
Results

CHAPTER III

RESULTS

3.1 PHYTOCHEMICAL SCREENING OF SECONDARY METABOLITES IN DIFFERENT SOLVENTS OF *SALVINIA MOLESTA*

Phytochemical screening of *S.molesta* leaf extracts in five different solvents *viz.*, aqueous, ethanol, acetone, chloroform and petroleum ether, confirmed the presence of tannins, saponins, quinones, terpenoids, steroids, flavonoids, phenols and alkaloids in different qualitative ranges. Results are recorded as positive, strong positive and negative values. The double positive confirmed the strong presence of phytochemicals whereas single positive sign depicts the presence of phytochemicals in moderate levels. The negative sign depicts the absence of phytochemical compounds (Table 2).

The ethanolic and acetone extract of *Salvinia molesta* showed strong presence of all major phytoconstituents with varying strength (Fig. 12). Whereas absence of saponins and alkaloids were observed in chloroform and petroleum ether solvents. Quantification of phytochemicals will reveal more in-depth information about their availability in each solvent. Thus all the solvent extractions with positive ranges was subjected to quantification assay to measure the concentration of significant phytochemicals. The quantification

parameters were performed for all the positive and double positive values and negatives values are not quantified and hence saponins and alkaloids in chloroform and petroleum ether solvents were not analysed.

3.2 QUANTIFICATION OF SIGNIFICANT PHYTOCHEMICALS

Among the eight phytoconstituents, phenol, flavonoid, alkaloid, tannin and saponins showed strong presence depicting the pharmaceutical property of the extracts. Hence all the five secondary metabolites in different extracts are quantified further to assay the best extraction possessing significant levels.

Total phenolics content in the aqueous leaf extract of *S.molesta* was 90.3 mg of Gallic Acid Equivalents(GAE)/g. Whereas the ethanolic extract measured 98.4 mg (GAE)/g. Acetone, chloroform and petroleum ether extracts contained 86.9, 53.4 and 58.4mg (GAE)/g respectively.

Total flavonoid content in the aqueous leaf extract of *S.molesta* was 6.14mg Quercetin Equivalents (QE)/g. Whereas the ethanolic extract measured 10.89 (QE)/g. Acetone, chloroform and petroleum ether extracts contained 7.11, 5.6 and 7.1(QE)/g respectively.

Total alkaloid content in the aqueous leaf extract of *S.molesta* was 81.0mg/g. The ethanolic extract measured 90.8mg/g. Whereas Acetone, and petroleum ether extracts contained 73.4 and 63.1mg/g respectively.

Total tannin content in the aqueous leaf extract of *S.molesta* was 5.6mg /TAE/g. Whereas the ethanolic extract measured 12.5mg/g. Acetone, chloroform and petroleum ether extracts contained 8.3, 5.2 and 4.26 mg /TAE/g respectively.

Total saponin content in the aqueous leaf extract of *S.molesta* was 37mg/g. The ethanolic extract measured 42mg/g concentration. Acetone and petroleum ether extracts contained 33 and 24mg/g respectively.

On quantification it was studied that the ethanolic extract possessed significant quantity of major phytochemicals and hence ethanolic leaf extract of *S.molesta* was further utilised for animal study to find the efficacy of *S.molesta* as a potent medicinal agent (Table 3).

3.3 ANTIOXIDANT ACTIVITY OF *S.MOLESTA* LEAF EXTRACTS

3.3.1 QUALITATIVE ANALYSIS

Qualitative analysis of antioxidant activity of *S.molesta* leaf extracts were performed using BHT (Butylated Hydroxy Toluene) as standard and relevant discoloration was observed. Samples which changed from purple to yellow and to pale pink were considered as strong and weak positive respectively. Ethanol and acetone extracts revealed the presence of significant levels of antioxidant with double positive indication. Aqueous extract showed single positive range indicating a weak response. Whereas, chloroform and

petroleum ether showed semi –positive indication. Still all the antioxidant positive samples were subjected for further quantitative analysis (Table 4).

3.3.2 QUANTITATIVE ANALYSIS

All the five different solvents *viz.,* Aqueous ethanol, acetone, chloroform ,and petroleum ether extracts of *S.molesta* leaves showed different ranges of antioxidant activity when estimated for free radical scavenging activity using diphenyl-2-picryl hydrazyl (DPPH) assay.

Among five different solvent extracts the aqueous extract exhibited 68.3%, whereas acetone, chloroform and petroleum ether exhibited 65%, 68% and 58 % antioxidant ability when compared to BHT standard. The ethanolic extract of *S.molesta* was recorded as the most effective in DPPH radical scavenging activity of 90.3% comparatively with BHT as control (Fig.13).

3.4 ANTIBACTERIAL ACTIVITY OF *SALVINIA MOLESTA* LEAF EXTRACT

As ethanolic leaf extract of *S.molesta* showed the maximum antioxidant activity, the same extract was further utilised to assay the antibacterial activity against selective gram negative and gram positive bacterial cultures. Antibacterial assay performed by disc diffusion method using varying concentration of ethanolic extracts of *S.molesta* showed maximum activity against test pathogens (Fig. 14).

According to the data obtained, it was recorded that the maximum zone of inhibition by ethanolic leaf extract of *S. molesta* against *A.hydrophila* was 30.12 ± 0.51 mm at 30mg/ml concentration, whereas 28.45 ± 0.32 mm zone of inhibition was recorded for *P.aeruginosa* at 30mg/ml (Fig. 15).

Following this maximum range, a moderate activity against *B.cereus* and *S.aureus* with 25.14 ± 0.32 mm and 19.13 ± 0.64 mm respectively were recorded and a least activity against *B.subtilis* of 10.12 ± 0.41mm and *E.coli* of 9.12 ± 0.46 mm was exhibited at 30 mg/ml concentration (Table 5).

By the obtained values it was confirmed that the ethanolic extract of *S. molesta* leaves had significant antibacterial property against major fish disease causing pathogens such as *A.hydrophila* and *P.aeruginosa*. Hence further bacterial challenges were performed to assay the antioxidant and immunomodulatory effect of *S. molesta* leaves in the animal model *Oziotelphusa senex senex.*

3.5 EXTRACTION OF THE ACTIVE COMPOUND FROM ETHANOLIC LEAF EXTRACT OF *SALVINIA MOLESTA*

The concentrated ethanolic extract was separated and analysed by column chromatography technique (Fig.16). Totally nine fractions were collected until a single crystal was obtained. Fraction IV was taken as the best fraction according to standard formula as it contained maximum of 94%

antioxidant activity with least reading of 0.06 . Since fraction IV possessed highest antioxidant activity it was further subjected for characterisation studies such as GC-MS, NMR, FT-IR to analyse the active compound present in the fraction.

3.6 GC-MS ANALYSIS OF THE ETHANOLIC LEAF EXTRACT OF *S. MOLESTA*

The GC-MS results of *S.molesta* extract confirmed the presence of major components highly responsible for its antioxidant, antibacterial, antiseptic and immunostimulant properties (Fig.17 and Table 6).

At retention time 13.921, the compound apiol appeared with molecular formula $C_{12}H_{14}O_4$. At retention time 17.160, the compound hexadecanoic acid, methyl ester appeared with molecular formula $C_{17}H_{34}O_2$. At retention time 18.846, compound Hexadecatrienoic acid, methyl ester appeared with molecular formula $C_{17}H_{28}O_2$.At retention time 19.091, compound heptadecanoic acid, 16-methyl-, methyl ester appeared with molecular formula $C_{19}H_{38}O_2$.

3.6.1 THIN LAYER CHROMATOGRAPHY -TLC

The concentrated ethanolic extract was subjected to TLC analysis using a solvent system n-butanol: acetic acid: water (2:1:1). The metabolite of *S.molesta* extract eluted with solvent system produced two and three bands

respectively when the chromatogram was visualized under iodine vapour. The active compound with R_f value of 0.59 cm were obtained (Fig.18).

3.6.2 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY - NMR

In the ^1H NMR spectrum, all the NMR signals appeared from 0.65 to 4.53 (δ, ppm) and there was no signals observed at 6-8 ppm. This confirmed that the signals in the NMR spectrum corresponded to an aliphatic compound. Signals for aliphatic groups –CH, $-CH_2$, and $–CH_3$ appeared between 0-2 ppm. Further, the signals for methylene ($-CH_2$) proton adjacent to C=O group was observed to appear between 2-3 ppm and methyl or methylene ($-CH_2$) protons in the ether moiety appeared at 3-4 ppm. In the ^1H NMR spectrum, the signals were observed at 2-4 ppm confirming the presence of aliphatic ester compound. Furthermore, (80 %) most of the signals observed at 0-2 ppm in the NMR spectrum confirmed the presence of the fatty acids groups (Fig 19 and 20). Also, merging of signals at every chemical shift range suggested that there is a possibility for more than one compound in the sample. From all the above observations ^1H NMR spectrum confirmed the presence of aliphatic fatty acid methyl ester compound (FAME).

3.6.3 FOURIER TRANSFORM INFRARED SPECTROSCOPY -FTIR

In the FT-IR spectrum the absorption peaks at 1642 and 1374 cm^{-1} are observed for C=O and C-O stretching, respectively, in the ester moiety [C(=O)

OCH3]. Furthermore, a strong absorption at 2937 cm^{-1} is observed which corresponds to the aliphatic C-H stretching frequency. All the observed peaks in the FT-IR spectrum of compound support the formation of aliphatic ester compound (Fig.21).

3.7 BIOCHEMICAL CHARACTERISATION OF TEST PATHOGENS

The procured bacterial cultures of *A.hydrophila and P.aeruginosa* showed positive results for Oxidase, Catalase, Indole, Citrate and Nitrate reduction test. The relevant colour changes were observed and recorded accordingly (Table 7).The characterisation results confirmed the identification parameters of both the cultures and thus used for further bacterial studies.

3.8 PHYSICAL CHANGES IN EXPERIMENTAL ANIMALS

WEIGHT

The weight of male and female crabs were measured in gms . Before treatment the weight of male crabs was 6.5±0.368 gms, whereas after treatment there was no significant change in weight except for few groups which gained few grams of weight of 1.4 ± 0.163 gms. Similarly before treatment weight of female crabs was 7.1 ± 0.586 gms and there was variation of weight in treated groups with gain in weight of 1.2 ± 0.37gms.

MORPHOLOGY

The appearance and morphological variations were studied for both male and_female crabs. The infected groups at 96 hrs duration started to develop white patches on the skin layer, whereas in treated groups the white patches on the skin layer significantly reduced during the treatment period.

MOVEMENT AND RESPONSE

After acclimatization the movement in aquatic environment and responses to feed and other climatic conditions were studied. The female crabs were more active than male groups before treatment. But both the groups remained inactive during infection period.

3.9 ACUTE TOXICITY STUDY OF PATHOGENS

3.9.1 Pathogenicity of *A.hydrophila* in *O.senex senex* -Lethal Dose (LD_{50})

At 96 hrs of infection with 10^8 CFU/0.1ml of *A.hydrophila* culture the observed mortality rates was 60% whereas when injected with 10^7 and 10^6 concentrations the mortality rate raised to 80% and 100% respectively. Hence 10^9 CFU/0.1ml was selected for bacterial challenge as the mortality rates diminished notably at this dosage concentration.

3.9.2 Pathogenicity of *P.aeruginosa* in *O.senex senex* -Lethal Dose (LD_{50})

At 96 hrs of infection with 10^8 CFU/0.1ml of *P.aeruginosa* culture the observed mortality rates was 48%, whereas when injected with 10^5 and 10^6 concentrations the mortality rate raised to 70% and 90% respectively. Hence 10^7 CFU /0.1ml was selected for bacterial challenge as the mortality rates diminished significantly at this concentration .

3.10 TOXICITY TEST BY ETHANOLIC LEAF EXTRACT OF *S.MOLESTA* ON *O.SENEX SENEX* (LC_{50})

Among each group of animals when administered with different concentrations of ethanolic leaf extracts of *Salvinia molesta* dissolved in saline solution, the crabs injected with 0.20mg concentration died immediately, whereas 0.15mg concentration was also lethal to crab groups .In 0.10 mg in 0.1ml concentration, none of the crabs died and there was no mortality in the groups injected with 0.1mg of *Salvinia molesta.* Hence 0.1mg/10ml *S.molesta* concentration was used for further studies.

3.11 BIOCHEMICAL PARAMETERS

3.11.1 TOTAL CARBOHYDRATE LEVELS

Total carbohydrate levels in hemolymph of *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The carbohydrate content in hemolymph of control male crabs was measured as 35.4 ± 0.321 mg/dl.

Group B: The carbohydrate content in hemolymph of control female crab was 29.3 ± 0.235mg/dl.

Infected Groups

Group C: Whereas in male groups infected with *A.hydrophila* at 96hrs, the levels of carbohydrates decreased and a relatively low concentration of 21.1 ± 0.461 was recorded.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the levels of carbohydrates levels decreased to 19.2 ± 0.354 mg/dl.

Treated Groups

Group E-E_3: *S.molesta* treated male groups were assayed for total carbohydrate levels and the values recorded were 22.2 ± 0.357 at 24 hrs (Group E) and there was an increase to 29.3 ± 0.416 mg/dl (Group E_3) at 96 hrs of treatment time .

This showed that male crabs were able to respond to the pharmacological properties of *S.molesta*. On infection there was a significant decrease ($P<0.05$) in carbohydrate level of group-C when compared to group-A. The treated group-E_3 exhibited significant increase ($P<0.05$) in carbohydrates level when compared with group-A.

Group F-F_3: Similar changes were observed in female groups where there was a significant decrease ($P<0.05$) in carbohydrates level in infected group-D and a significant increase ($P<0.05$) of carbohydrate levels in treated groups F-F_3 from 21.2 ± 0.324 mg/dl to 27.4 ± 0.265 mg/dl at 96 hrs. (Table 8 and Fig.22).

Total carbohydrate levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The carbohydrate content in hemolymph of control male crabs was measured as 32.3 ± 0.318mg/dl.

Group B: The carbohydrate content in hemolymph of control female crabs was 26.4 ± 0.336mg/dl.

Infected Groups

Group C: In *P.aeruginosa* infected male crabs at 96hrs the levels of carbohydrates decreased and relatively a lower concentration of 19.1 ± 0.413 mg/dl was recorded.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the levels of carbohydrates decreased and a low concentration of 18.3 ± 0.254 mg/dl was recorded.

Treated Groups

Group E-E_3: *S.molesta* treated groups were assayed for total carbohydrate levels and the values recorded ranged from 21.1 ± 0.147 mg/dl to 28.3 ± 0.316 mg/dl for 24, 48, 72 and 96 hrs respectively.

This showed that male crabs were able to respond to the pharmacological properties of *S.molesta*. On infection there was a significant decrease ($P<0.05$) in carbohydrates level of group-C when compared to group-A. The treated group-E_3 exhibited significant increase ($P<0.05$) in carbhohydrates level when compared with group-A.

Group F-F_3: Similar changes were observed in female groups where the concentration of carbohydrates decreased significantly ($P<0.05$) in group-D on infection and showed significant increase in treated groups and retained to closer physiological values *viz.*, from 20.3 ± 0.422 mg/dl to 27.4 ± 0.211 mg/dl at 96 hrs of treatment time.

The carbohydrate content retained to normal physiological values of male and female crabs elucidating the therapeutic role of ethanolic leaf extract of *S.molesta* (Table 9 and Fig.23).

3.11.2 TOTAL PROTEIN LEVELS

Total protein levels in hemolymph of *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The protein levels in hemolymph of control male crabs was measured as 6.8 ± 0.322gms/dl.

Group B: The protein levels in hemolymph of control female crabs were 5.9 ± 0.212 gms/dl.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96hrs the levels of protein decreased to 4.1 ± 0.423 gms/dl.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the levels of protein decreased and a lower concentration of 2.9 ± 0.321 gms/dl was recorded.

Treated Groups

Group E - E_3: *S.molesta* treated groups were assayed for the total protein levels and the values recorded were 3.2 ± 0.132 gms/dl to 6.91 ± 0.417 gms/dl from 24 hrs to 96 hrs of treatment time.

There was a significant decrease ($P<0.05$) of protein levels in infected group-C. Whereas, in the treated group-E_3 the protein levels were restored on par to the control group-A. The values showed that male crabs were able to respond to the pharmacological properties of *S.molesta* extract as the protein contents of group-E_3 was closer when compared to control group-A.

Group F-F_3 : Similar changes were observed in female groups with significant decrease ($P<0.05$) of protein levels in infected groups , whereas the concentration of protein increased closer to control group-A on treatment with

S.molesta leaf extract *viz.*, from 3.1 ± 0.142 gms/dl to 5.8 ± 0.311gms/dl at 96 hrs of treatment (Table 10 and Fig.24).

Total protein levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The protein levels in hemolymph of control male crabs was measured as 7.1 ± 0.321gms/dl.

Group B: The protein levels in hemolymph of control female crabs was 6.1 ± 0.251gms/dl.

Infected Groups

Group C: Whereas on infecting with *P. aeruginosa* at 96hrs the levels of protein decreased to 4.8 ± 0.413gms/dl.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the levels of protein decreased and a low concentration of 3.1 ± 0.211gms/dl was recorded.

Treated Groups

Group E-E_3: *S.molesta* treated groups were assayed for the total protein levels and the values recorded was 5.6 ± 0.336 gms/dl to 6.72 ± 0.431gms/dl at 96 hrs of treatment time.

There was significant decrease ($P<0.05$) in protein content on infected group-C when compared to group-A. Whereas there was an increase of protein content observed in group-E_3 when compared to group-A. The treated group-E_3 values was closer to control value of group-A. This showed that male crabs was able to respond to the pharmacological properties of ethanolic leaf extract of *S.molesta.*

Group F-F_3: Similar changes were observed in female groups where the concentration of protein increased and retained to closer control values *viz.*, from 4.2 ± 0.225 gms/dl to 5.9 ± 0.351 gms/dl at 96 hrs of treatment.

There was significant decrease ($P<0.05$) in protein content on infected group-D when compared to group-B. All the obtained values of group-F-F_3 were significant ($P<0.05$) when compared to group B. The protein content of group-F_3 at 96hrs of treatment was observed to be closer to control values indicating the therapeutic role of *S.molesta* (Table 11 and Fig.25).

3.11.3 TOTAL CHOLESTEROL LEVELS

Total cholesterol levels in hemolymph of *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The total cholesterol content in hemolymph of control (untreated)male crabs was measured as 23.9 ± 0.331mg/dl.

Group B: The total cholesterol content in hemolymph of control female crabs levels were recorded as 25.8 ± 0.960 mg/dl.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96hrs the levels of total cholesterol decreased and a low concentration of 12.5 ± 0.898 was recorded.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the levels of total cholesterol decreased and a low concentration of 15.2 ± 0.314was recorded.

Treated Group E-E_3: *S.molesta* treated groups were assayed for the total cholesterol levels and the values recorded was 17.9 ± 0.215 to 21.3 ± 0.335 mg/dl at 24 hrs to 96 hrs of treatment time respectively.

Group F-F_3: Similar changes were observed in female groups were the concentration of total cholesterol increased and reached closer to control values *viz.,* from 19.1 ± 0.116 to 23.4 ± 0.235 mg/dl.

There was significant decrease ($P<0.05$) in cholesterol level in infected groups C and D when compared to the control groups-A and B. Whereas in treated groups-E_3 and group-F_3 there was significant a increase ($P<0.05$) in cholesterol level retaining the physiological values in hemolymph of the crab groups at 96 hrs of treatment (Table 12 and Fig.26).

Total cholesterol levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The total cholesterol content in hemolymph of control male crabs was measured as 25.6 ± 0.216mg/dl.

Group B: The total cholesterol content in hemolymph of control female crabs was measured as 27.4 ± 0.312 mg/dl.

Infected Groups

Group C: On infecting with *P.aeruginosa* at 96hrs the levels of total cholesterol levels decreased and a low concentration of 14.6 ± 0.331 mg/dl was recorded.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the levels of total cholesterol decreased and a low concentration of 16.1 ± 0.343 mg/dl was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for the total cholesterol levels and the values recorded were 16.7 ± 0.241 to 26.8 ± 0.231 mg/dl at 96 hrs of treatment time.

Group F-F_3: Similar changes were observed in female groups were the concentration of total cholesterol was increased and retained to closer control values *viz.,* from 17.3 ± 0.226 mg/dl to 25.5 ± 0.461mg/dl (Table 13 and Fig.27).

In both male and female groups there was significant decrease ($P<0.05$) in cholesterol levels were observed at 96 hrs of infection. Whereas significant ($P<0.05$) increase of cholesterol were observed group-E_1 and F_1. The cholesterol levels were retained to closer control values at group E_3 and F_3 illustrating the pharmacological properties of ethanolic leaf extract of *S.molesta.*

3.11.4 TOTAL MICROPROTEIN LEVELS

Microprotein levels in hemolymph of *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The microprotein content in hemolymph of control male crabs was measured as 163.5±0.432mg/dl.

Group B: The microprotein content in hemolymph of control female crabs was 175.2 ± 0.453mg/dl.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96hrs the levels of micro protein decreased and a low concentration of 91.6 ± 0.325was recorded.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the levels of micro protein decreased and a low concentration of 97.2 ± 0.328 was recorded.

Treated Groups

Group E-E_3: *S.molesta* treated groups were analysed for the micro protein levels and the values recorded were 112.3 ± 0.239 to 161 ± 0.212 mg/dl at 96 hrs of treatment time.

This shows that male crabs were able to respond to the pharmacologically significant properties of *S.molesta* and hence the microprotein was restored on par to the control groups.

Group F-F_3: Similar changes were observed in female groups where the concentration of micro protein was increased and retained to closer control values *viz.,* from 121.3 ± 0.369 to 176.3 ± 0.424 mg/dl.

In both male and female crabs there was a significant decrease ($P<0.05$) of microproteins level in infected groups when compared to control group-A and B. All the treated groups showed significant increase ($P<0.05$) in micro protein levels at different time intervals *viz*.,24 hrs,48 hrs,72 hrs respectively and at 96 hrs of treatment the microproteins retained to closer control values of control groups (Table 14 and Fig.28).

Microprotein levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The micro protein content in hemolymph of control male crabs was measured as 169.5 ± 0.322 mg/dl.

Group B: The microprotein content in hemolymph of control female crabs was 171.2 ± 0.213 mg/dl.

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96hrs the levels of micro protein decreased and a lower concentration of 97.7 ± 0.352 was recorded.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the levels of micro protein decreased and a low concentration of 92.4 ± 0.221 was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for the micro protein levels and the values recorded were 120.3 ± 0.235 mg/dl to 165.1 ± 0.166 mg/dl from 24hrs to 96 hrs of treatment time.

Group F-F_3: Similar changes were observed in female groups where the concentration of micro protein was increased and retained to closer control values *viz.,* from 128.2 ± 0.389 mg/dl to 163.4 ± 0.238mg/dl.

There was an significant decrease ($P<0.05$) in microprotein levels in group-C and D when compared to group-A and group-B. Also significant increase ($P<0.05$) of microprotein levels were obtained in all the treated groups elucidating the role of *S.molesta* in retaining the microprotein levels on par to the control groups (Table 15 and Fig.29).

3.12 IMMUNOLOGICAL PARAMETERS

3.12.1 TOTAL HAEMOCYTE COUNT (THC)

THC in hemolymph of *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The total haemocyte count content in hemolymph of control male crabs was measured as 4231 ± 31.83mg/dl.

Group B: The total haemocyte content in hemolymph of control female crabs was measured as 3728 ± 27.83mg/dl.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96hrs the levels of total haemocyte decreased and a low concentration of 6341 ± 28.61was recorded.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the levels of total haemocyte count decreased and a low concentration of 6039 ± 26.41was recorded.

Treated Groups

Group E-E_3: *S.molesta* treated groups were assayed for the total haemocyte count levels and the values recorded were 6041 ± 24.31 to 4604 ± 34.92 mg/dl at 96 hrs of treatment time.

Group F-F_3: In female groups the concentration of total haemocyte count decreased and reached the control values by 96 hrs of treatment *viz.*, from 5389 ± 24.3 to 3556 ± 22.14mg/dl (Table 16 and Fig.30).

There was a significant decrease ($P<0.05$) of THC in infected groups-C and group-D when compared to control groups-A and group-B. Whereas there was an gradual increase of THC in hemolymph of both male and female treated groups. The values obtained at group E_3 and group-F_3 were closer to the range of control group values. This showed that male and female crabs were able to respond to the pharmacological properties of *S.molesta* and hence the total haemocyte count were restored on par to the control groups-A and group-B.

THC in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The total haemocyte count content in hemolymph of control male crabs was measured as 4232 ± 36.93mg/dl.

Group B: The total haemocyte count content in hemolymph of control female crabs was 3982 ± 42.15 mg/dl.

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96hrs the levels of total haemocyte count decreased and a low concentration of 6451 ± 58.62was recorded.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the levels of total haemocyte count decreased and a low concentration of 6128 ± 36.49was recorded.

Treated Groups

Group E-E_3: *S.molesta* treated groups were assayed for the total haemocyte count levels and the values recorded were 5421 ± 23.11 to 4032 ± 21.31mg/dl from 24hrs to 96 hrs of treatment time.

Group F-F_3: Similar changes were observed in female groups where the concentration of total haemocyte count increased on treatment and retained to closer control values *viz.,* from 5321 ± 46.13 to 3543 ± 28.17mg/dl.

In infected groups-C and D there was a significant decrease ($P<0.05$) in THC levels when compared to group A, whereas there was a significant increase ($P<0.05$) in THC levels at different time intervals and the levels of

THC in treated groups retained to closer control values at 96hrs (Table 17 and Fig.31).

3.12.2 DIFFERENTIAL HEMOCYTE COUNT

DHC levels in hemolymph of *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The levels of large granular cells, small granular cells and hyaline cells in control group was 23 ± 0.31, 51 ± 0.21 and 28 ± 0.23 respectively.

Group B: The levels of large granular cells, small granular cells and hyaline cells in control group was 25 ± 0.43, 42 ± 0.93 and 20 ± 0.36 respectively.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96hrs the levels of large granular cells, small granular cells in control group increased to 25 ± 0.65, 70 ± 0.32 respectively but hyaline cells count decreased to 15 ± 0.36.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the levels of large granular cells, small granular cells in control group increased as 32 ± 0.19 and 52 ± 0.34 respectively but hyaline cells count decreased to 12 ± 0.65.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for the differential hemocyte count and the values recorded for large granular cells ranged from 24 ± 0.19 to 21 ± 0.73, small granular cells ranged from 64 ± 0.65 to 53 ± 0.21 and hyaline cells ranged from 10 ± 0.26 to 24 ± 0.46 respectively.

Group F-F_3: The *S.molesta* treated groups were assayed for the differential hemocyte count and the values recorded for large granular cells ranged from 28 ± 0.92 to 23 ± 0.54, small granular cells ranged from 50 ± 0.32 to 45 ± 0.46 and hyaline cells ranged from 15 ± 0.92 to 23 ± 0.44 respectively .

There was a significant increase ($P<0.05$) of LGC, SGC and significant decrease ($P<0.05$) of HC in all the infected groups C and D when compared to group A and B. The values at 96 hrs for treated groups were closer to control group-A and B mentioning the therapeutic role of ethanolic leaf extract of *S.molesta* (Table 18 and Fig. 32).

DHC levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The levels of large granular cells, small granular cells and hyaline cells in control group was 24 ± 0.92, 54 ± 0.31and 26 ± 0.92 respectively.

Group B: The levels of large granular cells , small granular cells and hyaline cells in control group was 36 ± 0.33, 45 ± 0.23 and 22 ± 0.46 respectively.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96 hrs the levels of large granular cells, small granular cells in control group increased to 29 ± 0.45, 73 ± 0.33 respectively but hyaline cells count decreased to 7 ± 0.13

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the levels of large granular cells, small granular cells in control group increased to 41 ± 0.29,53 ± 0.48 respectively but hyaline cells count decreased to 14 ± 0.45.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for the differential hemocyte count and the values recorded for large granular cells ranged from 28 ± 0.21 to 25 ± 0.53, small granular cells ranged from 65 ± 0.66 to 45 ± 0.48 and hyaline cells ranged from 11 ± 0.36 to 23 ± 0.33 respectively.

Group F-F_3: The *S.molesta* treated groups were assayed for the differential hemocyte count and the values recorded for large granular cells ranged from 40±0.38 to 35±0.42, small granular cells ranged from 51 ± 0.96 to 44 ± 0.36 and hyaline cells ranged from 18 ± 0.36 to 24 ± 0.53 respectively.

In both male and female groups, there was a significant increase ($P<0.05$) of LGC, SGC and significant decrease ($P<0.05$) of HC in infected groups-C and group-D when compared to group-A and B. The values of DHC observed at 96 hrs for treated groups were closer to control group-A and B (Table 19 and Fig.33).

3.12.3 PROPHENOL OXIDASE ACTIVITY

ProPo levels in hemolymph of *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The prophenol oxidase activity in hemolymph of control male crabs was measured as 0.798 ± 0.021min/mg/protein

Group B: The prophenol oxidase content in hemolymph of control female crabs was 0.898 ± 0.016 min/mg/protein

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96 hrs the prophenol oxidase activity decreased and a lower concentration of 0.321 ± 0.036 min/mg/protein was recorded.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the prophenol oxidase activity decreased and a low concentration of 0.491 ± 0.026min/mg/protein was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for the prophenol oxidase activity and the values recorded was 0.411 ± 0.045 to 0.801 ± 0.02 min/mg/protein

Group F-F_3: Similar changes were observed in female groups where the concentration of prophenol oxidase activity was increased and retained to closer control values *viz.*, from 0.542 ± 0.032 to 0.853 ± 0.012min/mg/protein.

In both the infected group-C and group-D there was a significant decrease ($P<0.05$) in prophenol oxidase levels when compared to group-A. The values obtained in treated groups-E_3 and F_3 were closer to the control group-A values and hence it illustrates that treated groups were able to respond to the pharmacological properties of ethanolic leaf extract of *S.molesta* (Table 20 and Fig. 34).

ProPo levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The prophenol oxidase activity in hemolymph of control male crabs was measured as 0.818 ± 0.034 min/mg/protein

Group B: The prophenol oxidase content in hemolymph of control female crabs was measured as 0.768 ± 0.046 min/mg/protein.

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96hrs the prophenol oxidase activity decreased and a low concentration of 0.318 ± 0.083 min/mg/protein was recorded.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the prophenol oxidase activity decreased and a low concentration of 0.326 ± 0.041 min/mg/protein was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for the prophenol oxidase activity and the values recorded were 0.412 ± 0.015 to 0.799 ± 0.065min/mg/protein

Group F-F_3: Similar changes were observed in female groups where the concentration of prophenol oxidase activity increased and retained to closer control values *viz.,* from 0.412 ± 0.012 to 0.713 ± 0.036 min/mg/protein.

Both male and female groups showed significant decrease ($P<0.05$) in ProPo in infected groups. Whereas, the values of treated groups-E_3 and F_3 at 96hrs were observed to be closer to the control group-A and B .The prophenol oxidase levels are retained to closer control values in both the male and female treated groups at 96hrs of treatment (Table 21 and Fig. 35).

3.13 MARKER ENZYMES

3.13.1 TOTAL ACID PHOSPHATASE LEVELS (ACP)

ACP levels in hemolymph of *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The acid phosphatase levels in hemolymph of control male crabs was measured as 6.23 ± 0.516 IU/L.

Group B: The ACP levels in hemolymph of control female crabs was 8.13 ± 0.316 IU/L.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila at* 96 hrs the ACP levels increased and to a slight higher concentration of 11.13 ± 0.345 IU/L.

Group D: In female crabs on infection with *A.hydrophila* at 96 hrs the ACP levels increased and to a higher concentration of 12.46 ± 0.113 IU/L.

Treated Groups

Group E-E_3: *S.molesta* treated groups were assayed for ACP levels and the values recorded were 10.46 ± 0.213 to 5.24 ± 0.453 IU/L for 24hrs to 96 hrs respectively.

Group F-F_3: Similar changes were observed in female groups where the concentration of ACP levels decreased and retained to closer control values *viz.,* from 11.21 ± 0.331 to 7.31 ± 0.112 IU/L .

The infected groups-C and D showed significant increase ($P<0.05$) in ACP levels when compared to group-A and B. Both treated groups-E_3 and F_3 at 96hrs of treatment showed decrease in ACP levels illustrating the recovery of ACP levels closer to group-A and B (Table 22 and Fig. 36).

ACP levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The acid phosphatase levels in hemolymph of control male crabs was measured as 8.13 ± 0.326 IU/L.

Group B: The ACP levels in hemolymph of control female crabs was 10.33 ± 0.632 IU/L.

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96hrs the ACP levels increased and to a slight higher concentration of 10.16 ± 0.441.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the ACP levels increased to a higher concentration of 13.56 ± 0.414 IU/L.

Treated Groups

Group E-E_3: *S.molesta* treated groups were assayed for ACP levels and the values recorded were 9.13 ± 0.414 to 7.99 ± 0.113 IU/L for 24hrs to 96 hrs respectively.

Group F-F_3: Similar changes were observed in female groups where the concentration of ACP levels decreased and retained to closer control values *viz.,* from 12.32 ± 0.431 to 9.46 ± 0.131 IU/L.

In both male and female groups significant decrease ($P<0.05$) in ACP levels were observed and in the treated groups there was a gradual increase in ACP levels at different intervals. At 96 hrs, the group-E_3 and F_3 values were observed to be closer to group-A and group-B values illustrating the pharmacological role of ethanolic leaf extract of *S.molesta* (Table 23 and Fig. 37).

3.13.2 TOTAL ALKALINE PHOSPHATASE LEVEL

ALP levels in hemolymph of *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The alkaline phosphatase levels in hemolymph of control male crabs was measured as 10.46 ± 0.833 IU/L.

Group B: The ALP levels in hemolymph of control female crabs was 12.46 ± 0.751 IU/L.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96hrs the ALP levels increased and to a higher concentration of 78.48 ± 0.832 IU/L.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the ALP levels increased and to a higher concentration of 97.31 ± 0.854 IU/L.

Treated Groups

Group E-E_3: *S.molesta* treated groups were assayed for ALP levels and the values recorded were 45.31 ± 0.452 to 9.26 ± 0.321 IU/L for 24 hrs to 96 hrs respectively.

Group F-F_3: Similar changes were observed in female groups where the concentration of ALP levels decreased and retained to closer control values *viz.,* from 38.21 ± 0.893 to 11.46 ± 0.302 IU/L

Significant increase ($P<0.05$) in ALP levels were observed in infected groups C and D when compared with group A and B . On treatment with ethanolic leaf extract of *S.molesta* the ALP levels decreased significantly ($P<0.05$) in groups E to E_3 at different time intervals and reached closer values to group-A. Similar changes were observed in female groups-F to F_3 were significant increase in ALP levels were observed at 96hrs of treatment (Table 24 and Fig. 38).

ALP levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The alkaline phosphatase levels in hemolymph of control male crabs was measured as 12.26 ± 0.914 IU/L.

Group B: The ALP levels in hemolymph of control female crabs was 10.15 ± 0.951

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96hrs the ALP levels increased to a higher concentration of 75.48 ± 0.831 IU/L.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the ALP levels increased to a higher concentration of 87.61 ± 0.954 IU/L.

Treated Groups

Group E-E_3: *S.molesta* treated groups were assayed for ALP levels and the values recorded were 49.31 ± 0.852 to 11.17 ± 0.412 IU/L for 24 hrs to 96 hrs respectively.

Group F-F_3: Similar changes were observed in female groups where the concentration of ALP levels decreased and retained to closer control values *viz.*, from 37.21 ± 0.943 to 10.46 ± 0.812 IU/L

Both the male and female infected groups showed significant increase in ALP levels whereas the ALP levels decreased significantly by time from 24hrs to 96 hrs in both male and female groups elucidating the therapeutic role of ethanolic leaf extract of *S.molesta* (Table 25 and Fig. 39).

3.14 ANTIOXIDANT ENZYME ASSAYS

3.14.1 SUPEROXIDE DISMUTASE (SOD) ASSAY

SOD levels in hemolymph of *A.hydrophila infected* and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The SOD activity in hemolymph of control male crabs was measured as 4.31 ± 0.061 units/mg protein.

Group B: The SOD activity in hemolymph of control female crabs was 4.28 ± 0.058 units/mg protein.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96hrs the SOD activity decreased and a low concentration of 1.81 ± 0.097 units/mg protein was recorded.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the SOD activity decreased and a low concentration of 1.32 ± 0.0231 units/mg protein was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for the SOD activity and the values recorded was 2.04 ± 0.0145 to 4.23 ± 0.0514units/mg protein .

Group F-F_3: Similar changes were observed in female groups where the concentration of SOD activity was increased and retained to closer control values *viz.,* from 2.63 ± 0.0351 to 4.15 ± 0.0564 units/mg protein.

Both male and female infected group-C and D exhibited significant decrease ($P<0.05$) in SOD levels when compared with control groups-A and B .Whereas there was a prominent increase in SOD levels at 96 hrs of treatment time. As there was no significant variation between control and treated groups at 96 hrs the values obtained at treated groups illustrated the recovery of control group values during treatment period at 96 hrs (Table 26 and Fig.40).

SOD levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The superoxide dismutase activity in hemolymph of control male crabs was measured as 5.12±0.0241units/mg protein

Group B: The SOD activity in hemolymph of control female crabs was 4.95±0.021 units/mg protein.

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96hrs the SOD activity decreased and a low concentration of 1.92 ± 0.089 units/mg protein was recorded.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the SOD activity decreased and a low concentration of 1.56 ± 0.0651 units/mg protein was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for the SOD activity and the values recorded were 2.36 ± 0.0564 to 4.95 ± 0.1014 units/mg protein.

Group F-F_3: Similar changes were observed in female groups where the concentration of SOD activity was increased and retained to closer control values *viz.,* from 2.89 ± 0.089 to 4.83 ± 0.0521 units/mg protein (Table 27 and Fig.41).

Significant decrease ($P<0.05$) of SOD levels were observed in infected groups C and D when compared with control groups. On comparing control and treated groups at 24 hrs to 96 hrs there was an gradual increase in SOD levels depicting the pharmacological role of ethanolic leaf extract *S.molesta* in regulating the SOD levels.

3.14.2 TOTAL LEVELS OF CATALASE (CAT)

CAT levels in hemolymph of *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The catalase activity in hemolymph of control male crabs was measured as 36.51 ± 0.214 nmol H_2O2/min/mg protein.

Group B: The catalase activity in hemolymph of control female crabs was 35.15 ± 0.354 nmol H_2O2/min/mg protein.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila at* 96hrs the CAT activity decreased and a low concentration of 28.24 ± 0.178 nmol H_2O2/min/mg protein was recorded.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the CAT activity decreased and a low concentration of 27.34 ± 0.235 was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for catalase activity and the values recorded was 29.31 ± 0.314 to 36.54 ± 0.65 nmol H_2O2/min/mg protein.

Group F-F_3: Similar changes were observed in female groups where the concentration of catalase activity was increased and retained to closer control values *viz.,* from 28.65 ± 0.896 to 35.45 ± 0.547 .

In both male and female crabs there was a significant decrease ($P<0.05$) of CAT levels in infected groups-C and D were observed when compared with control groups-A and B. Whereas the levels of CAT gradually increased in treated groups and the values were retained to control group-A and B at 96hrs of treatment (Table 28 and Fig.42).

CAT levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The catalase activity in hemolymph of control male crabs was measured as 34.21 ± 0.314 nmol H_2O2/min/mg protein

Group B: The catalase activity in hemolymph of control female crabs was 38.31 ± 0.432

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa at* 96hrs the CAT activity decreased and a low concentration of 27.36 ± 0.586 nmol H_2O2/min/mg protein was recorded.

Group D: In female crabs, on infection with *P.aeruginosa* at 96hrs the CAT activity decreased and a low concentration of 29.54 ± 0.432 was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for catalase activity and the values recorded was 28.41 ± 0.314 to 35.64 ± 0.25 nmol H_2O2/min/mg protein.

Group F-F_3: Similar changes were observed in female groups where the concentration of catalase activity was increased and retained to closer control values *viz.,* from 30.45 ± 0.986 to 38.12 ± 0.31 .

In both male and female crabs there was a significant decrease ($P<0.05$) of CAT levels observed when compared with control groups. Whereas the levels of CAT gradually increased in treated groups and the values were retained to control group-A and B values at 96hrs of treatment. This showed that both male and female crabs were able to respond to the pharmacological properties of *S.molesta* and hence the CAT activity was restored on par to the control groups (Table 29 and Fig.43).

3.14.3 ESTIMATION OF GLUTATHIONE PEROXIDASE ACTIVITY

GPX levels in hemolymph of *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The GPX activity in hemolymph of control male crabs was measured as 295.31 ± 2.801 µmol NADPH oxidised /min/mg/protein.

Group B: The GPX activity in hemolymph of control female crabs was 321.12 ± 4.904 µmol NADPH oxidised /min/mg/protein.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96hrs the GPX activity decreased and a low concentration of 162.78 ± 1.75 was recorded.

Group D: In female crabs on infection with *A.hydrophila at* 96hrs the GPX activity decreased and a low concentration of 186.64 ± 8.50 was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups was assayed for GPX levels and the values recorded was 183.24 ± 5.33 to 318.54 ± 5.12 µmol NADPH oxidised /min/mg/protein.

Group F-F_3: Similar changes were observed in female groups where the concentration of GPX activity was increased and retained to closer control values *viz.,* from 197.54 ± 5.33 to 308.87 ± 1.51 µmol NADPH oxidised /min/mg/protein

There was significant decrease ($P<0.05$) in GPX levels observed in both male and female infected groups. Whereas there was significant increase in treated groups at different time intervals illustrating the significant treatment exhibited by *S.molesta* treatment retaining the physiological GPX values in treated groups (Table 30 and Fig.44).

GPX levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The GPX activity in hemolymph of control (untreated) male crabs was measured as 316.54 ± 6.12 µmol NADPH oxidised /min/mg/protein.

Group B: The GPX activity in hemolymph of control female crabs was 293.57 ± 8.1 µmol NADPH oxidised /min/mg/protein.

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96hrs the GPX activity decreased and a low concentration of 193.64 ±5.90 was recorded.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the GPX activity decreased and a low concentration of 154.64 ± 2.94 was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for GPX levels and the values recorded was 199.42 ± 5.24 to 324.43 ± 11.8.

Group F-F_3: Similar changes were observed in female groups where the concentration of GPX activity was increased and retained to closer control values *viz.*, from 168.31 ± 4.1 to 318.87 ± 5.68.

There was a significant decrease ($P<0.05$) in GPX levels observed in both male and female infected groups. Whereas, there was a significant increase in treated groups at different time intervals observed illustrating the significant treatment exhibited by *S.molesta*, therefore retaining the physiological GPX values in treated groups (Table 31 and Fig.45).

3.14.4 ESTIMATION OF GLUTATHIONE S-TRANSFERASE ACTIVITY

GST levels in *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The GST activity in hemolymph of control (untreated)male crabs was measured as 235.68 ± 7.71 µmol CDNB conjugate formed /min /mg protein.

Group B: The GST activity in hemolymph of control female crabs was 218.87 ± 2.49 µmol CDNB conjugate formed /min /mg protein.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila at* 96hrs the GST activity decreased and a low concentration of 183.32 ± 2.62 was recorded.

Group D: In female crabs on infection with *A.hydrophila* at 96 hrs the GST activity decreased and a low concentration of 162.21 ± 4.05 was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for GST levels and the values recorded was 198.54 ± 9.20 to 257.85 ± 5.54 µmol CDNB conjugate formed /min /mg protein.

Group F-F_3 :Similar changes were observed in female groups where the concentration of GST activity was increased and retained to closer control values *viz.,* from185.31 ± 7.93 to 222.85 ± 6.18 µmol CDNB conjugate formed /min /mg protein.

During infection at 96 hrs there was a significant decrease ($P<0.05$) in GST levels in both male and female groups-C and D when compared to control group-A. Whereas there was a steady increase in GST levels at different time intervals *viz.*,24, 48,72 and 96 hrs in treated groups .The values obtained for group-E_3 and F_3 showed closer values when compared to control groups since there is no large variation among the obtained values in both control and 96hrs treated groups. This showed that both male and female crabs were able to respond to the pharmacological properties of ethanolic leaf extract of *S.molesta.* (Table 32 and Fig. 46).

GST levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The GST activity in hemolymph of control male crabs was measured as 216.48 ± 3.68 µmol CDNB conjugate formed /min /mg protein

Group B: The GST activity in hemolymph of control female crabs was 236.02 ± 9.64 µmol CDNB conjugate formed /min /mg protein.

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96hrs the GST activity decreased and a low concentration of 175.64 ± 6.68 was recorded.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the GST activity decreased and a low concentration of 156.45±1.63 was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for GST levels and the values recorded were 189.31 ± 6.5 to 231.61 ± 8.54.

Group F-F_3: Similar changes were observed in female groups where the concentration of GST activity was increased and retained to closer control values *viz.,* from173.31 ± 2.44 to 239.46 ± 8.10.

Infected groups-C and D showed significant decrease ($P<0.05$) when compared to group-A and B. Values obtained in the treated groups-E_3 and F_3 illustrated that in treated groups the levels of GST were retained to closer control values as that of group A and B (Table 33 and Fig.47).

3.14.5 ESTIMATION OF GLUTATHIONE REDUCTASE ACTIVITY

GR levels in *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The Glutathione reductase activity in hemolymph of control male crabs was measured as 514.67 ± 8.21µmol NADPH oxidised /min/mg/protein.

Group B: The GR activity in hemolymph of control female crabs was 497.07 ± 16.6 µmol NADPH oxidised /min/mg/protein.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96hrs the GR activity decreased and a low concentration of 451.31 ± 7.34 was recorded.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the GR activity decreased and a low concentration of 448.26 ± 8.71 was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for GR levels and the values recorded was 476.02 ± 8.92 to 525.03 ± 3.74.

Group F-F_3: Similar changes were observed in female groups where the concentration of GR activity was increased and retained to closer control values *viz.,* from 462.03 ± 9.22 to 518.02 ± 10.49.

Significant decrease ($P<0.05$) of GR levels in infected groups were observed when compared to group-A. The values obtained for group-E_1 to E_3 and group-F to F_3 in different time period was closer to the control values of group A and B illustrating the recovery of normal physiological values in treated groups (Table 34 and Fig.48).

GR levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The Glutathione reductase activity in hemolymph of control male crabs was measured as 491.46 ± 3.26 µmol NADPH oxidised /min/mg/protein.

Group B: The GR activity in hemolymph of control female crabs was 517.31 ± 8.16 µmol NADPH oxidised /min/mg/protein.

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96 hrs the GR activity decreased and a low concentration of 354.61 ± 8.95 was recorded.

Group D: In female crabs on infection with *P.aeruginosa* at 96 hrs the GR activity decreased and a low concentration of 384.41 ± 9.20 was recorded.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for GR levels and the values recorded were 384.52 ± 4.32 to 512.01 ± 4.11.

Group F-F_3: Similar changes were observed in female groups where the concentration of GR activity was increased and retained to closer control values *viz.*, from 412.13 ± 6.31 to 518.12 ± 10.54

Significant decrease ($P<0.05$) of GR levels in infected groups were observed when compared to group A. The values obtained for group-E_1 to E_3 and group-F_1 to F_3 in different time period was closer to the control values of group-A and B illustrating the recovery of normal physiological values in treated groups (Table 35 and Fig.49).

3.15 ESTIMATION OF NON - ENZYMATIC ANTIOXIDANTS

3.15.1 ESTIMATION OF REDUCED GLUTATHIONE LEVELS

GSH levels in *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The reduced glutathione (GSH)levels in hemolymph of control male crabs was measured as 421.54 ± 2.816 nmol NADPH oxidised /min/mg protein..

Group B: The GSH levels in hemolymph of control female crabs was 431.01 ± 9.61 nmol NADPH oxidised /min/mg protein..

Infected Groups

Group C : Whereas on infecting with *A.hydrophila* at 96hrs the GSH levels activity decreased to a lower concentration of 382.46 ± 8.99 nmol NADPH oxidised /min/mg protein **.**

Group D: In female crabs on infection with *A.hydrophila* at 96 hrs the GSH levels decreased to 402.14 ± 9.21 nmol NADPH oxidised /min/mg protein .

Treated Groups

Group E-E_3: The *Salvinia molesta* treated groups were assayed for GSH levels and the values recorded was 395.14±2.05 to 421.03±8.62 respectively from 24 hrs to 96 hrs of treatment.

Group F-F_3: Similar changes were observed in female groups where the concentration of GSH activity was increased and retained to closer control values *viz.,* from 412.04 ± 8.64 to 436.45 ± 8.57.

Significant decrease ($P<0.05$) of GSH levels in infected groups were observed. Increase in GSH levels are observed in treated groups of male and female from 24 hrs to 96 hrs time period. This showed that both male and female crabs were able to respond to the pharmacological properties of ethanolic leaf extract of *S.molesta* and hence the reduced glutathione levels were restored on par to the control groups A and B (Table 36 and Fig.50).

GSH levels *in* hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The reduced glutathione (GSH) levels in hemolymph of control male crabs was measured as 431.21 ± 4.49 nmol NADPH oxidised /min/mg protein.

Group B: The GSH levels in hemolymph of control female crabs was 428.12 ± 7.93 nmol NADPH oxidised /min/mg protein.

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96hrs the GSH levels activity decreased to a lower concentration of 398.24 ± 4.83 nmol NADPH oxidised /min/mg protein **.**

Group D: In female crabs on infection with *P.aeruginosa* at 96 hrs the GSH levels decreased to 390.12 ± 6.54 nmol NADPH oxidised /min/mg protein**.**

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for GSH levels and the values recorded was 412.02 ± 6.944 to 436.08 ± 6.49 respectively from 24 hrs to 96 hrs of treatment.

Group F-F_3: Similar changes were observed in female groups where the concentration of GSH activity was increased and retained to closer control values *viz.,* from 402.06 ± 9.2 to 426.06 ± 8.7.

Significant decrease ($P<0.05$) of GSH levels in infected groups were observed. Increase in GSH levels were observed in treated groups of male and female from 24 hrs to 96hrs time period. This showed that both male and female crabs were able to respond to the pharmacological properties of

ethanolic leaf extract of *S.molesta* and hence the reduced glutathione levels were restored on par to the control groups A and B (Table 37 and Fig.51).

3.16 FREE RADICAL SCAVENGING ASSAY

3.16.1 LIPID PEROXIDASE ASSAY

LPO levels in *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The lipid peroxidase activity in hemolymph of control male crabs was measured as 2.61±0.041 nmol MDA/gm tissue.

Group B: The LPO activity in hemolymph of control female crabs was 2.95 ± 0.033 nmol MDA/gm tissue.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96hrs the LPO activity increased and a higher concentration of 5.33 ± 0.0547 nmol MDA/gm tissue was recorded.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the LPO activity increased to 4.66 ± 0.214 nmol MDA/gm tissue .

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for LPO levels and the values recorded was 4.54 ± 0.061 to 2.35 ± 0.213.

Group F-F_3: Similar changes were observed in female groups where the concentration of LPO activity was decreased and retained to closer control values *viz.,* from 3.98 ± 0.245 to 2.06 ± 0.241.

This shows that both male and female crabs were able to respond to the pharmacological significant compounds of *S.molesta* and hence the LPO activity was restored on par to the control groups.

There was significant increase ($P<0.05$) in both male and female infected groups were observed .Closer values to control groups were observed in treated groups illustrating the retaining cababilty of LPO levels in treated groups by ethanolic leaf extract of *S.molesta* (Table 38 and Fig.52).

LPO levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The lipid peroxidase activity in hemolymph of control male crabs was measured as 3.12 ± 0.131 nmol MDA/gm tissue

Group B: The LPO activity in hemolymph of control female crabs was 2.98 ± 0.214

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96hrs the LPO activity increased and a higher concentration of 5.95 ± 0.0921 nmol MDA/gm tissue was recorded.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the LPO activity increased and a higher concentration of 5.86 ± 0.0954 nmol MDA/gm tissue was recorded.

Treated Groups

Group E-E_3: The *Salvinia molesta* treated groups were assayed for LPO levels and decreased values recorded were recorded as 4.61 ± 0.0598 to 3.47 ± 0.052.

Group F-F_3: Similar changes were observed in female groups where the concentration of LPO activity was decreased and retained to closer control values *viz.,* from 4.45 ± 0.312 to 2.21 ± 0.091.

Significant increase ($P<0.05$) of LPO levels in infected groups-C and D were observed. On treatment with ethanolic leaf extract of *S.molesta,* gradual decrease in the LPO levels were observed. Values of group-E to E_3 and group-F

to F_3 illustrates the retaining of normal physiological values of LPO on par to control group-A and B (Table 39 and Fig.53).

3.16.2 NITRIC OXIDE ASSAY

NO levels in *A.hydrophila* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The nitric oxide activity in hemolymph of control male crabs was measured as 50.23 ± 0.893 µg/ml

Group B: The NO activity in hemolymph of control female crabs was 46.45 ± 2.45 µg/ml.

Infected Groups

Group C: Whereas on infecting with *A.hydrophila* at 96 hrs the NO activity increased and to a higher concentration of 68.13 ± 1.998.

Group D: In female crabs on infection with *A.hydrophila* at 96hrs the NO activity increased and to a higher concentration of 62.62 ± 1.690.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for NO levels and the values recorded were 64.20 ± 1.84 to 49.24 ± 0.53.

Group F-F_3: Similar changes were observed in female groups where the concentration of NO activity decreased and retained to closer control values *viz.,* from 63.14 ± 0.530 to 44.13 ± 2.860 µg/ml.

Significant increase ($P < 0.05$) in NO levels were observed in both male and female infected groups when compared to group-A and B. There was an decrease in NO levels from group-E to E_3 and group-F to F_3 depicting the therapeutic role of ethanolic leaf extract of *S.molesta*. Closer values between control and 96 hrs treated groups were markedly observed pertaining to the least difference between 96hrs treated and control group values (Table 40 and Fig.54).

NO levels in hemolymph of *P.aeruginosa* infected and *S.molesta* leaf extract treated crabs

Control Groups

Group A: The nitric oxide activity in hemolymph of control male crabs was measured as 45.14 ± 2.80µg/ml.

Group B: The NO activity in hemolymph of control female crabs was 42.46 ± 2.02 µg/ml.

Infected Groups

Group C: Whereas on infecting with *P.aeruginosa* at 96hrs the NO activity increased and to a higher concentration of 59.46 ± 2.05.

Group D: In female crabs on infection with *P.aeruginosa* at 96hrs the NO activity increased and to a higher concentration of 62.97 ± 1.05.

Treated Groups

Group E-E_3: The *S.molesta* treated groups were assayed for NO levels and the values recorded were 57.46 ± 2.16 to 43.16 ± 3.060.

Group F-F_3: Similar changes were observed in female groups where the concentration of NO activity decreased and retained to closer control values *viz.,* from 58.61 ± 2.261 to 44.56 ± 1.94.

Significant increase ($P < 0.05$) in NO levels were observed in both male and female infected groups when compared to group-A and B. Closer values between control and 96 hrs treated groups were markedly observed pertaining to the least difference between 96 hrs treated and control group values (Table 41 and Fig.55).