**Excretion/secretion of *Lucilia sericata* and *Chrysomya albiceps* (Diptera: Calliphoridae)maggots as potential anticancer agent and kinases inhibitor**

Ahmed Z.I. Shehata, Ahmed B.M. Mehany, Tarek M.Y. El-Sheikh

Department of Zoology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.

ahmedzeinhom00@gmail.com

**Abstract:** This study was performed to investigate the anticancer activity of sterile larval excretion/secretion **(ES)** of *Lucilia sericata* and *Chrysomya albiceps* on seven human parts tumor cell lines named: Human Liver Carcinoma cell line **(HepG-2)**, Human Breast Carcinoma cell line **(MCF-7)**, Human Colon Carcinoma cell line **(HCT-116)**, Human Lung Carcinoma cell line **(A-549)**, Human Intestinal Carcinoma cell line **(CACO)**, Human Prostate Carcinoma cell line **(PC-3)** and Human Cervical Carcinoma cell line **(HELA)**. The SulphoRhodamine-B (SRB) assay was applied to compare the antitumor activity of maggot's ES with the antitumor agent Fluorouracil **(5-FU)**. In vitro anticancerevaluation of *L. sericata* and *C. albiceps* maggot's ES revealed that, both maggot's ES possess moderate to high anticanceractivities against the different human tumor cell lines used, with IC50 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 and 17.3±0.26, 33.4±0.17, 32.1±0.37, 20.2±0.14, 34.8±0.25, 77.6±0.19 and 89.5±0.34 µg/ml for *C. albiceps* ES against(HepG-2), (MCF-7), (HCT-116), (A-549), (CACO), (PC-3), and (HELA) cell lines, compared to 28.3±0.32, 40.7±0.34, 19.8±0.11, 20.14±0.43, 31.82±0.25, 60.7±0.45 and 53.5±0.51µg/ml for the anticancer agent Fluorouracil  (5-FU), respectively. In addition, *L. sericata* and *C. albiceps* ESaffect the human epidermal growth factor receptor (EGFR), Human Insulin Receptor (IR), Human vascular endothelial cell growth factor receptor (VEGFR) and Fibroblast Growth Factor Receptor (FGFR) with IC50 6.57±0.09, 1.24±0.15, 5.41±0.18 and 3.22±0.22µg/ml for *L. sericata* ESand 9.62±0.11, 3.39±0.23, 8.01±0.17 and 6.72±0.16µg/ml for *C. albiceps* ES.

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**Keys words:** Anticancer,ES, *L. sericata*, *C. albiceps*, kinases.

**Introduction:**

Cancer is a scary disease, which has uncontrollable cellular growth, local tissue invasion, metastasis and cause several deaths per year worldwide, in cancer modern therapy such as chemotherapy, surgical and radiations there is a serious side effects, so many institutions have been engaged with the search for some safe anticancer agents in order to avoid the side effects of chemotherapy, surgical and radiation therapy, for example, Indian traditional system of medicine, using herbs, has been authorized as alternative form of medicine with less toxicity and more safety (Vasanthi *et al.,* 2014). In addition to, current pharmacological research focused on Chinese medicine showing prevent and cure cancer by improving human immunity (Suo *et al.,* 2016). Although the chemical therapeutic agents have a toxic effects on all dividing cells, the kinase inhibitors were more specific for tumor cells, with strong antitumor effects, the first molecular-targeted drug was recorded in 1998 for a chemical compound (imatinib) to inhibit the Bcr-Abl kinase and it revealed a strong effect in treatment of chronic myelogeneous leukemia (CML) and gastrointestinal stromal tumor (GIST) (Nishioka *et al.,* 2011). Since that time, researchers began to discover new kinase inhibitory therapeutic compounds.

The presence of insects in any ecological system, from waterway to extremely septic environments, has stimulated scientists to look for new therapeutic agents in this class of arthropods (Chernysh *et. al.,* 2002). A great part of efforts have been achieved for the investigation and re-examination of insect sources to obtain compounds that may have anticancer activity. The insertion of maggot debridement therapy into modern wound management stimulated further research into the antibacterial effectiveness of *Lucilia sericata* Maggots (Barnes *et al.,* 2010) and the maggot's ES have been shown to have a role in the success of maggot therapy (Bexfield *et al.,* 2010). In this study we focused on the anticancer activity of excretion/ secretion of *L. sericata* and *Chrysomya albiceps* maggots against seven human tumor cell lines (HepG-2, MCF-7, HCT-116, A-549, CACO, PC-3 and HELA) and the inhibitory effect of maggot's ES against four types of kinase receptors (EGFR, IR, VEGFR and FGFR).

**Materials and Methods:**

**Collection of larval ES:** *Lucilia sericata* and *Chrysomya albiceps* were collected and maintained for several generations at the Laboratory of Medical Entomology, Central laboratory building, Zoology Department, Faculty of Science, Al-Azhar University, under controlled laboratory conditions of 27±2ºC, 70±10% RH and 12-12 light-dark photoperiod. Larvae were reared on a diet of liver. Excretions/secretion (ES) of each *L. sericata* and *C. albiceps* maggots was collected by washing sterile 3rd instar larvae (5000 larvae) with 70% ethanol and sterile ultrapure water (ddH2O) then incubated overnight (10hrs) at 30°C (Kerridge *et al.,* 2005), after which ES was collected and centrifuged at 20,000 rpm for 15 min to remove large particles. The ES from each insect species was then stored frozen at -20°C until required.

**Cytotoxicity:** Human Liver Carcinoma cell line (HepG-2), Human Breast Carcinoma cell line (MCF-7), Human Colon Carcinoma cell line (HCT-116), Human Lung Carcinoma cell line (A-549), Human Intestinal Carcinoma cell line (CACO), Human Prostate Carcinoma cell line (PC-3) and Human Cervical Carcinoma cell line (HELA) were obtained from VACSERA- Cell Culture Unit, Cairo, Egypt. These cell lines originally obtained from the American Type Culture Collection (ATCC). The cell line was cultured in RPMI medium supplemented with 10 % inactivated fetal bovine serum (FBS). The reagents RPMI-1640 medium, SRB, DMSO and 5-fluorouracil were purchased from (sigma co., St. Louis, USA). Fetal bovine serum was obtained from (GIBCO, UK). The different cell lines mentioned above were used to determine the inhibitory effects of the two ES on cell growth using the SRB assay (SulphoRhodamine-B). This colorimetric assay is based on the ability of SRB to bind to protein components of cells that have been fixed in tissue culture plates by trichloroacetic acid (TCA).

**Cytotoxicity screening:** The cancer cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics were added 100 units/ml penicillin and 100µg/ml streptomycin at 37 °C in a 5% CO2 incubator. The cells were seeded in a 96-well plate at a density of 1.0x104 cells/well at 37 °C for 48 h under 5% CO2. After incubation, the cells were treated with different concentration (100.0, 50.0, 25.0, 12.5 and 6.25) of *L. sericata* and *C. albiceps* ES and incubated for 48h discard the medium, fixed with 10% trichloroacetic acid (TCA) 150 μl/well for 1 h at 4ºC, wash by water 3 times (TCA reduce SRB protein binding). Wells will be stained by SRB 70 μl/well for 10 min at room temperature in a dark place. Wash with acetic acid 1% to remove unbound dye (end point: colorless drainage). The plates will be air dried 24h. The dye will be solubilized with 50 μl/well of 10 mM tris base (PH 7.4) for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well will be measured at 570 nm with an ELISA microplate reader (EXL 800 USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100. Three replicates were applied and the IC50 values were calculated using sigmoidal concentration response curve fitting models (Sigmaplot software) (Skehan *et al.,* 2003).

**Kinases inhibitory activity:** The inhibitory activity of each *L. sericata* and *C. albiceps* ES to human Epidermal growth factor receptor (EGFR), Human Insulin Receptor (IR), Human vascular endothelial cell growth factor receptor (VEGFR) and Fibroblast Growth Factor Receptor (FGFR) was determined using kinase assay kit according to the manufacturer instructions. Ray Bio Human EGFR ELISA Kit (USA). Briefly, this assay employs an antibody specific for human kinase coated on a 96-well plate. Standards and samples are pipetted into the wells and kinase present in a sample is bound to the wells by the immobilized antibody(Balzano *et al.,* 2011).

**Results:**

*Lucilia sericata* and *Chrysomya albiceps* excretion/secretion (ES) were evaluated in vitro against seven human tumor cell lines. The results of the anticancer activity are presented in (Tables 1and 2). The basic measurement of anticancer activity used in this study was the cell viability average percentages of tumor cells in the test cultures compared to control for 24h of incubation.

The cell viability average percentages of tumor cells were recorded in (Table 1). The data obtained revealed that, the lowest percent of cell viability (9.74±0.19%) was recorded by *L. sericata* ES against (HepG-2), followed by 10.49±0.18% against (HCT-116) at the concentration 100µg/ml, meanwhile, ES of *L. sericata* maggots showed viability less than 25% against (HepG-2) and (A-549) cell lines at the concentrations 50µg/ml. At the lowest concentrations 12.5µg/ml, both *L. sericata* and *C. albiceps* ES showed more than 50% of the cell viability against tested tumor cells.

Median inhibitory concentrations **(IC50)** of *L. sericata* and *C. albiceps* ES against tumor cells tested in vitro are summarized in Table (2). The IC50 values were in the range of 14.8±0.05 to 89.5±0.34µg/ml. ES of *L. sericata* and *C. albiceps* maggots showed excellent anticancer activity against (HepG-2) cell line, with IC50 14.8±0.05 and 17.3±0.26µg/ml; respectively, compared to the anticancer agent (5-FU) 28.3±0.32µg/ml.

**Table 1. Cytotoxicity effect *Lucilia sericata* and *Chrysomya albiceps* maggot's excretion/secretion (ES) on different human parts (Liver, Breast, Colon, Lung, Intestinal, Prostate and Cervical) Carcinoma cell lines.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Concentration  (µg/ml) | Cell Viability Average Percentage (%) | | | | | | | | | | | | | |
| **(HepG-2) cell line** | | **(MCF-7) cell line** | | **(HCT-116 ) cell line** | | **(A-549) cell line** | | **(CACO) cell line** | | **(PC-3 ) cell line** | | **(HELA) cell line** | |
| ***L. sericata* ES** | ***C. albiceps* ES** | ***L. sericata* ES** | ***C. albiceps* ES** | ***L. sericata* ES** | ***C. albiceps* ES** | ***L. sericata* ES** | ***C. albiceps* ES** | ***L. sericata* ES** | ***C. albiceps* ES** | ***L. sericata* ES** | ***C. albiceps* ES** | ***L. sericata* ES** | ***C. albiceps* ES** |
| 100.0 | 9.74±0.19 | 21.24±0.21 | 25.13±0.22 | 33.96±0.11 | 10.49±0.18 | 12.54±0.30 | 13.82±0.27 | 16.26±0.12 | 26.47±0.34 | 31.24±0.28 | 20.53±0.12 | 40.71±0.19 | 40.92±0.23 | 48.22±0.19 |
| 50.0 | 19.78±0.23 | 32.51±0.13 | 39.45±0.34 | 46.22±0.25 | 27.54±0.25 | 30.82±0.18 | 19.87±0.14 | 25.32±0.23 | 38.04±0.25 | 42.36±0.37 | 34.71±0.15 | 66.87±0.24 | 78.16±0.19 | 80.45±0.26 |
| 25.0 | 32.69±0.18 | 46.33±0.16 | 70.87±0.15 | 79.15±0.19 | 57.31±0.36 | 62.11±0.22 | 40.56±0.19 | 49.63±0.31 | 73.89±0.28 | 86.57±0.16 | 65.47±0.19 | 79.93±0.13 | 94.16±0.12 | 95.65±0.31 |
| 12.5 | 61.48±0.31 | 70.76±0.27 | 89.43±0.41 | 90.36±0.27 | 74.49±0.29 | 83.67±0.26 | 58.91±0.20 | 66.19±0.17 | 91.48±0.22 | 97.35±0.11 | 85.72±0.13 | 94.69±0.22 | 98.73±0.16 | 100.0 |
| 6.25 | 89.16±0.17 | 93.12±0.33 | 99.58±0.26 | 100.0 | 87.63±0.17 | 94.44±0.32 | 73.85±0.32 | 88.21±0.20 | 97.62±0.26 | 100.0 | 94.16±0.16 | 98.65±0.34 | 100.0 | 100.0 |
| 0.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

**Table 2. IC50 values of *Lucilia sericata* and *Chrysomya albiceps* maggot's excretion/secretion (ES) and Fluorouracil (5-FU)** **on different human (Liver, Breast, Colon, Lung, Intestinal, Prostate and Cervical) Carcinoma cell lines.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Type of Cell Line** | **IC50 Concentrations**  **(µg/ml) ± SD** | | |
| *L. sericata*  ES | *C. albiceps*  ES | Fluorouracil  5-FU |
| **(HepG-2)** | 14.8 ± 0.05 | 17.3±0.26 | 28.3 ± 0.32 |
| **(MCF-7)** | 31.3 ± 0.09 | 33.4±0.17 | 40.7 ± 0.34 |
| **(HCT-116)** | 27.3 ± 0.11 | 32.1±0.37 | 19.8 ± 0.11 |
| **(A-549)** | 16.4 ± 0.07 | 20.2±0.14 | 20.1 ± 0.43 |
| **(CACO)** | 31.1 ± 0.31 | 34.8±0.25 | 31.8 ± 0.25 |
| **(PC-3)** | 30.4 ± 0.12 | 77.6±0.19 | 60.7 ± 0.45 |
| **(HELA)** | 85.6 ± 0.35 | 89.5±0.34 | 53.5 ± 0.51 |



**Fig.1. A typical cytotoxicity curve of ES from *Lucilia sericata* and *Chrysomya albiceps* against Liver, Breast, Colon Carcinoma cell lines.**



**Fig.2. A typical cytotoxicity curve of ES from *Lucilia sericata* and *Chrysomya albiceps* against Lung, Intestinal, Prostate and Cervical Carcinoma cell lines.**



**Fig.3. IC50 values of *Lucilia sericata* and *Chrysomya albiceps* maggot's excretion/secretion (ES) on different human parts (Liver, Breast, Colon, Lung, Intestinal, Prostate and Cervical) Carcinoma cell lines.**

*L. sericata* and *C. albiceps* ES exhibited a kinase inhibitory effect against four types of kinase receptors; human epidermal growth factor receptor (EGFR), Human Insulin Receptor (IR), Human vascular endothelial cell growth factor receptor (VEGFR) and Fibroblast Growth Factor Receptor (FGFR). Data given in Table (3) showed that, the IC50 of *L. sericata* and *C. albiceps* ES were 6.57±0.09, 1.24±0.15, 5.41±0.18, 3.22±0.22 and 9.62±0.11, 3.39±0.23, 8.01±0.17, 6.72±0.16µg/ml against (EGFR), (IR), (VEGFR) and (FGFR) kinases receptors.

**Table 4.** IC50 values of *Lucilia sericata* and *Chrysomya albiceps* maggot's excretion/secretion (ES) on kinase receptors; human epidermal growth factor receptor (EGFR), Human Insulin Receptor (IR), Human vascular endothelial cell growth factor receptor (VEGFR) and Fibroblast Growth Factor Receptor (FGFR).

|  |  |  |
| --- | --- | --- |
| Type of kinase | IC50 Concentrations  (µg/ml) | |
| ***Lucilia sericata***  **ES** | ***Chrysomya albiceps***  **ES** |
| EGFR | 6.57±0.09 | 9.62±0.11 |
| IR | 1.24±0.15 | 3.39±0.23 |
| VEGFR | 5.41±0.18 | 8.01±0.17 |
| FGFR | 3.22±0.22 | 6.72±0.16 |



**Fig.4. IC50 values of *Lucilia sericata* and *Chrysomya albiceps* maggot's excretion/secretion (ES) on kinase receptors; human epidermal growth factor receptor (EGFR), Human Insulin Receptor (IR), Human vascular endothelial cell growth factor receptor (VEGFR) and Fibroblast Growth Factor Receptor (FGFR).**

**Discussion:**

**Cytotoxic activity:**

*Lucilia sericata* and *Chrysomya albiceps* excretion/secretion (ES) were evaluated against a panel of cancer cell lines and 5-FU was used as a reference standard*. L. sericata* ES was more effective than that of *C. albiceps* against all tested tumor cell lines*,* its activity was arranged in the following order (HepG-2) > (A-549) > (HCT-116) > (PC-3) > (CACO) > (MCF-7) > (HELA). It has a potent activity on liver (HepG-2) and lung (A-549) cancer cell lines with IC50 values less than 20µg/ml. Its activity on Intestinal (CACO) cancer cell line is closely similar to its activity on both breast (MCF-7) and prostate (PC-3) cell lines (Table 2). Moreover, it has double potency of (5-FU) on both liver (HepG-2) and prostate (PC-3) cancer cell lines (Table 2). These results are in consistent with the previously mentioned suggestions by (Nakajima and Natori 1990; Itoh *et al.,* 1986) for lectin from the hemolymph of Sarcopha larvae which 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), as they reported that, the buffer extracts from *Tabanus,* *Mylabris* and *Huechys* demonstrated a strong anticancer activity. The IC50 values of five insect buffer fractions including *Huechys*, *Mylabris*, Red ant, Scorpion and Tabanus were lower than 1 mg/ml. In addition, 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 and F-7) fractions from *Protaetia* *brevitarsis* larva, where all fractions induce apoptosis activity against Colon 26 murine carcinoma cells and (Januszanis *et al.,* 2012) for *Galleria mellonella* hemolymph polypeptides on human brain glioblastoma multiforme cell line (T98G), where the treatment of the cells with *G. mellonella* polypeptides resulted in induction of cell death. Moreover Kustiawan *et al.,* (2015) tested Propolis from the Stingless Bee *Trigona incisa* against human cancer derived cell lines, the IC50 was 4.51±0.76μg/ml against human colon cancer (SW620), 6.06±0.39μg/ml against human gastric cancer (KATO-III), 0.71±0.22μg/ml against human liver cancer (Hep-G2), 0.81±0.18μg/ml against human lung cancer (Chago) and 4.28±0.14 μg/ml against human breast cancer (BT474) cell lines.

**Kinase inhibitory assay:**

Kinase inhibitors have played an increasingly prominent role in the treatment of cancer and other diseases (Gross *et al.,* 2016). Targeted therapy represent a new generation of anticancer treating compounds that are designed to interfere with a specific molecular target, the protein has a serious role in tumor growth or progression. This way of treating differs from using in classical cytotoxic chemotherapy, which lasted as cancer therapy for many decades (Sawyers, 2004). The discovery of small molecule kinase inhibitors was first reported more than 30 years ago (Gross *et al.,* 2016) and many types of targeted therapy are available (Arora and Scholar 2005). In this study we focused on the inhibitory effect of *L. sericata* and *C. albiceps* ES against four types of kinase receptors; The epidermal growth factor receptor (EGFR) is a type of Receptor Tyrosine Kinase (RTKs), its over activity are usually associated with a number of cancers as anal, lung and epithelial tumors in neck and head, so they are considered as a fatal target for stopping cancer (Kuan *et al.,* 2001); Insulin receptors (IR) which is important type of RTKs, they are transmembrane receptor and any decrease in their signaling may lead to cancer (Malaguarnera *et al.,* 2012); (VEGFR) which has an important role in both vasculogenesis (the formation of new vascularity) and angiogenesis (the growth of the newly formed blood vessels) (Holmes *et al.,* 2007) and targeting VEGFR represent a good strategy for preventing angiogenesis hence preventing the growth of cancer cells (Padró *et al.,* 2002) and finally, the fibroblast growth factor receptors (FGFR) which is considered a therapeutic target in breast cancer therapy (Brady *et al.,*2013). The present results in (table 3) were promising, as the *L. sericata* and *C. albiceps* ES showed inhibitory effect against tested RTKs, the IC50 value ranged from 1.24±0.15 to 9.62±0.11µg/ml. The best inhibitory effect was observed on (IR) with IC50 values equal 1.24±0.15and 3.39±0.23µg/ml for *L. sericata* and *C. albiceps* ES respectively, followed by (FGFR) with IC50 3.22±0.22 and 6.72±0.16µg/ml for the insect two species ES. Moreover, the inhibitory activity of the *L. sericata* and *C. albiceps* ES on (VEGFR) and (EGFR) was considered acceptable with IC50 values (5.41±0.18; 6.57±0.09µg/ml) for *L. sericata* ES and (8.01±0.17; 9.62±0.11µg/ml) for *C. albiceps* ES respectively. These results are confirmed with other results recorded by many authors for therapeutic compounds from natural sources, Skropeta and Zivanovic (2011) reported that, marine sponges have yielded over 70 novel compounds which exhibit significant inhibitory activity towards a range of protein kinases and Ansary *et al.,* (2016) for water and acetone extracts from 100 medicinal plants growing in Bangladesh, which have a selective inhibitor of Protein Kinase C (PKC).

**Conclusion:**

We conclude that, *Lucilia sericata* and *Chrysomya albiceps* maggot's excretion/secretion (ES) might have play a major role in anticancer activity and target therapy. Hence we can say that, *L. sericata* maggot's ES can be recommended for cancer treatment especially for Human Liver **(HepG-2)** and Prostate **(PC-3)** cancers. Further, in near future we need to initiate studies leading to find the bioactive compounds in *L. sericata* and *C. albiceps* maggot's ES which may responsible for anticancer activity.

**Corresponding Author:**

Dr. Ahmed Z.I. Shehata

Department of Zoology

Faculty of Science, Al-Azhar University

Cairo, Egypt.

Telephone: +201099525351

E-mail: [ahmedzeinhom00@gmail.com](mailto:ahmedzeinhom00@gmail.com)

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