Research Article

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Assessment of Mosquitocidal and Antibacterial Activities of Mixture of Different Extracts of *Curcuma amada* Rhizome and *Tamarindus indica* Leaves

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Abstract Present study aimed at evaluation of mosquito larvicidal and antibacterial activities of mixture of crude and solvent extracts of *Curcuma amada* rhizome and *Tamarindus indica* leaf against *Culex vishnui* larvae and eight pathogenic bacteria. Larvicidal bioassay was performed by graded concentrations of crude, methanol and aqueous extracts. Log-probit analysis for estimation of LC₅₀ and LC₉₀ values was performed. ANOVA analysis was also conducted. Effects of the extracts on non-target organisms were also examined. Antibacterial activity was examined through standard methods. Preliminary phytochemical analysis and Fourier Transform Infra Red (FT-IR) spectroscopic analysis of the most potent extract were also done. Highest mortality (100%) was recorded at 100 ppm concentration of methanol extract after 72 hours in 1st, 2nd and 3rd instars larvae. LC₅₀ and LC₉₀ values were 72.32 and 131.14 ppm respectively after 24 h for the 3rd instar larvae. No effects on non-target organisms found. Crude and methanol extracts showed antibacterial activity against all tested bacteria while aqueous extracts showed none. Highest inhibition zone (19.00±0.00 mm) was observed against *Pseudomonas fluorescens* MTCC 103 by methanolic extract. Preliminary phytochemical screening of most potent extract detected tannins, flavonoids, alkaloids and terpenoids. Presence of various functional groups was revealed by FT-IR analysis. Results confirm the potential mosquito larvicidal and antibacterial activities of mixture *C. amada* rhizome and *T. indica* leaf extracts. This approach of using mixture of extracts for getting better result against harmful biological agents is a promising finding which may further approve application of coactivity of bioactive compounds of plant origin.

Keywords *Curcuma amada* rhizome; *Tamarindus indica* leaf; Mixture of extracts; Mosquito larvicide and Bactericide; FT-IR analysis; Non-target organisms

Background

Among the well-known disease vectors mosquitoes are important in terms of public health concerns as they transmit a variety of fatal diseases like malaria, filariasis, dengue, Japanese encephalitis, yellow fever and many other diseases (Ghosh et al., 2012).

In India all the three members of *Culex vishnui* group viz., *Cx. vishnui*, *Cx. pseudovishnui* and *Cx. tritaeniorhynchus*, responsible for transmission of Japanese Encephalitis virus, are present in areas where paddy fields are abundant (Chandra, 2000; Rawani et al., 2017).

Recent mosquito control programme mainly focuses on avoidance of transmission by mosquito vectors either by killing adult mosquitoes by using fumigants or by destroying larvae at their breeding sites (Alouani et al., 2017). As mosquitoes are continuously gaining resistant against chemical insecticides, alternative measures such as applying botanical based insecticides are now practiced worldwide (Singh et al., 2016). Researchers are continuously searching new botanicals with more effectiveness as they could fulfill the criteria of being environment friendly, inexpensive, and easily available in nature (Sharma et al., 2006). Besides vector borne diseases, infectious diseases caused by bacteria are also our concern. Increasing resistance to commercial antibacterials or antibiotics in disease causing bacteria of human and livestock turned the focus of the researchers

to develop alternatives. Plants have several secondary metabolites which are reported to exhibit antibacterial, antifungal and insecticidal properties (Bhattacharya et al., 2014; Burman et al., 2018; Pahlaviani et al., 2018). *Tamarindus indica* (commonly called Tamarind), a plant under the family Leguminaceae is a tropical evergreen tree distributed throughout Africa and Southern Asia. It has a wide variety of biological effects including antimicrobial, antimalarial and antidiabetic effects (Doughari et al., 2006; Parvin et al., 2013; Satpute and Vanmare, 2017). In another plant, *Curcuma amada* (mango ginger) of family Zingiberaceae distributed throughout India, antioxidant, antibacterial, antifungal, anti-inflammatory, CNS depressant and analgesic activities have been reported (Policegoudra et al., 2011).

Larvicidal activities of *C. amada* rhizome and *T. indica* leaf have been evaluated on *Anopheles stephensi* individually (Vinayagam et al., 2008; Jegajeevanram et al., 2016). Antibacterial potency of *C. amada* rhizome and *T. indica* leaf was evaluated by several scientists (Kumar et al., 2009; Escalona-Arranz et al., 2010). The current study is focused on evaluating the combined biocidal effect of *C. amada* rhizome and *T. indica* leaf separately on mosquito vector *Culex vishnui* and some strains of human and fish pathogenic bacteria.

1 Materials and Methods

1.1 Collection and authentication of plants

Rhizome of *C. amada* was collected from local market of Burdwan and *T. indica* leaves were collected from outskirts of Burdwan (23°16'N, 87°54'E) in March 2018 and identified by Dr. Ambarish Mukherjee, former Professor, Department of Botany, The University of Burdwan, Burdwan, West Bengal, India. Specimen of the plants, bearing Voucher specimen No. GCP18-10 and GCP18-11 was kept in the Herbarium of Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan. The plant parts were thoroughly washed with tap water and then with distilled water and extra water was soaked in paper towel. Plant materials were then finely chopped and air dried for fifteen days.

1.2 Rearing of the larvae

Larvae of *Cx. vishnui* were collected from rice fields of Agriculture Farm, The University of Burdwan (23°16'N, 87°54'E) by applying standard dipping and scooping process. They were kept in plastic trays and fed with a mixture of dog biscuits and yeast powder at the ratio of 3:1. Pupae were transferred to insectary (45×45×40 cm) for adult emergence. Adults were fed with 10% sucrose solution combined with multivitamin syrup soaked in a cotton wick put in a Petri plate inside the cage as food. The adult mosquitoes were identified by the keys given by Christophers (1933), Barraud (1934), and Chandra G. (2000). Females were given blood meal from saved non motile rat in a different glass cage overnight. For oviposition Petri plates filled with water were kept inside the cage. After that, the eggs were allowed to hatch under laboratory conditions. First generation *Cx. vishnui* larvae were maintained at 25°C~30°C, 75%~85% relative humidity (RH), with a photoperiod of 14:10 h light/dark cycle were used for bioassay.

1.3 Test microorganisms

Test microorganisms comprised of four human pathogenic bacteria namely *Escherichia coli* MTCC 739, *Staphylococcus aureus* MTCC 2940, *Bacillus subtilis* MTCC 441 and *Pseudomonas aeruginosa* MTCC 2453 and four fish pathogenic bacteria namely *B. licheniformis* MTCC 530, *B. mycoides* MTCC 7343, *P. fluorescens* MTCC 103 and *P. putida* MTCC 1654 were obtained from Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, The University of Burdwan, Burdwan. All bacterial strains were cultured in nutrient broth Hi-Media, M002 (Hi-Media Laboratories Limited Mumbai, India) at 37°C and maintained on nutrient agar slants at 4°C with regular periods of subculture.

1.4 Preparation of plant extracts

1.4.1 Crude extract

Freshly collected *C. amada* rhizome and *T. indica* leaf were cleaned properly through distilled water and juices are produced by blender and then subjected to filtration by Whatman No. 1 filter paper. The filtrate was regarded as a stock solution (100% concentration).

1.4.2 Solvent extracts

For extraction mixture of air dried *C. amada* rhizome and *T. indica* leaf were taken in 1:1 ratio (50 g of each) in thimble of Soxhlet apparatus and extracted in 1000 mL of methanol and distilled water one after another. After 72 h of extraction of the plant material in each of the solvent, the extract was filtered with Whatman No. 1 filter paper and the excess solvent was evaporated *in vacuo*. The dried extract of each solvent, was stored in airtight bottles at 4°C in refrigerator.

1.5 Larvicidal bioassays

The larvicidal bioassays were done following the standard protocol of World Health Organization (WHO, 2005) in laboratory condition in the Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan. Twenty five larvae of each instar were placed in sterilized plastic bowls filled with 100 mL of distilled water. From stock solution of crude extracts of mixture of *C. amada* rhizome and *T. indica* leaves 0.1%~0.5% concentrations were prepared and added separately to different bowls. Five different working concentrations (60, 70, 80, 90 and 100 ppm) were prepared from both methanol and aqueous extracts and applied on different plastic bowls filled with water (100 mL each) each containing 25 larvae of different instars for the assessment of the larvicidal property under laboratory conditions at 25°C ~30°C and 80%~90% relative humidity. Each experiment was conducted thrice in laboratory condition. After 24 h, 48 h and 72 h of exposures larval mortality were noted. Death of larvae was confirmed when they failed to react on needle probing in the siphon or cervical region.

1.6 Test on non-target organism

Extracts were also tested for their toxicity against non-target organisms, *Chironomus circumdatus* larvae and *Tubifex* sp. Each experiment was repeated thrice.

1.7 Antibacterial bioassay

Agar well diffusion method was used to determine antibacterial activity of the extracts (Perez, 1990). For each bacterial strain, negative controls were maintained viz., 1% (v/v) DMSO (Dimethyl sulfoxide) as control for methanolic extract and sterile distilled water as control for crude and aqueous extracts. After sterilization through autoclave, Nutrient agar poured in 90 mm Petri plates and after solidification of the agar, wells of 6 mm diameter were made. Wells were filled with 30 µL each of crude extract (1%) and solvent extracts (30 mg/mL) for the bioassay along with respective controls. Tetracycline (10 µg/mL) was taken as positive control as it has broad spectrum antibacterial activity. Then each of the strains was allowed to grow on agar plate for 24 h incubation at 37°C temperature. Antibacterial activities were counted by measuring inhibition zones around the wells. The experiment was done three times.

1.8 Determination of minimum inhibitory concentration (MIC)

MIC was evaluated by dilution method as followed by the National Committee for Clinical Laboratory Standards (1993). The cultures were diluted in Müeller-Hinton broth at a density adjusted to turbidity of 0.5 MacFarland standards. Serially twofold diluted extracts ranging from 10 mg/mL to 0.0625 mg/mL were mixed with an equal volume of 0.5 mL inoculums prepared in the same medium in test tubes for each of the bacterial strains. The tubes were incubated at 37°C for 24 h. Two control tubes were maintained for each of the test i.e., one with only inoculums in broth and the other with 1% (v/v) DMSO in broth. The lowest concentration (highest dilution) of the extract that inhibits bacterial growth was regarded as MIC.

1.9 Fourier Transform Infra Red (FTIR) spectroscopic analysis

For functional group analysis by Fourier Transform Infrared Spectrophotometer (FT-IR) pellets were made by 10 mg of dried mixture of methanol extract of two plant parts and 100 mg of potassium bromide by using Hydraulic Press apparatus. Potassium bromide pellet without extract was taken as control. Loaded pellets (control and extract) were scanned in a range from 400 to 4500/cm in FTIR spectrometer (Jasco, FT/IR- 4700) to identify functional groups present in the mixture of two methanol extracts.

1.10 Phytochemical analysis

The freshly prepared extracts were subjected to standard phytochemical analyses for the presence of different phytochemicals like tannins, flavonoids, saponins, glycosides, alkaloids and steroids and terpenoids.

1.11 Statistical analyses

Larval casualty and diameter of inhibition zones are presented as mean $\pm$ SE. The statistical analysis of the data was done by using MS Excel 2007. Experimental data of larval death were statistically analysed through Log-probit analyses and regression analyses for determining LC₅₀ and LC₉₀ using the “STAT PLUS 2009 (Trial version)” and “MS EXCEL 2007” respectively. Details of statistical verification amongst three variables viz., different instars, different concentrations and different exposure time and the larval mortality were presented through completely randomized three-way ANOVA analyses.

2 Results

Crude and methanol extracts of mixture of *C. amada* rhizome and *T. indica* leaves was found to have notable mosquitocidal property against *Cx. vishnui* in our laboratory observations. The highest mortality i.e., 100.00% mortality was recorded at 0.5% concentration of crude extract against 1st instar larvae and 2nd and 3rd instars larvae after 48 h and 72 h of exposure respectively (Table 1). No larval mortality was observed against all larval instars after treatment with aqueous extract. On the other hand cent percent mortality of 1st instar larvae found in 90 ppm concentration of methanol extract at 48 h of exposure. In case of 2nd and 3rd instars larvae 100% mortality was achieved after three days of application of methanol extract of 100 ppm concentration. 4th instar larvae were less susceptible to both crude and methanol extract with highest mortality at 0.5 % and 100 ppm concentration of crude and methanol extract respectively (Table 1). LC₅₀ and LC₉₀ values (at 95% of confidence level) were given in Table 2 which decreases with exposure time. Results of Log probit analysis and Regression analysis of larval mortality by crude and methanol extract are presented in Table 2. The mortality rate (Y) had positively correlation with the concentration of exposure (X) and the regression coefficient (R) value is near 1 in each of the cases. The larval death was found statistically considerable ($p < 0.05$) through completely randomized three-way ANOVA analyses (Table 3). Non-target populations were not harmed throughout the experimental period.

Table 1 Dose response larvicidal activities of mixture of *Curcuma amada* rhizome and *Tamarindus indica* leaf crude and methanol extracts against *Culex vishnui* larvae

Crude extract					Methanol extract			
Larval instars	Concentration (%)	Percent Mortality (Mean $\pm$ SE)			Concentration (ppm)	Percent Mortality (Mean $\pm$ SE)		
		24 h	48 h	72 h		24 h	48 h	72 h
1 st	0.1	36.00 $\pm$ 0.58	46.68 $\pm$ 0.33	54.67 $\pm$ 0.33	60	49.32 $\pm$ 0.33	56.00 $\pm$ 0.00	70.68 $\pm$ 0.33
	0.2	50.68 $\pm$ 0.33	58.68 $\pm$ 0.33	69.32 $\pm$ 0.33	70	58.68 $\pm$ 0.33	65.32 $\pm$ 0.33	81.32 $\pm$ 0.33
	0.3	64.00 $\pm$ 0.58	68.00 $\pm$ 0.00	77.32 $\pm$ 0.33	80	66.68 $\pm$ 0.33	74.68 $\pm$ 0.33	90.68 $\pm$ 0.33
	0.4	80.00 $\pm$ 0.58	85.32 $\pm$ 0.33	93.32 $\pm$ 0.33	90	81.32 $\pm$ 0.33	86.68 $\pm$ 0.33	100.00 $\pm$ 0.00
	0.5	96.00 $\pm$ 0.58	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100	92.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
2 nd	0.1	49.32 $\pm$ 0.33	58.68 $\pm$ 0.33	68.00 $\pm$ 0.00	60	36.00 $\pm$ 0.00	50.68 $\pm$ 0.33	64.00 $\pm$ 0.58
	0.2	58.68 $\pm$ 0.33	66.68 $\pm$ 0.33	78.68 $\pm$ 0.33	70	45.32 $\pm$ 0.33	57.32 $\pm$ 0.33	73.32 $\pm$ 0.33
	0.3	70.68 $\pm$ 0.33	76.00 $\pm$ 0.00	85.32 $\pm$ 0.33	80	57.32 $\pm$ 0.33	70.68 $\pm$ 0.33	82.68 $\pm$ 0.33
	0.4	76.00 $\pm$ 0.00	85.32 $\pm$ 0.33	93.32 $\pm$ 0.33	90	68.00 $\pm$ 0.33	82.68 $\pm$ 0.67	93.32 $\pm$ 0.33
	0.5	88.00 $\pm$ 0.58	94.68 $\pm$ 0.33	100.00 $\pm$ 0.00	100	84.00 $\pm$ 0.00	94.68 $\pm$ 0.33	100.00 $\pm$ 0.00
3 rd	0.1	36.00 $\pm$ 0.00	45.32 $\pm$ 0.33	56.00 $\pm$ 0.58	60	29.32 $\pm$ 0.33	40.00 $\pm$ 0.00	57.32 $\pm$ 0.33
	0.2	46.68 $\pm$ 0.33	54.68 $\pm$ 0.33	66.68 $\pm$ 0.67	70	37.32 $\pm$ 0.33	50.68 $\pm$ 0.33	68.00 $\pm$ 0.58
	0.3	60.00 $\pm$ 0.33	65.32 $\pm$ 0.33	81.32 $\pm$ 0.33	80	50.68 $\pm$ 0.33	61.32 $\pm$ 0.33	84.00 $\pm$ 0.58
	0.4	70.68 $\pm$ 0.33	80.00 $\pm$ 0.58	90.68 $\pm$ 0.33	90	61.32 $\pm$ 0.33	74.68 $\pm$ 0.67	98.68 $\pm$ 0.33
	0.5	78.68 $\pm$ 0.33	89.00 $\pm$ 0.33	100.00 $\pm$ 0.00	100	74.68 $\pm$ 0.33	90.68 $\pm$ 0.33	100.00 $\pm$ 0.00
4 th	0.1	17.32 $\pm$ 0.33	24.00 $\pm$ 0.00	33.32 $\pm$ 0.33	60	21.32 $\pm$ 0.33	29.32 $\pm$ 0.33	57.32 $\pm$ 0.33
	0.2	25.32 $\pm$ 0.33	37.32 $\pm$ 0.33	44.00 $\pm$ 0.00	70	30.68 $\pm$ 0.33	42.68 $\pm$ 0.67	65.32 $\pm$ 0.33
	0.3	36.00 $\pm$ 0.58	45.32 $\pm$ 0.33	53.32 $\pm$ 0.33	80	37.32 $\pm$ 0.33	53.32 $\pm$ 0.33	77.32 $\pm$ 0.33
	0.4	49.32 $\pm$ 0.33	57.32 $\pm$ 0.33	65.32 $\pm$ 0.33	90	46.68 $\pm$ 0.33	61.32 $\pm$ 0.33	84.00 $\pm$ 0.58
	0.5	60.00 $\pm$ 0.58	72.00 $\pm$ 0.00	82.68 $\pm$ 0.33	100	53.32 $\pm$ 0.33	74.68 $\pm$ 0.33	97.32 $\pm$ 0.33

Table 2 Assessment of LC₅₀ and LC₉₀ values through log-probit and regression analyses using crude and methanol extracts of mixture of *Curcuma amada* rhizome and *Tamarindus indica* leaf against *Culex vishnui* larvae (x = concentration of crude extract (in %))

	Larval Instars	Period of Exposure (Hour)	LC ₅₀ (%)	LC ₉₀ (%)	Regression	R ² - value
Crude extract	1 st	24	00.17	00.58	Y=00.37x-13.53	0.99
		48	00.14	00.49	Y= 00.33x-08.72	0.98
		72	00.11	00.38	Y= 00.28x-03.19	0.98
	2 nd	24	00.13	00.38	Y= 00.19x+05.53	0.99
		48	00.14	00.86	Y=00.23x+00.94	0.99
		72	00.07	00.33	Y= 00.24x-01.80	0.98
	3 rd	24	00.20	01.36	Y=27.34x+06.40	0.99
		48	00.15	00.77	Y=28.33x+08.23	0.99
		72	00.10	00.41	Y=28.00x+11.33	0.99
	4 th	24	00.42	02.15	Y=27.34x+01.19	0.99
		48	00.30	01.68	Y= 29.00x+3.09	0.99
		72	00.22	01.29	Y= 30.01x+4.93	0.98
Methanol extract	1 st	24	69.22	99.11	Y=28.68x+04.06	0.99
		48	65.63	93.93	Y=31.34x+06.46	0.99
		72	61.05	86.93	Y=29.01x+11.69	0.95
	2 nd	24	63.55	112.28	Y=29.67+50.63	0.99
		48	58.09	100.19	Y=28.34x+09.30	0.99
		72	53.10	83.07	Y=23.00x+13.76	0.99
	3 rd	24	72.32	131.14	Y=28.68x+04.06	0.99
		48	66.32	108.58	Y=31.34x+06.46	0.99
		72	60.01	88.53	Y=29.01x+11.69	0.94
	4 th	24	91.48	153.24	Y=20.00x+03.46	0.99
		48	82.08	140.08	Y=27.34x+04.86	0.99
		72	74.87	128.01	Y=24.67x+11.66	0.99

Table 3 Completely randomized three-way ANOVA analyses of the larvicidal activities of crude and methanol extracts using concentration (C), hour (H) and instars (I) as three independent parameters

Crude extract						Methanol extract					
Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean of squares (MS)	F value	p-level	Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean of squares (MS)	F value	p-level
Instars (I)	1 462.72	3	487.67	1 236.10	0.00	Instars (I)	772.99	3	257.66	644.162	0.00
Hours (H)	555.41	2	277.70	704.04	0.00	Hours (H)	1 504.30	2	752.15	1 880.375	0.00
Conc. (C)	2 812.50	4	703.12	1 782.67	0.00	Conc. (C)	2 492.47	4	623.11	1557.79	0.00
I × H	13.52	6	2.25	5.71	0.00	I × H	108.85	6	18.14	45.35	0.00
I × C	66.74	12	5.56	14.10	0.00	I × C	23.47	12	1.95	4.89	0.00
H × C	6.86	8	0.85	2.17	0.03	H × C	26.08	8	3.26	8.15	0.00
I × H × C	19.75	24	0.82	2.08	0.00	I × H × C	32.75	24	1.36	3.41	0.00
Within groups	47.33	120	0.39	---	---	Within groups	48.00	120	0.40	--	--
Total	4 984.86	179	27.80	----	---	Total	5 008.95	179	27.98	--	--

All the test bacteria were susceptible to the crude and methanol extracts. Among the extracts used methanol extract was the most potent on the test microorganisms followed by the crude extract whereas the aqueous extract is ineffective against these strains (Table 4). The most inhibited fish pathogen by methanol extract was *P. fluorescens* MTCC 103 with inhibitory zones of 19.00±0.00 mm followed by *B. licheniformis* MTCC 530 and *P. putida* MTCC 1654 with inhibitory zones of 18.33±0.33 and 18.00±0.00 mm respectively. Human pathogens exhibited less susceptibility to the methanol extract with a maximum zone of inhibition of 17.33±0.33 mm against *P. aeruginosa* MTCC 2453. In comparison to the most potent plant extract with Tetracycline (10 µg/mL), the test

isolates in most cases were highly susceptible to the plant extract than Tetracycline (10 µg/mL) except in case of *E. coli* MTCC 739 and *S. aureus* MTCC 2940. The minimum inhibitory concentration (MIC) of methanol extract ranged from 0.062 5~10 mg/mL as shown in Table 5. Stronger activity of methanol extract against test organisms was observed at higher concentration. Therefore, concentration may play a role for the observed antibacterial activity. Preliminary phytochemical analysis exposed presence of tannins, flavonoids, terpenoids and alkaloids (Table 6). Results of FT-IR analysis were presented in Table 7 and Figure 1.

Table 4 Zone of inhibition of mixture of *Curcuma amada* rhizome and *Tamarindus indica* leaf extracts against some pathogenic bacteria

Bacteria	Crude extract (1%)	Methanol extract (30 mg/mL)	Aqueous extract (30 mg/mL)	Tetracycline 10 µg/mL	Sterile distilled water	1% (v/v) DMSO
Human Pathogens			Zone of inhibition (mm)			
<i>Escherichia coli</i> MTCC 739	15.33±0.17	14.00±0.00	0.00±0.00	16.67±0.00	0.00±0.00	0.00±0.00
<i>Staphylococcus aureus</i> MTCC 2940	14.17±0.17	14.33±0.33	0.00±0.00	22.33±0.33	0.00±0.00	0.00±0.00
<i>Bacillus subtilis</i> MTCC 441	15.00±0.00	15.33±0.33	0.00±0.00	12.33±0.33	0.00±0.00	0.00±0.00
<i>Pseudomonas aeruginosa</i> MTCC 2453	15.00±0.00	17.33±0.33	0.00±0.00	14.00±0.00	0.00±0.00	0.00±0.00
Fish Pathogens			Zone of inhibition (mm)			
<i>Bacillus licheniformis</i> MTCC 530	15.67±0.33	18.33±0.33	0.00±0.00	16.67±0.33	0.00±0.00	0.00±0.00
<i>Bacillus mycoides</i> MTCC 7343	15.17±0.17	16.67±0.33	0.00±0.00	14.33±0.33	0.00±0.00	0.00±0.00
<i>Pseudomonas fluorescens</i> MTCC 103	14.67±0.33	19.00±0.00	0.00±0.00	11.67±0.33	0.00±0.00	0.00±0.00
<i>Pseudomonas putida</i> MTCC 1654	15.5±0.28	18.00±0.00	0.00±0.00	9.00±0.00	0.00±0.00	0.00±0.00

Table 5 Minimum Inhibitory Concentration (MIC) of mixture of *Curcuma amada* rhizome and *Tamarindus indica* leaf methanol extracts against some pathogenic bacteria

Bacterial strains	Minimum inhibitory concentration(MIC) of Methanol extract				
	10 mg/mL	5.00 mg/mL	2.50 mg/mL	1.25 mg/mL	0.0625 mg/mL
<i>Escherichia coli</i> MTCC 739	-	+	+	+	+
<i>Staphylococcus aureus</i> MTCC 2940	-	+	+	+	+
<i>Bacillus subtilis</i> MTCC 441	-	+	+	+	+
<i>Pseudomonas aeruginosa</i> MTCC 2453	-	-	+	+	+
<i>Bacillus licheniformis</i> MTCC 530	-	-	-	+	+
<i>Bacillus mycoides</i> MTCC 7343	-	-	+	+	+
<i>Pseudomonas fluorescens</i> MTCC 103	-	-	-	+	+
<i>Pseudomonas putida</i> MTCC 1654	-	-	-	+	+

Table 6 Phytochemical analysis of mixture of *Curcuma amada* rhizome and *Tamarindus indica* leaf methanol extract

Phytochemicals	Methanol extract
Tannins	+ve
Flavonoids	+ve
Saponins	-ve
Alkaloids	+ve
Terpenoids	+ve
Glycosides	-ve
Steroids	-ve

Table 7 FT-IR Peak Values of mixture of methanol extract of *Curcuma amada* rhizome and *Tamarindus indica* leaf

Peak values (cm ⁻¹)	Functional groups
3300-3280, 1390-1330, 1260-1180	Ph-OH(Alcohols)
3000-2900, 1490-1430	Cyclohexyl (Alkanes)
3375-3325, 1638-1618, 1420-1400	R-CO-NH ₂ (Amides)
3100-2900, 1440-1395, 960-875	C=C-COOH(Carboxylic acids)
1300-1000, 1770-1720, 1740-1715	COO, C=C-COOR (Esters)
920-905	5-ring Ethers
1655-1635	C-(C=O)-Ph-βNH ₂ (Ketones)
1700-1600, 950-920, 3300-3100	C=N-OH(Oximes)
1285-1270, 870-840, 760-745, 710-690	R-O-NO (Nitrates)
1680-1600	(RCH=N-N=CHR) Azines
1650-1590, 3328-3250	RCH ₂ -NH ₂ Amines

Figure 1 FT-IR analysis of mixture of methanol extract of *Curcuma amada* rhizome and *Tamarindus indica* leaf

3 Discussion

Preference has been given to larval control in management of mosquito vector as larvae are more susceptible to insecticides due to their internment to their static habitat than to adults that may escape fogging of insecticidal agents. Plant extract could be a potential source of bioactive compounds to control mosquitoes. Many plant species have been noted and recognized to have mosquitocidal activity (Bhattacharya and Chandra, 2013). Jagajeevanram et al. (2016) reported only 70% death of 4th instar larvae of *Anopheles stephensi* after 72 h exposure to 0.5 % concentration of *C. amada* rhizome methanol extract. Whereas, Porto et al. (2017) tested larvicidal activity of several different plants at 0.5 mg/mL concentration against *Aedes aegypti* among which evaluation of larvicidal property of ethanolic extract of *T. indica* leaves was also included and showed no mortality at that concentration. Use of mixture of these two plant extracts which is first time reported as mosquitocidal agent in our experiment showed significant mortality at much lower concentration. LC₅₀ and LC₉₀ values were recorded 72.32 and 131.14 ppm respectively for the 3rd instar larvae after 24 h of exposure which is also significantly less when compared with the previous reports on individual plants. Non-target organisms have mortality percentage of zero, therefore the bioactive fraction of mixture of these plant extracts can be safely applied to environment and possess selective toxicity to target larval population.

On the other hand, numbers of multidrug resistant microbial strains are increasing day by day and convey a great concern at using antibiotics as these become ineffective due to their disproportionate and uncontrolled uses. This situation eventually claims the thrust to the search for new antibacterial substances from various sources like medicinal plants. Several works were done using aqueous and methanol extract of plants for their antibacterial efficacy. In the present study mixture of *C. amada* rhizome and *T. indica* leaf crude and methanol extracts exhibited broad spectrum activity against tested isolates. Aqueous extracts show no inhibitory property against any of the test microorganism *in vitro*. Harit et al. (2013) evaluated antimicrobial property of *C. amada* rhizome

against bacterial strains of *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC96) and fungal cultures of *Candida albicans* (MTCC 3017) and *Aspergillus flavus* (MTCC 277) and reported that ethanolic and aqueous extracts of *C. amada* were ineffective against all tested bacteria. Doughari (2009) have reported antibacterial activity of *T. indica* leaves where the MIC of the tested extracts were higher (ranged from 15.5~8 mg/mL) than the MIC of the methanol extract in present experiment which ranged from 5~2.5 mg/mL. Moreover, in our study, we found that mixture of the crude and methanol extracts of *C. amada* rhizome and *T. indica* leaf have broad spectrum antibacterial property against all the strains. Lowest MIC was 2.50 mg/mL against *Bacillus licheniformis* (MTCC 530), *Pseudomonas fluorescens* (MTCC 103) and *Pseudomonas putida* (MTCC 1654). Many reports have cited that the antibacterial activity of the plants is attributed to the presence of various phytochemicals such as alkaloids, flavonoids, tannins, saponins and phenols etc. (Garg and Grewal, 2015). Phytochemical analysis of methanolic extract in the present experiment revealed the presence of tannins, flavonoids, terpenoids and alkaloids in the mixture of methanol extract of *C. amada* rhizome and *T. indica* leaf which implies that these compounds might be responsible for the mosquitocidal and antibacterial properties of the methanol extract. However, the IR spectra indicated presence of functional groups like alcohols, alkanes, amides, ethers, esters, ketones, oximes, nitrate, azines, carboxylic acids and amines which also ascertain the presence of various phytochemicals that might be responsible for mortality of mosquito larvae and inhibition of bacterial growth. Previously some scientist proved that amount of phytochemicals may vary with the solvents used for extraction method and also suggested methanol as best solvent for extraction of certain group of biologically active chemicals (Alo et al., 2012; Dhawan and Gupta, 2017). As aqueous extract has no effect against all the tested bacterial strains, it may be concluded that the difference in active solvent extracts may be due to qualitative difference in the bioactive substances in two extracts. In many cases, though rich with phytochemicals, plant extracts sometimes showed astringent properties that can also be the reason for its ineffectiveness (Chukwuka et al., 2011). Thus this previous report also supports the ineffectiveness of aqueous extract as biocide in this particular study.

This is a preliminary study and has limitations as further investigations are needed to ascertain active ingredient which may fulfill the requirement of formulating an environment friendly commercial product as mosquito control as well as antibacterial agent.

4 Conclusion

In conclusion, the mixture of crude and methanol *C. amada* rhizome and *T. indica* leaf crude and methanol extracts tested in this study had potential mosquitocidal and antibacterial activities which are higher than their individual efficacy. This investigation has opened up the possibility of the use of these edible plant parts in drug development for human and fish consumption possibly for the treatment bacterial infections and as mosquito larvicide.

Authors' contributions

SB suggested the plant materials, carried out the laboratory experiments, analyzed the data, conducted statistical analysis and wrote the first draft of the manuscript. BS, SC, NS, RG, AM and BG collected the plant material and prepared the extracts. BS, NS and RG conducted the antibacterial bioassay; SC, AM and BG conducted the mosquito larvicidal bioassay experiments. SD conducted the IR analysis and helped in manuscript preparation. GC conceived of the study, and participated in its design and coordination, supervised the work, and did critical revision of the manuscript. All authors read and approved the final manuscript.

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