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Liquid Chromatography Of Hemolymph Of Adult Trachyderma Philistina (Coleoptera:Tenebrionidae)And Antitumor Effect Of Crude Hemolymph Against Different Cell Lines.

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Abstract: The liquid chromatography of hemolymph of adult Trachyderma philistine revealed the presence of a lot of individual free amino acids, amides, fattyacids, vitamins and other different compounds were also in the haemolymph .The hemolymph was subjected to liquid chromatography after challenge of holding insect during withdrawing hemolymph. The present study also evaluate the effect of in-vitro anti-cancer activity of crude hemolymph of adult stage of Trachyderma philistine against breast (MCF-7), hepatocellular (HEPG2) andLeukemia(M-NFS-60) carcinoma cells. The best overall antitumor activities (% average growth inhibition) were seen against Breast carcinoma cells with IC_{50} 1.63 ul/ml . The extract showed inhibitory activities against hepatocellular carcinoma HEPG2 and Mouse Myelogenous Leukemia carcinoma cellsM-NFS-60 cell line with IC50 values, 5.57and 12.2µl/ml, respectively .The crude hemolymph showed cytotoxic activity against the normal cell line used. The highest concentration used, 50 µl/ml killed about 61.46% of the normal cells.

Key words: Trachyderma philistine, hemolymph ,Liquid chromatography

1. INTRODUCTION

Insects are the largest group of living organisms. It has been estimated that more than one million species of insects have been described, comprising about 70% of all organisms **(Gullan and Cranston, 2005)**. Their richness and diversity means that insects have a close relationship with human beings and insect resources have been used by human for a long times as food, medicine and chemical materials. Medicinal insects have been used to cure diseases for more than 2000 years in traditional medicine by using insect bodies, eggs and secretions, but recently

modern scientific studies have revealed the therapeutic functions of insect extractions include anti-bacterial, anti-inflammatory and anti-tumor activities, immune regulations and reducing blood sugar. Numerous substances and compounds extracted from insects have been studied and tested as important resources for the discovery of new drugs **(Feng *et al.*, 2009).**

Hoskin and Ramamoorthy (2008) found that the development of a new class of anticancer drugs that lack the toxicity of conventional chemotherapeutic agents and are unaffected by common mechanisms of chemoresistance such as defense peptides of eukaryotic cells would be a major advance in cancer treatment.
Defense peptides are key factors in innate immunity against bacteria and fungi in vertebrates as well as invertebrates. In insects which lack an adaptive immune system, antimicrobial peptides play a crucial role infighting invading pathogens. They are synthesized in response to microbial infection or septic body injury mainly in insect fat body (functional equivalent of mammalian liver) and in certain blood cells, and then rapidly released into hemolymph where they act synergistically against microorganisms. From a large number of about 890 antimicrobial peptides of eukaryotic origin identified to date, more than 180 were described in insects (**Tzou*et al.*, 2002 ;Dunphy*et al.*, 2003 and Ivring*et al.*, 2004).**
The researches of **Chen and Feng (2009**) and **Lin *et al.* (2009)** have shown that the main kinds ofanticancer insects are catharis *Mylabris* spp. Bees, wasps, ants, house fly *Musca domestica* and silk worms *Bombyx mori*. Recently, some lepidopteran insects have been developed as model organisms for studying innate immunity mechanisms and also for pathogenic tests with different micro-organisms. The ancient medical application of insects is still explored. For example, the dried biomass of blister beetles (*Mylabris phalerata* and *M. cichorii)* containing cantharidin has been used in Chinese traditional medicine for the treatment of cancer for over 2,000 years, and studies are currently being conducted in this area **(Ratcliffe *et al.*, 2011)**. Cantharidin has been prescribed for numerous diseases including rabies, oedema, warts and impotence **(Ratcliffe *et al.*, 2011)**. It has been shown that cantharidin, or its derivatives, can destroy a variety of tumor cells *in vitro* and in animal models *in vivo* including hepatomas, leukaemia, breast cancer, melanoma and bladder or colorectal and pancreatic cancers.
The aim of the present study is to understand how a hemolymph in insects triggers immune response against tumors. In this view we have been attempt to induce the immune system of *Trachyderma beetle* through challenge of holding beetle and withdrawal of hemolymph. We were majorly interested in a aspects of biochemical components such as Proteins, amino acids free amino acids,fatty acids and other compounds by subjecting hemolymph of *Trachyderma philistine* to liquid chromatography.Also studying the in vitro anti-tumor effect of crude hemolymph against different cell lines.

2. MATERIALS AND METHODS

Collection of insect
Trachydermaphilistina are collected from Cairo and Siwa in Egypt **and identified according to Zumpt**

Haemolymph collection
Trachyderma beetle body surface cleaned with 70% alcohol. Then, in order to collect hemolymph, hind pair legs were cut and hemolymph fluid was extracted with a capillary tube placed into micro tubes containing EDTA. Hemolymph was centrifuged at 10000 × g for 10 minutes and the supernatant was collected and stored at 4°C .

Liquid chromatography mass spectrometry or hemolymph.
Haemolymph sample was made up 25ul and injected into liquid chromatography mass spectrometry.These samples were analyzed using an Aria TLX-1 LC system coupled with a TSQ Quantum.Ultra with electrospray ionization (ESI) source. (Thermo Scientific, Austin, TX, USA)

Aria TLX-1 System Parameters:
Two 0.5 x 50 mm Thermo Scientific Cyclone™ MAXTurboFlow columns with a C18 HPLC column(4.6 x 150 mm, 5 μm particle size).

Mass Spectrometer Parameters:
Ion Polarity: Positive ion mode-Vaporizer Temperature: 500 °C-Capillary Temperature: 300 °C
Sheath Gas Pressure (N2): 60 units-Auxiliary Gas Pressure (N2): 55 units-Scan Type: Full Scan. Mass range (50-2500).

Antitumor and cytotoxicity assays:
The crude hemolymph was tested against breast carcinoma MCF-7 cell line, Leukemia cancer cells and hepatocellular carcinoma HEPG2 cell line. It was also tested against Vero cells which are normal kidney cells for the evaluation of its cytotoxicity against the normal cells. The method of cytotoxic activity assay was carried out according to reported method **[Skehan*et al,* 1990]** in the Regional Center for Mycology and Biotechnology, Al-Azhar University. The Cells were seeded in 96-well plate at a cell concentration of 1x104 cells per well in 100 µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed micro titer plates (Falcon, NJ, USA) using a multichannel pipette. The micro titer plates were incubated at 37°C in a humidified incubator with 5% Co2 for a period of 48h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without dimethyl sulfoxide (DMSO). The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24hrs at 37°C, various concentrations of sample (100,50, 25, 12.5, 6.25,3.125,1.56&0.78µg) were added, and the incubation was continued for 48h and viable cells yield was determined by a colorimetric method. After the end of incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Micro plate reader (TECAN, Inc.), using a test wavelength of 490nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated **(Mosmann T, 1983), (Vijayan*et al,* 2004).**

3. RESULTS

Liquid chromatography results
The liquid chromatography of hemolymph of adult *Trachyderma philistine* revealed the presence of a lot of individual free amino acids (Table 3),amides (Table 4),fattyacids(Table 5),vitamins and other different compounds were also in the haemolymph (Table 6) .The

hemolymph was subjected to liquid chromatography after challenge of holding insect during withdrawing hemolymph.

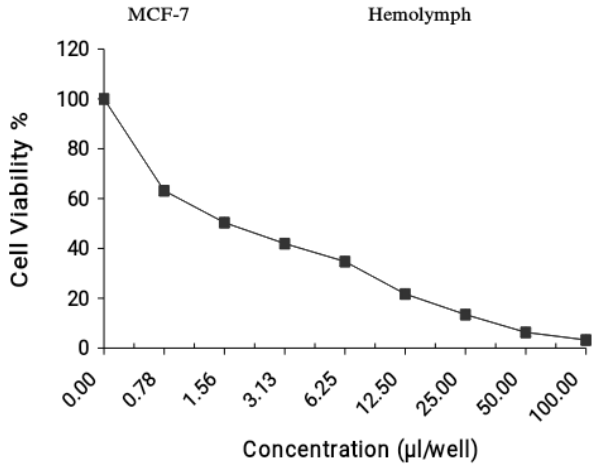
Antitumor and cytotoxic results

The present study evaluate the effect of in-vitro anti-cancer activity of hemolymph crude extract of adult stage of *Trachyderma philistine* against breast (MCF-7), hepatocellular (HEPG2) and*Leukemia(M*-NFS-60) *carcinoma cells*.

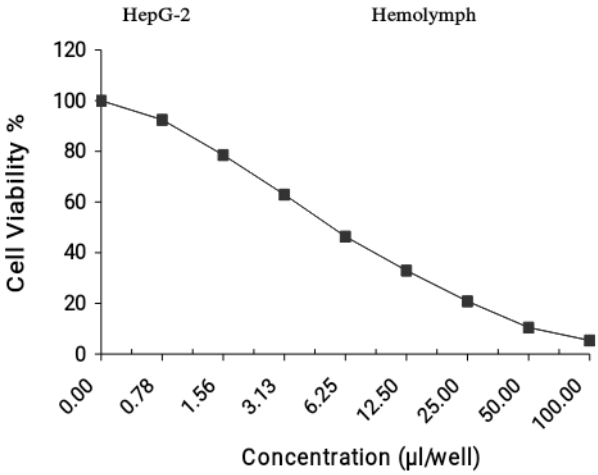
The best overall antitumor activities (% average growth inhibition) were seen *against* Breast carcinoma cells *with* IC_{50} 1.63. The extract showed inhibitory activities against hepatocellular carcinoma HEPG2 and *Mouse Myelogenous* Leukemia carcinoma cellsM-NFS-60 cell line with IC50 values, 5.57and 12.2µl/ml,respectively. The crude hemolymph showed cytotoxic activity against the normal cell line used. The highest concentration used, 50 µl/ml killed about 61.46% of the normal cells.(Fig 1(a,b,c,d),Table 1,2).

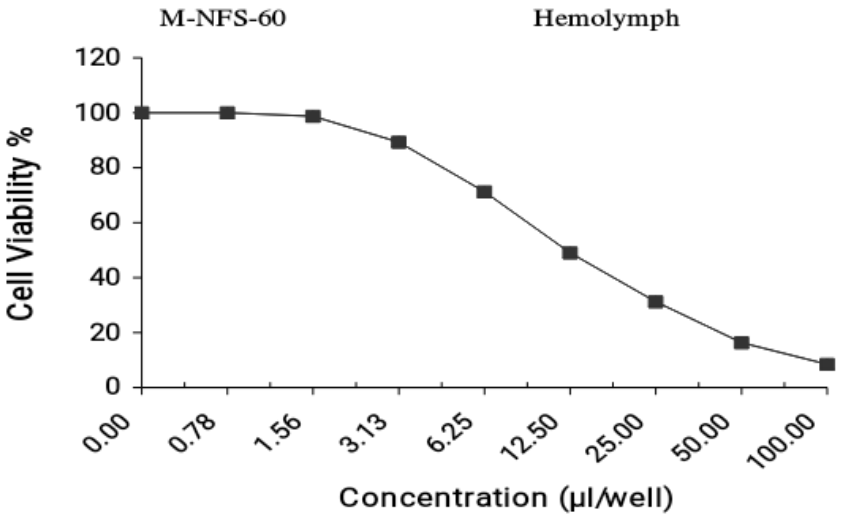
Fig. 1: Cytotoxic activities of the hemolymph of adult stage of *Trachyderma philistine*against (a) breast (MCF-7), (b) hepatocellular (HEPG2) , (c)*Leukemia(M*-NFS-60) *carcinoma cells*and (d) the effect on normalkidney cells.

(a)

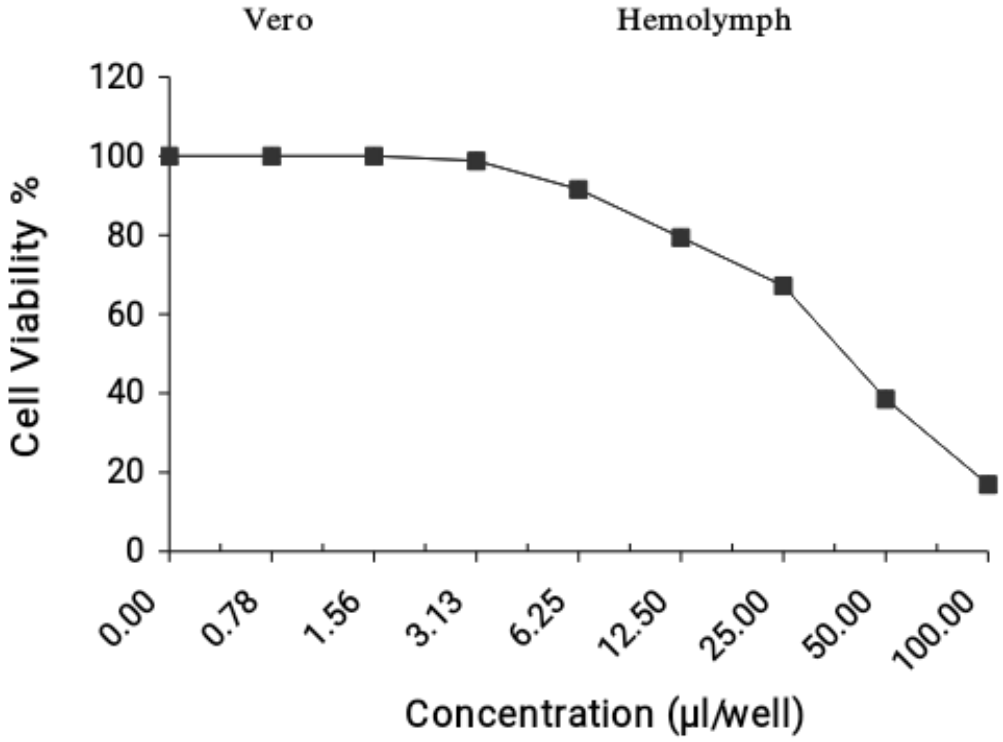


(b)




M-NFS-60
Hemolymph
Cell Viability %
120
100
80
60
40
20
0
0.00
0.78
1.56
3.13
6.25
12.50
25.00
50.00
100.00
Concentration (µl/well)

(c)


Vero
Hemolymph
Cell Viability %
120
100
80
60
40
20
0
0.00
0.78
1.56
3.13
6.25
12.50
25.00
50.00
100.00
Concentration (µl/well)

(d)

Table (1): Cytotoxic effect of the hemolymph of adult stage of *Trachyderma philistine*against breast carcinoma MCF-7 cell line, hepatocellular carcinoma HEPG2 cell line and leukemiac cell lines .IC50 (µl) (50% inhibitory concentration).

	Breast cancer			***Hepatocellular carcinoma cells***			***Leukemiac***		
Sample conc. (µl/well)	**Viability %**	**Inhibitory %**	**S.D. (±)**	**Viability %**	**Inhibitory %**	**S.D. (±)**	**Viability %**	**Inhibitory %**	**S.D. (±)**
100	**3.28**	**96.72**	**0.14**	**5.37**	**94.63**	**0.52**	**8.45**	**91.55**	**0.61**
50	**6.34**	**93.66**	**0.22**	**10.49**	**89.51**	**0.17**	**16.34**	**83.66**	**0.52**
25	**13.45**	**86.55**	**0.61**	**20.81**	**79.19**	**0.93**	**31.27**	**68.73**	**0.95**
12.5	**21.69**	**78.31**	**0.35**	**32.96**	**67.04**	**0.74**	**49.03**	**50.97**	**1.71**
6.25	**34.73**	**65.27**	**0.62**	**46.38**	**53.62**	**0.98**	**71.25**	**28.75**	**2.34**
3.125	**41.95**	**58.05**	**0.78**	**62.91**	**37.09**	**1.75**	**89.32**	**10.68**	**0.65**
1.56	**50.36**	**49.64**	**1.25**	**78.56**	**21.44**	**0.28**	**98.71**	**1.29**	**0.33**
0.78	**63.18**	**36.82**	**0.46**	**92.45**	**7.55**	**0.17**	**100**	**0**	**0**
IC50(µl/ml)	***1.63***			***5.57***			***12.2***		

Table (2): Cytotoxic effect of hemolymph ofadult stage of *Trachyderma philistine*against normal Vero cell lines. IC50 (µL) (50% inhibitory concentration).

Sample conc. (µl/well)	**Viability %**	**Inhibitory %**	**S.D. (±)**
100	**16.97**	**83.03**	**2.85**
50	**38.54**	**61.46**	**3.42**
25	**67.21**	**32.79**	**2.97**
12.5	**79.42**	**20.58**	**1.65**
6.25	**91.60**	**8.4**	**1.43**
3.125	**98.76**	**1.24**	**0.58**
1.56	**100**	**0**	
0.78	**100**	**0**	
0	**100**	**0**	**0**

Table 3: Total amino acids in hemolymph of adult stage of *Trachyderma philistina*

N	**Analytes**	**Molecular Formula**	**ecular Weight**	**RT**
1	**Homoserine, 3TMS derivative**	**C13H33NO3Si3**	**335**	**9.49**

2	**L-Valine, TMS derivative**	**C8H19NO2Si**	**189**	**11.41**
3	**L-Valine, TBDMS derivative**	**C11H25NO2Si**	**231**	**11.41**
4	**Glycyl-L-Valine, TMS derivative**	**C10H22N2O3Si**	**246**	**11.41**
5	**L-Homoserine, 2TMS derivative**	**C10H25NO3Si2**	**263**	**11.41**
6	**L-Leucine, 2TMS derivative**	**C12H29NO2Si2**	**275**	**13.12**
7	**L-LEUCINE, N-(TRIMETHYLSILYL)-, TRIMETHYLSILYL ESTER**	**C12H29NO2Si2**	**275**	**13.57**
8	**L-Norleucine, 2TMS derivative**	**C12H29NO2Si2**	**275**	**13.12**
9	**L-Isoleucine, 2TMS derivative**	**C12H29NO2Si2**	**275**	**13.12**
10	**L-PROLINE, 1-(TRIMETHYLSILYL)-, TRIMETHYLSILYL ESTER**	**C11H25NO2Si2**	**259**	**14.83**
11	**L-Ornithine, 3TMS derivative**	**C14H36N2O2Si3**	**348**	**14.83**
12	**3-Aminoisobutyric acid, 3TMS derivative**	**C13H33NO2Si3**	**319**	**16.69**
13	**á-ALANINE, N, N-BIS(TRIMETHYLSILYL)-, TRIMETHYLSILYL ESTER**	**C12H31NO2Si3**	**305**	**16.69**
14	**4-Aminobutanoic acid, 3TMS derivative**	**C13H33NO2Si3**	**319**	**27.89**
15	**N-à-Acetyl-L-Lysine, 3TMS derivative**	**C17H40N2O3Si3**	**404**	**30.28**
16	**L-PROLINE, 1-(TRIMETHYLSILYL)-4-[(TRIMETHYLSILYL)OXY]-, TRIMETHYLSILYL ESTER, TRANS-**	**C14H33NO3Si3**	**347**	**18.42**

17	**L-Hydroxyproline, (E)-, 3TMS derivative**	**C14H33NO3Si3**	**347**	**18.42**
18	**Glycylglycine, tris(trimethylsilyl)-**	**C13H32N2O3Si3**	**348**	**20.86**
19	**Putrescine, 4TMS derivative**	**C16H44N2Si4**	**376**	**20.99**
20	**l-Lysine, tris(trimethylsilyl)-, methyl ester**	**C16H40N2O2Si3**	**376**	**21.64**
21	**L-5-Oxoproline, 2TMS derivative**	**C11H23NO3Si2**	**273**	**22.58**
22	**L-PROLINE, 5-OXO-1-(TRIMETHYLSILYL)-, TRIMETHYLSILYL ESTER**	**C11H23NO3Si2**	**273**	**25.94**
23	**DL-Ornithine, 3TMS derivative**	**C14H36N2O2Si3**	**348**	**27.89**
24	**DL-Ornithine, 4TMS derivative**	**C17H44N2O2Si4**	**420**	**16.89**
25	**Cadaverine, 4TMS derivative**	**C17H46N2Si4**	**390**	**23.45**
26	**DL-Ornithine, 3TMS derivative**	**C14H36N2O2Si3**	**348**	**27.89**
27	**Dopamine, 4TMS derivative**	**C20H43NO2Si4**	**441**	**31.45**
28	**GLYCYLGLYCIN, N, N, N'-TRIS(TRIMETHYLSILYL)-, TRIMETHYLSILYLESTER**	**C16H40N2O3Si4**	**420**	**27.89**
29	**L-LYSINE, N2, N6, N6-TRIS(TRIMETHYLSILYL)-, TRIMETHYLSILYL ESTER**	**C18H46N2O2Si4**	**434**	**30.28**
30	**L-Tryptophan, 3TMS derivative**	**C20H36N2O2Si3**	**420**	**39.95**

Table 4: total amides in hemolymph of adult stage of *Trachydermaphilistina*

N	Analytes	Molecular Formula	ecular Weight	RT
1	N-Methyltrifluoroacetamide, TMS derivative	C6H12F3NOSi	199	6.27
2	ACETAMIDE, 2,2,2-TRIFLUORO-N-METHYL-N-(TRIMETHYLSILYL)-	C6H12F3NOSi	199	6.27
3	Acetamide, 2,2,2-trifluoro-N-methyl-	C3H4F3NO	127	8.90
4	ACETAMIDE, N-1,3,2-DIOXABOROLAN-2-YL-2,2,2-TRIFLUORO-N-METHYL-	C5H7BF3NO3	197	8.90
5	Acetamide, N-(2-hydroxyethyl) trifluoro-	C4H6F3NO2	157	8.90

Table 5: Total fatty acids in hemolymph of adult stage of *Trachydermaphilistina*

N	Analytes	Molecular Formula	ecular Weight	RT
1	1-PIPERIDINECARBOXYLIC ACID, 4-(2-METHYLPROPYL)-, METHYL ESTER	C11H21NO2	199	6.27
2	1-PIPERIDINECARBOXYLIC ACID, 4-(1-METHYLPROPYL)-, METHYL ESTER	C11H21NO2	199	10.26
3	2-PIPERIDINECARBOXYLIC ACID, 1-(TRIMETHYLSILYL)-, TRIMETHYLSILYL ESTER	C12H27NO2Si2	273	25.94
4	Palmitic Acid, TMS derivative	C19H40O2Si	328	32.79

5	**3á-(ACETYLOXY)-15à-HYDROXY-5à-CHOLESTA-8(14),9(11)-DIEN-7-ONE**	**C29H44O4**	**456**	**37.41**
6	**CHOLEST-9(11)-EN-3-OL, 4,14-DIMETHYL-, ACETATE, (3á,4à,5à)-**	**C31H52O2**	**456**	**37.41**
7	**Stearic acid, TMS derivative**	**C21H44O2Si**	**356**	**38.52**
8	**Oleic Acid, (Z)-, TMS derivative**	**C21H42O2Si**	**354**	**37.73**
9	**Linoelaidic acid, trimethylsilyl ester**	**C21H40O2Si**	**352**	**38.83**

Table 6: Totaldifferent compounds in hemolymph of adult stage of Trachyderma philistina

N	**Analytes**	**Molecular Formula**	**ecular Weight**	**RT**
1	**QUINOXALINE, 2-METHYL-3-PROPYL-, 1,4-DIOXIDE**	**C12H14N2O2**	**218**	**9.23**
2	**QUINOXALINE, 2-METHYL-3-(1-METHYLETHYL)-, 1,4-DIOXIDE**	**C12H14N2O2**	**218**	**9.23**
3	**2,6-DIAZAPHENOXATHIINE 2-OXIDE**	**C10H6N2O2S**	**218**	**9.23**
4	**Astemizole**	**C28H31FN4O**	**458**	**9.23**
5	**Mercaptoethanol, 2TMS derivative**	**C8H22OSSi2**	**222**	**12.31**
6	**1,4-Butanediol, 2TMS derivative**	**C10H26O2Si2**	**234**	**12.31**
7	**GLYCEROL-1,2,3-D3, TRIS-O-(TRIMETHYLSILYL)-**	**C12H29D3O3Si3**	**311**	**12.47**

8	**GLYCOLIC ACID-D2-O-(TRIMETHYLSILYL)-, TRIMETHYLSILYLESTER**	**C8H18D2O3Si2**	**222**	**12.47**
9	**Silanol, trimethyl-, phosphate (3:1)**	**C9H27O4PSi3**	**314**	**14.26**
10	**1,2-Butanediol, 2TMS derivative**	**C10H26O2Si2**	**234**	**15.89**
111	**NAPHTHALENE, 1-(METHYLTHIO)-**	**C11H10S**	**174**	**24.51**
12	**PHOSPHORIC ACID, 3-(ETHOXYIMINO)-2-[(TRIMETHYLSILYL)OXY] PROPYL BIS(TRIMETHYLSILYL) ESTER, (Z)- (. +-.)-**	**C14H36NO6PSi3**	**429**	**21.26**
13	**Malvidin 3-O-galactoside cation**	**C23H25O12**	**493**	**41.63**
14	**CHOLESTANE, PROPANEDIOIC ACID DERIV.**	**C32H50O5**	**514**	**43.07**
15	**Cholecalciferol, TMS derivative**	**C30H52OSi**	**456**	**57.09**
16	**Cholecalciferol, TBDMS derivative**	**C33H58OSi**	**498**	**57.09**
17	**8-HYDROXY-3-[[1',5'-DIMETHYL) HEXYL]3A,5B-DIMETHYL-3A,4,5,5B,6,7,8,9-OCTAHYDROINDANO[6,7-A] NAPHTHALENE**	**C27H44O**	**384**	**57.09**

4. DISSCUSSION

The present study revealed the presence of a lot of amino acids like homoserine,valine,leucine,norleucine,isoleucine,proline,ornithine,alanine, aminobutanoicacid,aminoisobutyricacid,glycylglycine,putrescine,lysine,cadverine, dopamine and tryptophan levels were present in the haemolymph as a result of hemolymph withdrawal from the adult *Trachyderma*. These amino acids are important as a stress response

indicator **(Tanguy *et al.*,2005; Leroy *et al.*, 2010)** and play a crucial role in managing oxidative stress also **(Michaelis, 1998; Matés*et al.*, 2002).**

Insects at all life stages are rich sources of proteins, fats and many other important nutrients **(Bovera et al., 2015).** Reviews on the nutritive value of different insects and their meals including black soldier fly larvae, housefly maggot and pupae, mealworms, silkworm pupae, as well as locusts, grasshoppers and crickets demonstrate their potential application as an alternative protein source in the nutrition of different livestock species **(Makkar et al., 2014;Sánchez- Muros et al., 2014).**
Finally, they are secreted into the hemolymph where they can diffuse thorough whole animal body. During larval-pupa and pupa-adult transformation the old tissue elimination occurs and then peptides and proteins may remove harmful or unnecessary cells **(Watanabe *et al*, 2004).**The bioactive peptides with anti-parasitic, antiviral or anti-tumoural activities can be very interesting for scientists because of their potential use as the therapeutic agents to support medicine.
Pant and Agrawal (1964) suggest that all amino acids found were likely to contribute a general pool for the synthesis of new proteins and for the products of protein breakdown, osmoregulation and buffering. They suggested that these results could be due to both protein degradation and protein synthesis. Haemolymph amino acids play an important role in the synthesis of cuticle constituents and in silk production.

Alanine and few other amino acids are believed to be involved in the cold and hardness. **Storey *et al.*, (1981)**and **Chen (1985)** stated that the insect haemolymph usually contains very high levels of total free amino acids. Similarly, **Liadouze *et al.*, (1995)** found that Lysine were related to nitrogen excretory metabolism. The free amino acids (FAA) content in the haemolymph can be attributed to high proteolytic activity also **(Gorres and Raines, 2010).**The FAA in the present study are, Proline and Glycine, may indicate that the possibility of active role of amino acids in Kreb's cycle and Glycolytic pathways to meet the emergency energy needs as well as their utilization in the production of some new proteins and their synthesize **(Colinet*et al.*, 2007).** Proline is an important amino acids for many other biological functions besides energy production. The presence of proline within the peptide chain regulates structural susceptibility against protease activity which in turn controls a multitude of biological functions **(Yaron*et al.*, 1993).**Thus, free amino acids levels in haemolymph were likely used for the synthesis of compounds related to the immune response or fatty acids transport and few FAA which were probably used as energy source in gluconeosis.
Insects protect themselves against bacterial infection by secreting a battery of antimicrobial peptides into the hemolymph. Insect hemolymph constitutes16-40% of the body weight of certain insects. The volume and component of hemolymph are different among species of insects and their developmental stages. The hemolymph circulation would help to transport the antimicrobial peptide to its target site **(Balzarini*et al*,2006; Kurata, 2006)**Insects survive against pathogenic infection depending on innate immunity because they lack the adaptive immunity of vertebrates that provides a host defense mechanism which is more potent and specific. Insects have various types of barriers which can be physical, chemical or biological weapons constituting first line of defense against invasion by pathogenic organisms. Upon a successful entry into the insects, the second line of innate immunity at the cellular and molecular levels is activated in the hemocoel. When this is successful, pathogens are eliminated from the infected insects, thus preventing their propagation and spread. Instead, in the hemocoel, pathogens have advanced variation of mechanism against host defense and inhibit the activated natural immune system **(Ikeda *et al*, 2013).**However, the innate immune responses are found to be very efficient mechanisms for safe guarding the insects against any

kind of infection, given the lifespan of the insects and their vast territorial successes in all ecospheres **(Lavine and Strand, 2002; Siva-Jothy*et al*,2005)**.

The mechanism by which AMPs exert their activity involves the destruction of the bacterial cell envelope. Most AMPs are cationic molecules which perturb the target cell membranes through the formation of ion channels or transmembrane pores and in this way destroy the bacterial cell **(Duclohier, 2002; Park and Hahm, 2005)**. They penetrate the cell through the negatively charged particles present in the bacterial cell envelopes with which the peptide can interact **(Bagnicka*et al*., 2011).** The main targets of AMPs are lipids in bacterial cell membrane. This action requires a high AMPs concentration and causes cell membrane dissolvence, similarly to the action of a detergent. Apart from the membrane destruction, some AMPs, i.e. pyrrhocoricin, drosocin and apidaecin, may exert antibacterial activity by interactions with intracellular targets thus disrupting intracellular processes. These belong to short proline-rich AMPs (PrAMPs). Insect-derived PrAMPs are typically 20–35 amino acid residues long.It has been suggested that AMPs exhibiting an antimicrobial effect at high concentrations act by permeabilizing/disrupting the microbial membrane, whereas AMPs, which are presumed to affect microbial viability at low to moderate concentrations, interact with intracellular targets **(Nicolas, 2009).**Insect AMPs may thus have potential for application as food preservatives. However, insect AMPs can also be constitutively produced in haemocytes and are released into the haemolymph in response to infection **(Fu *et al*., 2009).** Constitutive production of AMPs may also take place in different epithelia as a local protection against different pathogens, as for example in the gastrointestinal and reproductive tract. Constitutive production of AMPs in the salivary glands presumably has an additional role, i.e. the protection of eggs from infection (Isoptera: termite) **(Uvell and Engstrom, 2007; Kim et al., 2016).** AMPs may be also produced constitutively in the brain of insects as shown by **Lee *et al.* (2012)** in the American cockroach (*Periplanetaamericana*). In response to infection, AMPs can be produced following transcription of genes encoding the respective AMP and rapid release into the haemolymph and the entire body. Further, a constitutive production of AMPs has been observed, involving the storage of AMPs in particularly haemocytes and their release into the haemolymph after immune challenge **(Lambert*et al.*, 2001).** AMPs are mainly derived from the larval stage of insects. It has been shown that the genes encoding various peptides are rapidly induced after a septic injury. Their expression continues for at least three days. In insects with complete metamorphosis, the AMPs are rapidly and transiently synthesized by cells of the fat body (the functional equivalent of the mammalian liver) and by various epithelia. In insects nutrients they are stored in the fat body and act as a nutrient sensor, similarly to the mammalian liver and adipose tissue (**Buchon*et al*., 2014)**.

Putrescine was found in the hemolymph of adult stage *Trachyderma philistine* .Putrescine is involved in many fundamental processes of cell growth and survival including maintenance of protein and nucleic acid synthesis, stabilization of chromatin structure, differentiation, apoptosis, protection from oxidative damage and nucleic acid depurination and regulation of multiple ion channels necessary for cell-to-cell communication **(Pegg and Casero, 2011 ;Terui, 2018; Kurata*et al,* 2013; Rao, 2012)**. Putrescine is essential for normal cell growth, and their depletion results in cytostasis.
The present study also revealed that hemolymph of the adult *Trachyderma* contains some fattyacids that are piperidine carboxylic acid, palmitic acid,stearic acid,oleic acid and linoelaidic acid .Fatty acids (FA) are monocarboxylic acids containing a long hydrocarbon chain. They are formed by the cleavage of fats and oils derived from natural sources such as triacylglycerols or phospholipids and, in general, can be either saturated or unsaturated **((Beermann,2003; Denise, 2017).**The consumption of fatty acids can slow the growth of

tumors, by inducing the apoptotic processes in tumor cells**(Chamras, 2002; Serini, 2009)** or by inhibiting angiogenesis**(Wen, 2003; Spencer, 2009).** They also increase the efficacy of chemotherapeutic drugs and can reduce the chemotherapy or the cancer side effects (**Colquhoun and Curi, 1998; Hardman, 2002; Leaver, 2002; Siddiqui, 2011)**, at the same time being associated with a high level of safety **(Bégin, 1985)**.Also the effect of on the inhibition of growth of different types of pathogensis well known. Fatty acids show antibacterial **(Zheng, 2005; Urbanek, 2012; Bravo-santano, 2019)** and antifungal **(Pohl et al., 2011)** activities. Fatty acids are also used in chemotherapies. Linolenic acid suppressed cell growth of neuroblastoma cell lines **(Fujiwara *et al.*, 1986)**. Linolenic acid gained great attention as a chemotherapeutic agent after being displayed to have inhibitory effects on carcinogenesis **(Belury, 2002).**

The liquid chromatography of hemolymph of adult *Trachyderma* beetle also revealed the presence of some vitamins and other compounds like quinoxaline,astemizole,mercaptoethanol,butanediol,glycerol,glycolicacid,naphthalene,phosphoricacid,malvidin,cholestane,cholecalciferol.

Quinoxalines are considered as an important basis for anti-cancer drugs, quinoxalines with amide and sulphonamide moieties have been reported to inhibit the growth of human tumor cell lines **(Balderas-Renteria *et al*, 2012). Ghorab *et al*, 2011** designed (quinoxalin-2-yl) benzene sulphonamide derivative with a potent anti-cancer activity against human liver cancer cell line (Hep G2). Quinoxaline-bisarylurea has been reported by **(Göring*et al*, 2015)** to exhibit anti-tumor activity. Additionally, quinoxalines were found to induce apoptosis as a mechanism for their anti-cancer activity **(Gali-Muhtasib*et al*, 2018; Weng *et al*, 2008)**.

Several anti-histamine drugs, including astemizole, can inhibit tumor cell proliferation. Astemizole has gained enormous interest since it also targets important proteins involved in cancer progression. Astemizole decreases tumor cell proliferation in vitro and in vivo. It should be noted that some cardiovascular side effects have been reported for astemizole in a few rare cases. Nevertheless, astemizole stands as a very promising anti-cancer tool because it displays several anti-proliferative mechanisms, may serve as the basis to synthesize new anti-cancer agents, and has been previously administered clinically (**García-Quiroz andCamacho, 2011)**

Cholecalciferol(vitamin D3), has been found to interfere with the transduction pathways of various growth factor(s)-activated receptors (receptor tyrosine kinases) thereby modulating transcription and alteration of genomic functions resulting in inhibition of cell proliferation and angiogenesis and facilitation of cell differentiation and apoptosis. It also increases the level of an endogenous protein - cystatin D, which possesses antitumor property, by facilitation of the expression of the gene coding for it. Though not as a primary anticancer agent, this vitamin may be used for the prevention of cancer and included as an adjuvant in combination chemotherapy for the treatment of cancer **(Chakraborti, 2011).**

Malvidin glucoside inhibits growth and spread of various cancers, e.g., cancers of breast, lung, liver, oral cavity and larynx, and malignant ascites **(Govind , 2011)**.

Malvidin is one of the most wide-spread anthocyanidins which exhibits potential antioxidant, anti-cancer and anti-inflammatory activities. Malvidin mainly exists as Malvidin-3-glucoside (Mv-3-glc) and Malvidin-3-galactoside (Mv-3-gal)**(Huang*et al*, 2013).**

The present study also revealed that the crude hemolymph from the adult of *Trachyderma* beetle possessed antitumor activities (% average growth inhibition) were seen *against Breast carcinoma cells with* IC_{50} *1.63.* The crude hemolymph from the adult of *Trachyderma* beetle showed inhibitory activities against hepatocellular carcinoma HEPG2 and *Mouse Myelogenous Leukemiacarcinoma cells*M-NFS-60 cell line with IC50 values, *5.57*and *12.2*µl/ml, respectively. The crude hemolymph showed cytotoxic activity against the normal cell line used. This antitumor effect may be due to all these previous amino acids ,fatty acids and other

compounds that have anti-tumor and anti inflammatory properties that were found in the hemolymph of adult *Trachyderma* beetle**.** Also the toxicity of normal cell line may be due to that purification is needed for the active compounds .

5. CONCLUSION

Future studies are needed to purify the most active compounds from hemolymph *Trachyderma* beetle of against cancer and to apply it against other cell lines.

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