International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 02-06-2026, Accepted: 02-07-2026, Published: 17-07-2026
Volume 11, Issue 3, 2026, Page No. 103-107

In vitro cytotoxic action of scent gland secretions of true bug *Tessaratoma javanica* (Tessartomidae) Against HCT-116 cells

Pravin B Kale[1], Chandrakant V Sirsat[2]
[1] Research Student, Deogiri College, Chhatrapati Sambhajinagar, Maharashtra, India
[2] Professor and Head, Vinayakrao Patil College, Vaijapur, Chhatrapati Sambhajinagar, Maharashtra, India
Corresponding Author: Pravin B Kale
DOI: https://doi.org/10.66856/ijer.2026.11.3.11242

Abstract
The present investigation evaluated the *in vitro* cytotoxic activity of metathoracic scent gland secretions obtained from the Hemipteran true bug *Tessaratoma javanica* (Distant, 1902) against the HCT-116 cell line using the MTT assay. Adult insects are collected from host plants and maintained under natural environmental conditions. Molecular identification evaluated by using mitochondrial cytochrome-c oxidase subunit-I (COI) gene sequencing and phylogenetic analysis. The sequences were submitted to GenBank (PZ274831). The metathoracic gland secretion was collected aseptically using sterile capillary tubes and coded as PBK-6. HCT-116 cells are cultured in "Dulbecco's Modified Eagle Medium" (DMEM) supplemented with FBS and antibiotics under standard incubation conditions. Treatment of Cancer cells with sample ID- PBK-6 at concentrations ranging from 1 µg/ml to 1000 µg/ml. Cytotoxicity was evaluated by using the MTT assay, and absorbance was measured at 540 nm. PBK-6 exhibited concentration-dependent cytotoxicity with an IC_{50} of 932.5 ± 0.11 µg/ml. Morphological observations revealed reduced cell density, cellular shrinkage, and altered morphology at higher concentrations. Members of the family Tessaratomidae produce volatile defensive secretions containing aldehydes, ketones, esters, hydrocarbons, and other biologically active metabolites. The findings indicate that metathoracic scent gland secretions of *Tessaratoma javanica* (Distant, 1902) may exhibit anticancer properties through bioactive compounds. Further purification and molecular characterization studies are necessary to identify the active constituents which is responsible for cytotoxicity.

Keywords: Hemiptera, MTT assay, anticancer activity, bioactive compounds

Introduction
Cancer is one of the leading or major causes of mortality worldwide, and colorectal carcinoma is among the most leading forms of cancers associated with significant morbidity and mortality. Natural products continue to play a major role in anticancer drug discovery because numerous chemotherapeutic agents have originated from biological resources. Insects constitute one of the incredibly rich frontiers for biologically active compounds and possess remarkable biochemical diversity.

The Hemiptera is the largest insect order, comprising > 80,000 described species worldwide (Henry, 2017) [7]. Within Hemiptera, the superfamily Pentatomoidea includes several phytophagous families such as Pentatomidae, Dinidoridae, and Tessaratomidae (Rider, 2018) [15]. Members of family Tessaratomidae are commonly known as giant shield bugs and are characterized by relatively large body size, piercing-sucking mouthparts, strong odour-producing metathoracic scent glands, and phytophagous feeding behaviour. Tessaratomids are distributed mainly in tropical Asia, Africa, and Oceania. Several species are recognized as economically important agricultural pests and produce potent defensive secretions capable of causing skin irritation and temporary blindness in humans. *Tessaratoma javanica* (Distant, 1902) [3] belongs to the family Tessaratomidae, subfamily Tessaratominae, and tribe Tessaratomini. The genus *Tessaratoma* was established by Le Peletier and Serville (1825) and currently includes several species distributed in the Indo-Malayan region. Tessratomidae bug was reported from Chandwad tehsil of Nashik district (Kale & Sirsat, 2023) [13]. Modern insect taxonomy increasingly incorporates molecular approaches such as DNA barcoding used for accurate species identification. The mitochondrial cytochrome-c oxidase subunit-I (COI) gene is widely used for insect molecular identification and phylogenetic analysis (Lee *et al*., 2018; Tamura *et al*., 2021) [8, 9]. Species of *Tessaratoma* possess characteristic four-segmented antennae, a robust mesosternal ridge, spinous femora, and well-developed metathoracic scent glands. These glands produce volatile secretions containing aldehydes, ketones, hydrocarbons, and esters that function in defense against predators and microbial pathogens (Aldrich, 1988; Durak & Kalender, 2021) [1, 5].

Previous investigations have demonstrated the significant medicinal potential of insect-derived biomolecules, including defensive secretions, antimicrobial peptides, and microbial metabolites, exhibit antibacterial, antiviral, antioxidant, and anticancer activities (Dossey, 2010) [4]. Chernysh *et al*. (2002) [2] reported antiviral and antitumor peptides from insects, whereas Kim *et al*. (2021) [8] demonstrated anticancer activity of periplanetasin-5 isolated from Periplaneta americana. Nakabachi and Okamura (2019) [12] also demonstrated cytotoxic activity of diaphorin produced by bacterial symbionts associated with hemipteran insects. Such findings indicate that insects and their microbial symbionts may serve as important sources of pharmacologically active compounds. Kale & Sirsat (2024) review reported the presence of primary and secondary metabolites in Heteropteran bugs.

MTT assay is a worldwide used for calculating cellular viability and cytotoxicity method is a colorimetric method,

it is based on the mitochondrial reduction of tetrazolium salts to insoluble purple formazan crystals (Morgan, 1998; Van Meerloo *et al*., 2011) [11, 19]. The present experiment was conducted to evaluate *in vitro* cytotoxic activity of the metathoracic scent gland secretion sample PBK-6 obtained from *Tessaratoma javanica* (Distant, 1902) [3] against the HCT-116 cell line using MTT assay. Moderate cytotoxicity observed in PBK-6 indicates presence of biologically active metabolites within the secretion.

Taxonomic Position
Family: Tessaratomidae Stål, 1865
Subfamily: Tessaratominae Stål, 1865
Tribe: Tessaratomini Stål, 1865

Key to Genera
1. Antennae four segmented; pronotum posteriorly produced at the base of scutellum; scutellum apically spatulate; mesosternum with a robust ridge; femora usually spinous beneath. *Tessaratoma* Le Peletier and Serville.

Genus: *Tessaratoma* Le Peletier and Serville, 1825
Species: *Tessaratoma javanica* (Distant, 1902) [3] (Fig. 1)
1902 [3].
Tessaratoma javanica Distant, *Fauna of British India, Rhynchota* 1: 258.

Material and methods
Collection of Insects and Secretion Sample
The 3rd / 4th instars of *Tessaratoma javanica* (Distant, 1902) [3] (Image 1-a) were manually collected from host plants and maintained under the natural environmental conditions in a ventilated home gallery in a plastic jar (Image 1-b). Fresh host-plant material was supplied periodically for acclimatization. Collect Metathoracic scent gland secretions twice in the morning and evening from adult bugs (Image 1-b & c), continuing for three days to collect sufficient sample volume. For sample collection, disturb the bugs have to secretion, gently collect the secretions using sterile capillary tubes. The collected sample was shifted into sterilized microcentrifuge tubes and stored under refrigerated conditions at -20 ^{0}C until further experimental use. The sample was coded as PBK-6.

Fig 1: *Tessaratoma javanica* (Distant, 1902) [3]

(a) (b) (c)

Image: a - 3rd & 4th Instar, b – rearing jar; sample collection with the help capillary, c – Adult

Morphological Identification
Standard taxonomic keys are used for morphological identification of Heteropteran bugs, and descriptions provided in The Fauna of British India (Distant, 1902) [3] use a stereoscopic microscope for examining external morphology. The collected specimens showed characteristic features of *Tessaratoma,* including four-segmented antennae, spatulate scutellar apex, robust mesosternal ridge, spinous femora, and pungent metathoracic scent glands, confirming the specimens as *Tessaratoma javanica*.

Cell Line
The HCT-116 (Passage No. 25, procured from NCCS Pune, India, RRID-CVCL_D1ZN) was authenticated before being used for experimentation by an expert.

Cell Culture
The cell line HCT-116 was verified to be free of mycoplasma contamination. Then, cells were cultivated in D'sMEM (Dulbecco's Modified Eagle Medium -AT149-1L) supplemented with 10 % FBS (fetal bovine serum -

HIMEDIA-RM 10432) and 1 % antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781). Cultures were maintained at 37 °C, with 5 % CO_2.

MTT Cytotoxicity Assay

Approximately 1 × 10^4 HCT-116 cells were cultured is in sterile 96-well plates for 24 hr. Cells were treated with an different concentrations of PBK-6, ranging from concentration 1 µg/ml to 1000 µg/ml. Untreated cells served as the control, while Paclitaxel served as standard anticancer drug.

After incubation, MTT solution (5mg/ml) was added to cell-culture and further incubated (Air- jacketed CO2 incubator-Heal force-HF90) for 2 h. Viable cells converted tetrazolium salts into purple-formazan crystals through mitochondrial dehydrogenase activity. At the last step of the experiment, culture supernatant was removed and cell layer matrix was dissolved in 100 µl *Dimethyl-Sulfoxide* (DMSO –SRL- Cat no.- 67685) and read an ELISA plate reader (iMark, Bio-Rad, USA) at 540 nm. Percentage of cell viability was calculated using the following formula:

Formula: - % Viable cells=A testA Control×*100*

(**A** test = Absorbance of test sample)
(**A** Control = Absorbance of Control)

Statistical Analysis

IC_{50} was calculated by using GraphPad Prism -6. Data were expressed as 50% inhibitory concentration (*IC50*) was presented as Mean ± SEM (Standard Error of Mean).

Results

The metathoracic scent gland secretion sample PBK-6 exhibited concentration-dependent cytotoxic activity against HCT-116 human colorectal carcinoma cells. Increasing concentrations of PBK-6 resulted in a gradual reduction in cellular viability.

The calculated *IC_{50}* of PBK-6 was 932.5 ± 0.11 µg/ml, whereas the standard anticancer drug Paclitaxel exhibited an *IC_{50}* value of 9.79 ± 0.05 nM.

Table 1: *IC_{50}* values against HCT-116 cells

Sample	*IC_{50}* Value
Paclitaxel (Standard drug)	9.79 ± 0.05 nM
PBK-6 (Sample)	932.5 ± 0.11 µg/ml

Morphological observations under an inverted microscope revealed reduced cell density, cellular shrinkage, membrane damage, irregular morphology, and partial detachment of cells at higher concentrations of PBK-6.

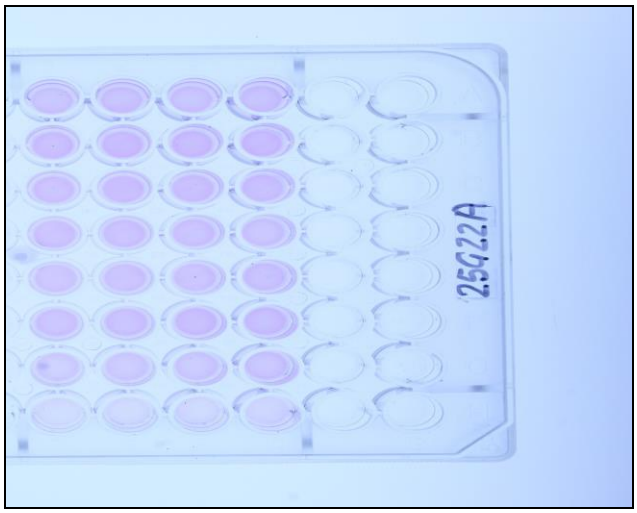


Fig 2: MTT Assay Plate Showing PBK-6 Activity

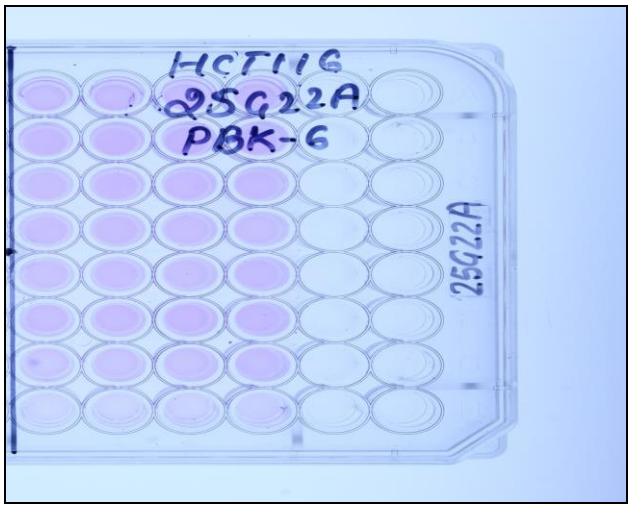
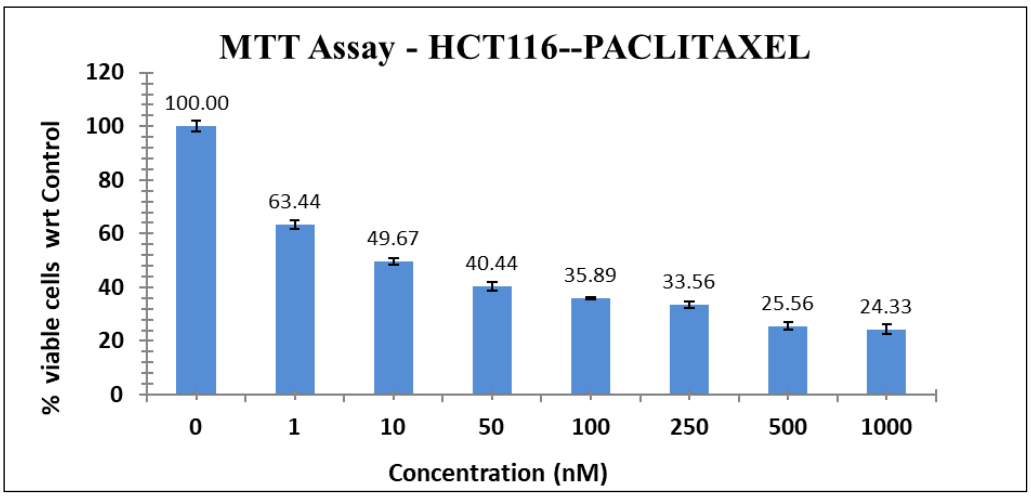
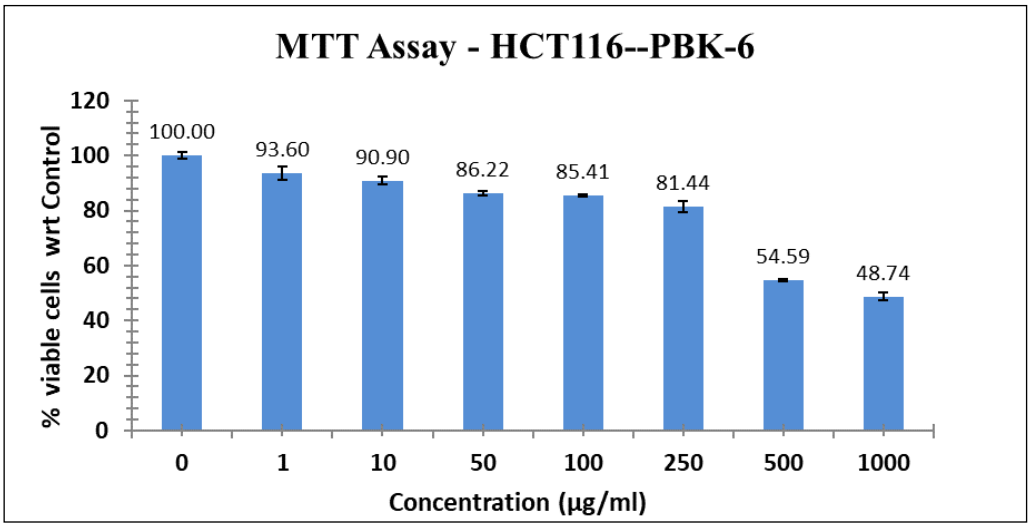


Fig 3: MTT Assay Plate of HCT-116 Cell Line





Discussion

The present study demonstrated measurable cytotoxic activity of metathoracic scent gland secretions from *Tessaratoma javanica* (Distant, 1902) [3] against the HCT-116 human colorectal carcinoma cell line. Members of Tessaratomidae possess specialized exocrine glands producing volatile defensive secretions rich in aldehydes, ketones, hydrocarbons, and esters involved in ecological defense mechanisms (Aldrich, 1988; Durak & Kalender, 2021) [1, 5].

Tessaratomids are important phytophagous insects distributed throughout tropical Asia and Australasia. The family contains approximately 240 species divided into three subfamilies and several tribes. The genus *Tessaratoma* includes several species economically associated with important plants and is characterized by large body size and strong defensive odours.

Previous investigations have demonstrated the medicinal significance of insect-derived biomolecules. Chernysh *et al*. (2002) [2] described antiviral and antitumor peptides from insects, while Kim *et al*. (2021) [8] reported anticancer activity of periplanetasin-5 isolated from *Periplaneta americana*. Nakabachi and Okamura (2019) [12] demonstrated that diaphorin synthesized by bacterial symbionts of Hemipteran insects against several types of human cancer cell lines exhibited cytotoxicity.

The moderate cytotoxicity observed in the sample PBK-6 suggests the presence biologically active metabolites within the secretion. Morphological alterations, including shrinkage and membrane damage, further support the cytotoxic effects of scent gland secretion on cancer cells. The activity may be associated with volatile aldehydes, ketones, hydrocarbons, peptide compounds, or microbial symbiont-derived metabolites present in the secretion sample.

Further investigations of the sample involving chromatographic purification, GC-MS analysis, peptide characterization, apoptosis assays, and molecular docking studies, are required to identification of the active constituents are responsible for anticancer activity.

Conclusion

The metathoracic scent gland secretion sample PBK-6 obtained from *Tessaratoma javanica* (Distant, 1902) [3] exhibited measurable *in vitro* cytotoxic activity against the HCT-116 cells using MTT assay. The secretion produced a concentration-dependent reduction in cell viability with an IC_{50} of 932.5 ± 0.11 µg/ml as 50% inhibitory concentration (Table-1). *IC50* is concentration of a sample PBK-6 at which viable cells are reduced by half.

The findings indicate that sample PBK-6 defensive secretions may represent potential sources of bioactive compounds that possess anticancer properties. The present study provides only baseline data. Further purification and molecular characterization studies are necessary to identify the active principles and evaluate their pharmaceutical significance.

Acknowledgement

The authors thanks to the Principal of Deogiri College, Chhatrapati Sambhajinagar for his invaluable support, and are also thankful to Professor Sirsat C. V., Supervisor, for guidance throughout the research. Also acknowledged to Mahatma Jyotiba Phule Research & Training Institute (MAHAJYOTI), Nagpur.

Author contribution statement

P.B.K. conceived and designed the research, conducted the experiments, and performed data collection. C.V.S. supervised the project and provided critical intellectual input. P.B.K. and C.V.S. analyzed the data. P.B.K. wrote the initial draft of manuscript. C.V.S. reviewed and edited the manuscript. All authors read & approved final manuscript.

Financial support

Mahatma Jyotiba Phule Research & Training Institute (MAHAJYOTI), Nagpur.

Conflict of interest

No conflict of interest

References

1. Aldrich JR. Chemical ecology of the Heteroptera. Annual Review of Entomology,1988:33:211-238.
2. Chernysh S, Kim SI, Bekker G, Pleskach VA, Filatova NA, Anikin VB, *et al*. Antiviral and antitumor peptides from insects. Proceedings of the National Academy of Sciences,2002:99(20):12628-12632.
3. Distant WL. The Fauna of British India, Including Ceylon and Burma: Rhynchota (Vol. 1). Taylor and Francis, 1902.

4. Dossey AT. Insects and their chemical weaponry: New potential for drug discovery. Natural Product Reports,2010:27(12):1737-1757.
5. Durak D, Kalender Y. Chemical composition and biological activities of heteropteran defensive secretions. Journal of Insect Science,2021:21(4):1-12.
6. Fotakis G, Timbrell JA. *In vitro* cytotoxicity assays: Comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicology Letters,2006:160:171-177.
7. Henry TJ. Biodiversity and classification of true bugs. Annual Review of Entomology,2017:62:379-398.
8. Kim IW, Choi RY, Lee JH, Seo M, Lee HJ, Kim MA, *et al*. Anticancer activity of periplanetasin-5, an antimicrobial peptide from *Periplaneta americana*. Journal of Microbiology and Biotechnology,2021:31(10):1343-1349.
9. Lee W, Lee Y, Kim N, Lee S. DNA barcoding of Hemiptera using mitochondrial cytochrome oxidase subunit I sequences. Molecular Ecology Resources,2018:18(2):377-387.
10. Meyer-Rochow VB. Therapeutic arthropods and other, largely terrestrial, folk-medicinally important invertebrates: A comparative survey and review. Journal of Ethnobiology and Ethnomedicine,2017:13(1):9.
11. Morgan DML. Tetrazolium (MTT) assay for cellular viability and activity. Methods in Molecular Biology,1998:79:179-184.
12. Nakabachi A, Okamura K. Diaphorin, a polyketide produced by a bacterial symbiont of the Asian citrus psyllid, kills various human cancer cells. PLOS ONE,2019:14(6):e0218190.
13. Kale PB, Sirsat C. Diversity of Pentatomoidea bugs (Hemiptera: Heteroptera) from Chandwad tehsil of Nashik district M.S. International Journal of Researches in Biosciences, Agriculture and Technology,2023:XI(Special Issue):118-122.
14. Kale PB, Sirsat CV. Heteropteran Bugs of the Order Hemiptera – A Review Article. International Journal of Advanced Research in Science, Communication and Technology,2024:4(3):561-567.
15. Rider DA. Higher classification of Pentatomoidea. Zootaxa,2018:4406(1):1-27.
16. Rolston LH, Aalbu RL, Murray MJ, Rider DA. Catalog of the Tessaratomidae of the World. Papua New Guinea Journal of Agriculture, Forestry and Fisheries, 1993.
17. Schuh RT, Weirauch C. True Bugs of the World (Hemiptera: Heteroptera): Classification and Natural History. Siri Scientific Press, 2020.
18. Tihauan B, Berca L, Adascălului M, Sanmartin A, Nica S, Cimponeriu D, *et al*. Experimental *in vitro* cytotoxicity evaluation of plant bioactive compounds and phytoagents: A review. Romanian Biotechnological Letters,2020:25:1832-1842.
19. Van Meerloo J, Kaspers GJL, Cloos J. Cell sensitivity assays: The MTT assay. Methods in Molecular Biology,2011:731:237-245.
20. Weirauch C, *et al*. Evolution and venom chemistry of assassin bugs (Reduviidae). Toxins,2019:11(5):292.
21. Zhong G, Hu M, Yi X, Li H, Wu H. Chemical constituents and biological activities of defensive secretions from stink bugs (Hemiptera: Pentatomidae). Journal of Asia-Pacific Entomology,2017:20(4):1177-1183.