33	750	0.92	1074600	--

ERF: Estimated reproductive factor; %IR: %Inhibition of reproduction

Table 2 and Figure 4 showed the acaricidal bio-efficacy of petroleum ether extract of *C. limetta* against *R. microplus* at 2, 4, 8, 16, 32 and 64 mg/ml exhibit 80, 66.66, 53.33, 40, 26.66 and 13.33 % survival 0.28, 0.24, 0.17, 0.16, 0.12 and 0.07 egg index, 340144, 162720, 60690, 30688, 5616 and 74.2 ERF, and 68.34, 84.85, 94.35, 97.14, 99.47 and 99.99 % IR, respectively. In case of control, survival% was 93.33 %, 0.92 EI and 1074600 ERF and no IR were recorded of the target organism. But % survival, EI, ERF were decreased and % IR increased by increasing concentration gradually.

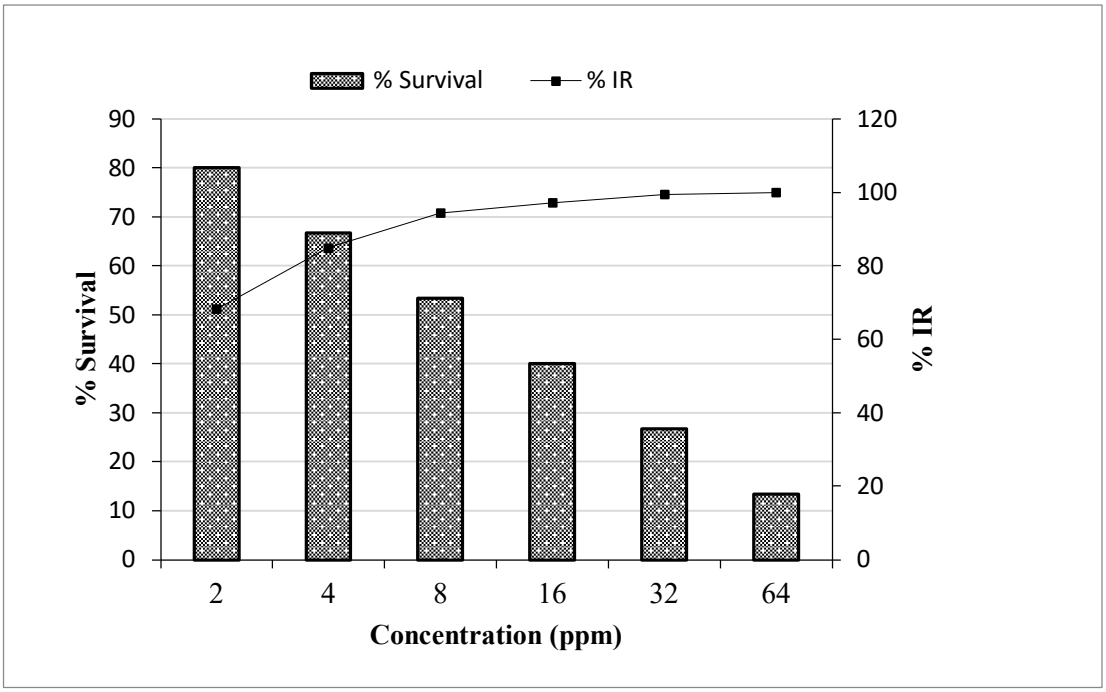

Figure 4: Acaricidal potential of petroleum ether extract of *Citrus limetta* peels on the survival and inhibition of reproduction of *R. microplus*.

Table 3: Adulticidal bio-efficacy of hexane extract of *Citrus limetta* peels.

Concentration (mg/ml)	Total (15) wt. of ticks (mg)	% Survival	Total wt. of egg laid survived ticks (mg)	Egg Index	ERF	% IR
20	1360	73.33	610	0.44	514641.6	53.70
40	1545	66.66	540	0.34	304572	72.60
50	790	53.33	265	0.33	192258	82.70
60	1550	26.66	200	0.13	13717.6	98.76
70	840	20	105	0.12	2584.8	99.76
100	1670	13.3	40	0.02	196.8	99.98
Control	1130	86.66	650	0.57	1111614	--

ERF: Estimated reproductive factor; %IR: %Inhibition of reproduction

Table 3 and Figure 5 showed the acaricidal bio-efficacy of hexane extract of *C. limetta* peels against *R. microplus* at 20, 40, 50, 60, 70 and 100 mg/ml exhibit 73.33, 66.66, 53.33, 26.66, 20 and 13.33 % survival 0.44, 0.34, 0.33, 0.13, 0.12 and 0.02 egg index, 514641.6, 304572, 192258, 13717.6, 2584.8 and 196.8 ERF, and 53.70, 72.60, 82.70, 98.76, 99.76 and 99.98 % IR, respectively. In case of control, survival% was 86.66%, 0.57 EI and 1111614 ERF and no IR were recorded of the target organism. But % survival, EI, ERF were decreased and % IR increased by increasing concentration gradually.

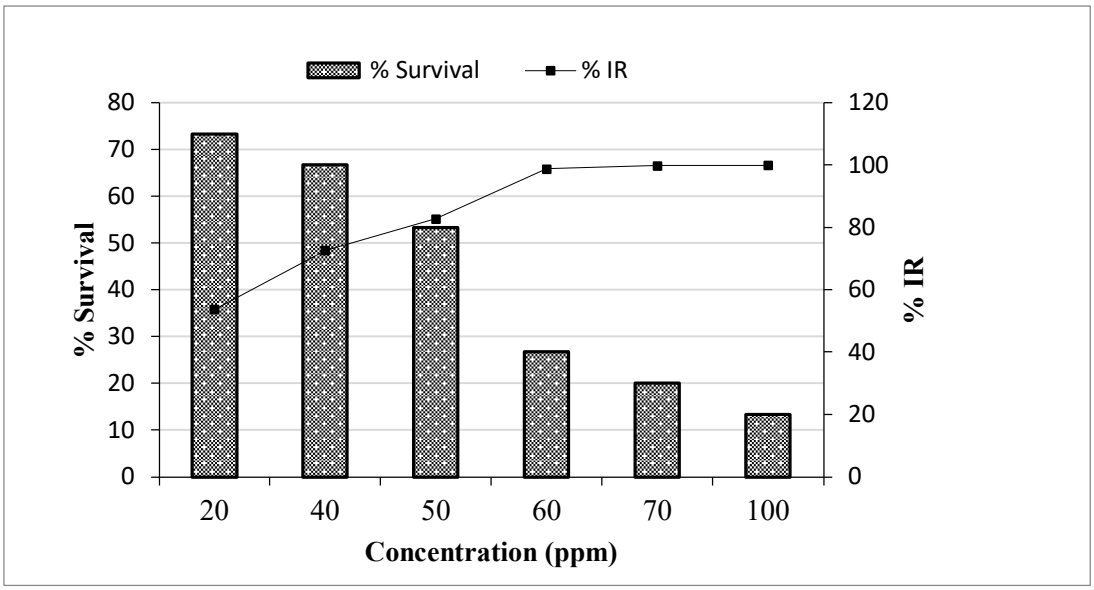

Figure 5: Acaricidal potential of hexane extract of *Citrus limetta* peels on the survival and inhibition of reproduction of *R. microplus*.

Table 4: Adulticidal bio-efficacy of methanol extract of *Citrus limetta* peels.

Concentration (mg/ml)	Total (15) wt. of ticks (mg)	% Survival	Total wt. of egg laid survived ticks (mg)	Egg Index	ERF	% IR
800	1230	53.3	560	0.45	458550	60.20
1000	1260	40	480	0.38	319656	72.25
1200	1320	26.66	450	0.34	219776	80.92
1400	1360	20	410	0.30	133200	88.43
1600	1460	6.66	340	0.23	6992	99.39
1800	1490	0	00	--	--	--
control	1620	100	970	0.59	1152152	--

ERF: Estimated reproductive factor; %IR: %Inhibition of reproduction

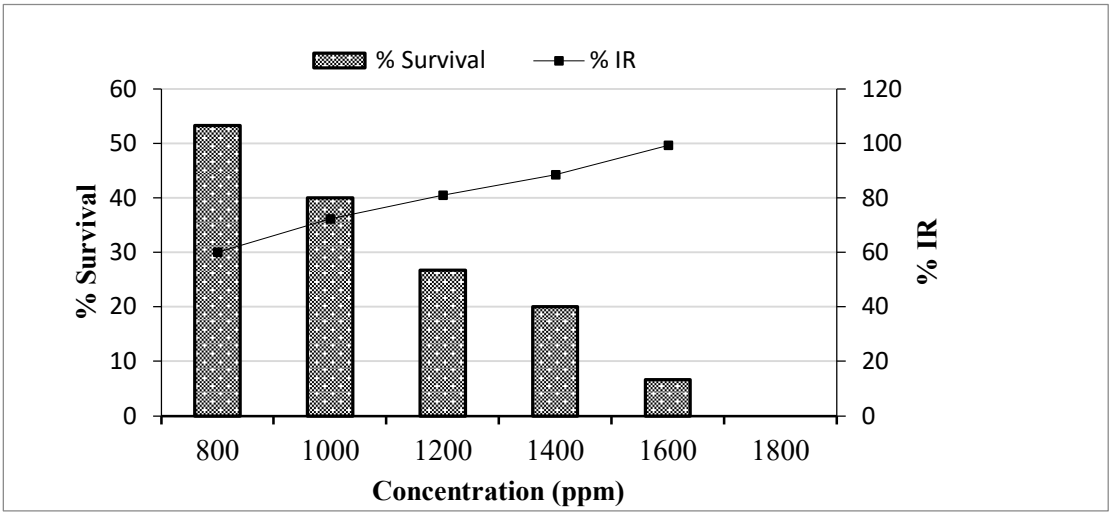

Figure 6: Acaricidal potential of methanol extract of *Citrus limetta* peels on the survival and inhibition of reproduction of *R. microplus*.

Table 4 and Figure 6 showed the acaricidal bio-efficacy of methanol extract of *C. limetta* peels against *R. microplus* at 800, 1000, 1200, 1400, 1600 and 1800 mg/ml exhibit 53.33, 40, 26.66, 20, 6.66 and 00 % survival 0.45, 0.38, 0.34, 0.30 and 0.23 egg index, 458550, 319656, 219776, 133200 and 6992 ERF, and 60.20, 72.25, 80.9, 88.43 and 99.39 % IR, respectively. In case of control, survival% was 100 %, 0.59 EI and 1152152 ERF and no IR were recorded of the target organism. But % survival, EI, ERF were decreased and % IR increased by increasing concentration gradually.

However, it was discovered that the methanol extract of *C. limetta* was the most effective natural pesticide for combating S*almonella typhimurium, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Escherichia coli* [9]. Essential oil of *Citrus limonum* shows the acaricidal efficacy on adults of *R. microplus* at 2.2% concentration [22]. Where, acaricidal activity of methanol extract of *Citurs sinensis* peels against spider mite, *Tetranychus urticae* and find that 34% mortality at 1250 ppm extract [23].

Essential oil obtained from *Citrus aurantium* found effective and showed remarkable acaricidal activity against *Tetranychus urticae*, phytophagous mite [24]. At a dosage of 100 mg/ml, an ethanolic extract from *Cassia fistula* leaves showed 58.33% death in adults of *Rhipicephlaus (Boophilus) annulatus* and 24.54% suppression of fecundity with 0% egg hatching [25]. Bagasse and seed extracts in ethanol of *C. limetta* possesses showed the antibacterial potential against *Escherichia coli* and *Staphylococcus aureus* but bagasse extract showed more potent bactericides than seed extract [14].

Phytochemical analysis

Further phytochemical investigation were performed and found that all the extracts (Petroleum ether, hexane and methanol) possesses alkaloids, coumarins, tannins, terpenes and volatile oils. While glycosides and tannins were present only in p. ether ans hexane extracts. However flavanoids was present only in p. ether extract while saponins was only present in hexane extract of *C. limetta* fruit peels (Table 5). Petroleum ether extract possesses alkaloids, coumarins, flavanoids, glycosides tannins, terpenes and volatile oils however, hexane extract possesses alkaloids, coumarins, glycosides, saponins, tannins, terpenes and volatile oils while methanol extract possesses alkaloids, coumarins, terpenes and volatile oils. While alkaloids, coumarins, terpenes and volatile oils were commonly present in all three extracts of *C. limetta* fruit peels. Our results was in agreement with the study conducted by Quidwai *et al.* and found that essential oil of *C. limetta* peels possesses various phytocompound alkaloids, phenols, tannins and steroids [7].

Table 5: List of phyto-chemical compounds present/absent in different extracts of *Citrus limetta* fruit peels.

Phytochemicals	Methods	Petroleum ether	Hexane	Methanol
Alkaloids	Mayer's test	++	+	++
Amino acid	Ninhydrin test	-	-	-
Carbohydrates	Fehlings's test	-	-	-
Coumarins	-	++	++	+
Flavanoids	Alkaline reagent's test	++	-	-
Glycosides	Keller-Kiliani's test	++	+	-
Saponins	Froath test	-	++	-
Tannins	Ferric chloride test	+++	+	-
Terpenes	Salkowski test	++	++	+
Volatile oils	-	+	++	++

(+) Present; (-) Absent

Present findings was similar to Shyam, as he found that hexane and chloroform extracts of *Citrus limetta* peels showed the presence of alkaloids, phenols, steroids, tannins and terpenoids whereas methanol extract showed the alkaloids, flavanoids, phenols, steroids, tannins and terpenoids [9]. Similarly, terpenes obtained from the essential oils showed the remarkable acaricidal activity against different stages (larvae, nymphs and adults) of *Psoroptes cuniculi*, rabbit mite [26]. Our results was also in agreement with Tabari *et al*. as they stated that monoterpenes and sesquiterpene extracted from essential oil of *Cannabis sativa* have acaricidal activity against eggs and larvae of *Dermanyssus gallinae* and *Hyalomma dromedarii* [27]. Similarly, different phytochemical compounds such as alkaloids, flavanoids, tannins and glycoside were also found in the methanol extracts of *Capsicum frutescens* and also responsible for acaricidal activity against *R. microplus* [6]. Similarly, essential oils of peels and leaves of *C. limetta* showed the presence of alkaloids, flavanoids, tannins and terpenes responsible for the insecticidal potential against *Anopheles stephensi* and *Culex quinquefasciatus* [12].

GC-MS analysis

Results obtained from the GC-MS analysis of petroleum ether extract of *Citrus limetta* peels revealed that compound, n-Hexadecanoic acid ($C_{16}H_{32}O_2$) found in highest concentration 28.90% area, while compound 9, 12-Octadecadienoic acid ($C_{18}H_{32}O_2$) found in 16.565% area and compound hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$) found in 10.875% (Table 6 and Figure 7).

Table 6: GC-MS analysis of petroleum ether extracts of *Citrus limetta* peels.

Retention Time	%Area	Name of Compound	Chemical Formula
14.468	6.171	1,2-Cyclohexanediol, 1-methyl-4-(methylethenyl)- OR Limonene-1,2-diol	$C_{10}H_{18}O_2$
14.617	0.255	1,6- Cyclodecadiene, 1-mrthyllene-8-(1-methylethyl)-	$C_{15}H_{24}$
17.006	0.586	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4- (1-methylethyl)-,	$C_{15}H_{26}O$
17.438	0.462	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	$C_{15}H_{24}$
17.842	0.394	Cyclohexanemethanol, 4-ethenyl-α, α, 4-trimethyl-3-((1-methylethyl)	$C_{15}H_{26}O$
19.369	0.547	Cyclopentanepropanol, 2-methylene	$C_9H_{16}O$
19.913	0.448	1, 9-Decadiyne	$C_{10}H_14$
20.703	0.541	Cyclopentaneundecanoic acid	$C_{16}H_{30}O2$
21.288	0.344	1-Cyclohenene-1-methanol, 4-(1-methyletheneyl)-	$C_{10}H_{16}O$
21.323	0.497	3,5-Nonadiene-7-yn-2-ol, (E, E)-	$C_9H_{12}O$
22.148	0.691	Phthalic acid, hex-2-yn-4yl isobutyl ester	$C_{18}H_{22}O_4$
22.162	0.405	Phthalic acid, nonyl tridec-2-yn-1yl ester	$C_{30}H_{46}O_4$
22.763	10.875	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
23.328	28.900	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
23.429	0.270	2,2,4-Trimethyl-3-pentanol	$C_8H_{18}O$
23.576	2.521	Heptanoic acid, 2-methyl	$C_9H_{18}O_2$
24.783	3.441	9, 12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O$
24.868	0.491	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$
25.334	16.565	9, 12-Octadecadienoic acid, (Z,Z)-	$C_{18}H_{32}O_2$
25.366	4.410	9,12-Octadecadienal	$C_{18}H_{32}O$
25.388	3.399	6(Z), 9(Z)-Pentadecadien-1-ol	$C_{15}H_{28}O$
25.411	7.974	Undec-10-ynoic acid	$C_{15}H_{18}O_2$

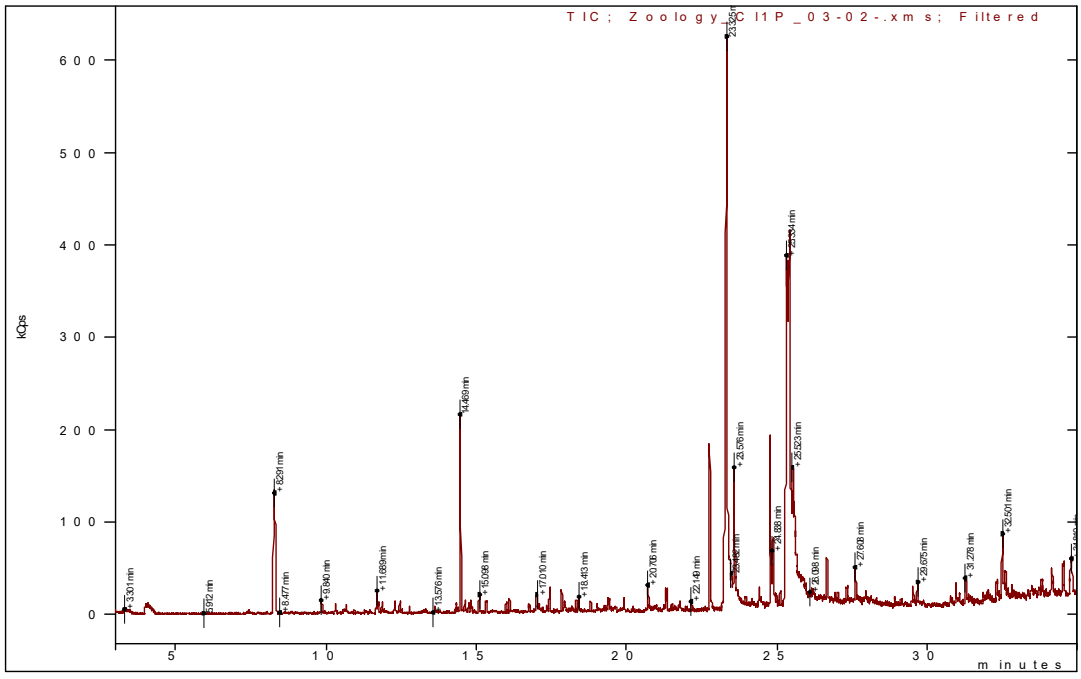

Figure 7: Chromatogram of petroleum ether extracts of *Citrus limetta* (peels).

In contrast to present findings, Gupta *et al.* identified 7 compounds in GC-MS MS profile of essential oil of *Citrus limetta* peels out of them 3 Allyl 6 methoxyphenol (12.86%), Phenol, 2 methoxy3 (2 propenyl) (10.70%), α-Terpineol (8.23%) and Terpinen 4-ol (8.02%) were the major constituents [28]. Compounds eugenol, thymol, and γ-terpinene were found in extracts of *Ocimum gratissimum* L. (three samples), *O. urticaefolium* Roth, and *O. canum* Sims which are responsible for acaricidal activity against *Rhipicephalus (Boophilus) microplus* [29]. Similarly, Khan *et al.* found 29 compounds from which, d-limonene (78.3%) found abundantly in GC-MS MS analysis of essential oil of *Citrus limetta* peels [30]. In contrary to present

investigation, essential oil of *Citrus limetta* peels showed that 10 important compounds from which, Limonene and *β*-Myrcene found abundantly in GC-MS MS analysis performed by Gautam [31]. While Aboelhadid *et al.* were found E-anethole and fenchone compounds from the GC-MS analysis of *Foeniculum vulgare* and possesses acaricidal efficacy against *Rhipicephalus annulatus* [32]. Kadapure *et al.* identified limonene (72.8%) as the dominant component from the results of GC-MS analysis of Sweet lime peel [33]. Similarly, the investigation of methanolic extract of Sweet lime peel using GC-MS, Deo and Sakhale found 12 compound Oleic Acid (9.62%) in higher concentration, n-Hexadecanoic acid (5.23%) and D-Limonene (4.70%) [34]. While, Kishore *et al.* were found N-Isobutyle-2(E), 6(Z), 8E decatrienamide compound (highest amount) in different fractions of the methanol extract of *Spilanthes acmella* flower and stated that this compound was responsible for the lethal effects on *Rhipicephalus microplus* [35].

CONCLUSION

Rhipicephalus (Boophilus) microplus is a crucial ectoparasite of cattle and a carrier of a number of infections that cause illnesses, which then have an impact on the agricultural sector as well as the economy. To combat the pesticide resistance that has evolved, a safer and more environmentally friendly alternative was produced. The findings of this investigation demonstrated that extracts from *C. limetta* peels had acaricidal action against *R. microplus.* In both larvicidal and adulticidal cases, petroleum ether extract was shown to be the most effective, whereas hexane extract was successful in ovicidal cases with a lower lethal dose at 50% mortality. According to a phytochemical analysis, all the extracts tested (Petroleum ether, Hexane, and Methanol) showed the presence of Alkaloids, Coumarins, Tannins, Terpenes, and Volatile Oils. GC-MS analysis of potent extract shows the presence of n-Hexadecanoic acid as a main compound which may responsible for acaricidal nature of peels extract. Therefore, these phytoextracts may be used as a safer alternative other than synthetic pesticides to remove that haematophagous and deadly ticks. Further, molecular analysis could also be performed the actual pathway of drug delivery system.

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