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High-performance Liquid Chromatography (HPLC) Analysis and Cytotoxic Activity of *Rhynchophorus ferrugineus* and *Spodoptera littoralis* Larval Extract

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Abstract

INSECTS PROVIDE a source of natural compounds that may have therapeutic benefits on many diseases including cancer. The present study aimed at investigating the cytotoxic activity of red palm weevil, *Rhynchophorus ferrugineus* and Egyptian cotton leaf worm, *Spodoptera littoralis* larval extract against adenocarcinoma-Breast cancer (MDA), Hepatocellular carcinoma (HepG2), and Normal cell line (Wi38), as well as High -performance liquid chromatography (HPLC) analysis of tested extracts. The extraction was carried out using n-butanol solvent. Generally, a comparison of cytotoxicity against MDA and HepG2 cancer cell lines with the Wi38 cell line, showed that the viability of fibroblast was higher than cancer cells after treated with n-butanol extract from *R. ferrugineus* and *S. littoralis* larvae different concentrations and have less cytotoxicity on fibroblast than cancer cell lines, it means that their side effects are low. The *R. ferrugineus* and *S. littoralis* larval n-butanol extracts recorded IC_{50} values of 315.17 and 188.78 µg/ml against Wi38; 81.19 and 96.55 µg/ml against MDA; 49 and 92.78 µg/ml against HepG2, respectively. Also, the HPLC analysis of n-butanol extract from *R. ferrugineus* and *S. littoralis* larvae revealed the presence of chlorogenic acid, catechin, and gallic acid with a moderate concentration, as well as methyl gallate, ellagic acid, ferulic acid and quercetin within the extracts, making substantial contributions to the therapeutic properties of tested extracts.

Keywords: *Culex antennatus, Rosa arabica, Eucalyptus citriodora,* Biosynthesis, Larvicidal.

Introduction

Cancer is widely recognized as one of the primary causes of mortality globally. Significant progress has been made in comprehending cancer biology in recent decades, resulting in improved diagnostic and therapeutic approaches. Insect-derived compounds can directly impact tumor cells by disrupting cell metabolism, inhibiting cell growth, or triggering cell death [1]. Insects' derived materials can act on tumor cells directly to interfere with cell metabolism, inhibit cell growth, or induce cell apoptosis. It also has an antitumor effect through improving the body's immune function [2-5].

Generally, arthropods including insects are still as a large, undiscovered, and unexploited natural sources of bioactive compounds for the modern medicine, particularly insects which comprise approximately more than 50.0% of total biodiversity and approximately more than 80.0% of all animal diversity and are present in many types of ecological systems [4, 6-9].

Recently, insects proved to have evolved innate immune systems that produce antimicrobial peptides for protection from several pathogens invasion, and these peptides are viewed as a strong antibiotic candidate and increase the possibility of using insects' body extracts as antimicrobial and anticancer agents [5,8,10-13].

Since more studies are needed to increase the knowledge of new natural materials to be used against cancer, the present study dealt with investigating the cytotoxic activity of red palm weevil, *Rhynchophorus ferrugineus* and Egyptian cotton leaf worm, *Spodoptera littoralis* larval n-butanol extract against adenocarcinoma-Breast cancer (MDA), Hepatocellular carcinoma (HepG2), and Normal cell line (Wi38), as well as High -

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performance liquid chromatography (HPLC) analysis of tested extracts.

Material and Methods

Rhynchophorus ferrugineus colony

The palm weevil larvae, *R. ferrugineus,* were gathered from the Central Laboratory for Date Palm Research and Development, Agricultural Research Center, Giza. They were subsequently bred for multiple generations in an insectary rearing laboratory located in the Animal House building of the Zoology Department, Faculty of Science, Al-Azhar University (Cairo). The larvae were raised under controlled conditions of temperature (25-27°C), relative humidity (60-70%), and photoperiods (12-12 hours light-dark rhythm) within a wooden cage measuring 200×120×300 cm. Briefly, three different media were used for feeding and egg production; cotton wool pieces saturated with 20.0% honey; five pieces of apple and five pieces of sugarcane (5×2 cm). Fifteen pairs of *R. ferrugineus* were cleaned with distilled water and kept together in 300 ml glass jars having the above-mentioned medium separately. About 100 eggs each were transferred with the fine hairbrush from above mentioned three media to four medium for hatching (Cotton wools saturated with 20.0% honey solution in plastic container, petri dishes lined with moistened filter paper, pieces of sugarcane and pieces of apple in plastic container). The larvae were transferred in cotton wools saturated with 60.0% honey, fresh apple, fresh sugarcane stem (20 cm) with the help of fine hairbrush by making small hole on one end of sugarcane stem and on apple. When the last larval stage reached the bottom of stem and about to pupate (pre-pupal stage) it was transferred to fresh sugarcane stem. The adults came out by rupturing the cocoon.

Spodoptera littoralis colony

Egg masses of a field strain of the cotton leaf worm Spodoptera littoralis were gathered from a cotton field in Benha, Al-Qalyubia Governorate, Egypt. The cotton leaf worm egg-masses were raised in the laboratory in a temperature range of 27-29°C and relative humidity of 65-70% on fresh cotton leaves to produce the larvae required for the experiment, following a process outlined in a previous study [15]. Approximately 50-60 pupae from egg-masses collected in the field were placed in wide glass jars until they emerged as adults. Adults were given blotting paper or branches of Tafla (Nerium oleander) for oviposition and were fed with 10.0% honey-water on a cotton wick as a feeding source.

After adult emergence (5-6 days post-pupation) and the start of oviposition, the blotting paper or Tafla leaves with eggs were taken out every 2 days. They were then immersed in 5% formaldehyde for 5 minutes and washed in running distilled water for 5 minutes. The blotting paper or Tafla leaves were then placed on paper towels and left to dry overnight. The next day, egg masses were transferred to a container with cotton leaves and the rearing process was repeated until they reached the necessary developmental stages.

Genetic identification of Rhynchophorus ferrugineus and Spodoptera littoralis

Extraction of DNA

The larval specimens of *R. ferrugineus* and *S. littoralis* were dissected into minute pieces and then stored in 1.5 L Eppendorf tubes. The DNA extraction was carried out using the PureLink® Genomic DNA Kits from Invitrogen, located in Waltham, Massachusetts, USA. Each sample was combined with tissue lysis buffer (about 180-250 µL) and then exposed to proteinase K (10 µL per 180 µL of tissue lysis buffer). Subsequently, the combination was kept at 56°C for 4 hours. The supernatant was moved to a fresh tube following the guidelines provided by the manufacturer (Invitrogen, Waltham, Massachusetts, USA). 200 µL of ethanol and 200 µL of Lysis/Binding Buffer were added to the lysate, and then the mixture was vortexed. The mixture was then placed into a spin column and centrifuged at 10,000 xg for one minute. Following two washes with wash buffers, DNA was extracted in 50 µL of elution solution and kept at -20°C for future use.

Polymerase chain reaction (PCR)

A primer designed to target the cytochrome oxidase COI subunit I region of mitochondrial DNA was used, as outlined in a prior study [16]. The forward primer, LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3'), and the reverse primer, LCO1490-R (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'), were used. The PCR amplification was conducted in a 50 µL final reaction volume containing 25 µL of 2X master mix solution (i-Taq, iNtRON, Seongnam, Korea), 0.2 µM (2 µL) of each primer, 4 µL of template DNA, 0.2 mg/mL of BSA, and 14.5 µL of nuclease-free water. The PCR amplification of ticks used a thermal cycling regimen consisting of an initial denaturation phase at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 46°C for 1 minute, and extension at 72°C for 1 minute. A final elongation step was carried out at 72°C for 10 minutes. The quality and quantity of the

PCR result were evaluated by seeing the amplified DNA on a 1% agarose gel stained with ethidium bromide, using a U.V. transilluminator (Spectroline, Westbury, USA).

Sequence analysis

The PCR products were purified with a Macrogen reagent from Seoul, Korea. Single-strand DNA sequencing was conducted, followed by aligning the nucleotide sequences of *R. ferrugineus* and *S. littoralis* COI.

Bioinformatics

The sequences were compiled using Chromas Pro 1.5 beta software developed by Technelysium Pty. in Tewantin, QLD, Australia. The recently obtained COI sequences of R. ferrugineus and S. littoralis (Accession number: OR958714 and OR958713) were analyzed against existing sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) accessible at http://blast.ncbi.nlm.nih.gov/Blast.cgi. The sequences were aligned using muscle alignment in MEGA 11.0 software. The Tamura-3-parameter method is used to determine sequence divergences [17]. N.J. trees utilize the Tamura 3-parameter approach to demonstrate species divergence patterns. Bootstrapping was conducted with 1000 replicates in MEGA 11.0 [18]. Improving visualization through the utilization of ITOl software [19]. We assessed the minimal spanning network for haplotype divergence using PopArt v.3.0.

Determination of n-butanol extraction' cytotoxicity on cells (MTT protocol):

The Lung fibroblast cell line (WI38), Adenocarcinoma-Breast cancer (MDA), and human liver cancer cell line (HepG2) were provided by the Department of Microbiology and Immunology at the National Research Centre in Dokki, Giza, Egypt. The cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum (FBS), 100 µg/ml penicillin, and 100 µg/ml streptomycin at 37°C in a 5% CO2 humidified incubator.

Samples were prepared for MTT analysis as described in reference [20]. The 96-well tissue culture plate was seeded with 1×105 cells/ml (100 µl/well) and then kept at 37°C for 24 hours to form a full monolayer. The growth medium was poured out of the 96-well microtiter plates after a confluent sheet of cells had formed. The cell monolayer was then washed twice with wash media. The analyzed sample was diluted by a factor of two in RPMI medium containing 2% serum (maintenance medium). 0.1 ml of each dilution was tested in several wells, with 3 wells serving as controls and receiving just maintenance medium. The plate was placed in an incubator at 37 degrees Celsius and inspected. Cells were examined for physical indications of toxicity such as partial or total loss of the monolayer, rounding, shrinkage, or cell granulation. MTT solution was produced at a concentration of 5 mg/ml in PBS from BIO BASIC CANADA INC. Approximately 20 microliters of MTT solution were applied to each well. Subjected to a shaking table at 150 rpm for 5 minutes to effectively blend the MTT into the medium. Incubated at 37°C with 5% CO2 for 4 hours to enable the metabolism of MTT. Remove excess media by dumping it, and if needed, dry the plate on paper towels to eliminate any residue. Reconstitute formazan (MTT metabolic product) in 200 µl DMSO. Place on a shaking table at 150 rpm for 5 minutes to ensure full mixing of the formazan with the solvent. opacity Measure the absorbance at 560 nm and then remove the background absorbance at 620 nm. Optical density is directly proportional to the number of cells. The cell viability % was determined using the following formula: The formula for cell viability is calculated by dividing the mean optical density (OD) of treated wells by the mean OD of the control well, and then multiplying the result by 100 to get the percentage.

Cell viability is linked to significant morphological alterations that take place at the cell surface or inside the cytoskeleton. Damage is characterized by significant reductions in volume caused by protein and intracellular ion losses due to changes in sodium or potassium permeability. Necrotic cells exhibit nuclear enlargement, chromatin flocculation, and lack of nuclear basophilia. Apoptotic cells exhibit cell shrinkage, nuclear condensation, and nuclear fragmentation.

High-Performance Liquid Chromatography (HPLC) of n-butanol extraction:

The HPLC analysis was conducted out using an Agilent 1260 series. The separation was carried out using Zorbax Eclipse Plus C8 column (4.6 mm × 250 mm i.d., 5 µm). The mobile phase comprised of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was sequentially coded in a linear gradient. 0 min (82% A); 0-1 min (82% A); 1-11 min (75% A); 11-18 min (60% A); 18-22 min (82% A); 22-24 min (82% A). The detector was monitored at a wavelength of 280 nm. Each sample solution was injected with a volume of 5 µl. The column temperature was held constant at 40°C [21,22].

Statistical analysis

The data was encoded and inputted with the statistical software SPSS V.22. Data were assessed for meeting the assumptions of parametric tests. Continuous variables underwent Shapiro-Wilk and Kolmogorov-Smirnov tests for normality. The probability and percentile data were normalized for normal distribution using Arcsine Square Root transformation. The data were given as the mean and standard deviation. ANOVA analyses were conducted on experimental groups to assess recorded mortalities and enzymatic activities. Each group had a minimum of three replicates. Post-hoc analysis was performed using Tukey pairwise comparison. Statistical significance was set at a P-value of <0.05. The analysis was conducted using MiniTab V 14. Data were shown graphically using R Studio version 2022.02.4.

Results

Genetic identification of Rhynchophorus ferrugineus and Spodoptera littoralis

Evolutionary history was inferred using the Neighbor-Joining method. The optimal trees are shown (Figures 1&2, *i*). The minimum haplotype spanning network represented the haplotypes of the nucleotide sequences evaluated in the present work for both species. Revealing the haplotype diversity. Nucleotide diversity shows a pi-value of 0.2474 and 0.14495 for *R. ferrugineus* and *S. littoralis* respectively; the number of segregating sites was 15 and 12 respectively, while 2 and 0 parsimony-informative sites were observed. The Tajima's D statistic was calculated to be -1.88604 and -2.38064 with P (D >= -1.88604 and -2.38064) = 0.999915 and 0.98524 (Fig. 1&2, *ii*).

Cytotoxicity of tested insects' n-butanol larval extracts

Human fetal lung fibroblast cells (Wi38)

Results given in Table (1) and Figures (3&4) showed the cytotoxicity of *R. ferrugineus* and *S. littoralis* larval n-butanol extracts on normal cells (Wi38). The cellular viability revealed that, 1000 µg/ml of *R. ferrugineus* and *S. littoralis* larval n-butanol extracts induced a drastic decreased in cellular viability with 4.76 and 3.42% cell viability, while at 125 µg/ml cell viability recorded 95.63 and 82.9%, respectively. The IC_{50} value recorded 315.17 and 188.78 µg/ml for *R. ferrugineus* and *S. littoralis* larval n-butanol extracts, respectively.

Adenocarcinoma-Breast cancer (MDA)

The cytotoxicity of *R. ferrugineus* and *S. littoralis* larval n-butanol extracts on MDA was recorded in Table (2) and illustrated in Figures (5&6). The cellular viability recorded 96.99 and 97.41% at 1000 µg/ml of *R. ferrugineus* and *S. littoralis* larval n-butanol extracts, respectively. The IC_{50} value recorded 81.19 and 96.55 µg/ml for *R. ferrugineus* and *S. littoralis* larval n-butanol extracts, respectively.

Hepatocellular carcinoma (HepG2)

The cytotoxicity of *R. ferrugineus* and *S. littoralis* larval n-butanol extracts on HepG2 cell line was recorded in Table (3) and illustrated in Figures (7&8). The cellular viability recorded 96.24 and 97.51% at 1000 µg/ml of *R. ferrugineus* and *S. littoralis* larval n-butanol extracts, respectively. The IC_{50} value recorded 71.49 and 92.78 µg/ml for *R. ferrugineus* and *S. littoralis* larval n-butanol extracts, respectively.

High-Performance Liquid Chromatography (HPLC) of insects' n-butanol larval extracts

The HPLC presents a quantitative analysis of phenolic and flavonoid compounds in n-butanol extraction from *R. ferrugineus* and *S. littoralis* larvae revealed the presence of compounds like Gallic acid, Chlorogenic acid, Catechin, and others are quantified in terms of their concentration in µg/ml and µg/g in both extracts. Remarkably, Gallic acid shows the presence in the *R. ferrugineus* and *S. littoralis* n-butanol extract (80.04 and 3.30 µg/ml), respectively. Also, Chlorogenic acid, found in moderate amounts (94.52 and 10.42 µg/ml) in both extracts. In addition, Catechin, a well-known flavonoid, has been recorded with small amounts in both extracts. These compounds, along with others like Ferulic acid and Quercetin, which are present in varying concentrations, contribute to the overall pharmacological potential of the extracts (Tables 4-7 and Fig. 10).

Discussion

The results showed that the red palm weevil, Rhynchophorus ferrugineus, and the Egyptian cotton leaf worm, Spodoptera littoralis, utilized in the study had over 100% COI sequence identity with their respective species. The significant sequence similarity in the cytochrome c oxidase subunit I (COI) gene, commonly employed for species identification and phylogenetic analysis, validates the precise identification of the specimens in the research. The COI gene is a dependable method for distinguishing species and has been widely used in DNA barcoding projects in many taxonomic groups,

such as insects. [23]. The accurate molecular identification of the target species is crucial, particularly in studies involving pest management or biological control strategies. The red palm weevil, *Rh. ferrugineus*, is a major pest that has caused significant damage to palm plantations worldwide, while *S. littoralis* is a polyphagous pest that poses a threat to various agricultural crops, including cotton [27]. By confirming the species identity through COI sequence analysis, we ensured the reliability of our findings and the applicability of their results to the intended target species.

The *in-vitro* assay findings of cytotoxicity of *R. ferrugineus* and *S. littoralis* larval n-butanol extract are very persuasive, specifically regarding the discernible distinctions in effects between the normal cell line (Wi38) and cancer cell lines (Adenocarcinoma-Breast cancer, MDA, and human liver cancer cell line, HepG2). Consistent with the previous reports, The IC_{50} value recorded 315.17 and 188.78 µg/ml for *R. ferrugineus* and *S. littoralis* larval n-butanol extract against Wi38 cell line, respectively. Meanwhile, The IC_{50} value recorded 81.19, 96.55 and 71.49, 92.78 µg/ml for *R. ferrugineus* and *S. littoralis* larval n-butanol extract against MDA and HepG2 cancer cell lines, respectively. The results from a previous study show that the excretion/secretion of *Lucilia sericata* and *Chrysomya albiceps* maggots have significant anticancer effects on various human tumor cell lines. The IC_{50} values for *L. sericata* ES range from 14.8±0.05 to 85.6±0.35 µg/ml, while for *C. albiceps* ES they range from 17.3±0.26 to 89.5±0.34 µg/ml against different cell lines (HepG-2, MCF-7, HCT-116, A-549, CACO, PC-3, and HELA). Additionally, the methanol crude extract from maggots of *Musca domestica, L. sericata*, and *C. albiceps* showed the highest anticancer effect on the human colon carcinoma cell line (Caco-2) with *L. sericata* maggots methanolic extract being the most effective, followed by *C. albiceps* and *M. domestica* at a concentration of 25 mg/ml. The IC_{50} values of the investigated extracts varied between 0.27 and 1.91 mg/ml [4].

On the other hand, The HPLC provided information offers significant insights into the potential pharmacological properties of n-butanol extraction from *R. ferrugineus* and *S. littoralis* larvae. Particularly the concentration and presence of compounds such as chlorogenic acid, catechin, and gallic acid. The antioxidant activity of gallic acid, which is present in significant amounts in the *R. ferrugineus* and *S. littoralis* n-butanol extract, have been widely reported. In (2014), Kumar and Pruthi reported that the significance ability of gallic acid as a potent antioxidant, eliminating free radicals and so aiding in the mitigation of oxidative stress inside the body. Considering the correlation between oxidative stress and a multitude of chronic ailments, such as cancer and cardiovascular disease, this activity is of the utmost importance [25]. The concentrations of chlorogenic acid found in the *R. ferrugineus* and *S. littoralis* (larvae) n-butanol extract have been recognized as having neuroprotective and anti-inflammatory properties [26]. Several biological pathways implicated in inflammatory responses have been observed to be modulated by this compound, which has demonstrated potential as a neuroprotectant, specifically in relation to neurodegenerative disorders. Catechin, a widely underscored flavonoid proved to have anti-cancer benefits [27]. Notably, catechin proved to inhibits cancer cells proliferation inducing apoptosis, rendering it a substance of potential therapeutic significance in the field of cancer research. Additional components such as methyl gallate, ellagic acid, ferulic acid and quercetin, however found in different amounts within the extracts, make substantial contributions to the therapeutic properties of *R. ferrugineus* and *S. littoralis* (larvae) n-butanol extract. A variety of biological actions, including anti-cancer, antioxidant, and anti-inflammatory, are attributed to these substances.

Conclusions

In conclusion red palm weevil, *Rhynchophorus ferrugineus* and Egyptian cotton leaf worm, *Spodoptera littoralis* larval n-butanol extract inhibited the proliferation of tumor cells. In addition, further studies are needed to elucidate the activity of various insects' extracts against other cancer cell lines. Nevertheless, in depth isolation and structural elucidation of the extracts should be accompanied with investigating the anticancer activity of the isolated compounds.

Conflicts of interest

The authors declare no conflict of interest.

Funding statement

This study was self-funded

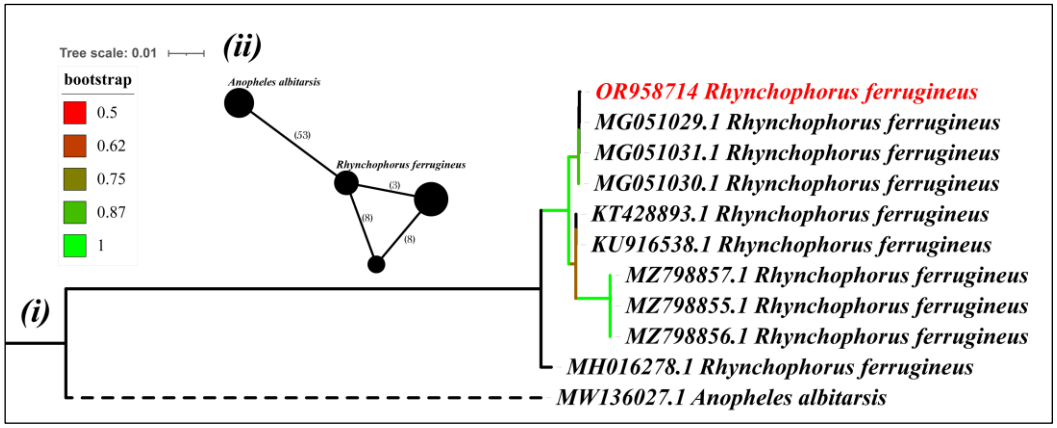

Fig. 1. ***(i)*** **Neighbor-Joining phylogenetic evolutionary tree** ***(ii)*** **Minimum haplotype spanning network for** ***Rhynchophorus ferrugineus*****.**

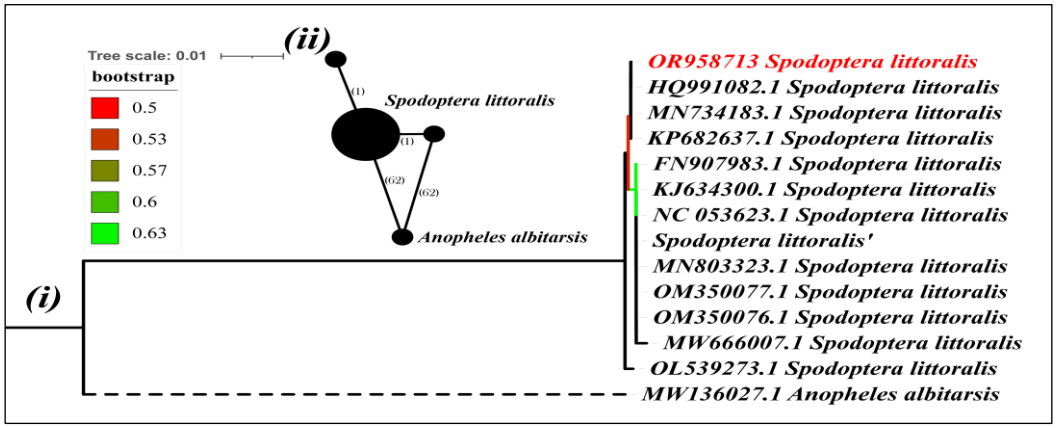

Fig. 2. ***(i)*** **Neighbor-Joining phylogenetic evolutionary tree** ***(ii)*** **Minimum haplotype spanning network for** ***Spodoptera littoralis.***

TABLE 1. Cytotoxicity of tested extract against Human fetal lung fibroblast cells (Wi38).

Tested extracts	Concentrations (µg/ml)	Viability (%)	IC_{50}±SD
Wi38	--------	100.0	
R. ferrugineus larval n-butanol extract	1000	4.761904762	315.17± 4.41
	500	18.03921569	
	250	51.2605042	
	125	95.6302521	
	62.5	99.43977591	
	31.25	99.49579832	
S. littoralis larval n-butanol extract	1000	3.417366947	188.78±1.08
	500	3.697478992	
	250	22.57703081	
	125	82.91316527	
	62.5	99.60784314	
	31.25	99.77591036	

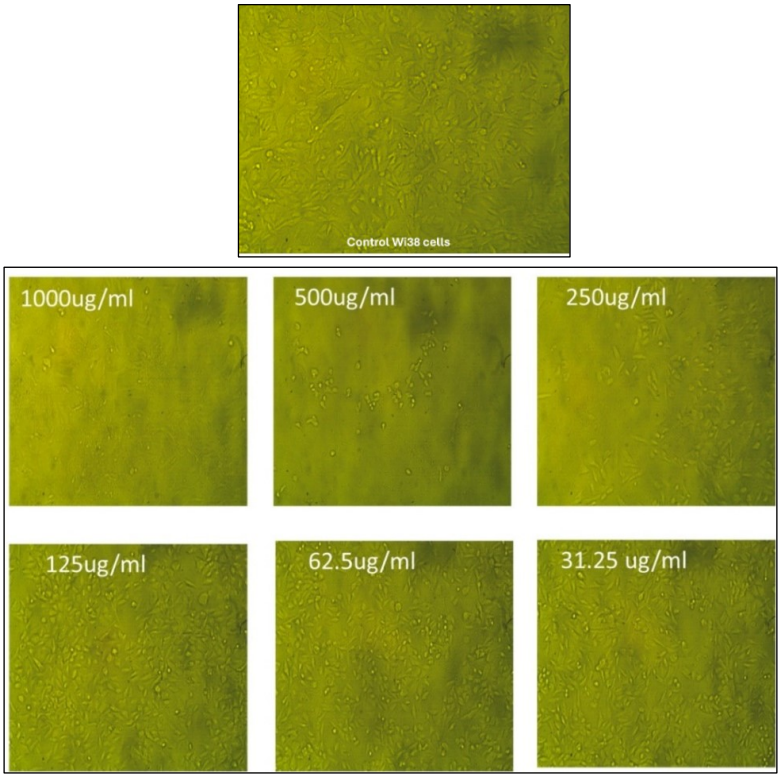

Fig. 3. Morphological changes of Wi38 cell lines treated with *Rhynchophorus ferrugineus* larval n-butanol extract, cell line displayed the atrophied and could not adhere, with effect of the different concentrations.

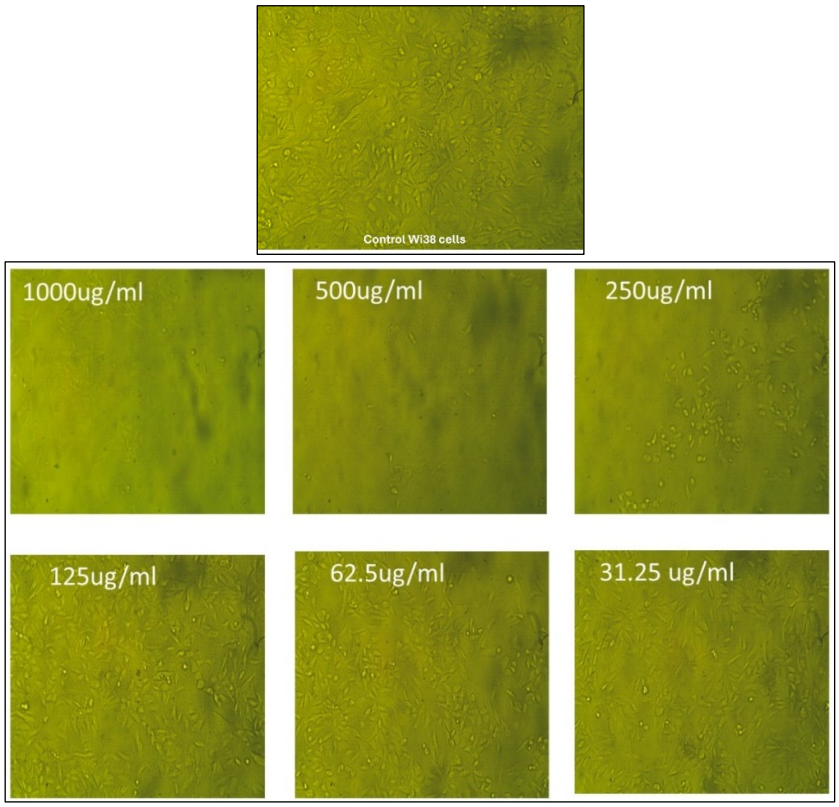

Fig. 4. Morphological changes of Wi38 cell lines treated with *Spodoptera littoralis* larval n-butanol extract, cell line displayed the atrophied and could not adhere, with effect of the different concentrations.

TABLE 2. Cytotoxicity of tested extract against Adenocarcinoma-Breast cancer (MDA)

Tested extracts	Concentrations (µg/ml)	Viability (%)	IC_{50}±SD
MDA	--------	100.0	
R. ferrugineus larval n-butanol extract	1000	3.013182674	81.19±0.65
	500	4.190207156	
	250	6.355932203	
	125	23.96421846	
	62.5	51.31826742	
	31.25	94.06779661	
S. littoralis larval n-butanol extract	1000	2.589453861	96.55±0.61
	500	2.777777778	
	250	7.721280603	
	125	21.56308851	
	62.5	98.96421846	
	31.25	99.85875706	

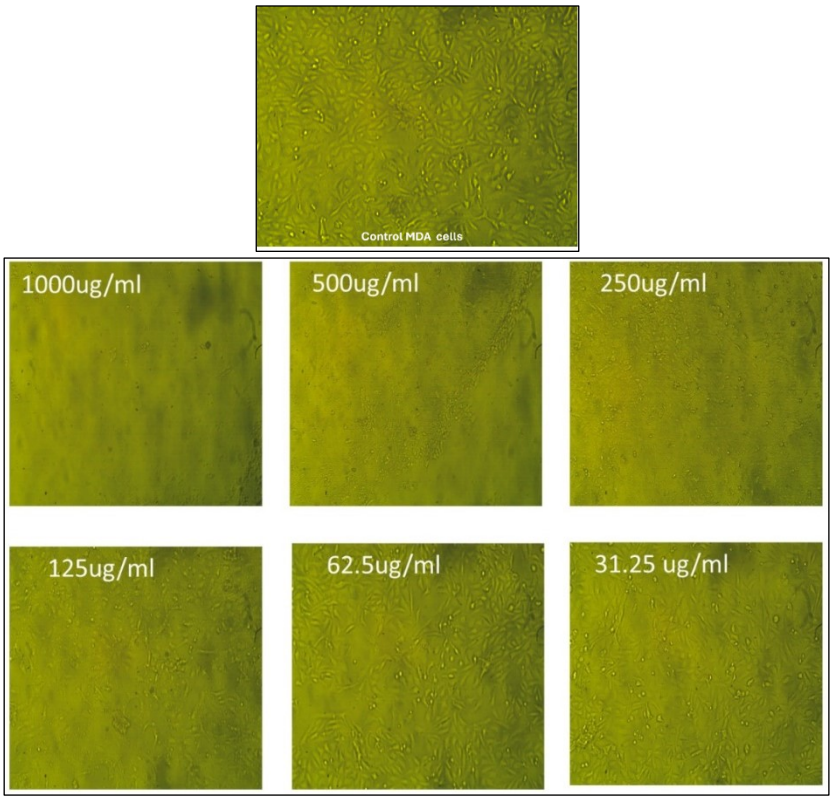

Fig. 5. Morphological changes of MDA cell lines treated with *Rhynchophorus ferrugineus* larval n-butanol extract, cell line displayed the atrophied and could not adhere, with effect of the different concentrations.

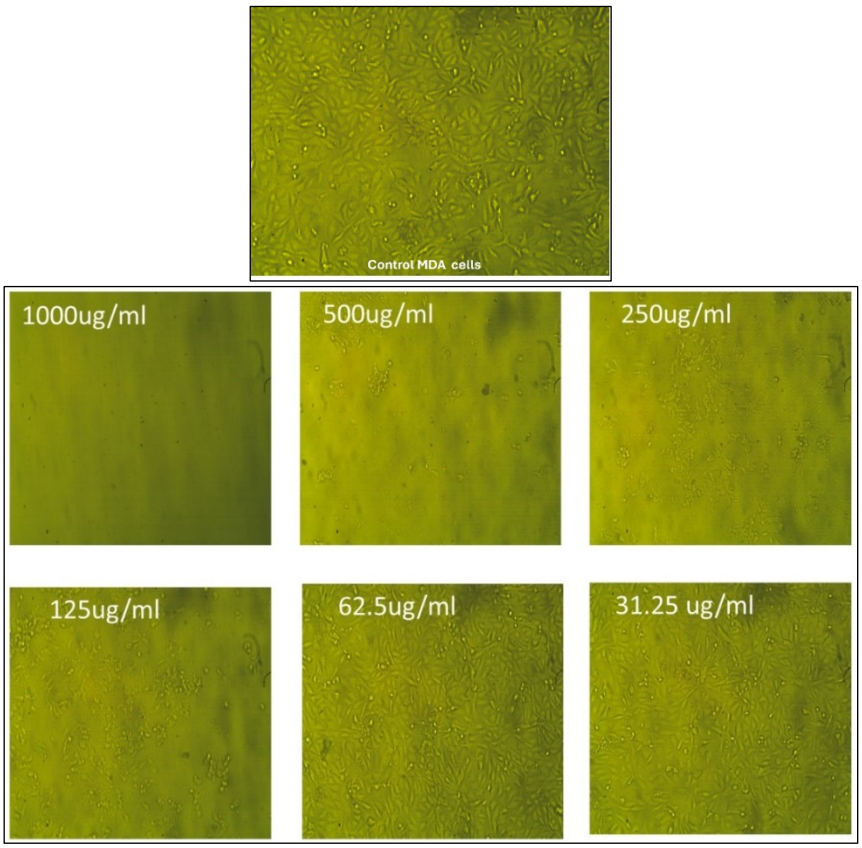

Fig. 6. Morphological changes of MDA cell lines treated with *Spodoptera littoralis* larval n-butanol extract, cell line displayed the atrophied and could not adhere, with effect of the different concentrations.

TABLE 3. Cytotoxicity of tested extract against Hepatocellular carcinoma (HepG2)

Tested extracts	Concentrations (µg/ml)	Viability (%)	IC_{50}±SD
MDA	--------	100.0	
R. ferrugineus larval n-butanol extract	1000	3.759057971	71.49±1.41
	500	5.570652174	
	250	5.842391304	
	125	14.80978261	
	62.5	47.32789855	
	31.25	84.64673913	
S. littoralis larval n-butanol extract	1000	2.490942029	92.78±0.97
	500	2.490942029	
	250	7.518115942	
	125	17.21014493	
	62.5	91.5307971	
	31.25	99.63768116	

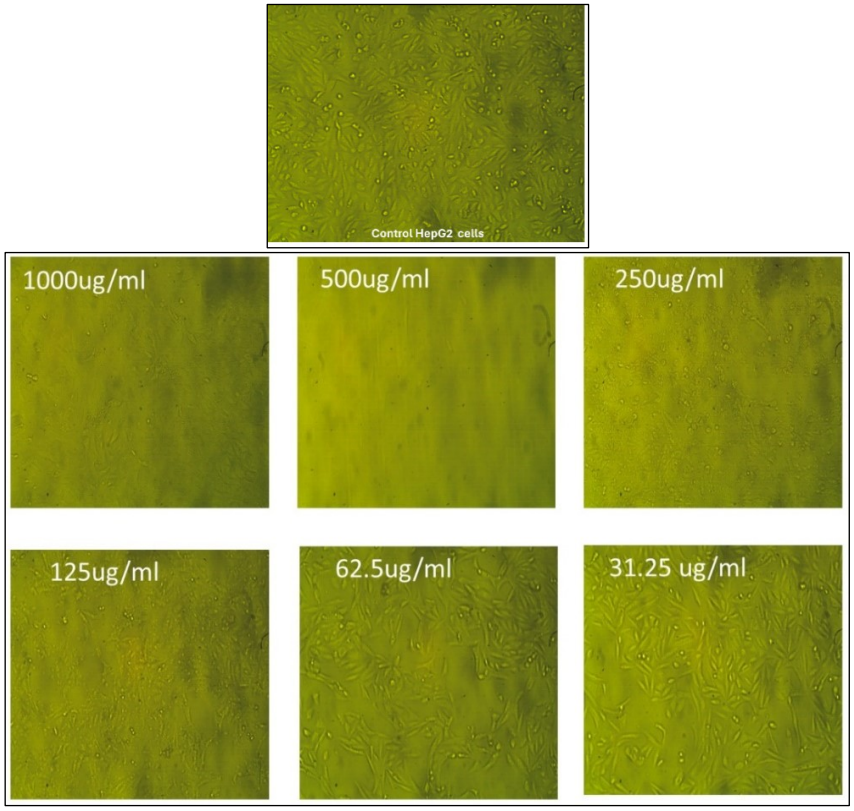

Fig. 7. Morphological changes of HepG2 cell lines treated with *Rhynchophorus ferrugineus* larval n-butanol extract, cell line displayed the atrophied and could not adhere, with effect of the different concentrations.

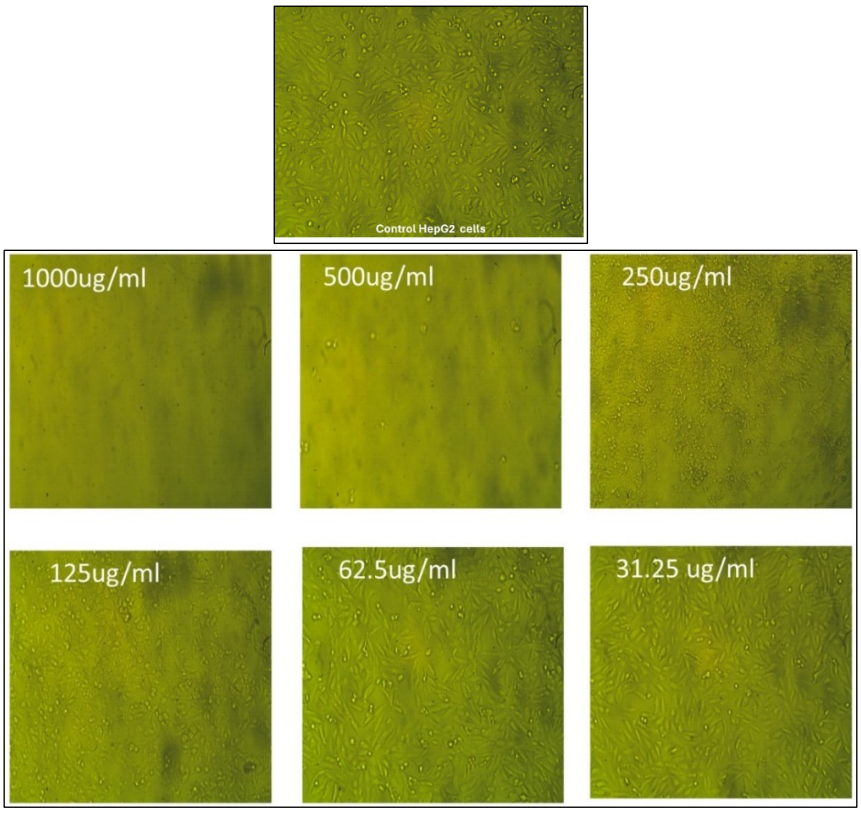

Fig. 8. Morphological changes of HepG2 cell lines treated with *Spodoptera littoralis* larval n-butanol extract, cell line displayed the atrophied and could not adhere, with effect of the different concentrations.

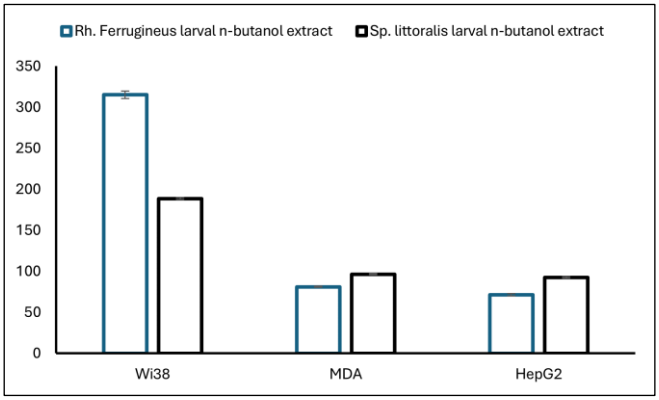

Fig. 9. Cytotoxicity of *Rhynchophorus ferrugineus* and *Spodoptera littoralis* larval n-butanol extracts against wi38, MDA and HepG2 cell lines.

TABLE 4. Phenolic and flavonoid compounds concentrations (µg/ml and µg/g) in *Rhynchophorus ferrugineus* larvae' n-butanol extract.

Compound	Area	Concentration (µg/ml)	Concentration (µg/g)
Gallic acid	964.51	80.04	4001.87
Chlorogenic acid	699.31	94.52	4726.06
Catechin	15.28	3.61	180.28
Methyl gallate	278.96	14.89	744.75
Coffeic acid	0.00	0.00	0.00
Syringic acid	58.07	4.07	203.48
Rutin	0.00	0.00	0.00
Ellagic acid	96.47	9.84	491.86
Coumaric acid	129.28	4.59	229.51
Vanillin	575.07	22.21	1110.58
Ferulic acid	127.48	7.50	374.89
Naringenin	127.93	12.06	603.25
Rosmarinic acid	3.37	0.35	17.74
Daidzein	2.68	0.18	8.82
Querectin	6.03	0.38	19.18
Cinnamic acid	53.42	0.97	48.39
Kaempferol	0.00	0.00	0.00
Hesperetin	0.00	0.00	0.00

TABLE 5. Area % in *Rhynchophorus ferrugineus* larvae' n-butanol extract in HPLC Profiles.

Peak #	Ret.Time (min.)	Type	Width (min.)	Area (mAu*s)	Area (%)	Name
1	3.588	BV	0.0723	964.50970	30.7380	Gallic acid
2	4.253	VB	0.1193	699.30530	22.2862	Chlorogenic acid
3	4.605	BB	0.1078	15.27958	0.4869	Catechin
4	5.667	MM	0.1274	278.95905	8.8901	Methyl gallate
5	5.936		0.0000	0.00000	0.0000	Coffeic acid
6	6.413	BV	0.1246	58.06845	1.8506	Syringic acid
7	6.928		0.0000	0.00000	0.0000	Rutin
8	7.174	BB	0.1813	96.46869	3.0744	Ellagic acid
9	8.735	BV	0.1525	129.28215	4.1201	Coumaric acid
10	9.108	MM	0.2304	575.06891	18.3269	Vanillin
11	9.599	BB	0.1852	127.48406	4.0628	Ferulic acid
12	10.628	BV	0.1556	127.92783	4.0769	Naringenin
13	11.680	VB	0.2073	3.37350	0.1075	Rosmarinic acid
14	15.790	BB	0.2493	2.67522	0.0853	Daidzein
15	16.923	VB	0.1411	6.02792	0.1921	Querectin
16	19.292	MM	0.1514	53.41569	1.7023	Cinnamic acid
17	20.671		0.0000	0.00000	0.0000	Kaempferol
18	21.257		0.0000	0.00000	0.0000	Hesperetin
	Totals:	**3137.84605**				

TABLE 6. Phenolic and flavonoid compounds concentrations (µg/ml and µg/g) in *Spodoptera littoralis* larvae' n-butanol extract.

Compound	Area	Concentration (µg/ml)	Concentration (µg/g)
Gallic acid	39.75	3.30	164.92
Chlorogenic acid	77.08	10.42	520.96
Catechin	5.80	1.37	68.40
Methyl gallate	12.81	0.68	34.21
Coffeic acid	19.32	1.64	81.84
Syringic acid	0.00	0.00	0.00
Rutin	13.28	2.16	108.20
Ellagic acid	75.22	7.67	383.53
Coumaric acid	3.72	0.13	6.60
Vanillin	12.95	0.50	25.02
Ferulic acid	1.14	0.07	3.36
Naringenin	16.89	1.59	79.63
Rosmarinic acid	159.21	16.75	837.41
Daidzein	3.87	0.26	12.76
Querectin	2.03	0.13	6.47
Cinnamic acid	17.27	0.31	15.65
Kaempferol	12.50	0.91	45.35
Hesperetin	8.11	0.37	18.39

TABLE 7. Area % in *Spodoptera littoralis* larvae' n-butanol extract in HPLC Profiles.

Peak #	Ret.Time (min.)	Type	Width (min.)	Area (mAu*s)	Area (%)	Name
1	3.574	MM	0.0833	39.74920	8.2645	Gallic acid
2	4.260	VB	0.1276	77.08461	16.0272	Chlorogenic acid
3	4.691	BB	0.1152	5.79746	1.2054	Catechin
4	5.681	BV	0.1718	12.81224	2.6639	Methyl gallate
5	5.933	VB	0.1612	19.31554	4.0160	Coffeic acid
6	6.427		0.0000	0.00000	0.0000	Syringic acid
7	6.929	BB	0.1467	13.27856	2.7608	Rutin
8	7.354	MM	0.1632	75.22213	15.6399	Ellagic acid
9	8.707	BV	0.1235	3.71996	0.7734	Coumaric acid
10	8.965	VV	0.1550	12.95487	2.6935	Vanillin
11	9.580	BB	0.1204	1.14312	0.2377	Ferulic acid
12	10.318	VV	0.1667	16.88608	3.5109	Naringenin
13	11.882	VB	0.1863	159.20859	33.1021	Rosmarinic acid
14	15.912	BB	0.2269	3.87095	0.8048	Daidzein
15	17.409	BV	0.1070	2.03480	0.4231	Querectin
16	19.282	BV	0.1870	17.27131	3.5910	Cinnamic acid
17	20.421	VB	0.2115	12.50391	2.5998	Kaempferol
18	21.173	MM	0.1109	8.10863	1.6859	Hesperetin
	Totals:	**480.96197**				

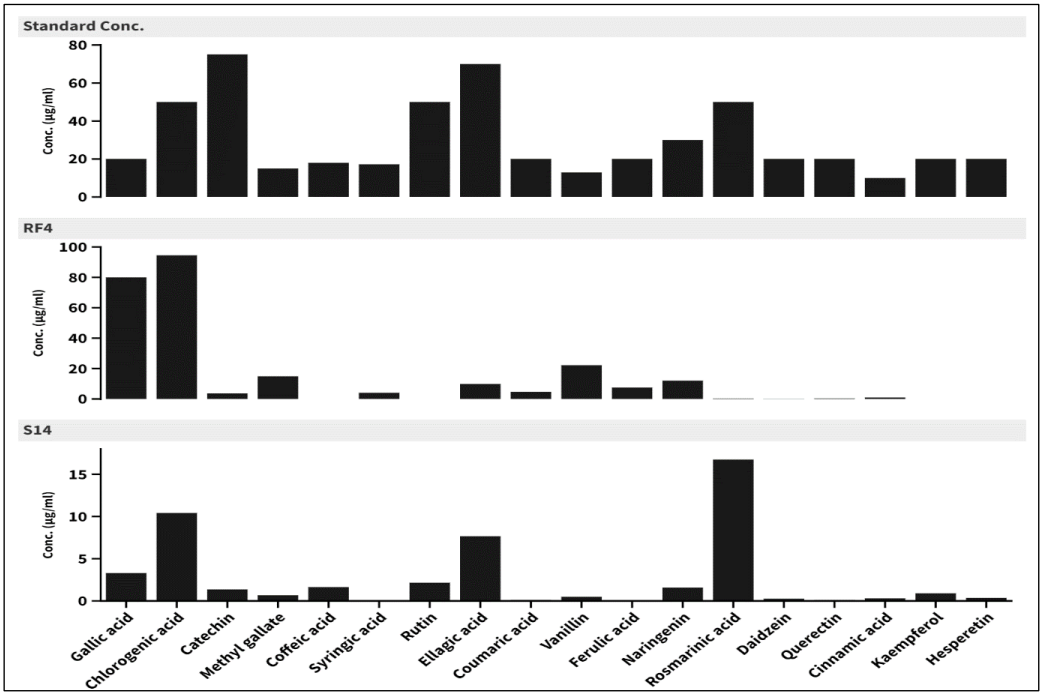

Fig. 10. Phenolic and Flavonoid Compounds' concentrations (µg/ml) in *Rhynchophorus ferrugineus* and of *Spodoptera littoralis* larvae' n-butanol extracts.

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النشاط السُمي الخلوي وتحليل اتش بي ال سي لمستخلص يرقات سوسة النخيل الحمراء، *رينكوفورس فيروجينيوس* ودودة ورق القطن المصري، *سبودوبترا ليتورالس*

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الملخص

توفر الحشرات مواد كيميائية طبيعية قد يكون لها تأثيرات علاجية في العديد من الأمراض، بما في ذلك السرطان. هدفت هذه الدراسة إلى دراسة التأثيرات السُمية الخلوية لمستخلصات يرقات سوسة النخيل الحمراء، *رينكوفورس فيروجينيوس* ودودة ورق القطن المصري، *سبودوبترا ليتورالس علي* سرطان الغدة- الثدي، وسرطان الخلايا الكبدية والخلايا الطبيعية. بالإضافة إلى ذلك، أجريت في هذه الدراسة تحليل كروماتوغرافيا سائلة عالية الأداء (اتش بي ال سي) على المستخلصات المختبرة. تم إجراء الاستخلاص باستخدام مذيب ن- بيوتانول. أكد تحليل تفاعلات البوليمرات المتسلسل (بي سي ار) التعريف الچيني لسوسة النخيل الحمراء ودودة ورق القطن المصري. التسلسلات التي تم الحصول عليها أضيفت بنجاح على بنك الچينات الدولي بأرقام: OR958714 و OR958713 علي التوالي. بشكل عام، أظهرت مقارنة السمية الخلوية ضد خطوط خلايا سرطان الغدة- الثدي وسرطان الخلايا الكبدية مع الخلايا الطبيعية ان قابلية بقاء الخلايا الليفية كانت أعلى من الخلايا السرطانية بعد علاجها بتركيزات مختلفة من مستخلص ن- بيوتانول من يرقات سوسة النخيل الحمراء ودودة ورق القطن المصري، ولها سمية خلوية أقل على الخلايا الطبيعية من خطوط الخلايا السرطانية، مما يعني أن آثارها الجانبية منخفضة. سجل مستخلص ن- بيوتانول من يرقات سوسة النخيل الحمراء ودودة ورق القطن المصري قيم تركيز مثبط نصفي تساوي 315.17 و188.78 ميكروغرام/مل ضد الخلايا الطبيعية؛ 81.19 و96.55 ميكروغرام/مل ضد خلايا سرطان الغدة- الثدي؛ 49.0 و92.78 ميكروغرام/مل ضد سرطان الخلايا الكبدية علي التوالي. ايضاً، تحليل كروماتوغرافيا سائلة عالية الأداء (اتش بي ال سي) لمستخلص ن- بيوتانول من يرقات سوسة النخيل الحمراء ودودة ورق القطن المصري كشف عن وجود حمض الكلوروچينيك، الكاتشين وحمض الغاليك بتركيزات معتدلة، بالإضافة إلى جالات الميثيل، حمض الإيلاچيك، حمض الفيروليك والكيرسيتين داخل المستخلصات مما ساهم بشكل كبير في الخصائص العلاجية للمستخلصات المختبرة

الكلمات الدالة: رينكوفورس فيروجينيوس، سبودوبترا ليتورالس، يرقات، مستخلصات، ضد السرطان.