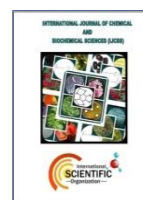
International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

© International Scientific Organization



Evaluation of the potential antitumor activities of some Egyptian spiders' venoms

Nehad Ali[1], Mona Eltonsy[1], Walaa Tantawy[1], Hamdy Aly[2], Waleed B. Suleiman[3]*

[1]Faculty of Science (girls), Al-Azhar University, Cairo-Egypt

[2]Faculty of Science, Al-Azhar University, Assiut Branch, Assiut-Egypt

[3] Faculty of Science (Boys), AlAzhar University, Cairo, Egypt

Abstract

Despite the diversity of strategies that treat cancer, it still poses a major threat to the world's population. Therefore, there is still a critical need to discover more safe anti-tumor drugs, especially those extracted from natural sources. Accordingly, the study focused on collecting several spiders from the Egyptian environment and identifying them morphologically. The venom glands were extracted, and the amount of total protein was estimated. A cytotoxicity test was conducted on 4 types of cancer cells hepatocellular (HEPG-2), lung (A549), colon (HCT-116), breast (MCF-7) versus a normal cell line WI-38, IC_{50} values were calculated, and the cell culture were examined. Four species were identified as follows: *Pardosa sp*., *Philodromus* sp., *Araneas sp*., and *Selenops sp.*, the total proteins ranged from 0.53 to 1.87 mg/ml. Both *Pardosa sp*., and *Selenops sp.* have the greater activity as antitumor agent against all tested cancer cell lines, their IC_{50} ranged from 186.5 to 592.8 and from 149.6 to 430.1 µg/ml, respectively in corresponding to those IC_{50} belong to the normal cell line which were > 1000 and 931.1 µg/ml, respectively. While the venom of *Philodromus* sp. gave the weakest results, it had a weak antitumor effect against HEPG-2 cell line. A moderate antitumor effect was reported by the venom of *Araneas sp*. which moderately inhibited the proliferation of both HEPG-2 and MCF-7 cell lines. Conclusively, spiders' venoms exhibited promising antitumor potential particularly against both liver and breast cancer cell lines with high safety profile against the normal cells.

Keywords: Spider venom; MTT assay; Cytotoxicity; Antitumor; Cancer.

Full length article **Corresponding Author*, e-mail: dr_wbs@azhar.edu.eg

1. Introduction

Despite recent advancements, cancer keeps a major cause of death worldwide and conveys significant treatment challenges. Multiple investigations are currently underway for developing anticancer medications that are less toxic, targeting intriguing possibilities and promising treatments for more than 100 conditions that have been characterized by rapid cell growth [1,2]. Cancer treatment is based on the patient's age and health, the location of the tumor, the disease's subtype, and stage. The combination of surgery, chemotherapy, hormonal, biological, radiation, transplantation, and immunotherapy, is currently the gold standard of care for cancer patients [3,4]. Chemotherapy is a major therapy for cancer method involving the administration of cytotoxic drugs that affect cells that divide rapidly. However, there are quite a few of challenges to the treatment, including nonselective drug distribution, multidrug resistance, amplified drug toxicity, and unfavorable side effects that make it difficult to distinguish between cancerous and normal cells [5]. Natural toxins are a source of valuable pharmacological components that may play an important role in the development of therapeutics. Between 1981 and 2019, natural products accounted for 25% of newly approved anticancer drugs [6,7]. Venom is a complicated mixture of powerful chemicals including ions, polypeptides, enzymes, polyamines, and other compounds. Since many of these compounds have been suggested to have powerful pharmacological effects, venom research is crucial to the design and development of novel medication [8]. Scorpions, sea snails, spiders, and a few other venomous animals are just a few instances [9]. Because spider venom peptides, in particularly, have been demonstrated to have antitumor properties, researchers are investigating with them as possible anticancer drugs [10]. No much information about abundance and biodiversity of spiders in Egypt, so according to the world spider map, Egypt could be considered as a white spot region. Wherefore, the current study attempted to knock out the door to study the spider diversity and their consequential applications. Moreover, there is a crucial need to continuously explore novel, safe,

efficient, and selective anticancer agents that can be used for cancer prevention and treatment. For all the mentioned reasons, the present study aimed to investigate the cytotoxic effects of four types of Egyptian spider venom on tumor-derived cell lines including hepatocellular (HEPG-2), lung (A549), colon (HCT-116), and breast (MCF-7), which provides a foundation for the development of new effective antitumor drug. Moreover, the effects on non-tumorigenic (WI-38) cells were investigated to evaluate the possible cancer cell-specific effects of spider venom.

2. Materials and methods

2.1 Spider collection

Spiders were collected using handpicking method from Giza, Fayoum, and Menoufia governorates. Specimens were alive transported back to the laboratory. Spiders have cannibalistic habits between each other; therefore, live specimens were kept each in its own container at the laboratory for rearing purpose. In the laboratory, specimens were examined under a binocular microscope and were preserved in 70% ethyl alcohol according to [11].

2.2 Identification of true spiders

In most cases the families are easy to distinguish in the field by position and number of eyes, overall shape, length of legs, and form of spinners. In the laboratory it is easy to separate the kinds of true spiders which are morphologically similar. Then, they were kept in a mixture of 70% alcohol and 5% glycerol until their identification.

2.3 Venom extraction

The venom was obtained from spiders using surgical separation of venom glands. After the spider is anesthetized, the carapace is removed. Then the location of the venom gland was determined and extracted. The venom gland was ground up to solubilized extract of venom in distilled water then centrifuged at 14,000 rpm for 10 min at 4°C. The supernatants were pooled, freeze-dried, and stored at −20°C. The lyophilized samples were dissolved in distilled water, centrifuged at 15,000 rpm for 15 min at 4°C and the supernatants were stored at −20°C until being used [12].

2.4 Total protein estimation

Protein concentrations of the samples were determined using BCA Protein Assay Kit (Pierice Biotechnology, USA) [13]. Protein in venom was determined by measuring the absorbance at 540 nanometer using spectrophotometer and commercial kit (vitroscient) according to manufacturer‘s instructions. Different concentrations of the samples were prepared by dissolving them in a specified volume of cell culture medium.

2.5 Cell lines and culture conditions

Four tumor-derived cell lines including hepatocellular (HEPG-2), lung (A549), colon (HCT-116), breast (MCF-7) and a non-tumorigenic cell line WI-38 were used to evaluate the cytotoxic effect of spider venom. The cancer cell lines were purchased from the Tissue Culture Unit at VACSERA Institute, Agouza, Giza, Egypt.

2.6 Assessment of cell viability via MTT assay

Cytotoxic effect of spiders was tested against human lung cancer cells (A-549), hepatocellular (HEPG-2), colon (HCT-116), breast (MCF-7) and human amnion epithelium (Wi38, normal cells) in vitro cell lines by the MTT assay [14]. Briefly, cancer cell lines and normal cell lines were cultured in RPMI-1640 supplemented with penicillin G (100 U/mL), streptomycin (100 µg/ml), L-glutamine, and 10% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO. The compound was dissolved in DMSO at a concentration of 1.0 mg/ mL as a stock solution. Further dilutions were made in culture medium. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide) substance, and all the other reagents and substances were obtained commercially (Sigma Aldrich, USA). IC_{50} was calculated by using Masterplex 2010 hitachia (GIRSS).

2.7 Cell culture examination

After treatment with different venom concentrations, monolayers of tumor cell lines per experimental group were photomicrographed. The morphological changes of cells were analyzed using Nikon bright field inverted light microscope (Japan) at 100X magnification and compared to the control group [15].

2.8 Statistical Analyses

All experiments were repeated at least three times, and data were presented as mean + SE. Student's t-test was used to test the significance of differences between controls and experimental values. Any variation with $p < 0.05$ was considered statistically significant. The IC_{50} values were determined using linear interpolation.

3. Results

3.1 Identification of true spiders

The identification of specimens was carried out according to the available taxonomical knowledge; the collected specimens were identified mostly at genus levels. Identification of the specimens (table 1 & 2) was done with the help of the following keys and papers: [16-18]. Under the heading lifestyle, a brief account is provided of what kind of predator the spider is. In addition, distribution of a family where spiders are found may be important because it will indicate whether it has been found in a particular region and can help with identification as shown in table (1) which described the area from which the spiders were collected, nature of their habitats, the prey, capture methods, and their behaviors. Additionally, spiders further differentiated from each other by descriptive characters that summarize those characters which define the family, such as body size, shape, legs, and eye pattern, summarized in table (2). *Pardosa sp.* is a small to very large araneomorph spider with carapace longer than wide, covered with dense setae, narrower and higher in cephalic region. It has eight eyes arranged in three rows (4:2:2), all dark in color; unequal in size; anterior row with four small eyes; second row with two large eyes and third row with two eyes of intermediate size situated on anterolateral surface of carapace (fig 1a,b). Legs have three claws; usually with scopula and spines and trochanters notched (fig 1c). They have six spinnerets and two booklungs as respiratory system; one pair of tubular tracheae; tracheal spiracle close to spinnerets (fig 1d). Cryptic color ranging from dull yellowish brown to grey or

almost black with broad bands over cephalothorax. Free-living ground-dwelling hunters and are supposed to have coevolved with grasslands. Egg cocoons are carried, attached to the spinnerets (fig.1e), and spiderlings spend the first days or weeks of their existence on the abdomen of the mother. *Philodromous sp.* is a small to medium-sized araneomorph spider with a slightly flattened carapace and clothed in soft recumbent setae. Abdomen is variable in shape, from heart-shaped to oval or elongate; covered with soft recumbent setae (fig.2f). The sternum shape correlated with carapace form, apex an obtuse point between coxae. Eyes are eight arranged in two rows (4:4); usually equal in size, sometimes with anterior eyes larger than posterior (fig.2a,b). Chelicerae usually without teeth. Legs have two claws; legs I, III and IV almost equal in length, leg II usually longer, sometimes much longer; trochanters with or without notches; anterior tibiae sometimes with a series of long spines (fig.2e).

Female palp: with small, toothed claw. Simple spinnerets and has two booklungs as respiratory system, tracheal spiracle close to spinnerets. Color varies from white to pale cream and reddish brown or greyish brown; frequently mottled, with longitudinal bands or chevrons (fig.2c). Free-living spiders are commonly found on plants or on the soil surface. *Araneas sp.* is a small to large araneomorph spider grouped with sticky orb-web weavers. Carapace is frequently flat. The Cephalic region is usually separated from thoracic region by oblique depression and sternum has heart-shape or triangular. Eight eyes arranged in two rows (4:4); lateral eye groups widely separated from median eyes (fig. 3a,b). Strong and vertical chelicerae with lateral condyle, simple spinnerets and two booklungs. The color is greyish brown to dark brown. *Selenops sp.* is a small to large araneomorph spider. Free-living, wandering spiders found under stones, rocks and on tree trunks or on walls. Flattened carapace and rounded sternum (fig.4c). Eight eyes arranged in two rows (6:2); anterior row wide with six eyes near edge of carapace, posterior row with two large eyes, one on each side (fig.4a, b). Both margins toothed chelicerae. Banded legs with two claws, setae on tibiae and metatarsi I and II and tarsal claw is smooth. Short spinnerets in compact group. Respiratory system: two booklungs; tracheal spiracle close to spinnerets. Creamy brown or grey in color and mottled with black, brown, and grey (fig. 4c).

3.2 Total estimated soluble proteins

Colorimetric determination was performed by using spectrophotometer. Equal amount of 50 µl venom extracts was used to determine total protein. Total protein for *Pardosa* sp., *Philodromus* sp., *Araneas* sp. and *Selenops* sp. is 0.53, 1.67, 1.87 and 1.85, respectively.

3.3 Antineoplastic activity of the selected spiders' venoms

The cell viability (table 3 & fig 5) of five cell lines after spider venom treatment, *Pardosa sp.* venom significantly inhibited cell viability of HEPG2, HCT-116 and A549 cells at concentrations of 500 µg/ml and above with IC_{50} of 309.4, 422.3 and 592.8µg/ml, respectively. However, it significantly affects MCF-7 viability at concentrations of 250 µg/ml and above in a concentration-dependent manner with IC_{50} of 186.5 µg/ml. *Philodromus* sp. venom induced a significant reduction of viability against HEPG-2 cells at concentration of 1000 µg/ml only with IC_{50} of 309.4 µg/ml. Also, *Araneas* sp. venom significantly affects cell viability of HEPG-2 cells at concentration of 1000 µg/ml with IC_{50} of 729.8µg/ml and MCF-7 cells at concentration of 500µg/ml and above with IC_{50} of 423.6µg/ml. Both A549 and HCT-116 viability were significantly affected with *Selenops* sp. venom at concentration of 500 and above with IC_{50} of 417.8 and 430.1µg/ml, respectively, while concentrations of 250µg/ml and above significantly inhibited MCF-7 viability in a concentration-dependent manner with IC_{50} of 149.66µg/ml.

To screen the selective role of spider venom treatment towards cancer cells over normal cells, all experiments were repeated by using WISH non-tumorigenic cells. Interestingly, the results showed a lower cytotoxic effect against normal cells than the cancer cell lines indicating cancer cell-specific effect. Results showed significant growth inhibition of only *selenops* sp. venom against Wi38 cell line at concentration of 1000µg/ml with IC50 of 931.1µg/ml. Taken together; the harvested data clearly represented that the venom has a significant reduction of cell viability when compared to normal cell line. The highest cytotoxicity was achieved when HEPG2 and MCF-7 cells were treated with *selenops* sp. and *pardosa* sp. venom.

4. Discussion

Morphological identification represents a significant tool for identifying all living organisms starting from those investigated under the microscope to those investigated by naked eye [19,20]. Natural products have many significant applications whether it was extracted from microbial cells [21-24], plant cell [25] or sea organisms [26,27] or other animal cells. In this study spider venom exhibited promising antitumor effect against various tumor cell lines, these results are in harmony with [28,29] who reported that spider venom peptides have been shown in several studies to be cytotoxic, either by preventing cell growth, degrading cell membranes, causing apoptosis, or reducing cell proliferation. There is enough data to demonstrate that lycosin-I can stop tumor growth both in vivo and in vitro by triggering two different signaling pathways that cause apoptosis and proliferation inhibition, respectively. Human tumor cell lines, including colon adenocarcinoma (HCT-116), cervix carcinoma (HeLa), fibrosarcoma (H1080), hepatocellular carcinoma (HepG2), lung adenocarcinoma (H1299, A549), and prostate carcinoma (DU145), all showed greater than 90% cell mortality upon treatment with lycosin-I (40 µM). On the other hand, non-tumor cells were less harmful to the same amount of lycosin-I [30]. Compared to scorpion venom, spider venom has less impact on cancer cells, in several cell lines and tumor models, spider peptides have shown general cytotoxicity as well as hemolytic, antifungal, antibacterial, and anticancer activities [31]. In agreement with the current study, GAO et al., [32] examined the impact of Macrothele raven spider venom on MCF-7 cell cytotoxicity and discovered that, following a 24-hour incubation period, spider venom at concentrations of 10, 20, and 40 mg/ml considerably enhanced MCF-7 cell cytotoxicity in comparison to the control group ($p<0.05$). Higher doses of spider venom were associated with increased cell cytotoxicity, inhibition of the cell cycle's progression and apoptosis induction. It's likely that the activity depends on both dose and time.

Fig 1: Morphological features of *Pardosa sp*. (a, b: eyes pattern, c: dorsal view showing carapace and legs, d: ventral view showing spinnerets and chelicera, e: female carry egg sac).

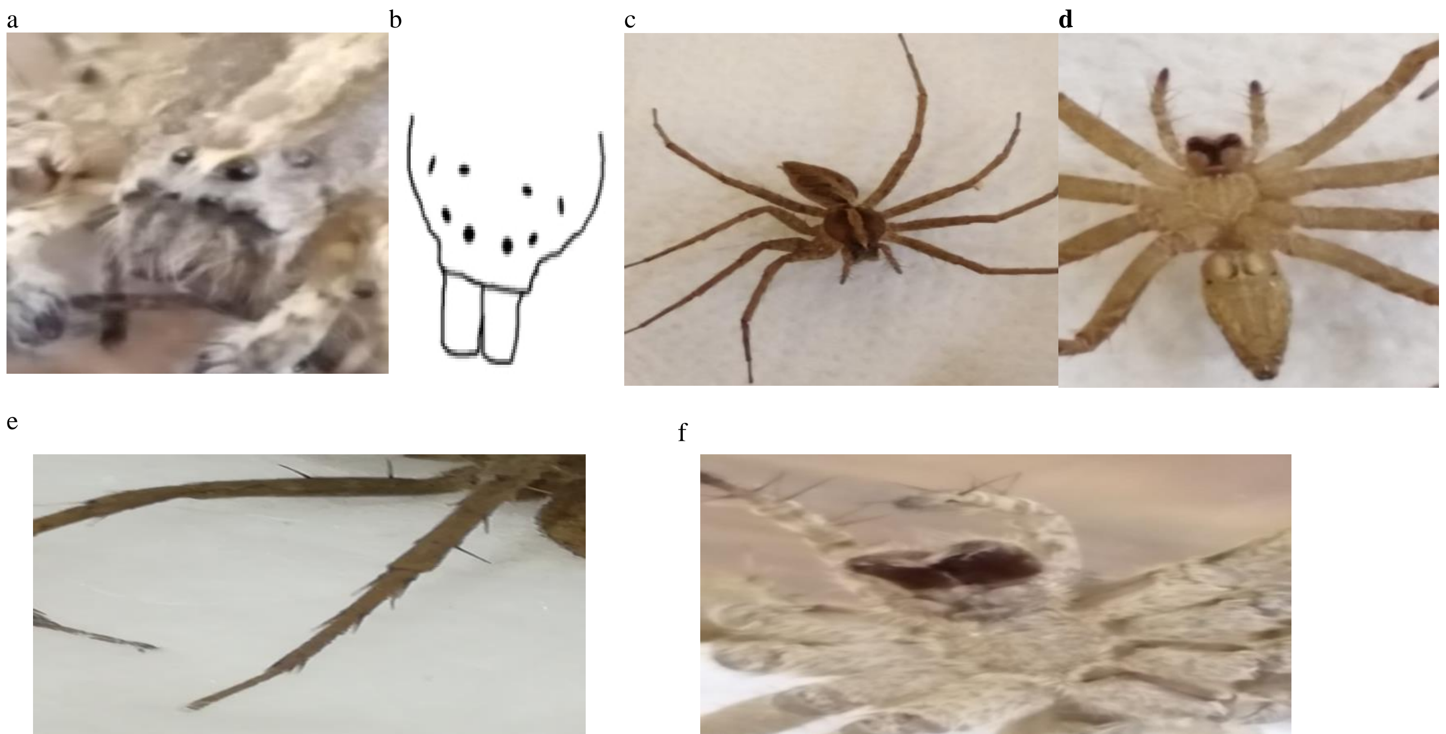


Fig 2: Morphological features of *Philodromous sp.* (a, b: eyes pattern, c: dorsal view showing carapace and legs, d: ventral view showing spinnerets and chelicera, e: legs, f: setae covered abdomen).

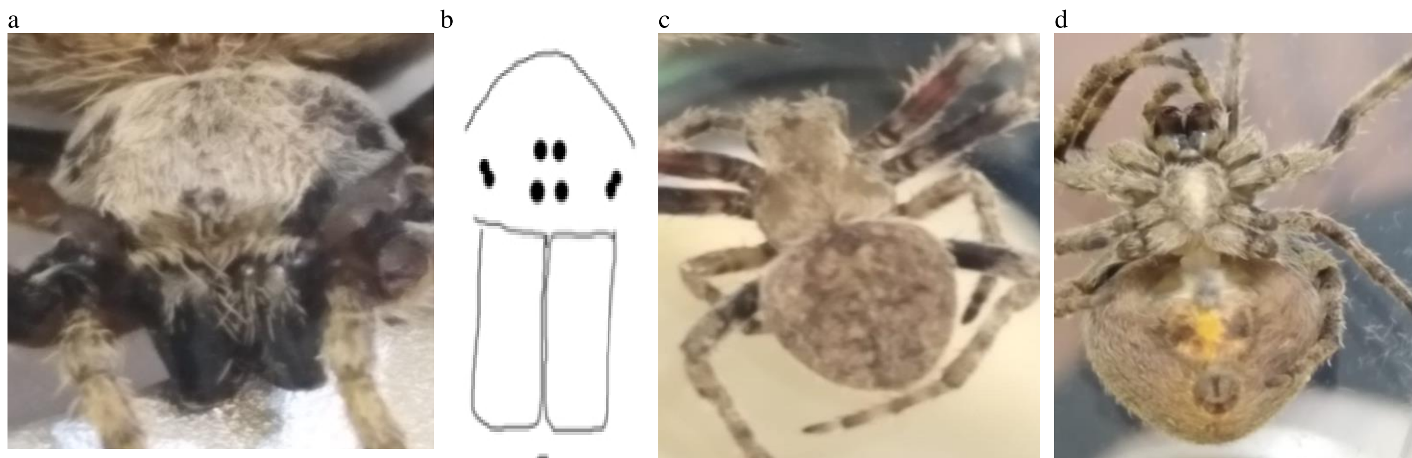


Fig 3: Morphological characteristics of *Araneas sp.* (a, b: eyes pattern, c: dorsal view showing carapace and legs, d: ventral view showing spinnerets and chelicera).

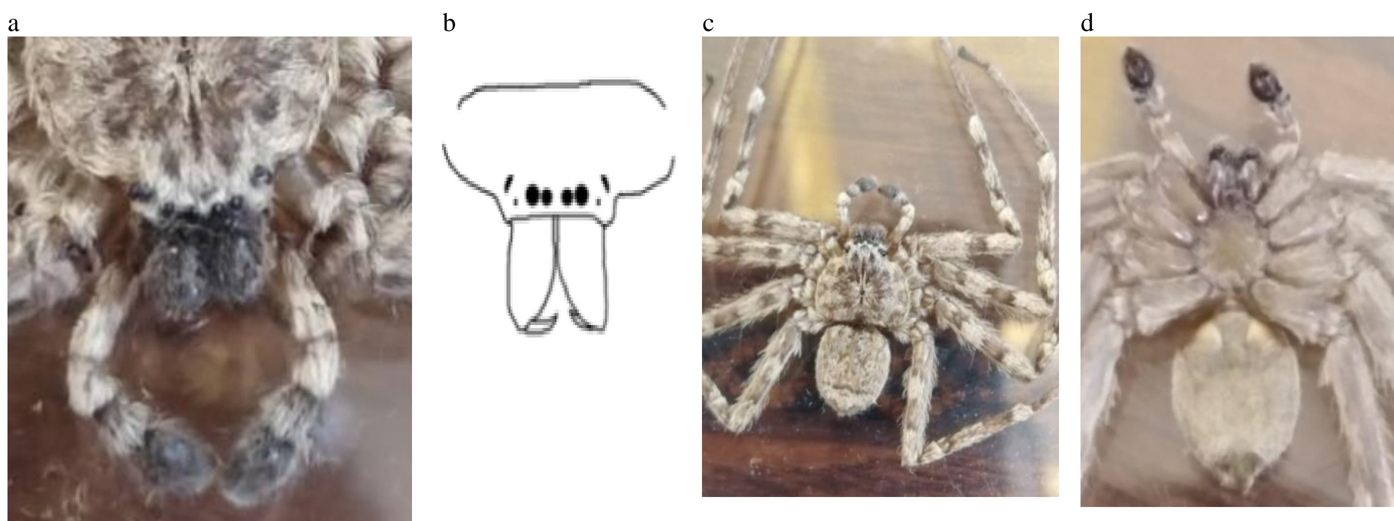


Fig 4: Morphological characteristics of *Selenops sp.* (a, b: eyes pattern, c: dorsal view showing carapace and legs, d: ventral view showing spinnerets and chelicera).

Table 1: Characterization of spiders according to environmental behavior

Family	Species	Sampling area	Habitat	Prey	Capture method	Behavior
Lycosidae (wolfspider) Sundevall, 1833	*Pardosa sp.*	Giza	Forests, field, and garden	Crickets, cutworms, and ground beetles	Active hunters	Nocturnal hunters
Philodromidae (Small Huntsman) Thorell, 1870	*Philodromus* sp.	Giza	Foliage and trunk of trees	Small flies and may flies	Active hunters	Nocturnal hunters
Araneidae (Orb web spiders) Simon, 1895	*Araneas sp.*	Fayuom	Forests, crop, and fields	Flies, moths, bees and may flies	Orb weaver	Active at night
Selenopidae (Crab spiders) Simon, 1897	*Selenops sp.*	Menofya	Wall cracks	Flies and moths	Active hunters	Active at night

Table 2: Description of remarkable identification characteristics of the collected spiders

Species	Body	Legs	Eyes
Pardosa sp.	Medium to large (3-45mm.) size, hairy oval, smoothly rounded abdomen, strong chelicerae , oval to scutiform sternum.	Long, stout, and hairy with spines.	8 in three rows (4:2:2); unequal in size: -1st with 4 small, closely spaced. -2nd with 2 large eyes. -3rd with intermediate size.
Philodromus sp.	Small to medium size (3-16 mm); with flattened carapace and abdomen covered with soft recumbent setae.	Long, agile for running, held laterally from body appearing crab-like.	8 small in 2 closely spaced, curved rows in (4:4); usually equal in size.
Araneas sp.	Small to large (3-30 mm.), large globular abdomen posterior strong and swollen chelicera.	Stout , spiny and have three claws	8 in two closely spaced (4:4), middle eyes widely separated from lateral ones, appear as a cluster of 4 in the center.
Selenops sp.	-Small to large (6-23 mm.), flattened abdomen, round to oval; clothed in dense setae. short spinnerets	Hold their legs out to their sides, like crabs.	8 in two rows (6:2); anterior row wide with six eyes near edge of carapace, posterior row with two fairly large eyes, one on each side.

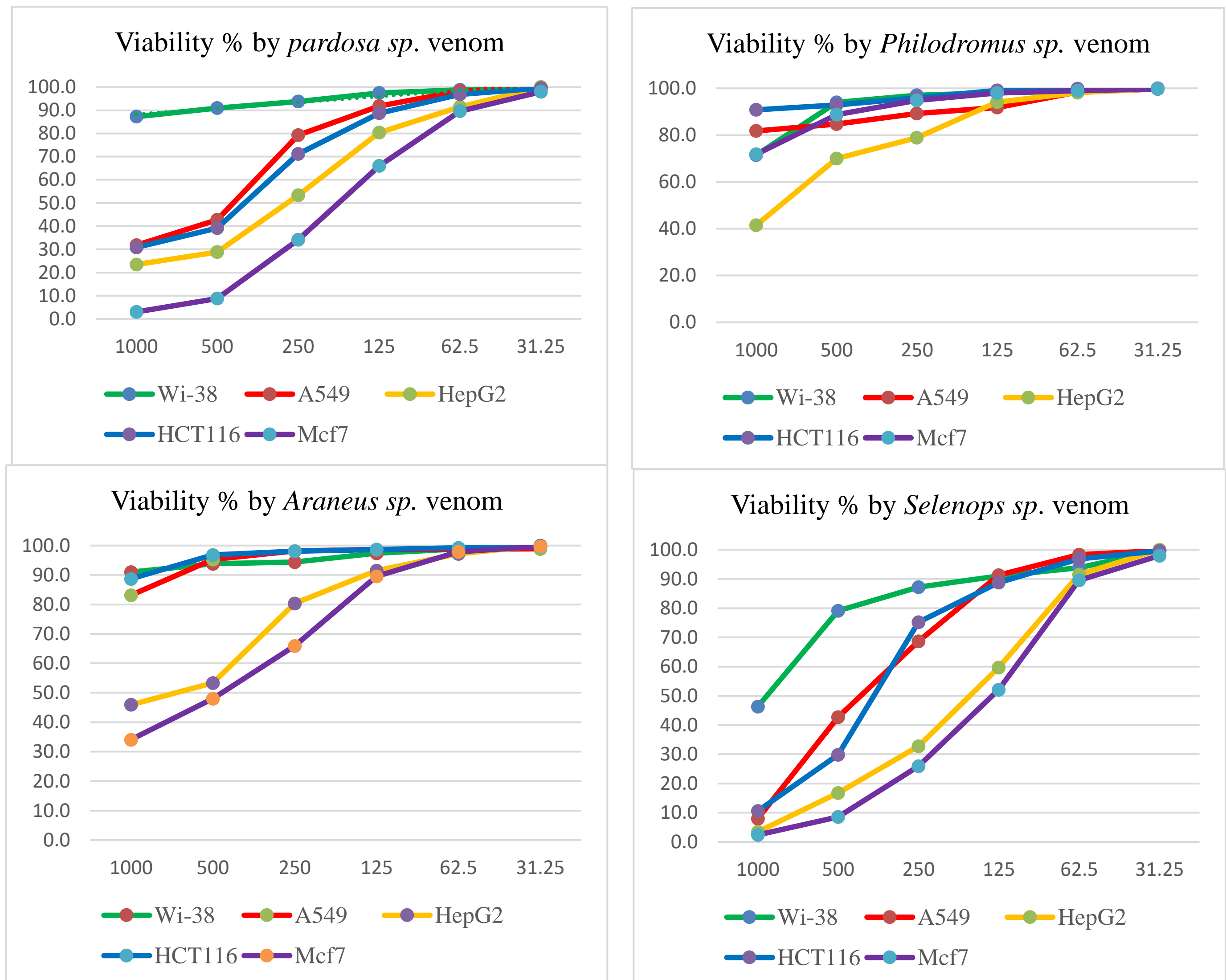


Fig 5: Viability of investigated cell lines by MTT Assay under the effect of 4 different spider venoms.

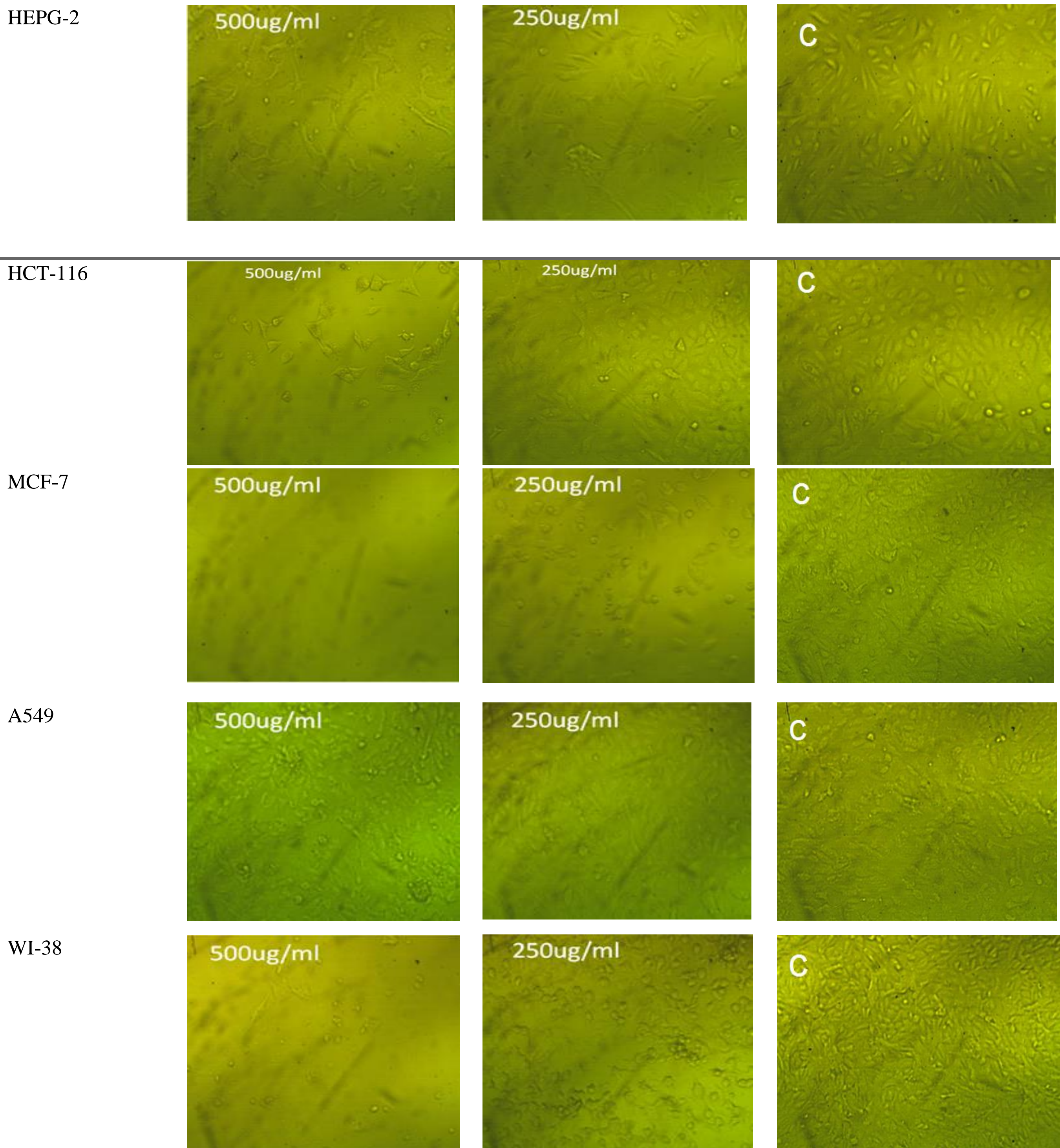


Fig 6: Phase-contrast imaging for demonstration of the cytotoxic effects of *Pardosa* sp. on cancer and normal cell lines. Magnification 100 x, C: control.

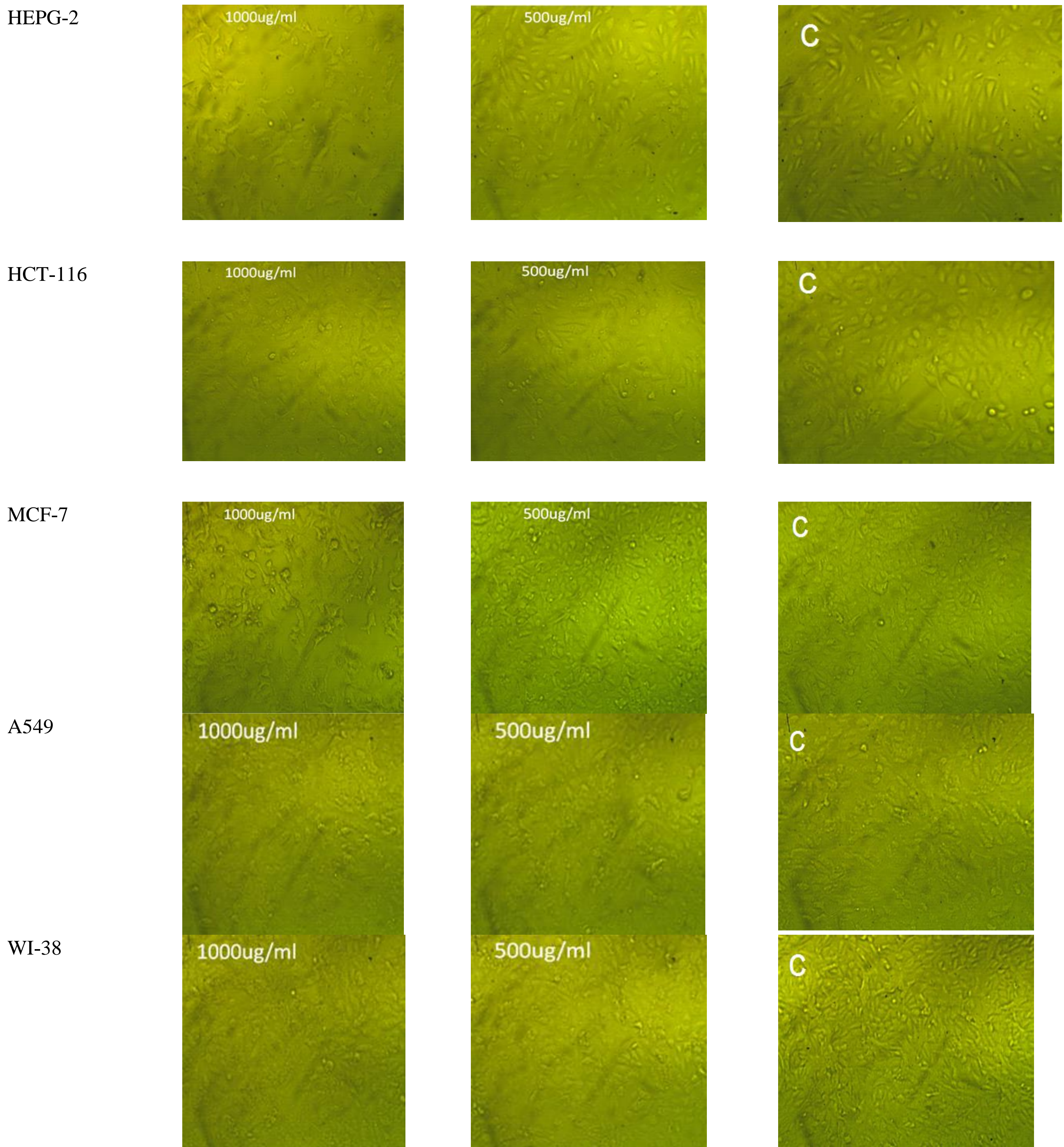


Fig 7: Phase-contrast imaging for demonstration of the cytotoxic effects of *Philodromous* sp. on cancer and normal cell lines. Magnification 100 x, C: control.

Table 3: IC_{50} values (µg/ml) of each cell line after treatment with spider venom

Cell line	*Pardosa sp.*	*Philodromus sp.*	*Araneas sp.*	*Selenops sp.*
HEPG-2	309.4	854.7	729.8	170.4
HCT-116	422.3	>1000	>1000	430.1
MCF-7	186.5	>1000	423.6	149.6
A549	592.8	>1000	>1000	417.8
Wi-38	>1000	>1000	>1000	931.1

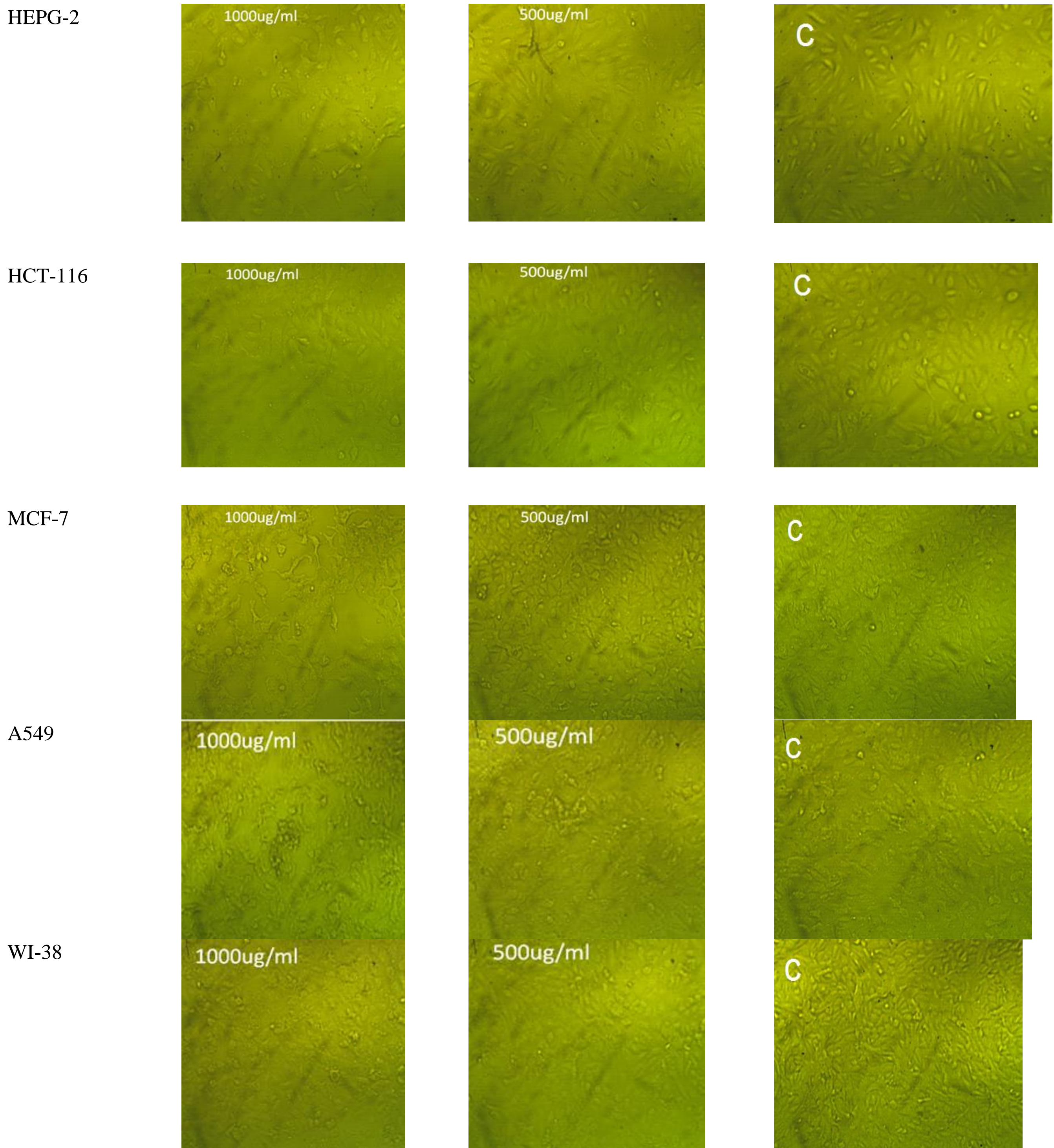


Fig 8: Phase-contrast imaging for demonstration of the cytotoxic effects of *Araneas* sp. on cancer and normal cell lines. Magnification 100 x, C: control.

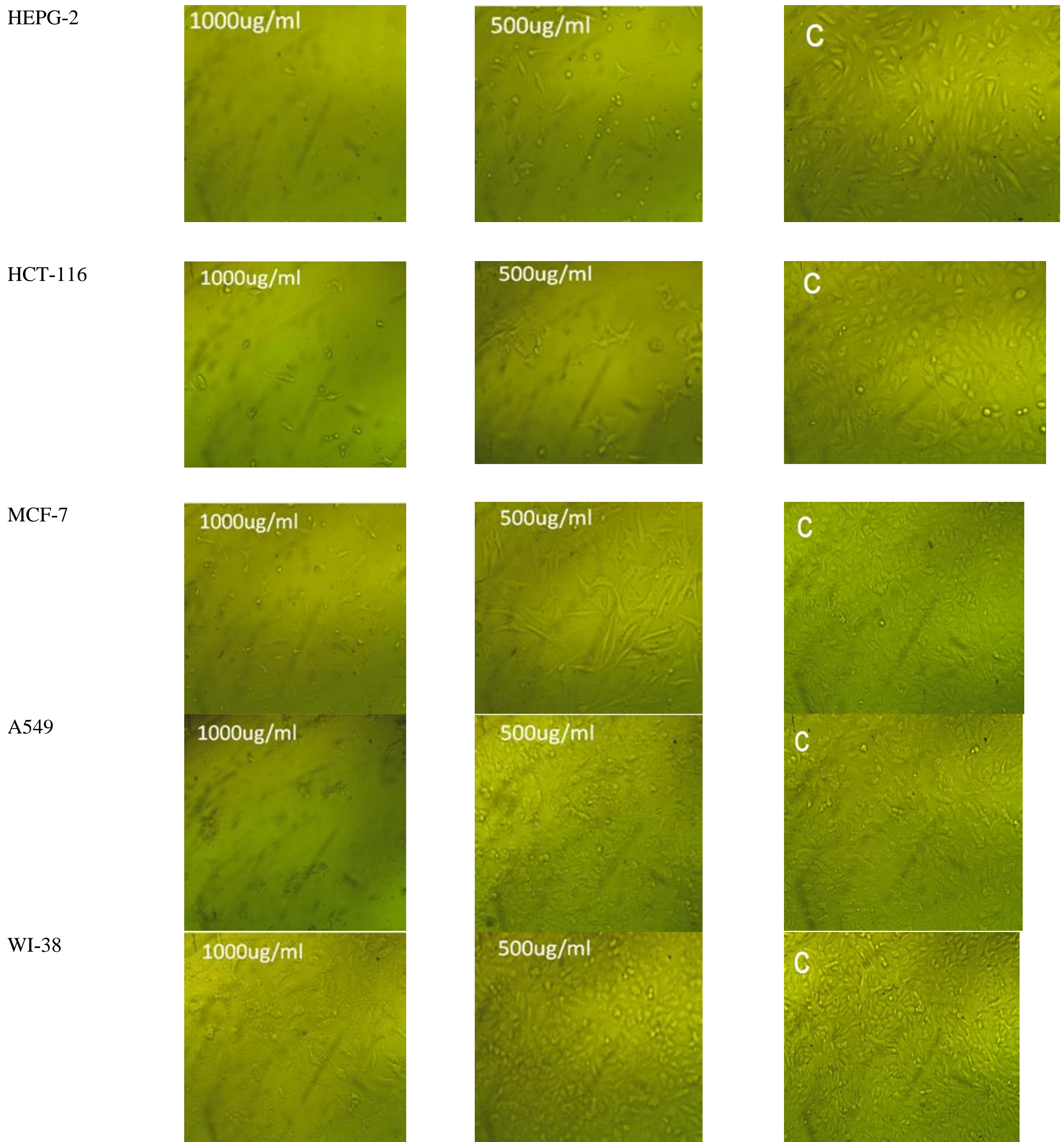


Fig 9: Phase-contrast imaging for demonstration of the cytotoxic effects of *Selenops* sp. on cancer and normal cell lines. Magnification 100 x, C: control.

At concentrations ranging from 10 to 40 mg/ml at 48 hours, spider venom significantly decreased accumulation in the S phase and caused a dose-dependent accumulation of MCF-7 cells in the G2/M and G0/G1 phases. When MCF-7 cells were treated with 10, 20, or 40 mg/ml of spider venom, the cell cycle distributions of the two groups differ statistically significantly ($p<0.01$). Similar results were obtained by Liu *et al*. [33] who studied the *Lycosa singoriensis* species and found that it was yielded a cationic peptide called Lycosin-I, which has been shown to be effective in inhibiting tumor growth in vivo and in vitro by activating two distinct signaling pathways that lead to apoptosis and proliferation inhibition, respectively. Lycosin-I treatment of the following human tumor cell lines (HCT-116), cervix carcinoma (HeLa), fibrosarcoma (H1080), and hepatocellular carcinoma (HepG2) resulted in more than 90% cell death. Spider venom is very effective at stopping A549 cells from proliferating, according to Guo *et al.* [31] suggesting that a significant portion of these cells are experiencing cell death. In a concentration-dependent manner, sulfur dioxide significantly decreased growth as measured by the MTT assay, inhibiting cell viability in the range of 0, 8, 16, and 32 mg/ml. The inhibition was obvious at concentrations between 8 and 32 mg/ml. In addition, Siedlakowski *et al*. [34] reported that the Pancratistatin (PST), a naturally occurring chemical derived from the Hawaiian spider lily, was recognized for its ability to selectively induce apoptosis in a range of cancer cell lines while sparing noncancerous cells and cell lines. Results reported that PST can specifically induce apoptosis in human breast cancer cell lines MCF-7 and Hs-578-T compared to noncancerous cell lines. Many of these cells are going through apoptotic cell death even though their cell membranes appear to be relatively intact. Spider venom dramatically reduced the rate of cell proliferation in HeLa cells in a time- and dose-dependent manner at doses of 40, 20, and 10 mg/l [35].

Moreover, it was revealed that A549 cells treated with high concentrations of fraction AT5-3 (200 µg/ml) of crude venom from the *Phlogiellus bundokalbo* spider showed reduced cellular adhesion, cell condensation, and membrane blebbing. When A549 cells were exposed to 10 µg/ml of cisplatin, these anatomical characteristics were more noticeable. On the other hand, A549 cells treated with 200 µg/ml of fraction AT5-6 exhibited membrane disintegration [36].

4.1 Cell culture examination

The activity of venom is indicated by the decrease in cell density. The cells were treated with descending concentrations of the venom, and then cells were imaged before the addition of the MTT salt. Changes in the cell morphology were monitored after exposure to 250 µg/ml of venom and above. The cancer cells changed from epithelial shape to polygonal with slightly to completely rupture of membrane and release of cytosolic contents. A1000µg/ml dose resulted in loss of cellular integrity with acute necrosis and increased cellular adhesiveness (Fig. 6-9). When spider venom (at dosages of 1.6, 1.8, and 2.0 µg/g mice) was used to prevent tumor growth in nude mice, the results showed that the tumor size dramatically decreased by the time the therapy reached day 21 and continued to decline at all stages of study for the next seven weeks ($p < 0.01$). Thus, we suggest that it may be possible to evaluate the effects of spider venom both in vivo and in vitro [32]. Anti-proliferation effects of spider venom include substantial apoptosis in TE13 cells, a sharp rise in reactive oxygen species (ROS) and a decrease in cellular mitochondrial membrane potential (MMP). Furthermore, P21 expression was found to be activated by SV as one of its pharmacological mechanisms, according to Western blotting research. In vivo studies demonstrated that tumor size was dramatically lowered after 21 days of therapy with the venom ($P < 0.01$) [37].

5. Conclusion

The present in vitro study examined the concentration-dependent response of four studied spiders on various human cell lines, corresponding to the normal cell line (Wi-38), including the breast tumor cell line (MCF-7), hepatocellular carcinoma cell line (HEPG-2), lung cancer cell line (A549), and colon carcinoma cell line (HTC-116). It demonstrated a less cytotoxic effect on normal cells compared to cancer cell lines, suggesting that the effect was specific to cancer cells. The study's early results suggest that even though spider venom is a veterinary medication, its ability to destroy human cancer cells indicates that it needed to be evaluated as a possible cytotoxic agent. Further research is needed to identify the functional compounds of the spider venom and its mechanism of action on cancer cells.

References

[1] A. Abdel-Razek, H. El-Sheikh, W. Suleiman, T.H. Taha, M. Mohamed. (2020). Bioelimination of phenanthrene using degrading bacteria isolated from petroleum soil: safe approach. Desalination and water treatment. 181: 131-140.

[2] M.S. Abdelhafez, K.F. Abd El-Wakeil, A.H. Mohamed. (2016). Spiders (Araneae) Inhabiting Elba Protectorate, Red Sea Governorate, Egypt. Indian Journal of Arachnology. 5(1-2): 92-99.

[3] H.M. Akef. (2018). Anticancer, antimicrobial, and analgesic activities of spider venoms. Toxicology research. 7(3): 381-395.

[4] L. Al Rabadi, R. Bergan. (2017). A way forward for cancer chemoprevention: think local. Cancer Prevention Research. 10(1): 14-35.

[5] K. Bukowski, M. Kciuk, R. Kontek. (2020). Mechanisms of multidrug resistance in cancer chemotherapy. International journal of molecular sciences. 21(9): 3233.

[6] G. Corso, P. Veronesi, G.I. Santomauro, P. Maisonneuve, C. Morigi, G. Peruzzotti, M. Intra, V. Sacchini, V. Galimberti. (2018). Multiple primary non-breast tumors in breast cancer survivors. Journal of Cancer Research and Clinical Oncology. 144: 979-986.

[7] K. Deiner, H.M. Bik, E. Mächler, M. Seymour, A. Lacoursière-Roussel, F. Altermatt, S. Creer, I. Bista, D.M. Lodge, N. De Vere. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Molecular ecology. 26(21): 5872-5895.

[8] A.M. El-Bitar, M. Sarhan, M.A. Abdel-Rahman, V. Quintero-Hernandez, C. Aoki-Utsubo, M.A. Moustafa, L.D. Possani, H. Hotta. (2020). Smp76, a scorpine-like peptide isolated from the venom of the scorpion Scorpio maurus palmatus, with a potent antiviral activity against hepatitis C virus and dengue virus. International journal of peptide research and therapeutics. 26: 811-821.

[9] L. Gao, S. Yu, Y. Wu, B. Shan. (2007). Effect of spider venom on cell apoptosis and necrosis rates in MCF-7 cells. DNA and cell biology. 26(7): 485-489.

[10] L. Gao, B.-e. Shan, J. Chen, J.-h. Liu, D.-x. Song, B.-c. Zhu. (2005). Effects of spider Macrothele raven venom on cell proliferation and cytotoxicity in HeLa cells. Acta Pharmacologica Sinica. 26(3): 369-376.

[11] L. Gao, J. Zhang, X. Liu, M. Zhao, L. Li, X. Liu, B. Zhao. (2013). Effect of spider venom on inhibition proliferation of TE13 cells in vivo and in vitro. Thoracic Cancer. 4(3): 306-311.

[12] J.E. Garb. (2014). Extraction of venom and venom gland microdissections from spiders for proteomic and transcriptomic analyses. JoVE (Journal of Visualized Experiments). (93): e51618.

[13] M.H. Hendy, A.H. Hashem, W.B. Suleiman, M.H. Sultan, M. Abdelraof. (2023). Purification, Characterization and anticancer activity of L-methionine γ-lyase from thermo-tolerant Aspergillus fumigatus. Microbial cell factories. 22(1): 8.

[14] S. Ilhan, K. Çiçek, C.V. Tok, H. Atmaca. (2021). Profiling of apoptosis-associated proteins in human prostate cancer cells in response to Montivipera bulgardaghica albizona venom by protein array. Toxin Reviews. 40(4): 1040-1047.

[15] R. Jocqué, A.S. Dippenaar-Schoeman. (2006). Spider families of the world. Royal Museum for Central Africa, 13, Leuvensesteenweg, 3080 Tervuren (Belgium) Second edition, ISBN:978-90-74752-11-4, Legal Deposit: D/2007/0254/11.

[16] A. Kamel, W.B. Suleiman, A. Elfeky, G.M. El-Sherbiny, M. Elhaw. (2022). Characterization of bee venom and its synergistic effect combating antibiotic resistance of Pseudomonas aeruginosa. Egyptian Journal of Chemistry. 65(5): 297-306.

[17] W. Lian, H. Lian, Q. Li, A. Hu, S. Liu. (2018). The venom of spider Haplopelma hainanum suppresses proliferation and induces apoptosis in hepatic cancer cells by caspase activation in vitro. Journal of ethnopharmacology. 225: 169-177.

[18] Z. Liu, M. Deng, J. Xiang, H. Ma, W. Hu, Y. Zhao, D.-C. Li, S. Liang. (2012). A novel spider peptide toxin suppresses tumor growth through dual signaling pathways. Current molecular medicine. 12(10): 1350-1360.

[19] A.B.R. Mayor, L.A. Guevarra Jr, M.R. Santiago-Bautista, L.A. Santiago. (2020). Phlogiellus bundokalbo spider venom: cytotoxic fractions against human lung adenocarcinoma (A549) cells. Journal of Venomous Animals and Toxins including Tropical Diseases. 26.

[20] C. Rapôso. (2017). Scorpion and spider venoms in cancer treatment: state of the art, challenges, and perspectives. Journal of clinical and translational research. 3 (2):233.

[21] N.J. Saez, V. Herzig. (2019). Versatile spider venom peptides and their medical and agricultural applications. Toxicon. 158: 109-126.

[22] A. Sharma, G. Singh, R. Singh. (2021). Faunal diversity of spider families Dictynidae, Dysderidae, Eresidae and Filistatidae (Araneomorphae: Araneae: Arachnida) in India. International Journal of Zoology and Applied Biosciences. 6(1): 1-9.

[23] P. Siedlakowski, A. McLachlan-Burgess, C. Griffin, S.S. Tirumalai, J. McNulty, S. Pandey. (2008). Synergy of Pancratistatin and Tamoxifen on breast cancer cells in inducing apoptosis by targeting mitochondria. Cancer biology & therapy. 7(3): 376-384.

[24] E. Simon. (1895). Histoire naturelle des Araignées. Roret, Paris. 1: 1084.

[25] E. Simon. (1897). Histoire naturelle des araignées. Paris. 2: 1-192.

[26] C.J. Sundevall. (1833). Conspectus Arachnidum. Londini Gothorum. 1-39.

[27] T. Thorell. (1870). On European Spiders. Nova Acta Regicae Societatis Scientiarum Uppsaliensis. 7: 109 -242.

[28] W.B. Suleiman. (2020). In vitro estimation of superfluid critical extracts of some plants for their antimicrobial potential, phytochemistry, and GC–MS analyses. Annals of Clinical Microbiology and Antimicrobials. 19 (1):1-12.

[29] W.B. Suleiman, E.E.-H. Helal. (2022). Chemical constituents and potential pleiotropic activities of Foeniculum vulgare (Fennel) ethanolic extract; in vitro approach. Egyptian Journal of Chemistry. 65(7): 617-626.

[30] W.B. Suleiman, R.M. Shehata, A.M. Younis. (2022). In vitro assessment of multipotential therapeutic importance of Hericium erinaceus mushroom extracts using different solvents. Bioresources and Bioprocessing. 9(1): 1-13.

[31] T. Wu, M. Wang, W. Wu, Q. Luo, L. Jiang, H. Tao, M. Deng. (2019). Spider venom peptides as potential drug candidates due to their anticancer and antinociceptive activities. Journal of Venomous Animals and Toxins including Tropical Diseases. 25.

[32] C. Rapôso. (2017). Scorpion and spider venoms in cancer treatment: state of the art, challenges, and perspectives. Journal of clinical and translational research 3 (2):233

[33] L. Gao, S. Yu, Y. Wu, B. Shan. (2007). Effect of spider venom on cell apoptosis and necrosis rates in MCF-7 cells. DNA and cell biology 26 (7):485-489

[34] Z. Liu, M. Deng, J. Xiang, H. Ma, W. Hu, Y. Zhao, D-C. Li, S. Liang. (2012). A novel spider peptide toxin suppresses tumor growth through dual signaling pathways. Current molecular medicine 12 (10):1350-1360

[35] P. Siedlakowski, A. McLachlan-Burgess, C. Griffin, S.S. Tirumalai, J. McNulty, S. Pandey.

(2008). Synergy of Pancratistatin and Tamoxifen on breast cancer cells in inducing apoptosis by targeting mitochondria. Cancer biology and therapy 7 (3):376-384

[36] N. Mach, J. Gao, L. Schaffarczyk, S. Janz, E. Ehrke-Schulz, T. Dittmar, A. Ehrhardt, W. Zhang. (2020). Spectrum-wide exploration of human adenoviruses for breast cancer therapy. Cancers 12 (6):1403

[37] A.B.R. Mayor, L.A. Guevarra Jr, M.R. Santiago-Bautista, L.A. Santiago. (2020). Phlogiellus bundokalbo spider venom: cytotoxic fractions against human lung adenocarcinoma (A549) cells. Journal of Venomous Animals and Toxins including Tropical Diseases 26

[38] L. Gao, J. Zhang, X. Liu, M. Zhao, L. Li, X. Liu, B. Zhao. (2013). Effect of spider venom on inhibition proliferation of TE13 cells in vivo and in vitro. Thoracic Cancer 4 (3):306-311