International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 15-06-2021, Accepted: 30-06-2021, Published: 15-07-2021
Volume 6, Issue 4, 2021, Page No. 42-48

Insecticidal and plant growth promoting activity of *Citrus aurantium* peel

A Suriya Daisy, R Maha Lakshmi, A Praveena*
Department of Biotechnology, Prathyusha Engineering College, Thiruvallur, Tamil Nadu, India

Abstract
Botanical insecticides are naturally occurring chemicals that are obtained from plants. This study aims to analyse the insecticidal activity of compounds present in the ethanolic extract of citrus aurantium peel. The solvent extraction method was used to get the ethanolic extract and the compounds were identified using GC-MS. The insecticidal activity of the ethanolic extract was studied by diet incorporation method. The feed deterrence index and their metabolic rates were assessed. At 40% concentration, the feed deterrence index was observed to be 46.6% and reveals that feed deterrence index increases with the increase in concentration. The consumption rate was observed to be 0.020g/day and denoted that, the consumption rate decreases with the increase in the concentration. The growth rate, efficiency of conversion of ingested food, approximate digestibility and efficiency of conversion of digested food were assessed to be 0.10 g/day, 10.7%, 61.36% and12.36% respectively. The insecticidal likeliness property of the compounds were evaluated using Tice rule and further the binding efficiency were studied using molecular docking against acetylcholinesterase, carboxylesterase and protease of Spodoptera litura. From the docking studies the compound Heptanoic acid,2-methyl, methyl ester from the ethanolic extract have the best interaction with Acetylcholinesterase and carboxylesterase with the least energy value of -79.6 and -76.7 respectively. The seed germination rate was higher at 5% & 10% concentration that is as the concentration of the ethanolic extract of Citrus aurantium decreases the seed germination rate increases. Thus the results could be used as the novel insecticide against the *Spodoptera litura*.

Keywords: *Citrus aurantium*, *Spodoptera litura*, GC-MS analysis, feed deterrence index, tice rule and docking

Introduction
The term biopesticides are the compounds that are used to manage agricultural pests by means of specific biological effects rather than by using certain chemical pesticides. The biopesticides are obtained from natural materials such as animals, plants, bacteria and some minerals.

The biopesticides are the form of pesticide based on the natural things or microorganism. The benefits of biopesticides may add the zero day pre-harvest interval and they may use for both moderate and severe crop problems. These pesticide can control the seed-borne fungal pathogens and that are on the surface of the seeds. These biopesticide can affect the pest via predatory, chemical and parasitic relations. Biopesticides are less toxic when compared to the conventional pesticides and may affect only certain target pest and closely related species. Whereas the conventional pests affects the birds, insects and mammals. Biopesticides are effective in small amount and also decomposes fastly when compared to the conventional pesticides. Biopesticides also increases the crop yield to a greater extent. It is the chief mode of preservation of genetic resources and land races by in situ and ex situ conservation. Biopesticides improves food quality. The defense chemicals or secondary metabolites of plants can serve several types of functions. They can be insecticidal [1] or antimicrobial to bacteria, fungi and viruses, some are also herbicidal and some possess other types of biological activities. These beneficial, bioactive chemical substances are found in abundance in plant species. Of the 5–10% of the higher plants which have been phytochemically analyzed, more than 30,000 secondary metabolites have been reported [2]. Plant essential oils exhibit biological activity against a wide spectrum of plant pests and may act as fumigants, contact insecticides, repellents, and antifeedant or they can affect the growth rate, reproduction and behavior of insect pests [3].

Materials and Method
Preparation of Plant Extract
The fruit, bitter orange (*citrus aurantium*) were collected from the sholinghur, Vellore district, Tamil Nadu. The peel of *citrus aurantium* were collected and shade dried for four weeks. The shade dried peels were powdered using electric blender and stored. From the powdered material, 35g of powder was taken for extraction and extracted with 250 ml of ethanol using soxhlet apparatus. Around three cycles where performed to get the clear solution. The ethanolic extract was stored in bottle in the refrigerator.

Phytochemical Screening
The various chemical constitutes present in the ethanolic extract of *citrus auratium* were analyzed, which was described by Allen (1974) and Harbone (1976) [4, 5]. The presence of Alkaloids, Saponins, Tannins, Phlobatannins, Flavonoids, Steroids, Terpenoids and Glycosides were studied.

Test for Alkaloids
To 1ml of the plant extract 2ml of freshly prepared Hager's reagent was added. An orange or red precipitate produced indicates the presence of alkaloids **(**Hager's test).

Test for Saponins
To 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The

frothing was mixed with 3 drops of olive oil and shaken vigorously, the formation of emulsion indicated the presence of saponins.

Test for Tannins
A few drops of 0.1% ferric chloride was added to the plant extract. A brownish green or a blue- black colouration was observed which indicated the presence of tannins.

Test for Phlobatannins
The 1ml of plant extract was dissolved in the distilled water and then filtered. The filtrate was allowed to boil with 2% HCl solution for few minutes. The appearance of red precipitate indicated the presence of phlobatannins.

Test for Flavonoids
The 1ml of the extract is dissolved in NaOH and HCl was added. A yellow colour was observed indicating the presence of flavonoids.

Test for Steroids
To 1ml of extract, 3-4 drops of H_2SO_4 was added and vigorously mixed. The red colour at the bottom indicated the presence of steroids.

Test for Terpenoids
The 1ml of extract was added with 2ml of chloroform and 3ml concentrated H_2SO_4 to get the layer. The reddish brown colour at the interface indicated the presence of terpenoids.

Test for Glycosides
The plant extract was diluted in water and 2ml of glacial acetic acid with ferric chloride solution along with the 1ml of H_2SO_4. The formation of brown ring at the interface indicated the presence of glycosides.

Gas Chromatography- Mass Spectrometry
Gas chromatography- Mass spectrometry (GC-MS) is an instrumental technique, consisting of gas chromatograph (GC) coupled to a mass spectrometer (MS), in which the compounds are separated, identified and quantified from the mixture. This is used for analysing the volatile and semi-volatile compounds.A Shimdzu GC-2010 Plus gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250µ I.D., 0.25µ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35°C, hold for 2minutes, then ramp at 20°C per minute to 450°C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).A Direct connection with capillary column metal quadupole mass filter prerod mass spectrometer operating in electron ionization (EI) mode with software GC-MS solution ver. 2.6 was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 1000 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 1000 at 1 second per scan. Identification of the components of the compound was matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software. GC-MS metabolomics Database was used for the similarity search with retention index.

Insecticidal Activity
The *Spodoptera litura* insects were collected from the elephant yam small (*Amorphophallus paeoniifolius*) field. The collected insects were kept in the 100ml cups closed with the transparent lids for observation of their behavior for 2 weeks under the temperature of 26˚C+2˚C and 57-67% of relative humidity. The insects were reared under the laboratory conditions (26˚C+2˚C, 57-67% RH) and they were provided with the fresh leaves placed in the plastic cups closed with the transparent plastic lid with the holes for aeration. The adults were reared to check whether the laboratory condition was suitable for its undisturbed life cycle. The adult insects were again collected and reared in groups of ten insects for acclimatization in laboratory conditions and these groups were used for testing.

Insecticidal Activity of Ethanolic Extract of *aurantium* on *Spodoptera litura*
Toxicity bioassay was carried out with *Spodoptera litura* using the ethanolic extract from *Citrus aurantium*. Insects of uniform size were taken from the mass culture maintained in the laboratory. By diet incorporation method the insecticidal bioassay was carried out. For each treatment insects at third instar were separated and put in the cups without the feed. The ethanolic sample on the other hand were applied on the leaf at different concentration such as 5%, 10%, 20% and 40%. These insects were fed with the leaves treated with the ethanolic sample of *Citrus aurantium*, thus the method of diet incorporation method. After 24 and 48 hours of feeding, the weight of the leftover feed and adult insects were measured for all the concentration of ethanolic extract. The results of percentage of Feed deterrence index, and their metabolic rate which includes the consumption rate, growth rate, efficiency of conversion of ingested food, approximate digestibility and efficiency of conversion of digested food were estimated using the following formulae with the data collected for each concentration of the ethanolic sample of *Citrus aurantium* [6].

Feeding Deterrence Index
Calculated for each treatment using the formula of Ben Jannet *et al*., 2000.

$$FDI=(C-T)/(C+T)*100$$

Where, C-Consumption in control, T-Consumption in Treated

Metabolic Rate
The following formula is used according to Waldbauer (1968) and Slansky and Scriber (1985) to calculate the consumption rate, growth rate, efficiency of conversion of ingested food, approximate digestibility and efficiency of conversion of digested food [7, 8].

1. Consumption rate
The consumption rate is given by,

CR= Weight of food eaten/ Duration of Experiment (Days)

2. Growth rate
The growth rate is given by,

GR= Weight gain/ Days

3. Efficiency of conversion of ingested food

The efficiency of conversion of ingested food is given by,

ECI= Weight gain/ Weight of food eaten * 100

4. Approximate digestibility

The approximate digestibility is given by,

AD= (Weight of food eaten – weight of feces)/Weight of food eaten* 100

5. Efficiency conversion of digested food

The efficiency of conversion of digested food is given by,

ECD= Weight gain/ (Weight of food eaten- Weight of feces) * 100

Bioinformatics Approach for Insecticidal Studies

The enzyme target (Acetylcholinesterase and carboxylesterase) of *Spodoptera litura* was chosen from the biological database such as NCBI (National Center for Biotechnology Information). The structure of the Acetylcholinesterase and carboxylesterase of *Spodoptera litura* was retrieved from the PDB database. The compounds obeyed Tice rule were selected for docking with the Acetylcholinesterase and Carboxylesterase of the insect using iGEMDOCK v 2.1 software. This *in silico* studies acts as an additional proof for molecular level action of prepared ethanolic extract compounds as a potent natural insecticide against the *Spodoptera litura.*

Target Selection

Cholinesterase enzymes belongs to the α/β hydrolase family and are divided into groups that catalyse the hydrolysis of acetylcholine to choline and acetate (acetylcholinesterase EC: 3.1.1.7) and those groups that catalyse the conversion of other acylcholines to choline and weak acid (cholinesterase EC: 3.1.1.8). Acetylcholinesterase is mostly chosen as the target due to its physiological role in neurotransmitter acylcholine. The Acetylcholinesterase has the high turnover number so that they are broken down easily. The active sites are located at the bottom of a deep and narrow cleft (active-site gorge). Thus the insect acetylcholinesterase is targeted by insecticides more frequently. The carboxylesterases (EC: 3.1.1.1) are the group of lipolytic enzymes (catalyses the hydrolysis of esters into acid and alcohol molecules) and are distributed widely in insects and play an important role in the metabolism with different functions. These enzymes play a major role in the xenobiotic defence system, biocatalysis and drug metabolism. Thus the carboxylesterase are chosen as the target. The PDB (Protein Data Bank) ID for acetylcholinesterase and carboxylesterase of *Spodoptera litura* are 1c7i and 2z7f respectively.

iGEMDOCK Software

iGEMDOCK is a Graphical Environment for Recognizing Pharmacological Interactions and Virtual Screening. Pharmacological interactions are useful for identifying lead compounds and understanding ligand binding mechanisms for a therapeutic target. Currently, these interactions are inferred from a set of active compounds that were acquired experimentally. Most docking programs loosely coupled the stages of structure-based virtual screening (VS) from preparation through to post-screening analysis.

The pharmacological interactions represent conserved interacting residues that often form binding pockets with specific physico-chemical properties to play the essential functions of the target protein. Experiment results shows that the success rate of iGEMDOCK is 78% (root-mean-square deviations below 2.0 angstrom) on 305 protein-compound complexes. For virtual screening, pharmacological interactions derived by iGEMDOCK often involve the biological functions [9].

Phytotoxicity Assay

The growth promoting activity was studied using red cow peas and the germination rate, speed of emergence was calculated using the below given formula.

Germination Rate Percent

GP= No. of germinated seeds at final count/ total no. of seeds set for bioassay *100

Speed of Emergence (SE)

SE= No. of germinated seeds at the starting day of germination/ No. of germinated seeds at the final day of germination *100

Results and Discussion

Ethanolic Extraction of *Citrus aurantium*

The ethanolic extract of *Citrus aurantium* was prepared by solvent extraction method. The prepared ethanolic extract was stored in dark brown bottle for further analysis.

Phytochemical Screening

The qualitative phytochemical screening were carried out by observing different color reaction that reflects the presence of compounds. The analysis showed the presence of various secondary metabolites namely Alkaloids, Tannins, Saponins, Glycosides, Terpenoids and Steroids in the ethanolic extract of *Citrus aurantium*.

Plants produce a high diversity of natural products or secondary metabolites with a prominent function in the protection against predators and microbial pathogens on the basis of their toxic nature and repellence to herbivores and microbes.

They are important for the communication of the plants with other organisms. There are three major groups of secondary metabolites namely the Terpenoids, Phenolics and N and S containing compounds. From the analysis the major groups namely the Terpenes and phenols are present in the plant extract of *Citrus aurantium*. In plants, saponins may serve as anti feedants, and to protect the plant against microbes and fungi. Some plant saponins may enhance nutrient absorption and aid in animal digestion. However, saponins are often bitter to taste and it can reduce the plant palatability or even imbue them with life- threatening animal toxicity. Data makes clear that some saponins are toxic to cold-blooded organisms and insects at particular concentration.

GC-MS Analysis

The 10 different compounds were identified from Gas

chromatography- Mass spectrometry analysis of the ethanolic extract. The ten compounds identified from the ethanolic extract of *Citrus aurantium* are 1,2,3-Propanetriol, 1-acetate, Isoamyl nitrite, 2,3,5,6-Tetrafluoroanisole, Octadecanoic acid, 2H-1-Benzopyran-2-one, 5,7-dimethoxy-, Hexadecanoic acid, ethyl ester, 2-Nonen-1-ol, 2-methyl-, Cyclohexaneethanol, E-11-Hexadecenoic acid, ethyl ester and Heptanoic acid, 2-methyl-, methyl ester. The Molecular properties of the obtained phytochemicals from the ethanolic extracts were analyzed based on Tice rule.

Insecticidal Activity

To analyze the insecticidal activity of the compounds from the ethanolic extract of *Citrus aurantium*, Diet-incorporation method of bioassay was carried out. This finding is in good agreement with that of who reported that aguerin B, Chlorojanerin and syringing, isolated from the chloroformic extract of *Rhaponticum pulchrum* were good antifeedent against *T.cconfusum, Sitophilus granaries* (L.) and *T.granarium.* The similar study was performed by the chloroformic extract of *Morinda tinctoria* against the *Helicoverpa armigera* of lepidopteran family [10]. *In-silico* studies performed using molecular docking of selected compounds using Tice rule from the ethanolic extract with the enzyme target Acetylcholinesterase and Carboxylesterase of *Spodoptera litura* [11].

Diet Incorporation Method

By diet incorporation method the insecticidal bioassay was carried out. For each treatment 10 larvae starved for 3 hours were introduced in separate containers with the feed. Three replicates were maintained for each treatment. The feed was applied with the ethanolic sample by dipping the leaf in the ethanolic sample. The insects were observed in 24 hours (Fig. 1). The insecticidal activity of the extracts was assessed by the Diet incorporation method of bioassay against adult insects of *Spodoptera litura* [12].

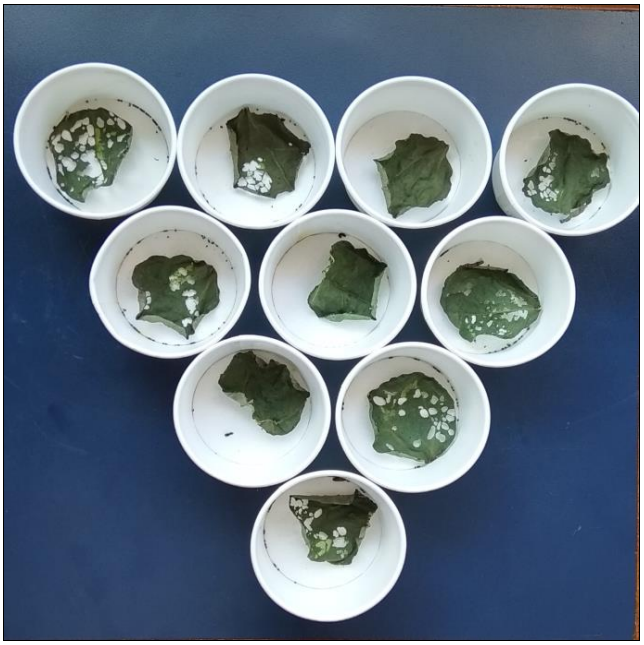

Fig 1: Diet incorporation method

Feed Deterrence Index

When the feed deterrence index was calculated on the bases of diet consumed on the respective treatment, it shows that the ethanolic extract of *Citrus aurantium* are feed deterrent to *Spodoptera litura.* With the increase in concentration of ethanolic extract an increased Feed deterrence index (FDI) was observed (Table 1).

The feed deterrence index was calculated for different concentrations of ethanolic extract of *Citrus aurantium* and plotted. This shows that at maximum concentration (40%), the feed deterrence index was (46.6%) maximum for the ethanolic sample of *Citrus aurantium* treated against *Spodoptera litura*.

Table 1: Feed deterrence index due to different concentration of ethanolic extract of *Citrus aurantium* after 24 hours of treatment.

Concentration (%)	Feed deterrence index
	24 Hours (%)
5	15.7±0.577
10	18.9±0.230
20	22.2±0.808
40	46.6±0.519
Control	0±0.577

Cosumption Rate

As the feed deterrence index is increasing with increasing concentration of the ethanolic extract, the consumption rate of *Spodoptera litura* is decreasing with increasing concentration of the ethanolic extract of *Citrus aurantium* (Table 2). The consumption rate of *Spodoptera litura* after treatment with the ethanolic extract of *Citrus aurantium* was calculated, tabulated and plotted. The results show that lower consumption rate, higher the efficiency of the insecticidal activity of ethanolic sample of *Citrus aurantium*. The consumption rate of *Spodoptera litura* was lower in the treated samples rather than the untreated samples. The maximum lower consumption rate (4.1%) was found to be with 40% ethanolic extract.

Table 2: Consumption and growth rate due to different concentration of ethanolic extract of *Citrus aurantium* treatment.

Conc. (%)	Consumption rate		Growth rate	
	24 Hours (%)	48Hours (%)	24 Hours (%)	48 Hours (%)
5	8.0±0.58	4.2±0.92	85±0.70	14±0.64
10	7.6±0.40	3.8±0.29	68±0.64	12±0.83
20	7.0±0.69	3.6±0.35	40±0.92	11±0.82
40	4.1±0.87	2.0±0.81	21±0.75	10±0.79
Control	11±0.81	1.0±0.58	91±1.09	19±0.84

Growth Rate

The growth rate (GR) of the insects results in the increase in the weight of the insects. Due to the insecticidal activity of ethanolic extract the weight of the insects were decreased (Table 2).

The growth rate was calculated for different concentrations of ethanolic extract of *Citrus aurantium* and plotted. This shows that at maximum concentration (40%) the growth rate was (21%) minimum for the ethanolic sample against the *Spodoptera litura*.

Efficiency of Conversion of Ingested Food

The efficiency of the conversion of the ingested food is the amount of food intake by the insects to survive. The table for the efficiency of the conversion of ingested food is given below (Table 3). The Efficiency of conversion of ingested food was measured for different concentrations of ethanolic

sample of *Citrus aurantium* and the graph was plotted between the concentration and the efficiency of conversion of ingested food. This reveals that at maximum concentration (40%) the Efficiency of conversion of ingested food was found to be (31.1%) minimum for the ethanolic sample against the *Spodoptera litura*.

Table 3: Efficiency of conversion and approximate digestibility of ingested food due to different concentration of ethanolic extract of *Citrus aurantium*

Conc (%)	Efficiency of conversion of ingested food		Approximate digestibility	
	24 Hours (%)	24 Hours (%)	48 Hours (%)	48 Hours (%)
5	68.1±0.84	90.23±0.93	88.80±0.51	31.0±0.721
10	53.8±0.18	89.37±0.69	82.46±0.83	22.4±0.831
20	45.4±0.81	88.37±0.66	70.45±0.80	13.9±0.490
40	31.1±0.91	86.60±0.35	61.36±0.82	10.7±0.496
Control	69.0±0.71	90.38±0.68	91.60±0.66	34.6±0.658

Approximate digestibility

The approximate digestibility (AD) is the ability of the each insect to digest the food. They are represented in terms of percentage of food digested inside the body of insects (Table 3).

The approximate digestibility was calculated for different concentrations of the ethanolic extracts of *Citrus aurantium* and plotted. Thus with the maximum concentration (40%) the approximate digestibility was observed to be (86.6%) minimum for the ethanolic sample treated against *Spodoptera litura.*

Efficiency of conversion of digested food

The efficiency of the conversion of the digested food is the amount of energy provided for the maintenance of physiological function to survive (Table 4).

Table 4: Efficiency of conversion of digested food due to different concentration of ethanolic extract of *Citrus aurantium* after 24 hours of treatment.

Concentration (%)	Efficiency of conversion of digested food	
	24 Hours (%)	48 Hours (%)
5	46.70±0.496	35.80±0.184
10	43.37±0.877	27.56±0.444
20	35.00±0.814	15.20±0.635
40	17.40±0.669	12.36±0.629
Control	51.60±0.658	38.20±0.548

The insecticidal activity of plant extract have been studied by many researchers and similar findings were reported [13, 14].

Molecular Property Analysis

All the 10 compounds identified from the ethanolic extract of *Citrus aurantium* through Gas chromatography- Mass spectrometry analysis were assessed for insecticidal activity. The molecular properties of the compounds were retrieved from PUBCHEM, SWISSADME and ZINC database. For the compounds to shows the insecticide-likeness, the molecular properties of the compounds needs to satisfy the Tice rule. The molecular weight of the below listed compounds from ethanolic extract of *Citrus aurantium* ranged from 117.15 g/mol to 284.48 g/mol. The number of rotatable bonds of the below listed compounds ranged from 0 to 16. The log P values of the below listed compounds ranged from -0.02 to 4.67 (Table 5). The molecular docking results also proved that the compounds isolated from the ethanolic extract were interacting against the Acetylcholinesterase and carboxyl-esterase enzyme with the least energy value of -79.6 and -76.7 respectively and the results so obtained showed that the ethanolic extracts of *Citrus aurantium* acts as insect repellent against *Spodoptera litura.* From the present study higher concentration (40%) of the ethanolic extract was effective when compared with low concentrations of the Ethanolic extract of 5%, but the seed germination rate was higher in the low concentration (5% and 10%) so, the 10% concentration is better with both the insecticidal activity and growth regulatory effects. These ethanolic extract of *Citrus aurantium* having compounds that possess insecticidal activity against *Spodoptera litura* (Tobacco caterpillar). The same findings were observed in the study, the toxicity of isoalantolactone on rice weevil was dependent on dosage amount. It showed the strong phytotoxic effects on seed germination and seedling growth at a concentration of 500 µg $ml^{-1}$for the duration of 60 hours [15].

Table 5: Compounds in ethanolic extract of *Citrus aurantium.*

S.No	Name of the compound	Molecular weight (g/mol)	Log P	No. of H bond donars	No. of H bond acceptors	No. of Rotatable bonds
1	1,2,3 propanetriol,1-Acetate	134.13	-0.02	2	4	3
2	Isoamyl nitrite	117.15	0.83	0	3	4
3	2,3,5,6- tetrafluroanisole	166.07	3.21	1	5	0
4	Octadecanoic acid	284.48	4.67	1	2	16
5	2-H-1-Benzopyran-2-one, 5,7-dimethoxy	178.14	0.45	2	4	0
6	Hexadecanoic acid,ethyl ester	284.48	4.67	0	2	16
7	2-Nonen-1-ol,2-methyl-	156.26	2.70	1	1	6
8	Cyclohexaneethanol	128.21	1.83	1	1	2
9	E-11-Hexadecanoic acid,ethyl ester	254.41	4.09	1	2	13
10	Heptanoic acid,2-methyl, methyl ester	158.24	2.28	0	2	6

Shortlisted Compounds Based on Tice Rule Comparison

There are 4 compounds from the ethanolic extract of *Citrus aurantium* possess the Molecular weight within 150 to 500 g/mol. Similarly, the number of rotatable bonds was below 12. The Log P values of the compounds were within 0 to 5. The Number of hydrogen bond donors was less than or equal to 2. The Number of hydrogen bond acceptors was within 1 to 8. The purpose of docking studies is to find the interaction of small molecules like drugs with their protein targets. The compounds that satisfy the Tice rule were docked with the enzyme target in *Spodoptera litura* such as Acetylcholinesterase and carboxyl esterase whose structure was retrieved through PDB database. The PDB (Protein Data Bank) ID for acetylcholinesterase and carboxylesterase of *Spodoptera litura* are 1c7i and 2z7f respectively. The software used for docking studies is iGEMDOCKv2.1 (Table 6) (Fig.2 and Fig.3).

Table 6: Energy values (kcal/mol) obtained from docking of compounds in ethanolic extract of *Citrus aurantium* with the Acetylcholinesterase (AChE) and Carboxylesterase (CE)

S.No	Name of the compound	AChE	CE
1	Heptanoic acid,2-methyl, methyl ester	-79.6	-76.7
2	2-Nonen-1-ol,2-methyl-	-62.7	-73.1
3	2-H-1-Benzopyran-2-one,5,7-dimethoxy	-57.5	-52.9
4	2,3,5,6- tetrafluroanisole	-21.0	-29.8

The docking of the ethanolic sample compounds with the Acetylcholinesterase possess the energy of -79.6 Kcal/mol with Heptanoic acid, 2-methyl, methyl ester and -21.0 Kcal/mol with 2, 3, 5, 6- tetrafluroanisole. The compound with the least energy is considered to be the best ligand to the target. The docking of the ethanolic sample compounds with the Carboxylesterase possess the energy of -76.7Kcal/mol with Heptanoic acid, 2-methyl, methyl ester and -29.8 Kcal/mol with 2, 3, 5, 6- tetrafluroanisole.

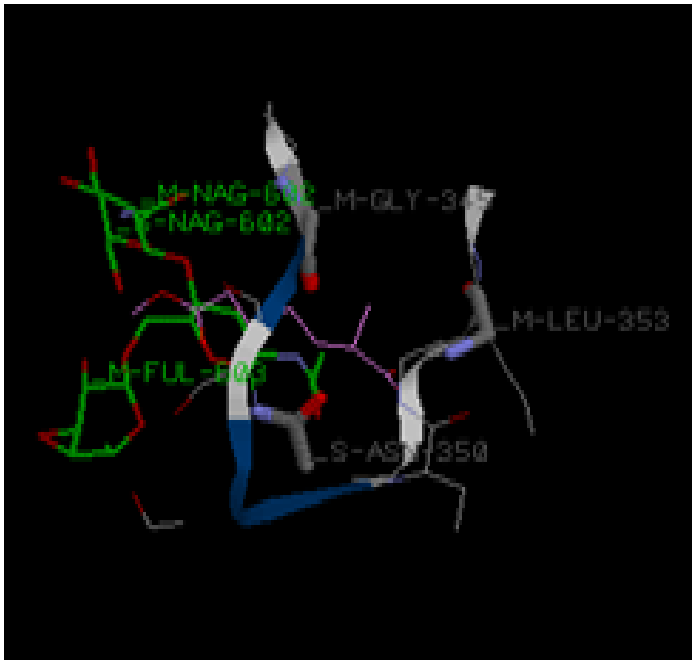

Fig 2: Compounds of *Citrus aurantium* with Acetylcholinesterase

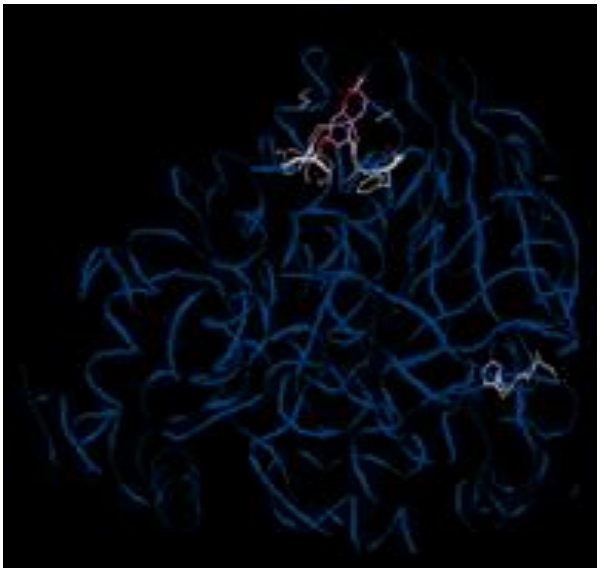

Fig 3: Compounds of *Citrus aurantium* with Carboxylesterase

Phytotoxicity Assessment of *Citrus aurantium* Extract

In the Phytotoxicity assay two different parameters were determined, namely root length and seed germination percent. The seeds root length was measured after five days period of dark treatment. Etiolated seedlings having long radical of different lengths were measured, showing differences in time of germination of seedlings with their cotyledons attached. The overall test period was 15 days. The percentage germination of the seeds in the different parameters like germination rate, speed of emergence and shoot length were significantly different from each other. The seeds treated with *Citrus aurantium* ethanolic extract showed maximum germination rate at 10% concentration. The germination rate was lower at higher concentration and acts as inhibitor whereas, at lower concentration it acts as the growth promoter (Table 7) (Fig.4). The seed germination rate of the red cow peas were assessed. The rate of germination was measured to be 20% at 10% concentration. Thus indicated that the germination rate increased with the decrease in the concentration.

Table 7: Speed of emergence and Germination rate percent of red cow peas

Conc (%)	Speed of emergence (%)		Germination rate (%)	
	Control	*Citrus aurantium* extract	Control	*Citrus aurantium* extract
5	0	33.33	0	30
10	0	50	0	20
20	0	100	0	10
40	0	0	0	0

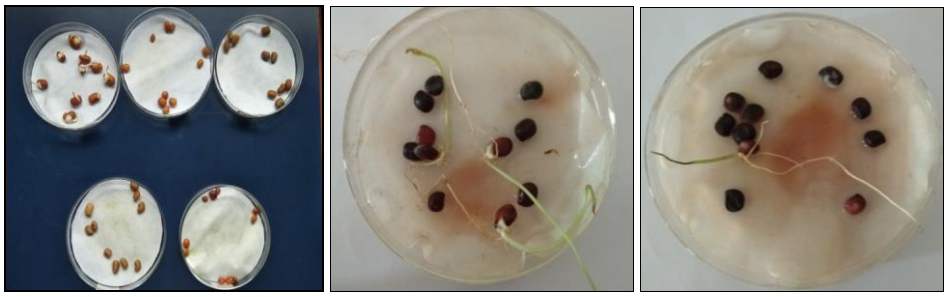

Fig 4: Seeds at different concentration (control, 5%, 10%, 20% and 40%), At 5% concentration and At 10% concentration

Conclusion

This study involves the characterization and insecticidal effect of bio-active compounds on pest enzyme Acetylcholinesterase via Molecular Docking using Bioinformatics tool. From the molecular docking studies the compound Heptanoic acid, 2-methyl, methyl ester have best interaction with Acetylcholinesterase and Carboxylesterase with least energy values. Hence the compounds present in prepared ethanolic extract shows insecticidal activity against *Spodoptera litura*. Scale up of the production shall also be considered for vast application of biopesticides using field trials. At higher concentration the ethanolic extract of *Citrus aurantium* shows potent insecticidal activity against *Spodoptera litura.* The ethanolic extract of *Citrus aurantium* may be considered to use as a novel potent insecticide against *Spodoptera litura* without affecting the plants and the environment.

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