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Assessment of toxic effects of solvent and aqueous extracts of *Capparis decidua* on biochemical and enzymatic parameters of *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

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Abstract. Different extracts of *Capparis decidua* stem was assessed to demonstrate their toxic effects on bio-molecules and certain metabolic enzymes of *Callosobruchus chinensis*. Solvent extracts i. e. acetone, chloroform, petroleum ether, methanol, hexane and water have given very low LD_{50} values 0.580 μg/gm, 0.290 μg/gm, 0.580 μg/gm, 0.370 μg/gm 0.590 μ g/gm and 3.05 μg/gm respectively. After 16 hr of extract exposure of *C. decidua* extracts the body contents of glycogen, protein, DNA, RNA, amino acid and lipid were depleted 26.72%, 40.60%, 37.14%, 30.31%, 29.52% and 58.25% compared to control. However it also suppressed the level of metabolically significant enzymes i.e. ACP (53.08%), ALP (67.18%), GPT (67.81%), GOT (75.75%), LDH (84.19%) and AChE (60.66%). Therefore solvent and aqueous extracts of *C. deciduas* is proved to have strong insecticidal activity against *C. chinensis* and can be used for control of stored grain insects.

Keywords: *Capparis decidua*, *Callosobruchus chinensis*, biochemical changes

1 Introduction

The pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) are far more diversified and are highly destructive stored grain insects in comparison to moths. Both grubs and adult insects attack the stored food material and cause loss of grain [1]. Among all important stored grain pests bruchids mainly the pulse beetle, *Bruchus chinensis* is highly serious pest of stored grains, cowpea, gram, arhar, soybean, moong and urd. It damages food grains, occurs in storehouses and godowns and has a worldwide distribution. The grubs eat the entire content of the grain and leave the shell behind. Adult beetles also reside in the circular holes of the grains. For controlling losses caused by the pulse beetle, farmer apply several biological control method such as treatment with burnt wood ash, sand with high amounts of quartz and crystal of NaCl, but it was not found more successful [2]. In most of the cases to control the stored grain insect synthetic pesticides are being used [3], that imposed certain hazardous and lethal effects on non-tagret organisms and put adverse impacts on the environment. Hence, there alternative and safer formulations should explored in form of bio-organic pesticides [4]. However, present plant species '*Capparis decidua*' selected for investigation that possess very high insecticidal activity and belong to family Capparidaceae 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 [5]. The powdered fruit of *C. decidua* is used in anti-diabetic formulations [6]. Upadhyay et al., [7] have demonstrated that compound isolated from C. decidua successfully inhibit the ovipositional responses of pulse beetle *Callosobruchus chinensis*. However, in the present study, insecticidal effects of *C. decidua* were observed on biochemical and enzymatic parameters of *Callosobruchus chinensis*.

2 Materials and methods

Insect culture

Adult insects of *Callosbruchus chinensis* L. were collected from the food grain store houses available in local market in Gorakhpur. The beetles were reared on healthy, clean and un-infested wheat seeds in glass jars and capped with muslin cloth for ventilation. Culture was maintained in laboratory under controlled temperature (28±2°C), relative humidity (75±5% RH) and a

photoperiod of 12: 12 (L:D) h in B.O.D. Insects were reared in glass jars on gram seeds and each time early age beetles were used for the experiments.

Collection of plant material

Stems of *Capparis decidua* were collected from different places of western part of India especially from state of Rajasthan. Specimens were identified by applying standard taxonomic key specially by observing inflorescence and family formula with the help of a taxonomic expert. Fresh plant material was used to prepare extracts. Plant material was dried, chopped, grounded and milled to make powder in domestic grinder.

Preparation of extracts

Stem of *C. decidua* was collected and chopped in to small pieces, dried and pulverized to make fine powder in an electric grinder. The powdered stem (200 gm) was then extracted with various solvent according to their polarity. Extracts were allowed to evaporate in a speed vac to get residue. It was dried and weighed and re-dissolved in known volume of different solvents. Dissolved residues were stored in cold at 4°C temperature for experimental purpose.

Toxicity bio-assays

Adults of *C. chinensis* were exposed with various increasing concentrations of each plant extracts separately. For this purpose, separate filter paper strips (1 cm^2) were coated with different concentrations of plant extracts were placed in the glass culture tubes and open ends were plugged with cotton balls. The coated filter paper strips were air-dried before application. Only solvent treated filter papers were strips used to set control. Ten adult insects were released culture in glass culture tubes (10 cm Height $\times$ 4 cm diameter). For each extract, five different concentrations were used and for each concentration six replicates were set. Mortality in *C. chinensis* was recorded after 24 hr in presence and absence of various plants extracts separately. LD_{50} values were determined by Probit method [8]. LD_{50} values were calculated in μg/gm body weight of the insect.

Determination of glycogen

Glycogen contents were measured according to method of Dubois et al., [9]. For this purpose 500 mg of *C. chinensis* were homogenized in 2 ml of 5% Tri-

chloro acetic acid with the help of glass-glass homogenizer and centrifuged. Optical density of the reactant was read at 530 nm. Glycogen contents in unknown (supernatant) were calculated by using standard curve drawn with known amount of glucose. The blank was set by taking 0.50 ml of 5% TCA and 6 ml of concentrate H_2SO_4. The amount of glycogen was expressed in gm/100 gm of body weight of *C. chinensis*. Three treatments were performed at three trials. Data obtained was statistically analyzed by using ANOVA method.

Determination of total free amino acid

Level of free amino acids was determined following Spies, [10]. A total 500 mg of *C. chinensis* were homogeninzed in 2 ml of 95% ethyl alcohol. Homogenate was centrifuged at 15,000 × g for 20 minutes and supernatant was separated. For estimation of total free amino acids 0.1 ml of supernatant was taken and to it 0.1 ml of distilled water and 2.0 ml Ninhydrin reagent were mixed. The reaction mixture was kept in boiling water for 15 minutes. A total of 2 ml of 5.0 % ethyl alcohol was added to the above boiled mixture. A violet color was developed in the reaction mixture which was measured at 575 nm. For calculating the total free amino acid content standard curve was prepared by using known amount of glycine and was expressed in gm/100 gm body weight of *C. chinensis*. Three replicates were used and data is statistically analyzed by ANOVA method.

Determination of nucleic acids

Level of nucleic acids in the whole body extracts of *C. chinensis* was estimated according to method of Scheidner [11]. For this purpose a total 500 mg of *C. chinensis* were fed with 40% and 80% of LD_{50} of different solvent extracts of *C. decidua* separately. Insects were scarified and homogenized in 5%TCA with glass-glass homogenizer at 15,000 × g for 25 minutes.

DNA estimation

For DNA estimation, 0.2 ml of supernatant was taken and it was diluted by adding 3.8 ml of distilled water. Then 4.0 ml of diphenylamine reagent (1 gm of diphenylamine, 100 glacial acetic acid and 2.5 ml of conc. H_2SO_4) were added to it. The mixtures were kept in boiling water bath for 10 minutes. A blue color was developed in the solution which is measured at 595 nm (O.D.).

RNA estimation

For RNA estimation 0.2 ml of supernatant was taken and it was diluted by adding 4.8 ml of distilled water. Now 2 ml of orcinol reagent (1 gm orcinol, 100 ml conc. HCl and 0.5 gm ferric acid) was added to it. The solution was kept in boiling water bath for 10 minutes, a green color was developed, which was measured at 660 nm. In both cases three replicates were set and data obtained was statistically analyzed by ANOVA method.

Determination of total protein

Total proteins of *C. chinensis* were estimated according to Lowry et al., [12]. For this purpose 500 mg of *C. chinensis* were treated with 40% and 80% of LD_{50} of different solvent extracts of *C. deciduas*. These treated *C. chinensis* were homogenized in 4.0 ml of 10% TCA with the help of glass-glass homogenizer. The obtained homogenate was centrifuged at 15,000 × g for 15 minutes. Each experiment was performed three times. Standard curve was prepared by using 10 μg, 20 μg, 40 μg, 80 μg and 100 μg of Bovine serum albumen. Data obtained was statistically analyzed by ANOVA method.

Determination of Total lipid

Level of total lipid in whole body extracts of *C. chinensis* was estimated according to method of Floch et al., [13]. A total of 500 mg of insects homogenized in 5 ml of chloroform and methanol mixture (2:1 v/v). Total lipid contents were weighted at the end and expressed in gm/100 gm body weight of insect. Three replicates were set and data was statistically analyzed by ANOVA method.

In vivo Determination of enzymatic parameters

To observe the effect on enzymatic parameters 500 mg of *C. chinensis* were provided sub-lethal doses (40% and 80% of LD_{50}) of different solvent extract of *C. decidua* was provided. Insects were sacrificed at the 4 h interval up to 16 h for measurement of various enzyme levels. Insects were homogenized in phosphate saline buffer (pH 6.9) in a glass-glass homogenizer and centrifuged at 4°C for 25 minutes at 15,000 × g. Supernatant was isolated in a glass tube and used as enzyme source.

Determination of acid and alkaline phosphatase

Level of alkaline phophatase level was determined according to the method of Bergmeyer, [14]. For this purpose 500 mg of *C. chinensis* were homogenized in 1 ml of PBS buffer at 4°C and centrifuged at 15,000 × g for 15 min. A 0.2 ml of supernatant was taken in a test tube and 1.0 ml of acid buffer substrate solution was added. Contents were mixed thoroughly and incubated for 30 minutes at 37°C. Now 4.0 ml of 0.10 N NaOH solution was added to the incubation mixture. Similarly, for determination of ALP, 0.10 ml of supernatant was taken in a test tube and 1.0 ml of alkaline buffer substrate was mixed with it. The mixture was mixed thoroughly and incubated for 30 minutes at 37°C. Now 5.0 ml of 0.02 N NaOH was added to the incubation mixture. The reaction was stopped by adding excess of NaOH. The p-nitrophenol formed as result of hydrolysis of p-nitrophenyl phosphate gave a yellow colour with NaOH. Optical density was measured at 420 nm. Standard curve was drawn with the help of different concentrations of p-nitrophenol. Enzyme activity was expressed as μ moles of p-nitrophenol formed /30min/mg protein.

Determination of lactic dehydrogenase

Activity of lactic dehydrogenase was measured according to the method of Annon, [15]. For this purpose, 100 mg of insects were homogenized in 1.0 ml of 0.1 M phosphate buffer (pH 7.5) in ice bath and centrifuged at 10000 × g for 30 minutes in cold centrifuge at 4°C. Supernatant was used as enzyme source. For determination of enzyme activity 0.05 ml of enzyme source was added to 0.50 ml of pyruvate substrate. Now the contents were incubated at 37°C for 45 minutes. Now 0.50 ml of 2,4- dinitrophenyl hydrazine solution was added and the contents were mixture and kept at the room temperature. After 20 minutes, 5.0 ml of 0.4 N NaOH was mixed and left for 30 minutes at room temperature. The optical density was measured at 540 nm and it was converted to LDH unit by drawing a standard curve. Enzyme activity has been expressed as moles of pyruvate reduced/45min/mg protein.

Determination of glutamate pyruvate transaminase and glutamic-oxaloacetic transaminase

GPT and GOT activity was measured according to the method of Reitman and Frankel, [16]. A total of 500 mg *C. chinensis* were homogenized in 2 ml ice cold PBS buffer and centrifuged at 15,000 × g for 15 min at 4°C. For determining the activity of GPT, 0.10 ml of enzyme source was taken and

0.50 ml of GPT substrate. Similarly, for determination of GOT, 0.10 ml of enzyme source was taken and 0.50 ml of GOT substrate was added to it. Now 0.50 ml of 2, 4-dinitrophenyl hydrazine solution was added and contents were left stand for 15 minutes at room temperature. Then 5.0 ml of 0.4 N NaOH was added and mixed well and allowed to stand at room temperature for 20 minutes. The optical density was read at 505 nm after setting the blank. Standard curve was prepared by using oxaloacetic acid as working standard. The enzyme activity was expressed in units of glutamate pyruvate transaminase or glutamate oxaloacetate transaminase activity/ hr/mg protein

Determination of acetylcholinesterase

Acetylcholinesterase activity was determined according to the method of Ellman et al., [17]. For this purpose 500 mg treated *C. chinensis* were homogenized 50 mM phosphate buffer (pH 8) in ice bath and centrifuged at 1000 × g for 30 minutes in cold centrifuge at 4°C. To the supernatant 0.10 ml (5×10^{-4} M) of freshly prepared acetylcholinethioiodide solution, 0.05 ml of DTNB reagent (chromogenic agent) and 1.45 ml of PBS (pH 6.9) were added. The changes in optical density were monitored at 412 nm regularly for three minutes at 25°C. Enzyme activity has been expressed as moles 'SH' hydrolysed per minute per mg protein.

Statistical analysis

The LD_{50} for each extract was determined by using Probit analysis. Mean, standard deviation, standard error and Student t-test were applied [18].

3 Results

Toxicity determination

The solvent extracts of *C. decidua* have shown a higher toxic potency a against the insect *C. chinensis* exhibiting very low LD_{50} i.e. 0.580 μg/gm, 0.290 μg/gm, 0.580 μg/gm, 0.370 μg/gm, 0.590 μg/gm, and 3.05 μg/gm of body weight of *C. chinensis* respectively for acetone, chloroform, petroleum ether, methanol, hexane and water extracts (Table 1).

Determination of bio-molecules

C. chinensis exposed with sub-lethal concentration of acetone, chloroform, petroleum ether, methanol hexane and water fraction of *C. decidua* have shown significant depleted in glycogen content up to 39.85%, 33.05%, 33.24%, 33.33%, 26.72 and 34.86% after 16 hr (Table2-7). The same treatment have retard the protein synthesis and protein content was found 49.79%, 53.73%, 49.11%, 50.24%, 40.27% and 49.60% in comparison to control insects (Table 2-7). It significantly cut down the DNA level up to 46.94%, 44.61%, 37.14%, 43.58%, 38.16% and 43.62%. In a similar consequence RNA content was also found to be decreased 35.62%, 30.31%, 43.15%, 48.28%, 49.27% and 52.82% (Table 2-7). Similarly, a remarkable suppression (49.49%, 43.78%, 40.49%, 48.61%, 29.52% and 53.22%) was reported in amino acid content, while a initial increase was observed in lipid content later it was cut down up to 66.34%, 74.59%, 74.67%, 75.08%, 74.75% and 58.25% compared to control (Table 2-7)

Determination of enzymes

Sub-lethal doses of acetone, chloroform, petroleum ether, methanol hexane and water fractions of *C. decidua* have expressed physiologically little or more toxic effects on certain enzymes in insect body. Concerned to this significant (p ¡ 0.05) deletion in acid phosphatase was recorded 83.97%, 71.83%, 64.37%, 71.65%, 53.08% and 81.38% in comparison to control respectively for the above fractions (Table 8-13). Similarly, alkaline phosphatase was cut down up to 77.28%, 73.15%, 68.58%, 70.25%, 67.18% and 83.53% after 16 hr (Table 8-13). Further, depletion was also noticed in GPT (75.87%, 72.18%, 72.47%, 72.06%, 67.81% and 97.87%) and GOT (89.46%, 77.54%, 76.31%, 76.25%, 75.75% and 86.15%). These extracts caused a slight decrease of 95.30%, 84.19%, 85.78%, 85.83%, 85.37% and 95.30 in lecatic dehydrogenase level (Table 8-13). However it have also caused neurotoxic effects and block AChE activity up to 75.15%, 75.26%, 64.39%, 75.15%, 60.66% and 84.32% in comparison to control (Table 8-13).

4 Discussion

Although stored grain insects are being controlled by synthetic pesticides, it is contaminating the environment and causing food poisoning. However in the present investigation natural extracts isolated from *C. deciduas* have used to observe its lethality in *C. chinensis*. Results obtained in the present investigation clearly demonstrate that both solvent and aqueous extracts of *C. decidua* are highly toxic to *C. chinensis* as each extract have shown high toxicity with very low LD_{50} values. However, maximum toxicity was obtained in chloroform extract i.e. 0.290 μg/mg, while acetone petroleum ether, methanol, hexane and water extracts have shown 0.580 μg/gm, 0.580 μg/gm, 0.370 μg/gm, 0.590 μg/gm and 3.05 μg/gm LD_{50} value. Similarly, active compounds isolated from Piper nigrum have shown strong insecticidal activity *Tribolium castaneum* [19]. Besides this, *Foeniculum vulgare* [20] and Azadirachta indica [21] have been reported to have strong toxic potential against stored grain insects. Fractions of *C. decidu*a have shown significant decrease in glycogen (26.72%), protein (40.27%), DNA (37.14%), RNA (30.31%), amino acid (29.52) and lipid (58.25%) contents after 16 hr of exposure.. Similarly, *Pimpla turionella* wasp treated with cypermethrin have dsisplayed remarkable changes in glycogen, protein and lipid [22]. It is the sign of stressful condition and to meet the energy demand glycogen was broken down [23]. It is evident that glycogen is primary reservoir of energy then protein and lipid compensates the energy demand. Parallel depletion in glycogen, protein and lipid indicates more and more utilization of food reserves to cope up the insecticide induced stress [24]. These changes provide ample stimulus for glycogenolysis in insect tissues and rapid utilization of glycogen units in response to stress caused by pesticide treatment [25]. Similarly protein and nucleic acid synthesis may also block at cellular level and catabolism get increased which results into low availability of proteins and nucleic acid.

More specifically, *C. decidua* extract have shown significant inhibition in certain enzymes i.e. ACP (53.08%), ALP (67.18%), GPT (72.18%), GOT (75.75%), LDH (84.19%) and AChE (60.66%). This reduction indicates the obstruction in their chemical pathways. This led to the formation of abnormal state in the insects and make insects unable to survive. Similarly solvent and aqueous extracts of *Gloriosa superba* [26], *Cassia obtusifolia* [27], *Artemisia annua* [28], *Teucrium royleanum* significantly inhibit certain enzymes like acetyl cholinesterase, lipoxygenase, urease and alkaline phosphatase, amino transferase of insects [29].

To fight with stress insect show significant induction in hydrolytic activities with in the body tissues which cut down the acid and alkaline phosphatase level [30]. Similarly in presence of toxicant transamination of amino acids get increase, hence the synthesis glutamate pyruvate transaminase, glutamate oxalo acetate transaminases get retard [31]. Similarly increase in lactic dehydrogenase level shows tissue necrosis in insects. Therefore, a decrease in the level of above enzymes effect oxygen consumption in insects. Solvent extracts significantly altered phosphatases, transaminase, dehydrogenase and esterase levels, which indicate very high toxic effects on body tissues of the insect. Hence it can be concluded that above plant species can be used for isolation of bio-pesticides to control pulse beetles (*C. chinensis*). For this purpose, constituent's level study along with structure activity relationships of natural products is to be required. Certainly active components from prepared plant species would show wider insecticidal performance and efficacy against *C. chinensis*.

Table 1: LD_{50} of different extracts of *Capparis decidua* against *C. chinensis*

Solvent extract	LD_{50} (μg/gm)	UCL	LCL	Slope function
Acetone	0.580	1.057	0.317	1.99
Chloroform	0.290	0.598	0.140	2029
Petroleum ether	0.580	1.141	0.294	2.17
Methanol	0.370	0.751	0.182	2025
Hexane	0.590	1.10	0.316	2.04
Water	3.05	5.758	1.615	2.07

Table 2: Effect of 40% and 80% of LD_{50} of *Capparis decidua* acetone fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.861±0.0081 (86.63)	1.782±0.0062 (82.95)	1.664±0.01 (77.46)	1.615±0.0048 (75.18)	1.338±0.0062 (62.28)	1.232±0.0046 (57.35)	0.933±0.0035 (43.43)	0.856±0.005 (39.85)
Protein (µg/gm)	10.512±0.0031 (100)	8.327±0.0064 (70.19)	7.366±0.004 (70.05)	7.173±0.0071 (68.21)	6.864±0.0056 (65.28)	6.782±0.0041 (64.49)	6.359±0.002 (60.47)	5.372±0.0069 (51.09)	5.2363±0.0048 (49.79)
DNA (µg/gm)	0.924±0.0042 (100)	0.8458±0.0056 (91.53)	0.7411±0.0059 (80.20)	0.7344±0.0068 (79.48)	0.6424±0.0045 (69.52)	0.5839±0.002 (63.19)	0.5404±0.0007 (58.48)	0.5057±0.0094 (54.73)	0.4338±0.053 (46.94)
RNA (µg/gm)	0.6848±0.0002 (100)	0.48535±0.004 (70.60)	0.4337±0.0053 (63.33)	0.3529±0.005 (51.04)	0.3643±0.007 (53.20)	0.3248±0.0002 (47.43)	0.475±0.002 (46.49)	0.2551±0.007 (37.25)	0.2439±0.004 (35.62)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.707±0.004 (77.58)	0.679±0.004 (74.51)	0.611±0.0035 (67.04)	0.609±0.0063 (66.82)	0.531±0.0023 (58.27)	0.475±0.009 (52.12)	0.47±0.0023 (51.57)	0.451±0.0035 (49.49)
Lipid (µg/gm)	1.212±0.0029 (100)	1.427±0.0145 (117.74)	1.605±0.0017 (132.43)	1.238±0.0019 (102.15)	1.303±0.002 (107.57)	0.803±0.0024 (66.86)	1.017±0.012 (83.91)	0.704±0.002 (58.08)	0.804±0.002 (66.34)

Values are mean ±SE of three replicates

Table 3: Effect of 40% and 80% of LD_{50} of *Capparis decidua* chloroform fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.765±0.0084 (82.16)	1.679±0.0017 (78.16)	1.589±0.0087 (73.97)	1.426±0.0077 (66.38)	1.477±0.007 (68.75)	1.369±0.0063 (63.73)	0.816±0.0049 (37.98)	0.71±0.0032 (33.05)
Protein (µg/gm)	10.512±0.0031 (100)	8.934±0.0058 (84.96)	8.4330±0.0084 (80.20)	7.565±0.006 (71.94)	7.407±0.008 (70.44)	6.986±0.003 (66.44)	6.6883±0.002 (63.60)	5.896±0.0081 (56.07)	5.6497±0.0049 (53.73)
DNA (µg/gm)	0.924±0.0042 (100)	0.8803±0.0056 (95.27)	0.7999±0.0054 (86.56)	0.7653±0.0048 (82.82)	0.6754±0.0037 (73.59)	0.6153±0.0017 (66.59)	0.5517±0.002 (59.70)	0.4830±0.0069 (52.27)	0.4122±0.0051 (44.61)
RNA (µg/gm)	0.6848±0.0002 (100)	0.5037±0.0036 (73.55)	0.4798±0.007 (70.06)	0.4386±0.0071 (64.05)	0.4133±0.0058 (60.35)	0.3476±0.0023 (50.76)	0.3013±0.003 (44.00)	0.2339±0.0094 (34.16)	0.2076±0.0054 (30.31)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.667±0.004 (73.19)	0.571±0.0018 (62.65)	0.598±0.0035 (65.62)	0.518±0.0035 (56.84)	0.526±0.0035 (57.72)	0.512±0.002 (56.18)	0.417±0.0043 (45.76)	0.399±0.0052 (43.78)
Lipid (µg/gm)	1.212±0.0029 (100)	1.507±0.004 (124.34)	1.603±0.002 (132.26)	1.302±0.0014 (107.43)	1.402±0.0012 (115.68)	1.033±0.024 (85.23)	1.002±0.0012 (82.67)	0.803±0.0014 (66.26)	0.904±0.0023 (74.59)

Values are mean ±SE of three replicates

Table 4: Effect of 40% and 80% of LD_{50} of *Capparis decidua* petroleum ether fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.785±0.0054 (83.09)	1.680±0.0046 (78.20)	1.625±0.0065 (75.64)	1.576±0.011 (73.36)	1.397±0.0052 (65.03)	1.203±0.0029 (56.00)	0.816±0.0049 (37.98)	0.714±0.0058 (33.24)
Protein (µg/gm)	10.512±0.0031 (100)	9.713±0.0078 (92.37)	8.095±0.031 (76.98)	8.135±0.0075 (77.36)	7.520±0.0084 (71.51)	6.812±0.0023 (64.78)	6.620±0.0023 (62.95)	5.233±0.0055 (49.76)	5.1642±0.0039 (49.11)
DNA (µg/gm)	0.924±0.0042 (100)	0.9163±0.0063 (99.16)	0.8645±0.0074 (93.56)	0.8287±0.0032 (89.68)	0.756±0.0073 (81.81)	0.5476±0.002 (59.26)	0.4519±0.001 (48.90)	0.3698±0.0053 (40.02)	0.3432±0.0077 (37.14)
RNA (µg/gm)	0.6848±0.0002 (100)	0.6179±0.0013 (90.23)	0.5440±0.0038 (79.44)	0.5532±0.0036 (80.78)	0.5155±0.005 (75.28)	0.4313±0.0018 (62.98)	0.3758±0.0013 (54.88)	0.3089±0.0039 (45.11)	0.2955±0.0058 (43.15)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.845±0.0024 (92.75)	0.817±0.0035 (89.65)	0.737±0.0041 (80.87)	0.716±0.0053 (78.57)	0.614±0.0026 (67.37)	0.593±0.003 (65.07)	0.379±0.0043 (41.59)	0.369±0.0032 (40.49)
Lipid (µg/gm)	1.212±0.0029 (100)	1.304±0.0027 (107.59)	1.409±0.0047 (116.25)	1.105±0.0017 (91.17)	1.205±0.0035 (99.42)	0.904±0.002 (74.59)	1.009±0.0048 (83.25)	0.807±0.0054 (66.58)	0.905±0.0035 (74.67)

Values are mean ±SE of three replicates

Table 5: Effect of 40% and 80% of LD_{50} of *Capparis decidua* methanol fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.731±0.0052 (80.58)	1.626±0.0053 (75.69)	1.635±0.004 (76.11)	1.543±0.01 (71.83)	1.445±0.0041 (67.26)	1.276±0.0035 (59.26)	0.78±0.0023 (36.31)	0.716±0.0049 (33.33)
Protein (µg/gm)	10.512±0.0031 (100)	9.223±0.0047 (87.71)	8.223±0.0059 (78.23)	8.773±0.0069 (83.43)	7.273±0.0081 (69.17)	7.516±0.0023 (71.48)	6.939±0.0017 (65.99)	5.926±0.0053 (56.36)	5.283±0.0075 (50.24)
DNA (µg/gm)	0.924±0.0042 (100)	0.7859±0.0048 (85.05)	0.7338±0.006 (79.41)	0.5803±0.0041 (62.80)	0.5292±0.0056 (57.27)	0.5367±0.0035 (58.08)	0.4846±0.0031 (52.44)	0.4262±0.005 (46.12)	0.4027±0.0052 (43.58)
RNA (µg/gm)	0.6848±0.0002 (100)	0.5528±0.004 (80.72)	0.5113±0.0043 (74.66)	0.4303±0.002 (62.84)	0.3849±0.0055 (56.21)	0.376±0.0011 (54.91)	0.3628±0.002 (52.98)	0.3436±0.0027 (50.17)	0.3306±0.0033 (48.28)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.809±0.0024 (88.77)	0.721±0.0029 (79.11)	0.733±0.0017 (80.43)	0.678±0.0032 (74.39)	0.609±0.0027 (66.82)	0.552±0.0035 (66.57)	0.469±0.0033 (51.46)	0.443±0.0054 (48.61)
Lipid (µg/gm)	1.212±0.0029 (100)	1.305±0.0029 (107.67)	1.406±0.0035 (116.01)	1.202±0.0015 (99.18)	1.303±0.0012 (107.51)	1.104±0.0026 (91.08)	1.105±0.0014 (91.17)	1.006±0.0035 (83.00)	0.91±0.0018 (75.08)

Values are mean ±SE of three replicates

Table 6: Effect of 40% and 80% of LD_{50} of *Capparis decidua* hexane fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	1.652±0.01 (76.90)	1.533±0.007 (71.36)	1.315±0.004 (61.21)	1.232±0.001 (57.35)	1.182±0.0042 (55.02)	1.071±0.0029 (49.85)	0.653±0.007 (30.40)	0.574±0.0035 (26.72)
Protein (µg/gm)	10.512±0.0031 (100)	8.326±0.005 (79.18)	8.243±0.0068 (78.39)	6.955±0.0068 (66.19)	6.5843±0.0043 (62.62)	5.2187±0.0018 (49.63)	5.143±0.0024 (48.91)	4.363±0.0082 (41.49)	4.2343±0.0063 (40.27)
DNA (µg/gm)	0.924±0.0042 (100)	0.7543±0.0047 (81.63)	0.6595±0.0006 (71.37)	0.7009±0.0004 (75.85)	0.5647±0.005 (61.11)	0.5468±0.0026 (59.17)	0.5158±0.0037 (55.82)	0.3026±0.0005 (42.49)	0.3526±0.007 (38.16)
RNA (µg/gm)	0.6848±0.0002 (100)	0.5513±0.0044 (80.50)	0.4289±0.0043 (62.63)	0.473±0.0048 (69.96)	0.3726±0.0074 (54.41)	0.3944±0.002 (57.59)	0.353±0.0011 (51.55)	0.3551±0.0076 (51.85)	0.3374±0.0019 (49.27)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.706±0.003 (77.47)	0.689±0.0035 (75.60)	0.674±0.0054 (73.96)	0.607±0.0046 (66.61)	0.524±0.0032 (57.50)	0.4913±0.0023 (53.91)	0.316±0.0064 (34.67)	0.269±0.0018 (29.52)
Lipid (µg/gm)	1.212±0.0029 (100)	1.504±0.0023 (124.09)	1.604±0.0023 (132.35)	1.304±0.0023 (107.59)	1.408±0.0023 (116.17)	1.106±0.0023 (91.26)	1.104±0.0031 (91.09)	0.704±0.0023 (58.09)	0.906±0.0021 (74.75)

Values are mean ±SE of three replicates

Table 7: Effect of 40% and 80% of LD_{50} of *Capparis decidua* aqueous fraction on glycogen, protein, DNA, RNA amino acid and lipid of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
Glycogen (µg/gm)	2.148±0.0046 (100)	2.058±0.01 (95.80)	1.99±0.0081 (89.84)	1.668±0.0081 (77.64)	1.53±0.01 (71.22)	1.237±0.004 (57.58)	1.191±0.0029 (55.44)	0.77±0.0053 (35.84)	0.749±0.0046 (34.86)
Protein (µg/gm)	10.512±0.0031 (100)	8.2733±0.0081 (78.68)	7.978±0.0078 (75.87)	7.231±0.0052 (68.77)	6.533±0.0081 (62.32)	6.71±0.0035 (63.81)	6.484±0.0023 (61.66)	5.2367±0.0066 (49.80)	5.2157±0.0087 (49.60)
DNA (µg/gm)	0.924±0.0042 (100)	0.8417±0.0055 (91.09)	0.7003±0.0067 (75.79)	0.7291±0.0038 (78.90)	0.6272±0.0059 (67.87)	0.5589±0.0029 (60.48)	0.5007±0.0035 (54.18)	0.4186±0.0012 (45.30)	0.4031±0.0056 (43.62)
RNA (µg/gm)	0.6848±0.0002 (100)	0.6736±0.0084 (98.36)	0.6209±0.0025 (90.67)	0.562±0.0044 (82.07)	0.4908±0.0043 (71.67)	0.4933±0.0018 (72.04)	0.4825±0.0044 (70.46)	0.3701±0.0045 (54.04)	0.3617±0.004 (52.82)
Amino acid (µg/gm)	0.9113±0.007 (100)	0.807±0.0035 (88.55)	0.799±0.0055 (87.67)	0.72±0.0038 (79.00)	0.692±0.005 (75.93)	0.608±0.0023 (66.71)	0.578±0.002 (63.42)	0.497±0.0052 (54.53)	0.485±0.0048 (53.22)
Lipid (µg/gm)	1.212±0.0029 (100)	1.304±0.003 (107.59)	1.503±0.0024 (124.01)	1.107±0.0035 (91.34)	1.307±0.0018 (107.84)	0.806±0.0038 (66.50)	1.005±0.0017 (82.92)	0.605±0.0029 (49.92)	0.706±0.0015 (58.25)

Values are mean ±SE of three replicates

Table 8: Effect of 40% and 80% of LD_{50} of *Capparis decidua* acetone fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	2.018±0.0024 (90.08)	1.921±0.057 (85.75)	1.918±0.013 (85.62)	1.917±0.0233 (85.58)	1.915±0.0083 (85.49)	1.913±0.012 (85.40)	1.887±0.01 (84.24)	1.881±0.003 (83.97)
ALP	1.792±0.01 (100)	1.517±0.0057 (84.65)	1.502±.0.0081 (83.81)	1.481±0.011 (82.64)	1.466±0.041 (81.80)	1.424±0.003 (79.46)	1.419±0.0013 (79.18)	1.391±0.0045 (77.62)	1.385±0.0009 (77.28)
GPT	4.145±0.007 (100)	4.031±0.0018 (97.24)	4.027±0.0012 (97.15)	4.005±0.012 (96.62)	3.912±0.003 (94.37)	3.909±0.002 (94.30)	3.886±0.0013 (93.75)	3.166±0.012 (76.38)	3.145±0.024 (75.87)
GOT.	3.019±0.002 (100)	2.849±0.004 (94.36)	2.839±0.0024 (94.03)	2.821±0.031 (93.44)	2.816±0.01 (93.27)	2.807±0.012 (92.97)	2.801±0.022 (92.77)	2.711±0.00081 (89.79)	2.701±0.0017 (89.46)
LDH.	8.301±0.019 (100)	8.281±0.0087 (99.75)	8.259±0.015 (99.49)	8.251±0.024 (99.39)	8.241±0.05 (99.27)	8.131±0.0017 (97.95)	8.001±0.011 (96.38)	7.941±0.0087 (95.66)	7.911±0.0057 (95.30)
AChE	0.938±0.012 (100)	0.909±0.031 (96.90)	0.891±0.035 (94.98)	0.879±0.0014 (93.71)	0.852±0.007 (90.83)	0.846±0.0013 (90.19)	0.831±0.003 (88.59)	0.722±0.031 (76.97)	0.705±0.004 (75.15)

Values are mean ±SE of three replicates

Table 9: Effect of 40% and 80% of LD_{50} of *Capparis decidua* chloroform fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	2.008±0.0019 (89.64)	1.891±0.018 (84.41)	1.788±0.003 (79.82)	1.751±0.0013 (78.16)	1.735±0.001 (77.45)	1.723±0.021 (76.91)	1.621±0.003 (72.36)	1.609±0.031 (71.83)
ALP	1.792±0.01 (100)	1.502±0.0012 (83.81)	1.479±.008 (82.53)	1.471±0.0031 (82.08)	1.455±0.006 (81.19)	1.413±0.05 (78.85)	1.411±0.0023 (78.73)	1.324±0.008 (73.88)	1.311±0.011 (73.15)
GPT	4.145±0.007 (100)	3.661±0.0018 (88.32)	3.437±0.002 (82.91)	3.405±0.012 (82.14)	3.212±0.023 (77.49)	3.189±0.013 (76.93)	3.127±0.0043 (75.44)	3.031±0.0019 (73.12)	2.992±0.002 (72.18)
GOT.	3.019±0.002 (100)	2.809±0.0019 (93.04)	2.793±0.002 (92.51)	2.721±0.013 (90.12)	2.696±0.011 (89.30)	2.687±0.0021 (89.00)	2.485±0.022 (82.31)	2.376±0.018 (78.70)	2.341±0.0019 (77.54)
LDH.	8.301±0.019 (100)	8.101±0.0021 (97.59)	7.959±0.015 (95.88)	7.851±0.016 (94.57)	7.641±0.011 (92.04)	7.432±0.037 (89.53)	7.111±0.031 (85.66)	7.009±0.007 (84.43)	6.989±0.01 (84.19)
AChE	0.938±0.012 (100)	0.885±0.031 (94.34)	0.871±0.025 (92.85)	0.857±0.0023 (91.36)	0.841±0.019 (89.65)	0.836±0.0013 (89.12)	0.811±0.008 (86.46)	0.713±0.031 (76.01)	0.706±0.023 (75.26)

Values are mean ±SE of three replicates

Table 10: Effect of 40% and 80% of LD_{50} of *Capparis decidua* petroleum ether fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	1.856±0.0019 (82.85)	1.674±0.018 (74.73)	1.629±0.0001 (72.72)	1.612±0.0013 (71.96)	1.595±0.0012 (71.20)	1.583±0.012 (70.66)	1.471±0.01 (65.66)	1.442±0.012 (64.37)
ALP	1.792±0.01 (100)	1.459±0.0022 (81.41)	1.439±.0081 (80.30)	1.421±0.0011 (79.29)	1.401±0.036 (78.18)	1.391±0.015 (77.62)	1.365±0.03 (76.17)	1.257±0.0045 (70.14)	1.229±0.018 (68.58)
GPT	4.145±0.007 (100)	3.632±0.0083 (87.62)	3.412±0.012 (82.31)	3.398±0.0021 (81.97)	3.201±0.0021 (77.22)	3.179±0.002 (76.69)	3.109±0.033 (75.00)	3.056±0.013 (73.72)	3.004±0.021 (72.47)
GOT	3.019±0.002 (100)	2.768±0.0019 (91.68)	2.756±0.002 (91.28)	2.702±0.033 (89.49)	2.659±0.001 (88.07)	2.652±0.003 (87.84)	2.414±0.002 (79.96)	2.317±0.05 (76.74)	2.304±0.0083 (76.31)
LDH	8.301±0.019 (100)	8.035±0.0083 (96.79)	7.788±0.045 (93.82)	7.771±0.016 (93.61)	7.523±0.011 (90.62)	7.369±0.017 (88.77)	7.242±0.011 (87.24)	7.154±0.021 (86.18)	7.121±0.033 (85.78)
AChE	0.938±0.012 (100)	0.772±0.031 (82.30)	0.765±0.045 (81.55)	0.731±0.014 (77.93)	0.712±0.019 (75.90)	0.703±0.03 (74.94)	0.687±0.011 (73.24)	0.612±0.04 (65.24)	0.604±0.003 (64.39)

Values are mean ±SE of three replicates

Table 11: Effect of 40% and 80% of LD_{50} of *Capparis decidua* methanol fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	1.898±0.009 (84.73)	1.787±0.018 (79.77)	1.766±0.0013 (78.83)	1.731±0.0023 (77.27)	1.715±0.002 (76.56)	1.703±0.011 (76.02)	1.611±0.004 (71.91)	1.605±0.01 (71.65)
ALP	1.792±0.01 (100)	1.492±0.005 (83.25)	1.468±.0009 (81.91)	1.454±0.03 (81.13)	1.447±0.016 (80.74)	1.403±0.05 (78.29)	1.391±0.0023 (77.62)	1.284±0.0083 (71.65)	1.259±0.009 (70.25)
GPT	4.145±0.007 (100)	3.641±0.0081 (87.84)	3.417±0.0032 (82.43)	3.401±0.021 (82.05)	3.209±0.003 (77.41)	3.181±0.0032 (76.74)	3.116±0.0013 (75.17)	3.005±0.013 (72.49)	2.987±0.002 (72.06)
GOT	3.019±0.002 (100)	2.779±0.009 (92.05)	2.764±0.0012 (91.55)	2.711±0.0013 (89.79)	2.676±0.01 (88.63)	2.666±0.034 (88.30)	2.425±0.021 (80.32)	2.312±0.016 (76.58)	2.302±0.013 (76.25)
LDH	8.301±0.019 (100)	8.041±0.0031 (96.86)	7.869±0.05 (94.79)	7.848±0.016 (94.54)	7.591±0.002 (91.44)	7.397±0.0009 (89.10)	7.261±0.0021 (87.47)	7.154±0.021 (86.18)	7.125±0.017 (85.83)
AChE	0.938±0.012 (100)	0.862±0.005 (91.89)	0.853±0.01 (90.93)	0.846±0.004 (90.19)	0.839±0.009 (89.44)	0.822±0.013 (87.63)	0.801±0.001 (85.39)	0.711±0.007 (75.79)	0.705±0.005 (75.15)

Values are mean ±SE of three replicates

Table 12: Effect of 40% and 80% of LD_{50} of *Capparis decidua* hexane fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	1.564±0.009 (69.82)	1.446±0.008 (64.55)	1.421±0.0013 (63.43)	1.382±0.0031 (61.69)	1.355±0.001 (60.49)	1.311±0.05 (58.52)	1.201±0.0001 (53.61)	1.189±0.024 (53.08)
ALP	1.792±0.01 (100)	1.431±0.0012 (79.85)	1.425±.0001 (79.52)	1.412±0.021 (80.13)	1.391±0.006 (77.62)	1.372±0.03 (76.56)	1.351±0.0023 (75.39)	1.221±0.015 (68.13)	1.204±0.0045 (67.18)
GPT	4.145±0.007 (100)	3.522±0.008 (85.96)	3.401±0.0012 (82.05)	3.359±0.015 (81.03)	3.195±0.003 (77.08)	3.172±0.002 (76.52)	2.979±0.0013 (71.86)	2.854±0.008 (68.85)	2.811±0.015 (67.81)
GOT	3.019±0.002 (100)	2.658±0.0019 (88.04)	2.639±0.0022 (87.41)	2.621±0.013 (86.81)	2.619±0.04 (86.75)	2.602±0.001 (86.18)	2.401±0.032 (79.52)	2.298±0.012 (76.11)	2.287±0.03 (75.75)
LDH	8.301±0.019 (100)	7.735±0.008 (93.18)	7.728±0.006 (93.09)	7.702±0.016 (92.78)	7.513±0.011 (90.50)	7.319±0.0009 (88.17)	7.214±0.0011 (86.90)	7.101±0.0001 (85.54)	7.087±0.0023 (85.37)
AChE	0.938±0.012 (100)	0.766±0.01 (81.66)	0.754±0.015 (80.38)	0.711±0.004 (75.79)	0.703±0.009 (74.94)	0.685±0.0013 (7302)	0.672±0.021 (71.65)	0.585±0.0031 (62.36)	0.569±0.011 (60.66)

Values are mean ±SE of three replicates

Table 13: Effect of 40% and 80% of LD_{50} of *Capparis decidua* aqueous fraction on ACP, ALP, GPT, GOT, LDH and AChE of *Callosobruchus chinensis*

Para-meters	Time (in h)								
	0 (Control)	4		8		12		16	
		40%	**80%**	**40%**	**80%**	**40%**	**80%**	**40%**	**80%**
ACP	2.240±0.004 (100)	1.941±0.003 (86.65)	1.938±0.013 (86.51)	1.931±0.023 (86.20)	1.925±0.038 (85.93)	1.913±0.019 (85.40)	1.903±0.003 (84.95)	1.841±0.023 (82.18)	1.823±0.021 (81.38)
ALP	1.792±0.01 (100)	1.686±.0001 (94.08)	1.671±0.0009 (93.24)	1.666±0.04 (92.96)	1.624±0.031 (90.62)	1.619±0.0002 (90.34)	1.594±0.0011 (88.95)	1.512±0.024 (84.37)	1.497±0.0045 (83.53)
GPT	4.145±0.007 (100)	4.127±0.0021 (99.56)	4.115±0.011 (99.27)	4.112±0.0013 (99.20)	4.109±0.002 (99.13)	4.056±0.013 (97.85)	3.991±0.02 (96.28)	3.901±0.031 (94.11)	3.891±0.0032 (93.87)
GOT	3.019±0.002 (100)	2.915±0.005 (96.55)	2.911±0.031 (96.42)	2.906±0.021 (96.25)	2.897±0.0034 (95.95)	2.891±0.012 (95.76)	2.671±0.0045 (88.47)	2.611±0.006 (86.48)	2.601±0.0009 (86.15)
LDH	8.301±0.019 (100)	8.269±0.001 (99.61)	8.256±0.006 (99.45)	8.248±0.03 (99.36)	8.149±0.017 (98.16)	8.041±0.031 (96.86)	7.981±0.01 (96.14)	7.914±0.023 (95.33)	7.911±0.05 (95.30)
AChE	0.938±0.012 (100)	0.911±0.024 (97.12)	0.909±0.0023 (96.90)	0.901±0.04 (96.05)	0.896±0.0023 (95.52)	0.881±0.006 (93.92)	0.863±0.003 (92.00)	0.813±0.031 (86.67)	0.791±0.003 (84.32)

Values are mean ±SE of three replicates

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