

## Review Article

# Antitermite Activities of *C. decidua* Extracts and Pure Compounds against Indian White Termite *Odontotermes obesus* (Isoptera: Odontotermitidae)

Ravi Kant Upadhyay,<sup>1</sup> Gayatri Jaiswal,<sup>1</sup> Shoeb Ahmad,<sup>1</sup>  
Leena Khanna,<sup>2</sup> and Subhash Chand Jain<sup>3</sup>

<sup>1</sup> Department of Zoology, D. D. U. Gorakhpur University, Gorakhpur 2730 09, India

<sup>2</sup> University School of Basic and Applied Sciences, Guru Gobind Singh Indraprastha University, Sector 16C Dwarka, New Delhi 110075, India

<sup>3</sup> Department of Chemistry, University of Delhi, Delhi 110 007, India

Correspondence should be addressed to Ravi Kant Upadhyay, rkupadhya@yahoo.com

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In the present investigation, we have tested antitermite responses of *Capparis decidua* stem, root, flower, and fruit extracts and pure compounds to *Odontotermes obesus* in various bioassays. Crude stem extract has shown very high susceptibility and very low LD<sub>50</sub> values, that is, 14.171 µg/mg in worker termites. From stem extract, three pure compounds were isolated in pure form namely, heneicosylhexadecanoate (CDS2), triacontanol (CDS3), and 2-carboxy-1, 1-dimethylpyrrolidine (CDS8) which have shown very low LD<sub>50</sub> value in a range of 5.537–10.083 µg/mg. Similarly, one novel compound 6-(1-hydroxy-non-3-enyl)-tetrahydropyran-2-one (CDF1) was isolated from flower extract that has shown an LD<sub>50</sub> 8.08 µg/gm. Repellent action of compounds was tested in a Y-shaped glass olfactometer in which CDF1 compounds have significantly repelled termites to the opposite arm. Besides this, *C. decidua* extracts have shown significant reduction ( $P < 0.05$  and  $0.01$ ) in termite infestation in garden saplings when it was coated on cotton tags and employed over tree trunks. Further, *C. deciduas* stem extract was used for wood seasoning, which gave very good results as test wood sticks have shown significantly ( $P < 0.05$  and  $0.01$ ) very low termite infestation.

## 1. Introduction

The Indian white termite, *Odontotermes obesus* Rambur (Isoptera: Odontotermitidae), is highly destructive polyphagous insect pest, lives in huge mounds, and feeds on cellulose material and almost anything which contains carbohydrate. It causes economic damage to commercial wood, fibers, cellulose, sheets, papers, clothes, woolens and mats, and woody building material and infests green standing foliages, cereals stored in godowns. Both worker and soldier termites harm nonseasoned commercial wood and its formed materials. Whether it is a rural area or an urban domestic site, termite menace is everywhere. However, for controlling termite population in the field, various synthetic pesticides such as

chlorodane [1], cypermethrin [2], hydroquinone, and indoxacarb [3] have been used. But all such synthetic pesticides are highly poisonous and kill nontarget organisms. Due to their longer residual persistence in the environment, these have been banned and new alternatives are discovered in form of natural pesticides.

These plant-origin natural pesticides provide wide range of control and efficiently cut down the population of all kinds of pests even applied in very low quantity. These plant-origin pesticides are much safer and easily biodegradable in the medium and show no residual effect. So far numbers of plant species have been screened to explore potential antitermite agents by the researchers to control termite menace. Few natural products such as flavonoids [4], sesquiterpenes [5],

and thiophenes [6] isolated from different plants species were found effective against termites [7]. In addition, for enhancing the insecticidal potential of crude plant extracts and its target specificity, few synergists were applied in form of poison baits which successfully exploit feeding, tunneling [8], and reproductive behavior in termites [9]. Similarly, application of Summon disks and filter paper disks coated with few chitin synthesis inhibitors, that is, diflubenzuron, hexaflumuron, and chlorfluazuron [10] controlled the aggregation, feeding, and recruitment behavior in *Coptotermes formosanus* termites.

We, in continuation to our phytochemical studies on various Indian medicinal plants, have already evaluated *Capparis decidua* for insecticidal and ovipositional inhibitory activity against *Bruchus chinensis* [11]. We observed that this plant is not attacked by termites at all, and this inspires us to study its antitermite activity, in order to find out some potent antitermite components of this plant. In the present study, different extracts of *Capparis decidua* and compounds isolated from its various parts have been evaluated for antitermite activity. *C. decidua* belongs to the family Capparidaceae [12] and is an indigenous medicinal plant, commonly known as “Kureel” in Hindi. It is a densely branching shrub with scanty, small, caduceus leaves. Barks, leaves, and roots of *C. decidua* have been claimed to relieve variety of ailments such as toothache, cough, asthma, intermittent fever, and rheumatism [13]. The powdered fruit of *C. decidua* is used in antidiabetic formulations [14], while the bark of its leafless shrub is used for the treatment of asthma, cough, inflammation, and acute pain [15]. Seeds of *C. decidua* showed antibacterial activity against *Vibrio cholerae* *ogava*, *inaba*, and *etor* [16].

In this study, we conducted preliminary investigation on the antitermite activity of *C. decidua* extracts and pure compounds. For this purpose, active components were isolated and fractionated to obtain and pure compounds employed to test the toxicity and repellent action in workers of *O. obesus* both in laboratory and field conditions. The overall aim of this study was to identify potential anti-termite compound that could be used to develop an effective repellent and toxic formulation to kill field termites. Further, we have also used active ingredients from *C. decidua* in spray, repellent tags, and wood seasoning to protect the wood from termite infestation on garden trees and wood sticks.

## 2. Materials and Methods

**2.1. Insects and Plant Material.** Termite *O. obesus* were collected from infested logs found at the University of Gorakhpur, India, and nearby forest area of eastern Uttar Pradesh, India. Termites removed from plant biomass and logs were maintained in glass jars (height 24", diameter 10") in complete dark conditions at  $28 \pm 2^\circ\text{C}$ ,  $75 \pm 5$  RH. Termites were fed on green leaves.

Stems, flowers, and fruits of *Capparis decidua* were collected from different places of Rajasthan and western Uttar Pradesh, India. These were separately shaved, dried, and subsequently pulverized to obtain fine powder.

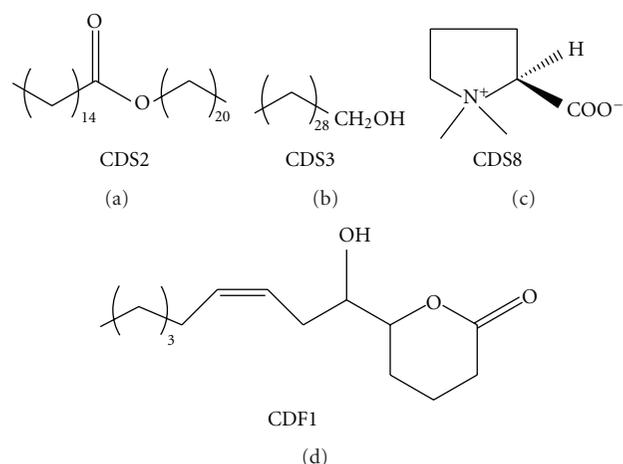


FIGURE 1: Pure compounds obtained from *C. decidua* after solvent fractions being eluted and purified by column chromatography. Compounds CDS3 (25 mg) and CDS8 (50 mg) were identified as triacontanol and 2-carboxy-1,1-dimethylpyrrolidine. While compound CDS2 is characterized as heneicosylhexadecanoate. The column was prepared in petroleum ether using silica gel (60–80) as an adsorbent and eluted with petroleum ether/chloroform, chloroform, chloroform/methanol mixtures of increasing polarity. CDF1 was isolated (15 mg) from flowers from the chloroform fraction of the extract. It was identified as 6-(1-hydroxy-non-3-enyl) tetrahydropyran-2-one and is colorless.

**2.2. Extraction of Plant Foliage Chemicals.** The powdered stems (200 g) were extracted using  $\text{CHCl}_3/\text{MeOH}$  (1 : 1), cold MeOH, and hot MeOH sequentially to obtain dry extracts CDS1 (14 g), CDS2 (5 g), and CDS3 (2 g), respectively, while its flowers (40 g) and fruits (50 g) were only extracted with  $\text{CHCl}_3/\text{MeOH}$  (1 : 1) to obtain dry extracts CDS7 (3 g) and CDF1 (0.2 g), respectively.

**2.3. Isolation and Characterization of Active Components from 50% Methanol/Chloroform Extract (CDS1) of Capparis Stems.** The solvent free extract CDS1 from *C. decidua* stem was found to be a mixture of several components of varying polarity on TLC and further fractionated by column chromatography to isolate the minor active components. The column was prepared in petroleum ether using silica gel (60–80) as an adsorbent and eluted with petroleum ether/chloroform, and chloroform, chloroform/methanol mixtures of increasing polarity. Twelve fractions were obtained, and three compounds, namely, CDS2, CDS3, and CDS8 have been isolated and characterized. Compounds CDS3 (25 mg) and CDS8 (50 mg) were identified (4) as triacontanol and 2-carboxy-1,1-dimethylpyrrolidine (Figure 1). While compound CDS2 is characterized as heneicosylhexadecanoate, and this is the first report of the isolation from the genus *Capparis* and species *decidua*.

**2.4. Characterization of Compound CDS2.** Compound CDS2 (20 mg) was obtained as colorless oil. Its  $^1\text{H}$  NMR spectrum revealed its aliphatic nature. Its IR spectrum showed absorptions at  $1735$  and  $1175\text{ cm}^{-1}$  which were characteristic

for C=O and C–O stretching of an ester linkage. Its  $^1\text{H}$  NMR spectrum exhibited a triplet, at  $\delta$  2.29 integrating for two protons of  $\alpha$ -methylene linked to carbonyl group. Also, downfield at  $\delta$  4.05, another triplet was observed which was assigned to the methylene directly attached to oxygen atom. Apart from this,  $^1\text{H}$  NMR spectrum exhibited a triplet at  $\delta$  0.88 corresponding to two terminal methyl groups indicating it to be a middle ester. Compound CDS2 displayed a molecular ion peak at  $m/z$  550 and hence analyzed for  $\text{C}_{37}\text{H}_{74}\text{O}_2$ . Its mass spectrum showed prominent peaks for McLafferty rearrangement at  $m/z$  355 and 257 thereby revealing the presence of hexadecanoate as the acid moiety and heneicosane as the alcohol moiety in the molecule.

On the basis of above-mentioned spectral data, compound CDS2 was identified as heneicosylhexadecanoate. This is the first report of its isolation from the genus *Capparis*.

**2.5. Heneicosylhexadecanoate (CDS2).** Compound CDS2 was obtained as colorless oil (12 mg). It showed a single spot on TLC using petroleum ether as the developing solvent,  $R_f = 0.3$ . IR  $\nu_{\text{max}}$  (KBr): 2918, 2850, 1735, 1463, 1175, 758, 719  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\delta$ ,  $\text{CDCl}_3$ , 300 MHz): 0.88 (t, 6H, 2x- $\text{CH}_3$ ), 1.25 (m, 60H, 30x- $\text{CH}_2$ ), 1.58 (m, 4H,  $-\text{CH}_2\text{CH}_2-\text{C}=\text{O}$  &  $-\text{CH}_2\text{CH}_2-\text{O}$ ), 2.29 (t, 2H,  $-\text{CH}_2-\text{C}=\text{O}$ ), 4.05 (t, 2H,  $-\text{O}-\text{CH}_2-$ ). Mass Spectral data, EIMS  $m/z$  (%): 550 (10,  $\text{M}^+$ ), 453 (5), 355 (24), 257 (31), 240 (8,  $\text{M}^+-\text{O}(\text{CH}_2)_{20}\text{CH}_3$ ), 202 (15), 174 (10), 111 (20), 97 (35), 71 (48), 57 (100), 41 (80).

Extract from *Capparis decidua* flowers when checked on TLC gave one major spot. It was subjected to column chromatography using silica gel (60–80) as an adsorbent and eluted with petroleum ether/chloroform, chloroform, and chloroform/methanol mixtures of increasing polarity. One major compound CDF1 (15 mg) was isolated from the chloroform fraction of the extract. It was assigned constitution as 6-(1-hydroxy-non-3-enyl)-tetrahydropyran-2-one. This is the first report of isolation of this compound from any natural and synthetic source.

Extract CD10 (0.2 g), when checked on TLC, gave one major spot, similar to that of compound CDF1. Some more amount of CDF1 was isolated from the extract by column chromatography for bioassays.

**2.6. Triacontanol (CDS3).** White solid, mp 82–85°C IR  $\nu_{\text{max}}$  (KBr): 3398, 2918, 2849, 1463, 1360, 1061, 720  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\delta$ ,  $\text{CDCl}_3$ , 300 MHz): 0.88 (t, 3H,  $-\text{CH}_3$ ), 1.25 (brs, 54H), 1.54 (m, 2H,  $-\text{CH}_2\text{CH}_2\text{OH}$ ), 3.63 (t, 2H,  $-\text{CH}_2\text{OH}$ ).  $^{13}\text{C}$  NMR ( $\delta$ ,  $\text{CDCl}_3$ , 75.47 MHz): 14.01 ( $-\text{CH}_3$ ), 25.67–32.76 ( $-\text{CH}_2$ ), 63.04 ( $-\text{CH}_2\text{OH}$ ). EIMS  $m/z$  (%): 420 ( $\text{M}^+-18$ , 36), 392 (8), 364 (5), 167 (18), 153 (20), 125 (950), 57 (100).

**2.7. 2-Carboxy-1,1-Dimethylpyrrolidine (CDS8).** White solid, mp 268°C. It gave a single spot on TLC,  $R_f = 0.5$  using chloroform/methanol (60:40) as the developing solvent. IR  $\nu_{\text{max}}$  (KBr): 3401, 1621, 1531, 1479, 1400, 1368, 1326, 1002, 961  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\delta$ ,  $\text{CD}_3\text{OD}$  300 MHz): 2.13 (m, 2H, H-4), 2.32 (m 1H, H-3'), 2.49 (m, 1H, H-3), 3.14 (s, 3H, N- $\text{CH}_3$ ),

3.31 (s, 3H, N- $\text{CH}_3$ ), 3.67 (m, 1H, H-5), 3.71 (m, 1H, H-5'), 4.01 (t, 1H, H-2).  $^{13}\text{C}$  NMR ( $\delta$ ,  $\text{CD}_3\text{OD}$ , 75.47 MHz): 20.22 (C-4), 27.23 (C-3), 46.80 (N- $\text{CH}_3$ ), 53.17 (N- $\text{CH}_3$ ), 68.43 (C-5), 78.17 (C-2).  $^{13}\text{C}$  NMR DEPT 135 ( $\delta$ ,  $\text{CD}_3\text{OD}$ , 75.47 MHz): 20.21 (C- $\text{CH}_2$ ), 27.02 ( $-\text{CH}_2$ ), 46.73 ( $-\text{CH}_3$ ), 53.09 ( $-\text{CH}_3$ ), 68.42 ( $\text{CH}_2$ ), 78.14 ( $-\text{CH}$ ). EIMS  $m/z$  (%): 144 ( $\text{M}^+$ , 2), 117 (7), 115 (6), 101 (5), 99 (3), 85 (7), 59 (100), 55 (13), 45 (42), 43 (70).

**2.8. Toxicity Bioassay.** For evaluation of observation of toxic responses in termites, serial concentrations, that is, 1.0, 2.0, 4.0, 8.0, 16, and 32  $\mu\text{g}$  of different extracts were loaded on separate Whatman paper strips ( $1 \times 1 \text{ cm}^2$ ) and air dried to remove the solvent. These precoated solvent free strips were placed in the center separate Petri dishes (42 mm diameter) as tests and uncoated as control. Twenty-five worker termites were released in the Petri dish to observe the mortality. After setting the experiment, green leaves were provided as food for both tests and control insects and containers were covered with black paper sheets. Mortality was recorded on the basis of dead and living termites, and observations were made in triplicate for each extract and pure compounds up to 24 hrs. Insects were treated as dead when they become immobile and have shown no further activity to the external stimuli. The  $\text{LD}_{50}$  after 24 hrs of exposure to each was calculated by using Probit analysis tested using the method of Finney [17].

**2.9. Repellency Bioassay.** Repellent responses were observed in a glass Y-tube olfactometer by using serial concentrations 0.001, 0.002, 0.004, 0.008, 0.016, and 0.032 mg of different extracts loaded on separate Whatman paper strips ( $1 \times 1 \text{ cm}^2$ ) and air-dried to remove the solvent. These precoated solvent free strips were placed in right arm of Y-tube olfactometer (16 mm diameter  $\times$  90 cm length) as tests, while similar strips uncoated were placed in left arm as control. Twenty-five worker termites were released inside the opposite triarm to observe the repellent activity. After introduction of termites tube, openings were closed by Teflon tape and number of termites oriented towards uncoated strips or nonscented area were counted as repelled. Individuals that did not enter at least one of the arms were scored as unresponsive. Tests were conducted for 18 hrs at 27°C temperature. Same tests were conducted after reversing the arms to test directional bias. A  $\text{Chi}^2$  test was used to compare the number of termites responding to the olfaction generated by *C. decidua* active ingredients.

### 3. Field Experiments

**3.1. Thread-Binding Assay.** For control of termite infestation in garden plants, presoaked cotton threads were tagged around the tree trunks at a height of 5-6 feet above the ground. For this purpose, threads were soaked in *Capparis decidua* aqueous extract for 24 hr and dried in shade. Early age saplings of *Tectona grandis* (4 year old) trees in 8 different rows each having 24 plants were selected and tagged with the cotton threads and sprayed regularly at 15 days interval with same extract. In controls, the uncoated threads were tagged at

TABLE 1: LD<sub>50</sub> values obtained in solvent extracts and pure compounds isolated from *Capparis decidua* against Indian white termite *Odontotermes obesus*.

Extracts Single	hr	LD <sub>50</sub> values ( $\mu\text{g}/\text{mg}$ ) ( $P < 0.05$ ) <sup>a</sup>	LCL <sup>b</sup>	UCL <sup>b</sup>	t-ratio	Slope value	Heterogeneity	Chi test
Root	24	14.515	11.421	17.459	11.215	0.081	2.070	6.210
Stem	24	14.171	10.48	17.566	12.422	0.093	3.3741	0.124
Fruit	24	14.781	11.106	18.350	11.933	0.088	3.204	9.612
Flower	24	15.274	11.878	18.778	11.982	0.088	2.995	8.986
<i>Mixture</i>								
CSTM1 (stem)	24	13.246	5.663	21.147	6.159	1.62	0.796	3.982
CSTM2 (stem)	24	6.616	0.563	0.825	4.796	1.058	0.470	2.348
CSMM3 (stem)	24	4.973	0.057	16.028	4.088	0.769	0.736	3.679
<i>Pure compounds</i>								
CDS1*	24	7.514	2.054	10.995	7.123	0.093	1.614	4.833
CDS2	24	7.290	3.178	10.295	8.799	0.135	2.154	6.462
CDS3	24	7.434	5.277	9.179	6.864	0.087	0.533	1.60
CDS4	24	6.531	2.920	9.106	8.655	0.141	1.7475	5.24
CDS5	24	5.537	1.192	8.157	4.853	0.059	0.782	2.345
CDS6	24	8.655	5.711	11.405	9.578	0.149	1.9415	5.824
CDS8	24	10.083	8.39	11.759	7.353	0.093	0.906	2.717
CDF1*	24	8.086	5.40	10.47	9.366	0.147	1.545	4.636

LD<sub>50</sub> values represent lethal dose that causes 50% mortality in the test insects. <sup>b</sup>LCL and UCL mean lower confidence limit, and upper confidence limit respectively. <sup>c</sup>t-ratio, slope value, and heterogeneity were significant at all probability levels (90, 95, and 99%). t-ratio: difference in degree of freedom at 0.5, 0.05, and 0.005 levels; slope value shows the average between LD<sub>50</sub> and LD<sub>80</sub>, from which LD<sub>50</sub> value is calculated; heterogeneity value, shows the effect of active fraction on both susceptible and tolerant insects among all of the treated insects. CSTM indicates combined tincture of *Capparis decidua*, coconut oil, terpene oil, glycerol, elemental sulphur, and liter water. CDS\* represents pure compound isolated from *C. decidua* stem fraction, while CDF1\* represents compound isolated from flower.

similar height without coating any active fraction on threads. Separate rows were chosen for spray, thread binding, and both.

**3.2. Wood Seasoning.** For evaluation of termiticidal action of *C. decidua* stem aqueous extract dried solid wood sticks of *Tectona grandis* each having 3 feet length were used to dug and treated wood sticks were planted into the pits. For this purpose, set of six wood sticks were seasoned with three different concentrations of *C. decidua* named as CSTM1, CSTM2, and CSTM3. Antitermite mixture or tincture was prepared by mixing different ingredients (90 gm *Capparis decidua*, 50 mL coconut oil, 50 mL terpene oil, 50 mL glycerol, and 11 gm elemental sulphur in 15 liter water). In CSTM2 and CSTM3, mixtures *C. decidua* powder was mixed 132 gm and 180 gm while rest of the ingredients were the same. Seasoning of the wood sticks was done by dipping them in the above mixtures separately for 24 hours. Then, seasoned wood sticks were dried for 12 h and planted inside soil by making separate pit of 2.75 feet depth at a distance of 3 feet. Similarly, six control wood sticks were also used which were unseasoned. After 30-day interval, each one of control and test wood stick was dug out for evaluation of antitermite activity. % weight loss and % infestation, exposure period, and concentration of ingredients were considered for determination of antitermite activity in wood sticks in garden soil. Experiments were run up to 180 days,

and wood sticks were marked with colored marker for corresponding control.

#### 4. Statistical Analysis

Standard deviations chi-square, t-significance, correlation, and ANOVA were calculated from the means of two replicate, using three equal subsamples from each replicate by using method of Sokal and Rohlf [18]. In the experiments, analysis of variance (ANOVA) was done whenever two means were obtained at a multiple test range and  $P < 0.05$  probability level. The LD<sub>50</sub> after 24 hrs of exposure to each was calculated by using Probit analysis tested using the method of Finney [17].

#### 5. Results

Toxic and repellent responses of various extracts and pure compounds isolated from *C. decidua* were evaluated against Indian white termite *O. obesus*. For this purpose, insects were treated with increasing dose of both extracts and compounds separately. The mortality rate was found dose and time dependent as it was found to be increase with an increase in dose and exposure period. The LD<sub>50</sub> values for different extracts of 24 h are given in Table 1. Solvent extracts have shown LD<sub>50</sub> in a range of 14.171–15.274  $\mu\text{g}/\text{mg}$ , while combined mixtures of *C. decidua* have shown synergistic activity

TABLE 2: Percent repellency obtained in solvent extracts and pure compounds isolated from *Capparis decidua* against Indian white termite *Odontotermes obesus*.

Compounds	Concentration in mg	Mean number. of Insects repelled	Expected number. of insect repelled	$\chi^2$ Value	ED <sub>50</sub>
<i>Single fractions (acetone)</i>					
Root	0.001–0.016	14.0	10	NS <sup>a</sup>	0.009
Stem	0.001–0.016	12.4	10	NS <sup>a</sup>	0.011
Fruit	0.001–0.016	14.4	10	NS <sup>a</sup>	0.006
Flower	0.001–0.016	13.2	10	NS <sup>a</sup>	0.008
<i>Mixture</i>					
CSTM1 extract	0.005–0.08	11.44	10	NS <sup>a</sup>	0.015
CSTM2 extract	0.005–0.08	10.96	10	NS <sup>a</sup>	0.042
CSTM3 extract	0.005–0.08	11.24	10	NS <sup>a</sup>	0.038
<i>Pure compounds</i>					
CDS1	0.001–0.016	10	10	0.50 <sup>b</sup>	0.012
CDS2	0.001–0.016	8.6	10	0.80 <sup>b</sup>	0.017
CDS3	0.001–0.016	12.2	10	NS <sup>a</sup>	0.008
CDS4	0.001–0.016	10	10	0.50 <sup>b</sup>	0.013
CDS5	0.001–0.016	10	10	0.20 <sup>b</sup>	0.012
CDS6	0.001–0.016	12	10	NS <sup>a</sup>	0.010
CDS8	0.001–0.016	12.2	10	NS <sup>a</sup>	0.009
CDF1	0.001–0.016	10.4	10	0.20 <sup>b</sup>	0.011

<sup>a</sup>Not significant as the calculated values of  $\chi^2$  were less than the table values at all probability levels (90%, 95%, and 99%).

<sup>b</sup>Significant at all probability levels (90%, 95%, and 99%).

The data responses lines would fall within 95% confidence limits, and thus the model fits the data adequately. UCL-LCL\*: upper confidence limit and lower confidence limit. CSTM indicates combined tincture of *Capparis decidua*, coconut oil, terpene oil, glycerol, elemental sulphur, and liter water.

against termites and caused comparably high mortality with LD<sub>50</sub> 4.973–13.246  $\mu\text{g}/\text{mg}$  (Table 1).

From fractionation of stem extract, three compounds were isolated in pure form, which were identified as henicicosyl-hexa decanoate (CDS2), tricantanol (CDS3), and 2 carboxy-1-1-dimethylpyrrolidine (CDS8) (Figure 1). All these three compounds were evaluated for their antitermite activity which have shown very low LD<sub>50</sub> values, that is, 7.290, 7.434, and 10.083  $\mu\text{g}/\text{mg}$  body weight of termite (Table 1). Similarly, flower extract was fractionated and a single major compound CDF1 was identified as 6-(1-hydroxy-non-3-enyl)-tetrahydropyran-2-one. It has shown very high antitermite potential against *O. obesus* with an LD<sub>50</sub> value of 8.086  $\mu\text{g}/\text{mg}$  (Table 1). It is highly noticeable that *C. decidua* fractions in termites remain active for longer duration and cause high lethality. The index of toxicity estimation indicates that the mean value was within the limit at all probabilities (90, 95, and 99%) as it is less than 0.05 values of t-ratio. Besides this, regression was also found significant. The steep slope values indicate that even small increase in the dose causes high mortality. Values of the heterogeneity less than 1.0 denote that, in the replicate test of random sample, the dose response time would fall within 95% confidence limit, and, thus, the model fits the data adequately.

In olfactometry tests, solvent extracts prepared from stem, flower, fruits, and pure compounds have shown significant repellency at a very low dose. Interestingly, solvent

extract has repelled mean number of insects 12.4, while 8.6 mean numbers of insects were repelled by pure compound in olfactometer. ED<sub>50</sub> values obtained in pure compounds (CDS1-CDF1) range 0.008–0.017  $\mu\text{g}/\text{mg}$  body weights, and solvent extracts have shown ED<sub>50</sub> in between 0.006–0.042  $\mu\text{g}/\text{mg}$  (Table 2). Besides this, for control of termites in the garden, presoaked cotton threads impregnated with *Capparis decidua* stem extract were tagged around tree trunks of *Tectona grandis*. By employing these precoated threads, termite infestation and tunneling activity were significantly decreased ( $P < 0.05$  and 0.01) (Table 3). However,  $F$ -values obtained in experiments have shown successful random control of termites in the groups. ( $F_{0.05} = 2.895$ ,  $F_{0.01} = 4.455$ );  $F$  is significant for  $X$  value, while, for  $Y$  values, it is nonsignificant and  $F_{xy} = 5.38$ . It was also tried to adjust the values by computation for adjustment of SS for  $Y$  that shows the termite killing was significant ( $df = 31$ ,  $t_{0.05} = 2.04$ ,  $t_{0.01} = 2.75$ ) (Table 3). There was observed a significant decrease in mud plastering after regular spray on the infested trees as it was found, and no further termite infestation was observed even after 6 months of experiment.

Besides this, *C. decidua* extracts were also used in wood seasoning for the protection of wood from termite infestation. *C. decidua* fractions have shown good termiticidal action as almost no infestation was observed in test wood sticks up to 6 months. The percent weight loss obtained was also minimized up to 3.25%, while, in untreated sticks 52.82% weight was lost (Table 4). Infestation was found to

TABLE 3: Termite management after employment of tag binding and spray on infested garden plants.

Treatment	Number of termites		% infestation		% inhibition in tunneling activity	
	Mean + SE		Mean + SE		Mean + SE	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Spray	21.50 ± 0.021 (100)	15.5* ± 0.017 (41.89)	76.75 ± 0.14 (100)	19.0* ± 0.03 (19.8)	55.4 ± 0.01 (100)	21.73* ± 0.04 (28.17)
Tag binding	19.4 ± 0.019 (100)	10.75* ± 0.09 (35.65)	63.2 ± 0.07 (100)	11.25* ± 0.02 (15.11)	38.6 ± 0.05 (100)	13.75* ± 0.01 (26.26)
Spray and tag binding	20.6 ± 0.03 (100)	3.375* ± 0.004 (14.07)	67.8* ± 0.07 (100)	8.97* ± 0.01 (11.68)	37.2 ± 0.06 (100)	9.2* ± 0.08 (19.82)

Observations were made at every 15-day time interval.

\*Significant at  $P < 0.01$  levels.

TABLE 4: Effect of *C. decidua* stem extract on weight loss and infestation in seasoned wood sticks planted in garden soil.

Fractions	Time duration						
	0 month	1 month	2 month	3 month	4 month	5 month	6 month
Control (-ve)	543 ± 2.09	500.1 ± 2.74 (7.90)*	469.3 ± 1.60 (13.5)*	449 ± 2.50 (17.31)*	368.5 ± 2.66 (32.13)*	286.5 ± 1.72 (47.23)*	256.16 ± 1.75 (52.8)*
	0.00 (0.00)	79.66 ± 1.37 (100) <sup>+</sup>	113.83 ± 1.33 (142.89) <sup>+</sup>	122.6 ± 1.55 (153.93) <sup>+</sup>	135.33 ± 1.43 (169.88) <sup>+</sup>	152.66 ± 1.22 (191.63) <sup>+</sup>	192.16 ± 1.03 (241.22) <sup>+</sup>
Control (+ve)	532 ± 2.01	524.8 ± 1.81 (1.35)*	516.8 ± 1.63 (2.85)*	497.3 ± 1.74 (6.52)*	493.5 ± 1.12 (7.2)*	469 ± 1.74 (11.84)*	424.16 ± 2.68 (20.27)*
	0.00 (0.00)	9.3 ± 0.97 (100) <sup>+</sup>	13.8 ± 1.03 (148.38) <sup>+</sup>	21.83 ± 1.25 (134.73) <sup>+</sup>	24 ± 1.05 (158.06) <sup>+</sup>	25.66 ± 0.97 (175.91) <sup>+</sup>	31.16 ± 1.20 (235.05) <sup>+</sup>
Capparis T1	533 ± 1.51	527.5 ± 0.97 (1.03)*	485.3 ± 1.43 (8.9)*	472.6 ± 1.40 (11.33)*	467 ± 1.49 (12.38)*	461.1 ± 1.05 (13.48)*	447.5 ± 1.65 (16.04)*
	0.00 (0.00)	0.00 (0.00) <sup>+</sup>	0.00 (0.00) <sup>+</sup>	0.00 (0.00) <sup>+</sup>	9.3 ± 0.86 (7.2) <sup>+</sup>	11.33 ± 0.86 (8.01) <sup>+</sup>	16.3 ± 0.86 (9.2) <sup>+</sup>
Capparis T2	532.6 ± 1.46	523.3 ± 1.26 (1.74)*	519.8 ± 1.70 (2.29)*	513 ± 1.41 (3.68)*	509.5 ± 1.81 (4.33)*	513.8 ± 1.70 (3.52)*	510 ± 1.13 (4.24)*
	0.00 (0.00)	0.00 (0.00) <sup>+</sup>	0.00 (0.00) <sup>+</sup>	0.00 (0.00) <sup>+</sup>	5.8 ± 0.82 (4.47) <sup>+</sup>	8.0 ± 0.903 (5.53) <sup>+</sup>	8.66 ± 0.97 (4.71) <sup>+</sup>
Capparis T3	553.5 ± 1.48	551.6 ± 1.05 (0.003)*	545.6 ± 1.43 (1.42)*	542.8 ± 1.82 (1.93)*	539.5 ± 3.28 (2.52)*	537.1 ± 2.62 (2.96)*	535.5 ± 0.97 (3.25)*
	0.00 (0.00)	0.00 (0.00) <sup>+</sup>	0.00 (0.00) <sup>+</sup>	0.00 (0.00) <sup>+</sup>	0.00 (0.00) <sup>+</sup>	0.00 (0.00) <sup>+</sup>	0.00 (0.00) <sup>+</sup>

\* Values in bracket depict percent weight loss represented in grams.

<sup>+</sup> Values in brackets depict percent weight loss and percent termite infestation.

% Wt loss is mean of weight loss obtained in six wood sticks planted in soil after seasoning. It is represented in grams.

% infestation represents damage caused by the termites in six wood sticks. It is also mean of number of termites available on six wood sticks.

be decreased with increasing concentration of *C. decidua*. Statistical analysis of infested and uninfested data have shown significant correlation between tests and control, as the values of correlation were found positive (0.5416) in the weight loss and infestation in comparison to tests. Test wood sticks have shown significantly very low termite infestation after wood seasoning ( $P < 0.05$  and  $0.01$ ).

## 6. Discussion

In present time, termite menace is a serious problem in tropical and subtropical regions. Indian white termite is a dreadful insect pest which causes economic damage to

commercial wood, fibers, paper sheet, clothes, woollens, and mats and seriously infests agricultural crops and forest products. In the present study, we have tried to control termite infestation in garden soil by applying nonchemical plant-based extracts and pure compounds isolated from *C. decidua*. Our results show that *C. decidua* solvent extracts and pure compounds possess enough antitermite potential. By applying very small dose of these natural products orientation, movement, feeding, and tunneling behavior in termites were found to be significantly suppressed. Further, infestation was to be significantly decreased in seasoned wood sticks even after six months of treatments. In toxicity bioassays, *C. decidua* solvent extracts have shown very high lethality which is proved by very low LD<sub>50</sub> value, that is,

14.171–15.274  $\mu\text{g}/\text{mg}$  obtained. Further, addition of coconut and terpene oil, glycerol, and sulphur in *C. decidua* have shown synergistic activity. In such combinations,  $\text{LD}_{50}$  was found in a range of 4.973–13.246  $\mu\text{g}/\text{mg}$  (Table 1). On the other hand,  $\text{LD}_{50}$  in pure compounds isolated from *C. decidua* obtained was in a range of 5.537–10.083  $\mu\text{g}/\text{mg}$  (Table 1). Among pure compounds, CDF1 identified as 6-(1-hydroxy-non-3-enyl)-tetrahydropyran-2-one has shown very high antitermite potential against *O. obesus* with an  $\text{LD}_{50}$  value of 8.086  $\mu\text{g}/\text{mg}$  (Table 1).

In addition to it, pure compounds isolated from *C. decidua* (CDS1, CDS2, CDS3, CDS4, CDS5, CDS6, CDS8, and CDF1) have shown significant ( $P < 0.05$ ) repellent activity at a very low dose with an  $\text{EC}_{50}$  ranging between 0.008 and 0.017  $\mu\text{g}/\text{gm}$  (Table 2). Repellency provided extra advantage to wood seasoning which significantly decreases the infestation and damage done by *O. obesus* in the field. Similar toxic and repellent activity of plant products have been reported by Blaske and Hertel [19]. Besides this, *Chamaecyparis nootkatensis*, *Sequoia sempervirens*, and *Pseudotsuga menziesii* show high antifeedant and toxic activities against termites due to presence of potential antitermitic ingredients [20]. Further, treatment of infested saplings by both spray and tag binding has significantly reduced the number of termites (14.0%), % infestation (11.68), and tunneling activity (19.825) in garden saplings (Table 3). Further, active ingredients of *C. decidua* repelled large number of termites in seasoned wood sticks that were planted in three-foot deep small pits made in garden soil. It has protected the wood weight loss up to 3.25%, and no infestation was observed even after 6 months of digging (Table 4). Similarly, *Aleurites fordii* have shown anti-termite potential (Tung tree) extracts against *Reticulitermes flavipes* at 0.1 to 5.0% w/w [21]. 2'-acetonaphthone also obstructed tunneling and feeding behavior in Formosan subterranean termite *Coptotermes formosanus* Shiraki at 8.33 mg/kg concentration [22]. Besides this, natural amides such as nootkatone [23], valencenoid derivatives [24], and imidacloprid [25] also deter feeding in termites and suppress adult survival [26]. Similarly, larch wood flavonoids [27] and stilbene-rich compounds isolated from bark of *Picea glehnii* such as piceid (3,4,5 trihydroxystilbene glucoside), isorhapontin (3-methoxy-3,4,5 trihydroxystilbene-3-d-glucoside), and astringin (3,3,4,5-tetrahydroxystilbene-3-d-glucoside) also deter termites at a very low concentration 0.63 to 2.5  $\mu\text{mol}/\text{disc}$  [28]. Similarly, limonoids from meliaceae and rutaceae family showed strong antifeedant activity in *Reticulitermes speratus* Kolbe in no-choice bioassay. Both obacunone and nomilin showed a drastic antifeedant effect at 510 and 1360 ppm concentration [29]. Isorhapontin exhibited better antioxidant potential than toxifolin (3,3,4,5,7 pentahydroxyflavone) in no-choice tests [28]. Amides isolated from piperaceae family (*Piper nigrum*) have shown high-insecticidal activity against *Coptotermes formosanus* Shiraki [30]. It is a biodegradable environmental friendly natural product with minimal mammalian toxicity [30]. Similarly, in a filter paper-based bioassay for termiticide, guineesine, a minor constituent isolated from *Piper nigrum*, has shown >90% mortality in termites at 1% wt/wt application.

Moreover, root extracts of *Diospyros sylvatica* impose significant repellent activity and cause high mortality in subterranean termite, *Odontotermes obesus* in filter paper disc bioassay due to presence of plumbagin, isodiospyrin, and microphyllone or quinones [31]. Similarly, diterpene acids are screened as good antifeedants [32], while pine resin and its derivatives, cis/trans-deisopropyl dehydroabietanol, showed promising performance against termite [32]. Similarly leaf extracts of *Polygonum hydropiper* (L) and *Pogostemon paviflorus* (Benth) have shown high toxicity and mortality in tea termite *Odontotermes assamensis* (Holm) [33]. Similarly, both heartwood and sapwood of *Taiwania cryptomerioides* were found effective against *C. formosanus* at 10 mg/g concentration [34], while solvent and aqueous extracts of *Gloriosa superba* [35], *Paeonia emodi* [36], *Corydalis incise* [37], *Cassia obtusifolia* [38], *Artemisia annua* [39], *Teucrium royleanum* [40], *Andrachne cordifolia* [41], *Angelica archangelica* and *Geranium sylvatica* significantly killed certain harmful insects by inhibiting enzyme activity [42].

Besides plant extracts, essential oils have also shown very strong repellent and toxic activity against Formosan subterranean termite due to presence of volatile compounds [43]. Moreover, plant origin monoterpenes were proved highly toxic to *Coptotermes formosanus* [44]. Similarly, essential oils such *Calocedrus formosana* (Cupressaceae) effectively work against *Coptotermes formosanus* at very low dose 27.6 mg/g [45], while maca (*Lepidium meyenii*) essential oil effectively kills *Coptotermes formosanus* at 1% (w/w) concentration [46]. Similarly, clove bud oils kill Japanese termite *R. speratus* at 7.6 mL/liter air by fumigation [47]. Vulgarone B isolated from *Artemisia douglasiana* Besser, apiol isolated from *Centaurea maculosa* have shown higher mortalities in Formosan subterranean termites *Coptotermes formosanus* [30]. Similarly, patchouli oil and patchouli alcohol have shown high toxicity and repellency against same species [48]. Both have caused tissue destruction inside exoskeleton of the termites due to contact activity [48]. Similarly, vetiver oil, nootkatone, and disodium octaborate tetrahydrate affect termite tunneling, feeding, and wood digestion by symbiont protozoa resides inside the termite gut [30]. Vetiver oil is a confined novel termiticide with reduced environmental impact for use against subterranean termites [23].

Similarly is a similar bioassay 40% CNSL + 1%  $\text{CuCl}_2$  and 40% CNSL + 2%  $\text{CuCl}_2$  which have shown least damage done by the termites [49] after 10-day exposure. Similarly, boron was established as a termiticide [50]. Sulfonated wattle tannins alone combined with copper chloride at different concentrations and cashew nut shell liquid without or with copper chloride have successfully prevented termite attack [49]. Similarly, wood treated with copper II compounds tri- and dialkylamine-boric acid complex showed lesser termite damage and acted as good preservatives [27]. Nootkatone affect wood consumption termite survival and affects growth of flagellate symbionts [23]. Similarly, in the present study, sulfur, added in *C. decidua* combinatorial mixture significantly decreased the termite attack and damage. It may be due to effect of combinatorial mixture on exoskeleton of termites and antimicrobial effect of sulfur on termite gut

microfauna. It is also possible that sulfur inhibits growth of microbes in the soil if used in seasoned woods, or sprayed over tree trunks, it destructs microbial population that may work as an attractant for termites. Because in rainy season after gaining extra humidity due to pouring rain water, tree bark provides substratum for growth of fungi and bacteria. It becomes extra soft due to rain water, fungal and bacterial activity that might be palatable for termites and induce mud plastering in termites. As presence of sulfur in natural products such as amide caused higher mortality in termites [51], many commercial termiticides are available in the market to combat the destructive termites but none are entirely natural. The main purpose of present work was to contribute to the development of new termiticide from plant natural resource that may have better activity than synthetic termiticides and might be environmentally more acceptable than any other synthetic pesticide.

No doubt, *C. decidua* possesses enough antitermite potential to control Indian white termite, *O. obesus* population. However, it can be concluded that *C. decidua* active components can be used for controlling the damage and termite infestation if used as spray, fumigant or in form of poison baits. Hence, strong recommendations are being made to develop ecofriendly antitermite formulation from *C. decidua* plant for effective control of field termites.

## Conflict of Interests

The authors declare that they have no conflict of interests.

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