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ANTIBACTERIAL, ANTIVIRAL AND CYTOTOXIC ACTIVITIES OF *RHYNCHOPHORUS FERRUGINEUS* (COLEOPTERA: DRYOPHTHORIDAE) AND *SPODOPTERA LITTORALIS* (LEPIDOPTERA: NOCTUIDAE) LARVAL EXTRACTS

By

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Abstract

The present study dealt with evaluating the effect of different crude extracts from the larvae of palm weevil, *Rhynchophorus ferrugineus* and cotton leaf worm, *Spodoptera littoralis* on different pathogenic strains of bacteria and viruses. The results revealed that, ethyl acetate extracts of *R. ferrugineus* and *S. littoralis* larvae were more effective against different pathogenic bacterial strains tested than petroleum ether, chloroform, methanol and ethanol extract. Also, The MICs' determination showed that all different extracts of *R. ferrugineus* and *S. littoralis* larvae were active against tested bacterial strains. Minimum Inhibitory Concentration (MIC) values were ranged from 25 to 6.25mg/ml depended on tested species. Ethyl acetate extract from *R. ferrugineus* and *S. littoralis* larvae exhibited antiviral activity against hepatitis A virus (HAV) and Herpes simplex virus (HSV-1) cell lines at 625 & 312.5mg/ml. Ethyl acetate extract from *R. ferrugineus* and *S. littoralis* larvae recorded 5.19 & 4.07% antiviral activity against HAV at 625 & 312.5mg/ml, respectively; while, antiviral activity percentage was 12.42 & 26.17 at 625 and 312.5mg/ml, respectively against HSV-1. Tested extracts exhibited no antiviral activity against Coxsackie B virus (Cox B4) cell lines. Crude extracts of *R. ferrugineus* & *S. littoralis* were not cytotoxic to Vero (CCL-81) cells at 312.5 and/or 625µg/ml.

Keys words: Antibacterial activity, *Rhynchophorus ferrugineus*, *S. littoralis*.

Introduction

The discovery of penicillin, antibiotics were instrumental in treating infectious diseases. But, emerging antibiotic multi-resistance coincided with a nearly exhausted drug pipeline was a major concern for future of therapy of infections (Netzker *et al*, 2018). But antimicrobial resistance was a major global issue, the rate of emergence of antimicrobial resistance has superseded the rate of discovery and introduction of new effective drugs (The *et al*, 2017). Antimicrobial peptides of insects play an important role in innate immune systems and host defense mechanisms attracted attention as a novel class of antibiotics, in particular for antibiotic resistant pathogens, because of their action mechanism of non- selective interaction with cell surface membranes of microbes (Hancock and Rozek, 2002; Zasloff 2002; Boman 2003; Bulet *et al*, 2004), insect peptides/polypeptides a broad spectrum of activity against Gram-positive & negative bacteria and fungi (Hoffmann *et al*, 1996). Antibacterial proteins were isolated from insects (Cociancich *et al*, 1994) of five main groups (Hultmark 1993): cecro-pins, insect defensins, attacin-like (glycine-rich) proteins, proline-rich peptides and lysozymes. But, only a few number of antifungal peptides/polypeptides were reported from insects; drosomycin (Fehlbaum *et al*, 1994), metchiniko-win, cecropin A & B from *Hyalophora cecropia* (Levashina *et al*, 1995) and heliomicin from *Hellothis virescens* (Lamberty *et al*, 1999).

The present study aimed to evaluate crude extracts activity from larvae of palm weevil, *Rhynchophorus ferrugineus* and cotton leaf worm, *Spodoptera littoralis* against some pathogenic strains of bacteria and viruses.

Materials and Methods

Insects used: Last instar larvae of *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) and *Spodoptera littoralis* (Lepidoptera: Noctuidae) were collected from Ismailia and Beheira Governorates and maintained for several generations in Entomology Insectary, Animal House, Department of Zoology (Shahina *et al,* 2009; El-Sheikh and Aamir, 2011).

Extraction and sample preparation: The larvae were washed with 80% ethanol as a disinfectant for three seconds, then rinsed with deionized water and excess water was removed by filter paper. Fifty grams of each insect species were thoroughly homogenized in 50ml of 40mM tris-HCl (pH 7.4). The homogenate was centrifuged at 10,000rpm for 30min at 4°C and supernatant was used as buffer extract. The residue was soaked in pure methanol and centrifuged again. The supernatant was the methanolic extract. The residue was soaked in ethanol 70% and centrifuged again. The supernatant was the ethanolic 70% extract. The residue was soaked in petroleum ether and centrifuged again. The supernatant was the petroleum ether extract. The residue was soaked in ethyl acetate and centrifuged again. The supernatant was the ethyl acetate extract. Lastly, residue was soaked in chloroform and centrifuged to have the chloroform extract. All extracts were dried by evaporation and tested antimicrobial activity (Meylears *et al,* 2002).

Antimicrobial bioassay: Four pathogenic organisms were used for antimicrobial assay; *Bacillus subtilis* and *Staphylococcus aureus* as Gram-positive bacterial strains, and *Escherichia coli* & *Enterobacter faecalis* were used as Gram-negative strains. Antimicrobial assay was broadly based on the well diffusion and Microdilution methods at Mycology and Biotechnology Antimicrobial Department, Faculty of Science.

Evaluation of antibacterial activity (Well diffusion method): Microbial growth inhibition was assayed using agar well diffusion method (Valgas *et al,* 2007).

Minimum inhibitory con-centration (MIC) by microdilution method: MIC was determined by broth microdilution method using 96-well micro-plates (Irith *et al,* 2008).

Antiviral assay (MTT assay protocol): A 10,000 cells in 200µl media per well were plated in a 96 well plate. Three wells were leaved empty for blank controls. Cells were incubated (37^{o}C, 5% CO_2) overnight to allow cells to attach to wells. Non-lethal dilution of tested sample and virus suspension were incubated for an hour (volume 1:1 v/v). A 100µl was added from viral/ sample suspension and placed on a shaking table, 150 rpm for 5 minutes. Cells were incubated (37^{o}C, 5% CO_2) for 1- days to allow the virus to take effect. Two ml or more of MTT solution per 96 well plated at 5mg/ml in PBS. A 20µl of MTT solution was added to each well and placed on a shaking table, 150rpm for 5 minutes, to thoroughly mix the MTT into the media. The wells were incubated (37C, 5% CO_2) for 1-5 hours to allow the MTT to be metabolized. The media was dumped off. (Dry plate on paper towels to remove residue if necessary. formazan resuspend (MTT metabolic product) in 200µl DMSO and placed on a shaker, 150rpm for 5 minutes, to thoroughly mix the formazan into the solvent. Optical density was read at 560nm and subtracts background at 620nm, which directly correlated with cell quantity.

Determination of samples cytotoxicity on VERO cell using MTT assay: A 100mg of tested sample was dissolved in 1ml DMSO. Growth medium was decanted from 96 well micro titer plates after confluent sheet of VERO cell was formed; cell monolayer was washed twice with wash media. Double-fold dilutions of samples were made in MEM. A 0.1ml of each dilution was tested starting from 5mg/ml in different wells leaving 3 wells as control with maintenance medium. Plate was incubated at 37°C and examined frequently for up to 2 days. Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of monolayer, rounding, shrinkage, or cell granulation.

MTT solution was prepared (5mg/ml in PBS, BIO Basic Canada Inc.). A 20µl MTT solution were added to each well and placed on a shaker, 150rpm for 5 minutes, to thoroughly mix the MTT into media and incubated (37^{o}C, 5% CO2) for 1-5 hours to allow the MTT to be metabolized. The media was dumped off (Dry plate on paper towels to remove residue if necessary. Formazan resuspend (MTT metabolic product) in 200ul DMSO and placed on a shaker, 150rpm for 5 minutes, to thoroughly mix the formazan into the solvent. Optical density was read at 560nm and subtract background at 620nm that directly correlated with cell quantity. Maximum non-toxic concentration [MNTC] of each extract was determined and was used for further biological studies. MNTC for sample (*R. ferrugineus*) ethyl acetate extract = 625 µg/ml. MNTC for sample (*S. littoralis)* ethyl acetate extract = 312.5 µg/ml.

Statistical analysis: Data was done (Armitage, 1974) and analysed by Quattropro for windows program version 2. Microsoft, windows version 7 and graphic were drawn using Harvard Graphics program version 4. Data were assessed by calculating mean, standard deviation and student t-test.

Results

Antibacterial activity using well diffusion method: For Gram-positive bacteria, methanol extract of *R. ferrugineus* had the highest effect against *Bacillus subtilis*. The growth-inhibition zone was 13.0mm vs. 11.4mm for *Kanamycin* antibiotic which used as control. On the other hand, the lowest antibacterial activity against *B. subtilis* was recorded by chloroform extract. The growth-inhibition zone was 10.0mm. As regards the activity of petroleum ether, ethyl acetate and ethanol extracts against *B. subtilis*, the growth-inhibition zones were 11.0, 12.0 & 12.0mm, respectively. For *S. aureus*, ethyl acetate extract of *R. ferrugineus* gave the highest effect with 11.0mm growth-inhibition zone vs. 11.4mm for *Kanamycin*. Also, ethanol and methanol extracts showed no antibacterial activity against *S. aureus.* Petroleum ether and chloroform extracts activity against *S. aureus* were equal 10.0mm (Tab. 1, Fig. 1). On the other hand, chloroform extract of *Sp. littoralis* have the highest effect against *B. subtilis* where, growth-inhibition zone was 13.0mm vs. 11.4mm for *Kanamycin* antibiotic (control). The lowest antibacterial activity against *B. subtilis* was recorded by petroleum ether & ethyl acetate extracts, growth-inhibition zone was 11.0 & 11.0mm, respectively. Activity of ethanol & methanol extracts against *B. subtilis* recorded 12.0 and 12.0mm, respectively. For *S. aureus*, the results showed that, ethyl acetate extract of *Sp. littoralis* had the highest effect against *S. aureus* with 14.0mm growth-inhibition zone vs. 10.3mm for *Kanamycin*. Also, methanol extract showed no antibacterial activity against *S. aureus* (Tab. 2, Fig. 2).

For Gram negative bacteria, ethyl acetate extract of *R. ferrugineus* had the highest effect against *Escherichia coli* as the growth-inhibition zone recorded 10.0mm vs. 7.3mm for control *(Kanamycin*). Also, petroleum ether and chloroform extracts recorded 9.0 and 8.0mm against *E. coli*, respectively. Ethyl acetate extract of *R. ferrugineus* had the highest effect against *Enterobacter faecalis* where, the growth-inhibition zone was 11.0mm compared with 9.0mm for *Kanamycin* and the lowest antibacterial activity against *E. faecalis* was recorded by ethanol extract where, the growth-inhibition zone was 7.0mm. Petroleum ether, methanol and chloroform extracts recorded 10.0, 8.0 & 9.0mm inhibition zones against *E. faecalis*, respectively. Ethyl acetate extract of *S. littoralis* have the highest effect against *E. coli*, as growth-inhibition zone recorded 15.0mm vs. 11.0mm for *Kanamycin* (control). While, the lowest antibacterial activity against *E. coli* was recorded by petroleum ether extract; the growth-inhibition zone was 7.0mm. Activity of ethanol, methanol and chloroform extracts against *E. coli* was 14.0, 12.0 & 11.0mm, respectively. For *E. faecalis*, ethyl acetate extract of *S. littoralis* gave the highest effect against *E. faecalis.* Grow-

th-inhibition zone was 14.0mm compared with 9.0mm for *Kanamycin*. Lowest antibacterial activity against *E. faecalis* was recorded by petroleum ether extract; growth inhibition zone was 8.0mm. Activity of ethanol, methanol and chloroform extracts against *E. faecalis* was 11.0, 10.0 & 11.0mm, respectively.

Determination of minimum inhibitory concentration (MIC) by microdilution method: All pervious different crude extracts of *R. ferrugineus* were active against *B. subtilis*, *S. aureus*, *E. coli* & *E. faecalis* showed different antibacterial activity. MIC values were ranged from 25 to 6.25mg/ml depending on tested species. Petroleum ether and methanol fractions showed a discrete activity against *B. subtilis* with MIC at 6.25mg /ml; whereas only ethanol fraction presented MIC at 25mg/ml (Tab. 3; 4). Crude extracts of *R. ferrugineus* against *S. aureus* showed that all fractions have a discrete activity with MIC at 6.25mg/ml except ethanol fraction presented MIC at 12.5mg/ml. Ethyl acetate and chloroform fractions showed a discrete activity against *E. coli* with MIC at 6.25mg/ml. Crude extracts of *R. ferrugineus* against *E. faecalis* showed that all fractions a discrete activity with MIC at 12.5mg/ml except chloroform fraction presented MIC at 25mg/ml. But, chloroform fraction of *S. littoralis* showed a discrete activity against *B. subtilis* with MIC at 6.25mg/ml. Crude extracts of *S. littoralis* against *S. aureus*, ethyl acetate, ethanol and methanol fractions gave a discrete activity with MIC at 6.25mg/ml. MIC for different crude extracts of *S. littoralis* against *E. coli* showed that chloroform and ethyl acetate fractions gave a discrete activity against *E. coli* with MIC at 6.25mg/ml; Petroleum ether fraction was presented MIC at 25mg/ml (Tabs. 5, 6). Petroleum ether fraction of *S. littoralis* gave a discrete activity against *E. faecalis* with MIC at 6.25mg/ml.

Antiviral activity: Ethyl acetate extract from *R. ferrugineus* and *S. littoralis*, ethyl acetate extract from both *R. ferrugineus* and *S. littoralis* larvae showed a little antiviral activity against HAV, the activity was 5.2 and 4.1% for *R. ferrugineus* and *S. littoralis*, respectively. But, no antiviral activity displayed by tested extracts against Cox B4 virus. Ethyl acetate extract from *R. ferrugineus* and *S. littoralis* larvae gave a good antiviral activity against HSV-1 gave activities 12.5 & 26.2% for *R. ferrugineus* and *S. littoralis*, respectively (Tab. 7).

Cytotoxic assay: Crude extracts of *R. ferrugineus* did not show cytotoxicity against Vero cells when incubated with 312.5µg/m, as the cytotoxicity values were 0.0, 0.0, 0.1, 0.0 &1.5% for petroleum ether, ethyl acetate, ethanol, methanol and chloroform, respectively. Crude extracts of *S. littoralis* did not show cytotoxicity against Vero cells when incubated with 312.5µg/m, as the cytotoxicity values were 0.6, 1.8, 1.5, 3.2 & 1.5% for petroleum ether, ethyl acetate, ethanol, methanol and chloroform, respectively (Fig. 3). Crude extracts of *R. ferrugineus* did not show cytotoxic activity against Vero cells when incubated with 625µg/ml except for petroleum ether extract, as the cytotoxicity of petroleum ether extract was 40.0%. Crude extracts of *S. littoralis* did not show cytotoxicity against Vero cells when incubated with 625µg/mL except for ethyl acetate extract; cytotoxicity of ethyl acetate extract the results were 16.0% (Fig. 4).

Table 1: Growth-inhibition zone of different bacterial strains caused by *R. ferrugineus* larval extracts.

Bacterial strain	Gram +/-	Growth-inhibition zone in mm					Standard *Kanamycin*
		Pet. ether	Eth. acetate	Ethanol	Methanol	Chloroform	
Bacillus subtilis	+ve	11.0	12.0	12.0	13.0	10.0	11.4
Staphylococcus aureus	+ve	10.0	11.0	NA	NA	10.0	10.3
Escherichia coli	-ve	9.0	10.0	NA	NA	8.0	7.3
Enterobacter faecalis	-ve	10.0	11.0	7.0	8.0	9.0	9.0

NA: No activity.

Table 2: Growth-inhibition zone of different bacterial strains caused by *S. littoralis* larval extracts.

Bacterial strain	Gram +/-	Growth-inhibition zone in mm					Standard *Kanamycin*
		Pet. ether	Eth. acetate	Ethanol	Methanol	Chloroform	
B. subtilis	+ve	11.0	11.0	12.0	12.0	13.0	11.4
S. aureus	+ve	10.0	14.0	13.0	NA	11.0	10.3
E. coli	-ve	7.0	15.0	14.0	12.0	11.0	11.0
E. faecalis	-ve	8.0	14.0	11.0	10.0	11.0	9.0

Table 3: Minimal Inhibitory Concentrations (MIC) in mg/ml of *R. ferrugineus* larval extracts.

Bacterial strain	Petroleum ether	Ethyl acetate	Ethanol	Methanol	Chloroform
B. subtilis	6.25	12.5	25	6.25	12.5
S. aureus	6.25	6.25	12.5	6.25	6.25
E. coli	12.5	6.25	12.5	25	6.25
E. faecalis	12.5	12.5	12.5	12.5	25

Table 4: Antibacterial activity indicated by microdilution plate at 480nm of *S. littoralis* larval extracts

Bacteria strain	Conc. (mg/ml)	Petroleum ether	Ethyl acetate	Ethanol	Methanol	Chloroform
B. subtitles	Control	1.75±0.1	1.33±0.3	1.05±0.6	1.62±0.9	1.49±0.4
	25	1.5±0.1	0.7±0.1	0.6±0.2	1.3±0.1	1.5±0.03
	12.5	1.6±0.1	1.2±.06	1.1±0.2	1.5±0.1	1.4±0.06
	6.25	1.8±0.1	1.3±.06	1.1±0.1	1.6±0.1	1.4±0.05
S. aureus	Control	1.44±0.09	1.59±0.08	1.26±0.1	1.4±0.02	1.89±0.5
	25	1.1±0.2	0.7±0.07	0.5±0.05	1.2±0.05	0.7±0.2
	12.5	1.2±0.05	1.0±0.1	0.7±0.2	1.2±0.02	1.6±0.1
	6.25	1.4±0.03	1.2±0.05	0.9±0.1	1.3±0.1	1.9±0.2
E. coli	Control	1.45±0.8	0.98±0.4	0.88±0.2	1.56±0.4	1.12±0.8
	25	0.8±0.3	0.7±0.4	0.6±0.1	1.2±0.1	0.9±0.1
	12.5	1.2±0.3	0.5±0.1	0.7±0.1	1.5±0.1	1.0±0.1
	6.25	1.4±0.2	0.7±0.2	0.8±0.1	1.5±0.1	0.8±0.1
E. faecalis	Control	1.89±0.7	0.87±0.5	0.71±0.3	1.69±0.4	1.56±0.8
	25	1.6±0.1	0.5±0.1	0.6±0.2	1.3±0.1	1.4±0.1
	12.5	1.3±0.1	1.4±.03	0.7±0.4	1.5±.1	1.5±0.1

Table 5: Minimal inhibitory concentrations (MIC) in mg/ml of *S. littoralis* larval extracts.

Bacterial strain	Petroleum ether	Ethyl acetate	Ethanol	Methanol	Chloroform
B. subtilis	12.5	12.5	25	12.5	6.25
S. aureus	12.5	6.25	6.25	6.25	12.5
E. coli	12.5	6.25	12.5	25	6.25
E. faecalis	6.25	25	12.5	12.5	25

Table 6: Minimal inhibitory concentrations (MIC) in mg/ml of *S. littoralis* larval extracts.

Bacterial strain	Petroleum ether	Ethyl acetate	Ethanol	Methanol	Chloroform
B. subtilis	12.5	12.5	25	12.5	6.25
S. aureus	12.5	6.25	6.25	6.25	12.5
E. coli	12.5	6.25	12.5	25	6.25
E. faecalis	6.25	25	12.5	12.5	25

Table 7: Antiviral activity of *R. ferrugineus* and *S. littoralis* larval Ethyl acetate extracts.

Extract	Dil. (mg/ml)	O D	Viability	Toxicity	Viral activity %	Anti-viral effect %
Control Vero	---	0.217	100.0	0.0	---	---
HAV	---	0.127	58.52535	41.47465	100.0	0.0
R. ferrugineus	625	0.131667	60.67588	39.32412	94.81482	5.1851751
S. littoralis	312.5	0.130667	60.21505	39.78495	95.92594	4.0740639
coxB4	---	0.107333	49.46237	50.53763	100.0	0.0
R. ferrugineus	625	0.106	48.84793	51.15207	101.2158	0.0
S. littoralis	312.5	0.106667	49.15515	50.84485	100.6079	0.0
HSV1	---	0.117667	54.22427	45.77573	100.0	0.0
R. ferrugineus	625	0.13	59.90783	40.09217	87.58389	12.416108
S. littoralis	312.5	0.143667	66.20584	33.79416	73.8255	26.174497

Discussion

In the present study, extracts from the larvae of *Rhynchophorus ferrugineus* and *Spodoptera littoralis* exhibited variable antibacterial activities against Gram-positive namely; *Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative namely; *Escherichia coli* and *Enterobacter faecalis* by two methods used.The ethyl acetate extract from *R. ferrugineus* and *S. littoralis* larvae caused

the highest antibacterial activity against bacterial strains. Gram-positive bacteria strains were affected by the extracts more than the Gram-negative strains. These results agreed with Leem *et al*. (1999) for the saw fly, *Acantholyda parki* extract which have a broad antibacterial spectrum against both Gram-negative and Gram-positive bacteria. Hou *et al*. (2007) found that the extract of the housefly larvae at high concentration showed higher antibacterial activity against both Gram- positive and Gram- negative bacteria. They concluded that extract housefly possess wide broad antibacterial activity against Gram- positive and Gram- negative bacteria. This agreed with the present study. In spite of such a response, the susceptible insects within the host range of a given pathogen were killed by the pathogen. However, insect resistant against pathogen was out of the host range (Yamauchi, 2001). Most of insect extracts exhibited antibacterial activity against Gram-positive and Gram-negative bacteria, the silk worm *Bombyx mori* (Hara and Yamakawa, 1995a; b), the European bumblebee, *Bombus pascuorum* (Rees *et al*, 1997) and *Tenebrio molitor* larvae (Lee *et al*, 1998). But, some insects showed activity only against Gram-positive bacteria as *Aedes aegypti* (Lowenberger *et al*, 1995), *Chironomus plumosus* (Lauth *et al*, 1998) and *Anopheles gambiae* (Vizioli *et al*, 2001).

In the present study, ethyl acetate extracts of *R. ferrugineus* and *S. littoralis* larvae displayed a variable effect on Hepatitis A virus (HAV), and Herpes simplex virus (HSV-1) Vero cell lines. But, these extracts showed no antiviral activity against Coxsackie B virus (Cox B4). These results are in agreement with such results recorded by many authors for using different insect materials against different Vero cell lines. Esser *et al.* (1979) proved that treatment of murine virus capsid with melittin, the viral membrane peeled off the surface, generating membrane that could be stabilized by melittin and prevent virus cell entry. Baier *et al.* (2000) found that the nasal application of lipopeptide increased protection against a lethal influenza infection. Wachinger *et al.* (1998) found that the reduction of viral infectivity was not due to an effect of melittin on virus particles but due to the peptide intracellular action of readily taken up into the cells. Fenard *et al.* (2001) reported that the honeybee venom phospholipase A_2 (p3bv) peptide (amino acids 21 to 35 of bvPLA$_2$) inhibited the replication of T-lymphotropic (T-tropic) HIV-1 isolates (ID_{50}= 2 mM) but without effect on monocytotropic (M-tropic) HIV-1 isolates. P3bv was capable to prevent cell-cell fusion process mediated by T-tropic HIV-1 envelope. Chernysh *et al.* (2002) isolated alloferon from wild-type of *Calliphora vicina* and reported that the activity spectrum of alloferon as an antiviral agent was not restricted to influenza viruses. Wu *et al.* (2017) stated that naturally derived phenolic acids especially caffeic acid/gallic acid and their derivatives might be regarded as novel promising antiviral leads or candidates. They added that scarcely any of these compounds was used as antiviral treatment in clinical practice. Thus, these phenolic acids with diverse skeletons and mechanisms gave an excellent resource for finding novel antiviral drugs.

In the present study, crude extracts of *R. ferrugineus* and *S. littoralis* evoked a slight effect on (CCL-81) Vero lineage. These results agreed with Nakajima and Natori (1990) and Itoh *et al.* (1986) for lectin from *Sarcophaga* larvae hemolymph induced cytotoxic effects on tumor cells in the presence of murine macrophage (Ahn *et al*, 2000) for the buffer, methanol and ethylacetate extracts from 26 insects against Human cervical carcinoma cell line (HELA) where, the buffer extracts from *Gryllotalpa orientalis* and *Apriona germari* larvae showed greater/more rapid (HELA) cell growth than that of other insects; (Yoo *et al*, 2007) for isolated (F-2, F-4, F-5 & F-7) fractions from *Protaetia brevitarsis* larva, where all fractions induce apoptosis activity against Colon 26 murine carcinoma cells, Januszanis *et al.*

(2012) for *Galleria mellonella* hemolymph polypeptides on human brain glioblastoma multiforme cell line (T98G), treated the cells with *G. mellonella* polypeptides inducted cell death; Kustiawan *et al.,* (2015) for Sting less Bee *Trigona incisa* Propolis against human cancer derived cell lines, IC_{50} was 4.51±0.76µg/ml against human colon cancer (SW620), 6.06±0.39µg/ml against human gastric cancer (KATOIII), 0.71±0.22µg/ml against human liver cancer (Hep-G2), 0.81±0.18µg/ml against human lung cancer (Chago) and 4.28±0.14µg/ml against human breast cancer (BT474) cell lines. In the same line, Shehata *et al.* (2016) found that *Lucilia sericata* and *Chrysomya albiceps* maggots excretion/secretion (ES) gave anticancer activity against seven human tumor cell lines: liver carcinoma cell line (HepG-2), breast carcinoma cell line (MCF-7), colon carcinoma cell line (HCT-116), lung carcinoma cell line (A-549), intestinal carcinoma cell line (CACO), prostate carcinoma cell line (PC-3) and cervical carcinoma cell line (HELA) with IC_{50} values: 14.8±0.05, 31.3±0.09, 27.3 ±0.11, 16.4±0.07, 31.1±0.31, 30.4±0.12, 85.6±0.35µg/ml for *L. sericata* ES & 17.3± 0.26, 33.4±0.17, 32.1±0.37, 20.2±0.14, 34.8 ±0.25, 77.6±0.19 & 89.5±0.34µg/ml for *C. albiceps* ES against (HepG-2), (MCF-7), (HCT-116), (A-549), (CACO), (PC-3), and (HELA) cell lines.

Conclusion

Emerging and reemerging viral infections represent a major concern to man and domestic animals and there is an urgent need for the development of broad-spectrum antivirals

Petroleum ether, ethyl acetate, ethanol, methanol and chloroform extracts from the palm weevil, *Rhynchophorus ferrugineus* and cotton leaf worm, *Spodoptera littoralis* larvae gave variable antibacterial activities against Gram-positive and Gram-negative bacterial strains, as well as antiviral activity against hepatitis A virus and Herpes simplex virus (HSV-1).

References

Abu-Shanab, B, Adwan, G, Abu-Safiya, D, Jarrar, N, Adwan, K, 2004: Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. Turk. J. Biol. 28, 1:99-102.

Armitage, P, 1974: Paired student t-test in statistical methods in medical research black well scientific pub. Oxford, London.pp:116-20.

Baier, W, Masihi, N, Huber, M, Hoffmann, P, Bessler, WG, 2000: Lipopeptides as immune adjuvants and immune stimulants in mucosal immunization. J. Immun. Biol. 201, 3/4:391-405.

Boman, HG, 2003: Antibacterial peptides: basic facts and emerging concepts. J. Int. Med. 254: 197-215.

Bulet, P, Stocklin, R, Menin, L, 2004. Antimicrobial peptides: from invertebrates to vertebrates. Immunol. Rev. 198:169- 84.

Chernysh, S, Kim, SI, Bekker, G, Pleskach, V A, Filatova, NA, *et al*, 2002: Antiviral and antitumor peptides from insects. JABS 99, 20: 12628-32.

Cociancich, S, Dupont, A, Hegy, G, Lanot, R, Holder, F, *et al*, 1994: Novel inducible antibacterial peptides from a hemipteran insect, the sap-sucking bug *Pyrrhocoris apterus*. Biochem. J. 300:567-75.

El-Sheikh, EA, Aamir, MM, 2011: Comparative effectiveness and field persistence of insect growth regulators on a field strain of the cotton leafworm, *Spodoptera littoralis*, Boisd (Lepidoptera: Noctuidae). Crop Protect. 30, 6:645-50.

Esser, AF, Bartholomew, RM, Jensen, FC, Muiler-Eberhard, HJ, 1979: Disassembly of viral membranes by complement independent of channel formation. Proc. Nat. Acad. Sci. U.S.A., 76:5843-7.

Fehlbaum, P, Bulet P, Michaut L, Lagueux, M, Broekaert, WF, *et al*, 1994: Insect immunity: septic injury of *Drosophila* induces the synthesis of a potent antifungal peptide with sequence homology to plant antifungal peptides. J. Biol. Chem. 269: 33159-63.

Fenard, D, Lambeau, G, Maurin, T, Lefebvre, JC, Doglio, A, 2001: A peptide derived from bee venom-secreted phospholipase A2 inhibits replication of T-cell tropic HIV-1 strains via interaction with the CXCR4 chemokine receptor. Mol. Pharmacol. 60:341-7.

Hancock, REW, Rozek, A, 2002: Role of membranes in the activities of antimicrobial cationic peptides.FEMS Microbiol. Lett. 206:143-9.

Hara, S, Yamakawa, M, 1995a: A novel antibacterial peptide family isolated from the silkworm, *Bombyx mori*. Biochem. J. 310, 2:651-5.

Hara, S, Yamakawa, M, 1995b: Moricin, a novel type of antibacterial peptide isolated from the silk worm, *Bombyx mori*. J. Biol. Chem. 270, 50:29923-27.

Hoffmann, JA, Reichhart, JM, Hetru, C, 1996: Innate immunity in higher insects. Curr. Opin. Immunol. 8:8-13.

Hou, L, Shi, Y, Zhai, P, Le, G, 2007: Antibacterial activity and in vitro anti-tumor activity of the extract o the larvae of the housefly (*Musca domestica*) J. Ethnoph. 111:227-31.

Hultmark, D, 1993: Immune reactions in *Drosophila* and other insect: Amodel for innate immunity. Tren.Genet. 9:178-83.

Irith, W, Kai, H, Hancock REW, 2008: Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances, Nature Protocols 3, 2: 163-75.

Itoh, A, Ohsawa, E, Ohkuma, Y, Natori, S, 1986: Participation of tumor killing factor in the antitumor effect of *Sarcophaga* lectin. FEBS. Lett. 20:137-40.

Januszanis, B, Staczek, S, Barabas, AZ, Badziul, D, Gil, JJ, et *al*, 2012: Effect of Galleria mellonella hemolymph polypeptides on human brain *Glioblastoma multiforme* cell line– a preliminary study. Ann. UMCS, Biol. 67, 2:53-62.

Kustiawan, PA, Phuwapraisirisan, P, Puthong, S, Palaga, T, Arung, ET, *et al*, 2015: Propolis from the Stingless Bee *Trigona incisa* from East Kalimantan, Indonesia, induces in vitro cytotoxicity and apoptosis in cancer cell lines. Asian Pac. J. Cancer Prev. 16, 15:6581-9.

Lamberty, M, Ades, S, Joseph, US, Brookhart, G, Bushey, D, *et al*, 1999: Insect Immunity: Isolation from the lepidopteran *Heliothes virescense* of a novel insect defensin with potent antifungal activity. J. Biol. Chem. 274, 14:9320-6.

Lauth, X, Nesin, A, Briiand, JP, Roussel, JP, Hetru, C, 1998: Isolation, characterization and chemical synthesis of a new insect defensin from *Chironomus plumosus* (Diptera).Insect Biochem. Mol. Biol. 28, 12:1059-66.

Lee, KH, Hog, SY, Oh, JE, Kwon, M, Yoon, JH, *et al*, 1998: Identification and characterization of the antimicrobial peptide corresponding to C- terminal beta-sheet domain of tenecin l, an antibacterial protein of larvae of *Tenebrio molitor*. Biochem. J. 334:99-105.

Leem, JY, Jeong, IJ, Prak, KT, Park, HY, 1999: Isolation of P-hydroxycinnamaldehyde as an antibacterial substance from the saw fly, *Acantholyda Parki* S. FEBS Lett: 442:53-6.

Levashina, EA, Ohresser, S, Bulet, P, Reichhart, JM, Hetru, C, *et al*, 1995: Metchinikowin, a nobel immune-inducible proline- rich peptide from *Drosophila* with antibacterial and antifungal properties Eur. J. Biochem. 233: 694-700.

Lowenberger, C, Bulet, P, Charlet, M, Hetru, C, Hodgeman, B, *et al*, 1995: Insect immunity: Isolation of three noel inducible antibacterial defensins from the vector mosquito, *Aedes egypti*. Insect Biochem. Mol. Biol. 25, 7:867-73.

Meylears, K, Cerstiaens, A, Vierstraete, E, Baggerman, G, Michiels, CW, *et al*, 2002: Antimicrobial compounds of low molecular mass are constitutively present in insects: Characterisation of β-Alanyl-Tyrosine. Curr. Pharma. Design 8: 99-110.

Nakajima, Y, Natori, S, 1990: Anti-tumor factor in insect' hemolymph. Hum. Cell 3:131-6.

Netzker, T, Flak, M, Krespach, MK, Str-oe, MC, Weber, J, *et al*, 2018: Microbial interactions trigger the production of antibiotics. Curr. Opin. Microbiol. 45:117-23.

Rees, JA, Moniatte, M, Bulet, P, 1997: Novel antibacterial peptides isolated from a European Bumblebee *Bumbus* pascurum (Hymenoptera, Apoidea). Insect Bioch. Mol. Biol. 27, 5:413-22.

Shahina, F, Salma, J, Mehreen, G, Bhatti, M I, Tabassum, KA, 2009: Rearing of *Rhynchophorus ferrugineus* in laboratory and field conditions for carrying out various efficacy studies using epns. Pak. J. Nematol. 27, 2:219-28.

Shehata, AZI, Mehany, ABM, El-Sheikh, TM Y, 2016: Excretion/secretion of *Lucilia sericata* and *Chrysomya albiceps* (Diptera: Calliphoridae) maggots as potential anticancer agent and kinases inhibitor. New York Sci. J. 9, 12:95-101.

Teh, CH, Nazni, WA, Nurulhusna, AH, Norazah A, Lee, HL, 2017: Determination of antibacterial activity and minimum inhibitory concentration of larval extract of fly via resazurin-based turbidometric assay. BMC. Microbiol. 17, 1:36-42.

Valgas, C, de Souza, SM, Smania, EFA, Smania, AJr, 2007: Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 38, 2:369-80.

Vizioli, J, Richman, A, Joseph, US, Blass, C, Bulet, P, 2001: The defensin peptide of the malaria vector mosquito *Anopheles gambiae*: antimicrobial activities and expression in adult mosquitoes- Insect Biochem. Mol. Biol. 31:241-8.

Wachinger, M, Kleinschmidt, A, Winder, D, von Pechmann, N, Ludvigsen, A, *et al*, 1998: Antimicrobial peptides melittin and cecropin inhibit replication of human immunodeficiency virus 1 by suppressing viral gene expression. J. Gen. Virol. 79:731-40.

Wu, YH, Zhang, BY, Qiu, LP, Guan, RF, Ye, ZH, *et al*, 2017: Structure properties and mechanisms of action of naturally originated phenolic acids and their derivatives against human viral infections. Curr. Med. Chem. 24, 38: 4279-302.

Yamauchi, H, 2001: Two novel insect defensins from larvae of the cupreous chafer, *Anomala cuprea*: purification, amino acid sequences and antibacterial activity. Insect Biochem. Mol. Biol. 32:75-84.

Yoo, YC, Shin, BH, Hong, JH, Lee, J, *et al*, 2007: Isolation of fatty acids with anticancer activity from *Protaetia brevitarsis* Larva. Arch Pharm Res.30, 3:361-5.

Zasloff, M, 2002: Antimicrobial peptide of multicellular organisms. Nature 415: 389-95.

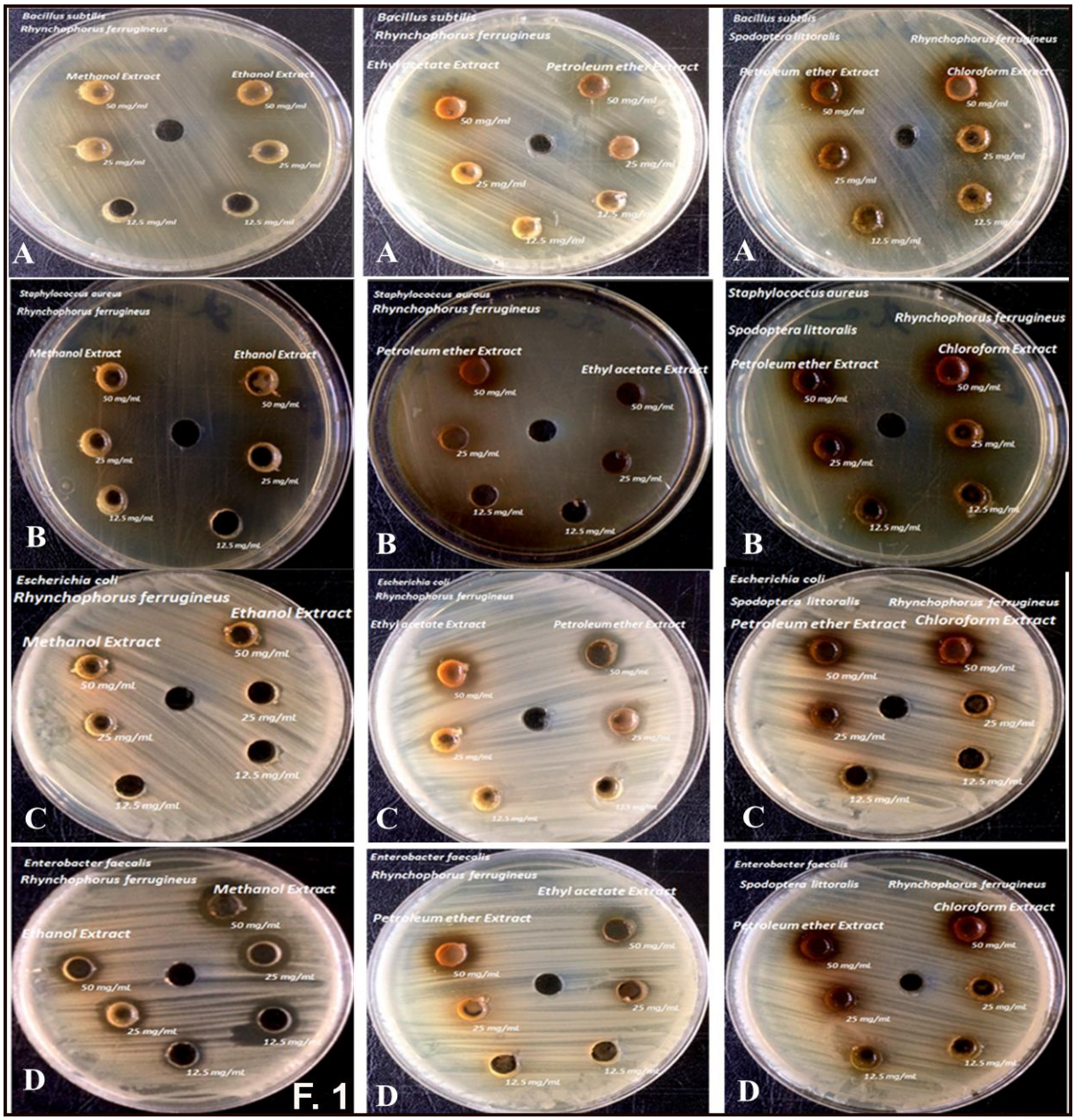

Fig. 1: Effect of *R. ferrugineus* larval extracts against Gram–positive and Gram–negative bacteria (A) *Bacillus subtilis*. (B) *Staphylococcus aureus*. (C) *Escherichia coli*. (D) *Enterobacter faecalis* represented by inhibition zone (mm).

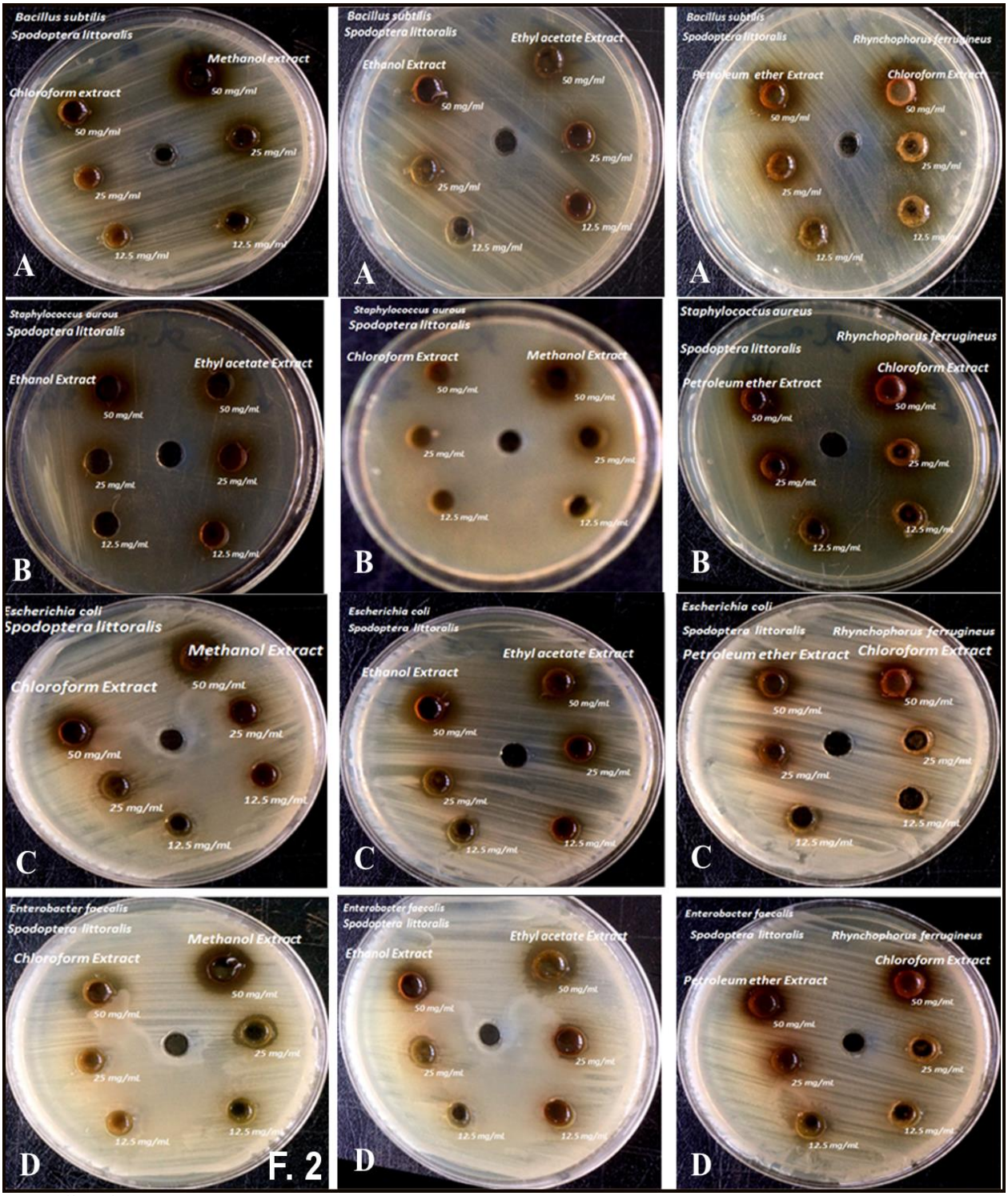

Fig. 2: Effect of *S. littoralis* larval extracts against the Gram–positive and Gram–negative bacteria (A) *Bacillus subtilis*. (B) *Staphylococcus aureus*. (C) *Escherichia coli*. (D) *Enterobacter faecalis* represented by inhibition zone (mm).

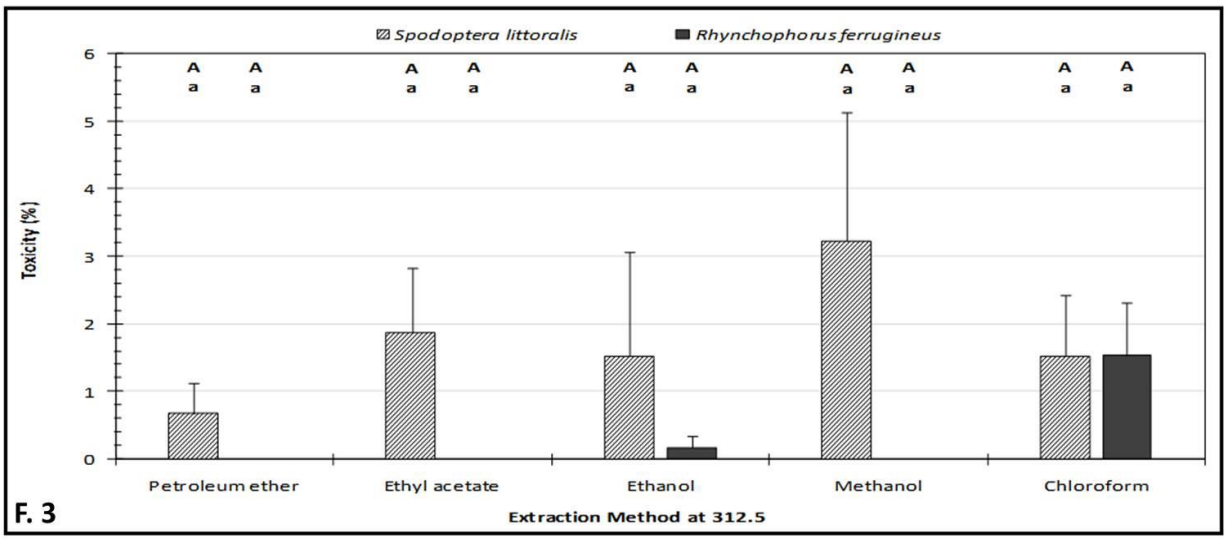

Fig. 3: Cytotoxicity assay of Vero cells in different crude extracts of *S. littoralis* and *R. ferrugineus* larvae at 312.5µg/mL concentration. Capital letters represent comparisons between extracting methods.

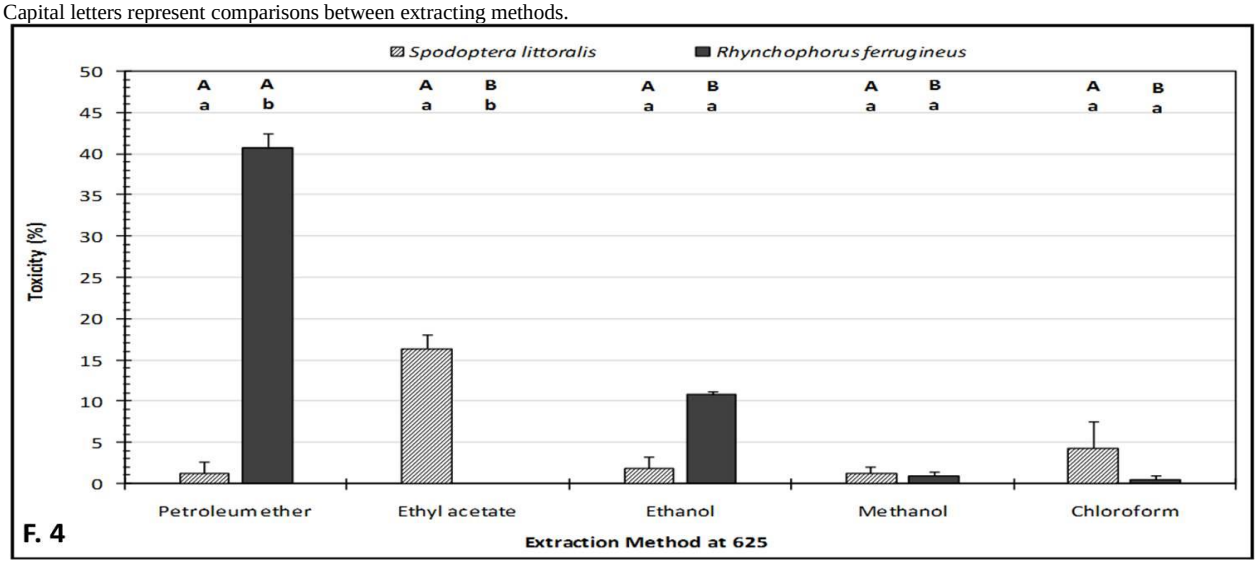

Fig. 4: Cytotoxicity assay of Vero cells in different crude extracts of *S. littoralis* and *R. ferrugineus* larvae at 625µg/mL concentration. Capital letters represent comparisons between extracting methods.