International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 06-04-2024, Accepted: 26-04-2024, Published: 20-05-2024
Volume 9, Issue 5, 2024, Page No. 24-28

In vitro cytotoxicity activity of hexodecanoic acid extracted from red fire ant *(Solenopsis Invicta)*

Kavitha M, Poomani VM, Sharmila Banu G*
Department of Zoology, NKR Government of Arts College for Women, Namakkal, Tamil Nadu, India

Abstract
Breast cancer is a leading cause of death among women worldwide, and current therapies have limitations and adverse effects. Bioactive compounds found in natural sources have shown potential as alternative therapies. This study aimed to evaluate the *in vitro* cytotoxic activity of hexodecanoic acid extracted from red fire ants Solenopsis invicta, particularly against breast cancer. Solenopsis invicta was isolated using gas chromatography-mass spectrometry (GC-MS), and up to 11 bioactive compounds were identified in the ethanolic extract. The MCF-7 cell line, known for its hormone receptor expression, was used as a model to study breast cancer. The MTT assay was used to evaluate the anti-breast cancer efficacy of the bioactive compounds found in Solenopsis invicta. The bioactive compounds extracted from Solenopsis invicta demonstrated significant cytotoxic activity against MCF-7 and MDA-MB-231 cancer cells. The CTC50 value was 73.86 g/ml for MCF-7 and 71.22 g/ml for MDA-MB-231. The study suggested that the Solenopsis invicta ant extract has high anti-breast cancer efficacy on MCF-7 cells. The results of the study suggest that bioactive compounds extracted from red fire ants have potential as alternative therapies for breast cancer. Further research is needed to identify the specific compounds responsible for the observed anticancer properties and to determine their mechanisms of action. Nonetheless, these findings provide a promising direction for future studies on natural sources for cancer treatment.

Keywords: Breast cancer, *Solenopsis invicta*, MDA-MB-231, MCF-7, hexodecanoic acid

Introduction

Cancer is one of the most widespread and deadly diseases globally, affecting millions of people. It is a complex group of disorders characterized by abnormal cells in the body multiplying and spreading uncontrollably. Among cancers, breast cancer is the most common type affecting women. Tragically, every year, many women lose their lives to breast cancer (Wang *et al.*, 2023; Zhu *et al.*, 2021) [35, 40]. The incidence of cancer remains high due to limitations and restrictions of currently available cancer drugs and treatments (Li *et al.*, 2022; Xu *et al.*, 2022) [21, 36]. According to previous study, approximately 60% of breast cancer cases are hormone-dependent, meaning they rely on estrogen for their growth (Ganesan & Xu, 2017; Jia *et al.*, 2021) [5, 8]. Oestrogen receptor beta (ERβ) has been identified as a potential contributor to breast cancer development. In recent years, it has become evident that breast cancer is not a single disease but rather a group of molecularly diverse tumors originating from the epithelial cells of the breast (Ganesan & Xu, 2020; Zhang *et al.*, 2021) [3, 4].

Hexadecanoic acid, also known as palmitic acid, is a saturated long hydrocarbon chain carboxylic acid consisting of 16 carbon atoms. It is widely present in vegetable oils and animal fats (Xu *et al.*, 2023) [7]. Palmitic acid is the most abundant saturated fatty acid found in microbes, plants, and mammals (Sui *et al.*, 2024) [32]. Additionally, palmitoleic acid, also known as hexadecenoic acid, is a monounsaturated omega-7 fatty acid with 16 carbon atoms. Although hexadecanoic acid is not a pharmaceutical in itself, there is growing interest in its sources and potential medical applications due to its perceived health benefits. Rather than being directly used as medicine, these potential uses often revolve around consuming foods that are high in hexadecanoic acid (Zeng *et al.*, 2023) [38].

Cell lines play a crucial role in molecular detection and research of breast cancer, serving as valuable tools in laboratory experiments, particularly in cancer research. The MCF-7 (Michigan Cancer Foundation - 7) wild-type cell line has proven to be a valuable model for studying hormone-responsive breast cancer. On the other hand, the MDA-MB-231 (M.D. Anderson and MB for Metastasis Breast cancer) cell line is well-known in cancer research, specifically for investigating breast cancer. These cells exhibit high aggressiveness due to the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression, making them triple-negative. The aggressive nature of MDA-MB-231 cells makes them an important model for studying breast cancer biology and exploring potential therapeutic strategies. The MDA-MB231 cell line was initially isolated from a metastatic site in a breast cancer patient, specifically the human breast adenocarcinoma (Ganesan *et al.*, 2022; Ganesan *et al.*, 2021; Ganesan *et al.*, 2023) [2, 4, 7].

Solenopsis invicta, commonly known as the red imported fire ant or Buren, is a highly notorious invasive species globally. It is a voracious, aggressive, and widely distributed ant species (Menchetti *et al.*, 2023) [28]. This invasive ant species has caused significant damage to agriculture, public health, and ecosystems (Valles *et al.*, 2021) [33]. Interestingly, red fire ants have been observed to protect plants with aphid infestations, as they serve as effective defenders against potential predators of the aphids. This behavior of fire ants can offer some ecological benefits by helping to control pest populations in gardens and agriculture, as they prey on other pests. However, their aggressive nature can also have negative impacts on native species and disrupt ecosystems (Kumar, Sharmila Banu, & Rajasekara Pandian, 2007; Latha *et al.*, 2023; Latharaja & Banu, 2022; Matheswaran & Banu, 2021, 2022) [15,19,20, 23-24].

The objective of the present study is to investigate the *in*

vitro cytotoxic activity of hexodecanoic acid, extracted from the red fire ant Solenopsis invicta.

Fig 1: Red Fire Ant (*Solenopsis invicta*)

Materials and Methods

Collection and preparation of ant (*solenopsis invicta*) extracts

In this study, the Soxhlet extraction method was employed to obtain the ant extract. Approximately 10g of the sample material was packed evenly into a thimble, and 150 ml of solvent was used for each extraction. The extraction process was performed for a full day or until the solvent in the syphon tube of the extractor turned yellow. Afterward, the extract was transferred to a beaker and heated on a hot plate at 80˚C until the solvent evaporated. The resulting dried extract was then stored at 4˚C in a refrigerator for future use.

Cell lines and culture medium

The MDA-MB-231 cell line and the MCF-7 human breast cancer cell line were provided by the National Centre for Cell Sciences (NCCS) in Pune, India. The stock cells were cultured using Dulbecco's modified Eagle's medium (DMEM) as the culture medium. The medium was supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml), and amphotericin B (5 µg/ml) in a humidified environment with 5% CO2 at 37°C until reaching confluence. To separate the cells, a TPVG solution (0.02% glucose in PBS, 0.02% EDTA, and 0.2% trypsin) was used. Each experiment was carried out on 96-well microtitre plates, and the stock cultures were grown in 25 cm2 culture flasks from Tarsons India Pvt. Ltd., located in Kolkata, India.

Preparation of test solutions

To prepare a stock solution with a concentration of 1 mg/ml and sterilize it through filtering, each weighed test medication was dissolved individually in distilled dimethyl sulfoxide (DMSO). The volume was then adjusted with DMEM supplemented with 2% inactivated Fetal Bovine Serum (FBS) for the cytotoxicity investigations. Serial two-fold dilutions were created from the stock solution for cytotoxic research purposes.

Determination of cell viability by MTT assays

In accordance with the instructions provided by earlier studies (Ganesan *et al.*, 2023) [7], the monolayer cell culture was trypsinized, and the cell count was adjusted to 1.0 x 105 cells/ml using media containing 10% FBS. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was then performed to assess the cell viability. The absorbance at 540 nm was measured using a microplate reader. To calculate the percentage growth inhibition, the following formula was used: % Growth inhibition = {100 – (OD of sample/ OD of Control)} X 100.

Statistical analysis

The dose-response curves for each cell line were utilized to determine the concentration of the test medication required to inhibit cell growth by 50% (CTC50) values. All experiments in the study were conducted at least three times in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism (V5.0, Graphpad Software, La Jolla, CA, USA). One-way analysis of variance (ANOVA) followed by multiple comparisons using Tukey's test was employed to assess IC50 values and cytotoxicity data. A significance level of $P < 0.05$ was considered statistically significant (Ganesan *et al.*, 2020) [3].

Result and Discussion

In the context of biological or medical research, the term "cytotoxicity activity" refers to the ability of a substance to induce cell death. This can include chemicals and drugs that have the potential to cause harm to cells. The interpretation of cytotoxicity activity depends on the specific objectives of the research and the types of cells or organisms being studied (Kumar *et al.*, 2006; Kumar, Sharmila Banu, Murugesan, & Rajasekara Pandian, 2007; Pandian *et al.*, 2006) [10, 15, 10]. In our study, we focused on investigating the dose-response relationship of cytotoxicity. Cytotoxicity experiments typically involve exposing cells or organisms to different concentrations or doses of the test material. The cytotoxicity response is often concentration-dependent, meaning that the level of cell death or toxicity may increase with higher concentrations of the substance (Kumar *et al.*, 2004) [13]. To understand the toxicity profile of a compound, it is important to analyze the dose-response relationship. However, it is crucial to consider the specific cell type and species used in our investigation, as the cytotoxicity activity can vary depending on these factors. The same chemical may exhibit different effects on different cell types and species (Banu & Raja, 2023; Matheswaran & Banu, 2022) [1, 24]. Therefore, the biological context needs to be taken into account when interpreting cytotoxicity results. There are two main types of cytotoxicity: selective and non-selective. Non-selective cytotoxicity affects a range of cell types, while selective cytotoxicity specifically targets certain cells or regions. Selectivity in cytotoxicity can be advantageous for the development of drugs that target specific diseases or conditions (Murugesan *et al.*, 2020; Vijayapriya *et al.*, 2019) [29, 34].

In the study conducted on the impact of the ethanol extract of red fire ant from Kattuputhur, Trichy district, on the MCF-7 breast cancer cell line, the cytotoxicity of the extract was assessed using the micro culture tetrazolium MTT test. The red fire ant ethanol extract was tested at various concentrations to determine its cytotoxicity against the

MCF-7 breast cancer cells. The concentration response curve was used to calculate the effective concentration, and the CTC50 value was found to be 73.86µg/ml. This indicates that the red fire ant extract significantly increased cell death in the MCF-7 breast cancer cells. The dose-response relationship curve (Figure 2 and Table 1) demonstrated the impact of the red fire ant extract on the MCF-7 breast cancer cells. The extract was found to activate caspase, induce chromatin condensation and fragmentation, cause inter nucleosomal DNA cleavage, membrane blebbing, and movement of phosphatidylserine from the inner to the outer leaflet of the plasma membrane. These are characteristic features associated with apoptosis or programmed cell death (Banu & Raja, 2023; Kumar, Banu, & Pandian, 2005; Matheswaran & Banu, 2021; Pandian *et al.*, 2006) [1, 12, 23, 10]. Apoptosis activation is considered an effective strategy in cancer treatment (Kumar, Sharmila Banu, Maheswaran, *et al.*, 2007; Logeswari *et al.*, 2021) [15, 22]. The examination of cell cycle flow cytometry and Annexin V/PI revealed that the red fire ant ethanol extract inhibits the growth of MCF-7 cells by inducing apoptosis without causing cell cycle arrest. Similar findings were observed in prior research where Corallina pilulifera ethanolic extracts induced apoptosis in HeLa cells without causing cell cycle arrest (Kumar *et al.*, 2008; Kumar *et al.*, 2009; Pandian *et al.*, 2006) [9, 16, 10].

Table 1: Cytotoxicity analysis of the ethanol extract of the ant sample in the MCF-7 cell line

S. No	Concentration (µl)	OD Values	CTC_{50} %	CTC_{50} (µg/ml)	Cell Viability
1	20	0.367	24.95	73.86	75.05
2	40	0.326	33.33		66.67
3	60	0.271	44.58		55.42
4	80	0.231	52.76		47.24
5	100	0.186	61.96		38.04

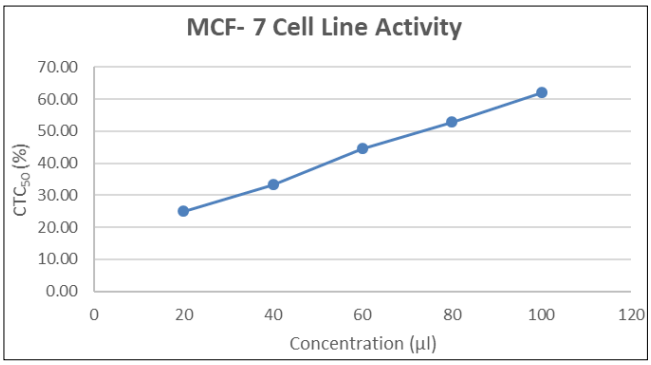

Fig 2: Cytotoxicity analysis of the ethanol extract of the ant sample in the MCF-7 cell line

In the study conducted on the impact of the ethanol extract of red fire ants from Kattuputhur, Trichy district, on the MDA-MB-231 breast cancer cell line, the cytotoxicity of the extract was evaluated using the micro culture tetrazolium MTT test. The red fire ant ethanol extract was tested at various concentrations to determine its cytotoxicity against the MDA-MB-231 breast cancer cells. The concentration response curve was used to calculate the effective concentration, and the CTC50 value was found to be 71.22µg/ml. This indicates that the red fire ant extract resulted in a degradation of cell death in the MDA-MB-231 breast cancer cells. The dose-response relationship curve (Figure 3 and Table 2) illustrates the impact of the red fire ant extract on the MDA-MB-231 breast cancer cells. However, the specific details of the curve are not described in the given information. In other studies involving dendritic cells transfected with anti-CTLA-4 antibody mRNA, the effectiveness of this immune modulator was associated with a decrease in antigen-specific cytotoxic T lymphocytes (CTL) responses against breast cancer cells, including MCF-7, MDA-MB-231, and T4D7 cell lines (Kumar, Banu, *et al.*, 2007; Kumar, Sharmila Banu, *et al.*, 2005; Matheswaran *et al.*, 2019; Pandian *et al.*, 2006; Rajasekara Pandian *et al.*, 2007) [11, 14, 34, 10, 15]. This suggests that the immune modulator had an impact on the cytotoxicity response of these breast cancer cells.

The study indicates that the tumor microenvironment is inactivated when anti-CTLA-4 (partially anti-PD-1) antibodies are applied. This inactivation disrupts the interaction between CD4+ and CD8+ cells and breast cancer cells. However, it is noted that in this case, MDA-MB-231 breast cancer cells showed alterations, while MCF-7 cells did not. The lack of alterations in MDA-MB-231 breast cancer cell proliferation in the context of PD-1 suppression is consistent with other findings that have shown no impact on tumor survival and metastasis when tumors are treated solely with anti-PD-1 antibodies (Matheswaran *et al.*, 2020a, 2020b) [26, 27]. These findings suggest that the response to PD-1 suppression may vary depending on the specific breast cancer cell line. In this particular study, MDA-MB-231 cells did not show alterations in proliferation, indicating that anti-PD-1 treatment alone may not have a significant impact on the survival and metastasis of these cells. It's important to note that the provided information references findings from another study, and further details from that study would be needed to fully understand the implications and significance of these observations.

Table 2: Cytotoxicity analysis of the ethanol extract of the ant sample in the MDA-MB-231 Cell Line

Sl. No	Concentration (µl)	OD Values	CTC_{50} %	CTC_{50} (µg/ml)	Cell Viability
1	20	0.388	26.93	71.22	73.07
2	40	0.339	36.16		63.84
3	60	0.294	44.63		55.37
4	80	0.241	54.61		45.39
5	100	0.199	62.52		37.48

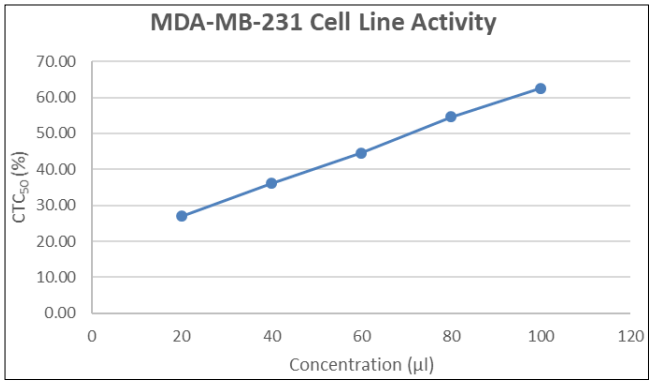

Fig 3: Cytotoxicity analysis of the ethanol extract of the ant sample in the MDA-MB-231 Cell Line

Conclution

Based on the exploration conducted on the anti-breast cancer activity of hexadecenoic acid from red fire ants, it can be concluded that the compound demonstrates anti-breast cancer activity against the MCF-7 and MDA-MB-231 cell lines. The MTT assay revealed that hexadecenoic acid possesses cytotoxicity activity, indicating its ability to inhibit the proliferation of breast cancer cells. The study suggests that further research should be conducted to investigate the mechanism of action and potential use of hexadecenoic acid in cancer treatment. The findings of the study provide new insights into the potential therapeutic applications of hexadecenoic acid as a cytotoxic compound with anti-breast cancer activity. It is mentioned that hexadecenoic acid, also known as palmitic acid, is the main bioactive compound with anticancer properties. The cytotoxicity test conducted using the MTT assay on MCF-7 and MDA-MB231 cancer cells resulted in a higher CTC50 value, indicating a greater potency of hexadecenoic acid in inhibiting the growth of these breast cancer cells. Overall, the anti-breast cancer activity of hexadecenoic acid from red fire ants holds promise for its potential use in cancer treatment, but further research is needed to fully understand its mechanism of action and explore its therapeutic applications.

References

1. Banu G, Raja L. Extraction and quantification of 1-Deoxynojirimycin in Silkworm during metamorphosis and moulting stages. Research Journal of Biotechnology,2023:18:15-21. https://doi.org/10.25303/1801rjbt15021
2. Ganesan K, Du B, Chen J. Effects and mechanisms of dietary bioactive compounds on breast cancer prevention. Pharmacological Research,2022:178:105974. https://doi.org/https://doi.org/10.1016/j.phrs.2021.105974
3. Ganesan K, Ramkumar KM, Xu B. Vitexin restores pancreatic β-cell function and insulin signaling through Nrf2 and NF-κB signaling pathways. *Eur J Pharmacol*,2020:888:173606. https://doi.org/10.1016/j.ejphar.2020.173606
4. Ganesan K, Wang Y, Gao F, Liu Q, Zhang C, Li P, *et al.* Targeting Engineered Nanoparticles for Breast Cancer Therapy. *Pharmaceutics*, 2021, 13(11). https://doi.org/10.3390/pharmaceutics13111829
5. Ganesan K, Xu B. Molecular targets of vitexin and isovitexin in cancer therapy: a critical review. *Ann N Y Acad Sci*,2017:1401(1):102-113. https://doi.org/10.1111/nyas.13446
6. Ganesan K, Xu B. Deep frying cooking oils promote the high risk of metastases in the breast-A critical review. *Food Chem Toxicol*,2020:144:111648. https://doi.org/10.1016/j.fct.2020.111648
7. Ganesan K, Xu C, Liu Q, Sui Y, Chen J. Unraveling the Role of Hepatic PGC1α in Breast Cancer Invasion: A New Target for Therapeutic Intervention? *Cells*, 2023, 12(18).
8. Jia L, Guo L, zhang XF, Yu Q, you J, Ganesan K, *et al.* Music Therapy in Traditional Chinese Medicine Attenuates the Depression-Associated Breast Cancer Development in MMTV-PyMT Mice and Clinics. International Journal of Medical Research & Health Sciences,2021:10(3):110. http
9. Kumar G, Banu GS, Murugesan AG. Effect of Helicteres isora bark extracts on heat antioxidant status and lipid peroxidation in streptozotocin diabetic rats [Article]. Journal of Applied Biomedicine,2008:6(2):89-95. https://doi.org/10.32725/jab.2008.012
10. Kumar G, Banu GS, Murugesan AG, Pandian MR. Hypoglycaemic effect of Helicteres isora bark extract in rats [Article]. Journal of Ethnopharmacology,2006:107(2):304-307. https://doi.org/10.1016/j.jep.2006.05.007
11. Kumar G, Banu GS, Murugesan AG, Pandian MR. Antihyperglycaemic and antiperoxidative effect of Helicteres isora L. bark extracts in streptozotocin-induced diabetic rats [Article]. Journal of Applied Biomedicine,2007:5(2):97-104. https://doi.org/10.32725/jab.2007.014
12. Kumar G, Banu GS, Pandian MR. Evaluation of the antioxidant activity of Trianthema portulacastrum L. [4] [Letter]. Indian Journal of Pharmacology,2005:37(5):331-333. https://doi.org/10.4103/0253-7613.16861
13. Kumar G, Banu GS, Pappa PV, Sundararajan M, Pandian MR. Hepatoprotective activity of Trianthema portulacastrum L. against paracetamol and thioacetamide intoxication in albino rats [Article]. Journal of Ethnopharmacology,2004:92(1):37-40. https://doi.org/10.1016/j.jep.2003.12.009
14. Kumar G, Sharmila Banu G, Kannan V, Rajasekara Pandian M. Antihepatotoxic effect of β-carotene on paracetamol induced hepatic damage in rats [Article]. Indian Journal of Experimental Biology,2005:43(4):351-355. https://www.scopus.com/inward/record.uri?eid=2-s2.0-17644365492&partnerID=40&md5=16cd0c5859836113f8df880dbf57e4be
15. Kumar G, Sharmila Banu G, Maheswaran R, Rema S, Rajasekara Pandian M, Murugesan AG. Effect of Plumbago zeylanica L. on blood glucose and plasma antioxidant status in STZ diabetic rats [Article]. Journal of Natural Remedies,2007:7(1):66-71. https://www.scopus.com/inward/record.uri?eid=2-s2.0-34047264593&partnerID=40&md5=00812a22398f8a7c7547cbd8a55d1cc4
16. Kumar G, Sharmila Banu G, Murugesan A. Attenuation of Helicteres isora L. bark extracts on streptozotocin-induced alterations in glycogen and carbohydrate metabolism in albino rats [Article]. Human and Experimental Toxicology,2009:28(11):689-696. https://doi.org/10.1177/0960327109350797
17. Kumar G, Sharmila Banu G, Murugesan AG, Rajasekara Pandian M. Preliminary toxicity and phytochemical studies of aqueous bark extract of Helicteres isora L [Article]. International Journal of Pharmacology,2007:3(1):96-100. https://doi.org/10.3923/ijp.2007.96.100
18. Kumar G, Sharmila Banu G, Rajasekara Pandian M. Biochemical activity of Selenium and glutathione on country made liquor (CML) induced hepatic damage in rats [Article]. Indian Journal of Clinical Biochemistry,2007:22(1):105-108. https://doi.org/10.1007/BF02912891

19. Latha R, Gani S, Banu G. 1-Deoxynojirimycin Mitigates Glycogen and Carbohydrate Metabolic Enzyme Alterations in High Glucose-Induced Diabetic Tilapia: Implications for Therapeutic Intervention, 2023, 301-314. https://doi.org/10.48165/bpas.2023.42A.2.9
20. Latharaja R, Banu G. Hypoglycemic and antioxidant potential of 1-deoxynojirimycin in high glucose-induced experimental diabetic Tilapia (Oreochromis niloticus). The Uttar Pradesh State dental journal: an official publication of the State U.P. Dental Branch of the Indian Dental Association,2022:43:7-14. https://doi.org/10.56557/UPJOZ/2022/v43i163135
21. Li Y, Ganesan K, Chen J. Role of Biological Mediators of Tumor-Associated Macrophages in Breast Cancer Progression [Review]. Current Medicinal Chemistry,2022:29(33):5420-5440. https://doi.org/10.2174/0929867329666220520121711
22. Logeswari D, Kaliyammal K, Facika A, Rajasekara M, Banu G. Effect of Sublethal Concentrations of Commercial Detergents on the Protein Content of Selective Freshwater Fishes. Bulletin of Pure & Applied Sciences- Zoology,2021:40:127-139. https://doi.org/10.5958/2320-3188.2021.00016.4
23. Matheswaran P, Banu G. Evaluation of *In-vitro* free radicals scavenging activities of edible insects. The Uttar Pradesh State dental journal: an official publication of the State U.P. Dental Branch of the Indian Dental Association,2021:42:1127-1134.
24. Matheswaran P, Banu G. Nutritional Composition of Seven Edible Insects. Jundishapur Journal of Microbiology,2022:15:1776-1789.
25. Matheswaran P, Raja L, Banu G. Antioxidant and anti-inflammatory efficacy of functional proteins obtained from seven edible insects,2019:4:24-31.
26. Matheswaran P, Raja L, Banu G. Anti-diabetic and anti-obesity effect of functionally active proteins obtained from seven edible insects. International Journal of Pharmaceutical Sciences and Research,2020a:11:1000-1009. https://doi.org/10.13040/IJPSR.0975-8232.11(9).1000-09
27. Matheswaran P, Raja L, Banu G. Anti-Hypertensive and Anti-Microbial Activity of Protein Hydrolysate Obtained from Seven Edible Insects. *Bulletin of Pure and Applied Sciences - Section F Geological Sciences*,2020b:39:206-216. https://doi.org/10.5958/2320-3188.2020.00024.8
28. Menchetti M, Schifani E, Alicata A, Cardador L, Sbrega E, Toro Delgado E, *et al.* The invasive ant Solenopsis invicta is established in Europe. *Curr Biol*,2023:33(17):R896-r897. https://doi.org/10.1016/j.cub.2023.07.036
29. Murugesan M, Chinnappu J, Manoharan P, Matheswaran P, Raja L, Banu G. Assessment of diversity and relative richness of aquatic entomofauna in Jedarpalayam Dam, Namakkal District, Tamil Nadu. *Journal of Entomology*,2020:5:103-110.
30. Pandian M, Banu G, Kumar G. A study of the antimicrobial activity of Alangium salviifolium [Article]. *Indian Journal of Pharmacology*,2006:38(3):203-204. https://doi.org/10.4103/0253-7613.25810
31. Rajasekara Pandian M, Banu GS, Kumar G. Antimicrobial activities of natural honey from medical plants on antibiotic resistant strains of bacteria [Article]. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*,2007:9(2):219-224. https://www.scopus.com/inward/record.uri?eid=2-s2.0-34547498715&partnerID=40&md5=54daff0a7d991e709495a05f50ff3bcc
32. Sui Y, Liu Q, Xu C, Ganesan K, Ye Z, Li Y, *et al.* Non-alcoholic fatty liver disease promotes breast cancer progression through upregulated hepatic fibroblast growth factor 21. Cell Death & Disease,2024:15(1):67. https://doi.org/10.1038/s41419-023-06386-8
33. Valles SM, Oliver JB, Addesso KM, Perera OP. Unique venom proteins from Solenopsis invicta x Solenopsis richteri hybrid fire ants. *Toxicon X*, 2021, 9-10, 100065. https://doi.org/10.1016/j.toxcx.2021.100065
34. Vijayapriya S, Raja L, Malathi A, Matheswaran P, Kiruthika P, Banu G. Assessment of Zooplankton Diversity in Kosavampatti Lake at Namakkal District, Tamil Nadu. International Journal of Biological & Pharmaceutical Research,2019:10:1-5.
35. Wang Y, Zhang C, Xiao M, Ganesan K, Gao F, Liu Q, *et al.* A tumor-targeted delivery of oral isoliquiritigenin through encapsulated zein phosphatidylcholine hybrid nanoparticles prevents triple-negative breast cancer. Journal of Drug Delivery Science and Technology,2023:79:103922. https://doi.org/https://doi.org/10.1016/j.jddst.2022.103922
36. Xu C, Ganesan K, Liu X, Ye Q, Cheung Y, Liu D, *et al.* Prognostic Value of Negative Emotions on the Incidence of Breast Cancer: A Systematic Review and Meta-Analysis of 129,621 Patients with Breast Cancer. *Cancers (Basel)*, 2022, 14(3). https://doi.org/10.3390/cancers14030475
37. Xu C, Zhang C, Ganesan K, Chen Q, Tang H, Gao F, *et al.* Anti-migratory Properties of Cryoprotective Isoliquiritigenin-zein Phosphatidylcholine Nanoparticles Prevent Triple-negative Breast Cancer through PI3K-mTOR and MMP2/9 Pathways. *Curr Med Chem*, 2023. https://doi.org/10.2174/0109298673259973231023110945
38. Zeng X, Gong G, Ganesan K, Wen Y, Liu Q, Zhuo J, *et al.* Spatholobus suberectus inhibits lipogenesis and tumorigenesis in triple-negative breast cancer via activation of AMPK-ACC and K-Ras-ERK signaling pathway. *Journal of Traditional and Complementary Medicine*, 2023. https://doi.org/https://doi.org/10.1016/j.jtcme.2023.09.002
39. Zhang F, Liu Q, Ganesan K, Kewu Z, Shen J, Gang F, *et al.* The Antitriple Negative Breast cancer Efficacy of Spatholobus suberectus Dunn on ROS-Induced Noncanonical Inflammasome Pyroptotic Pathway. *Oxid Med Cell Longev*, 2021, 5187569. https://doi.org/10.1155/2021/5187569
40. Zhu H, You J, Wen Y, Jia L, Gao F, Ganesan K, *et al.* Tumorigenic risk of Angelica sinensis on ER-positive breast cancer growth through ER-induced stemness *in vitro* and *in vivo*. *J Ethnopharmacol*,2021:280:114415. https://doi.org/10.1016/j.jep.2021.114415