Review of Literature
2. REVIEW OF LITERATURE

Review of literature in this chapter highlight the occurrence of cytolytic molecules with lytic properties on erythrocytes and bacteria in various groups of insects for their salient features, strategies adopted for their purification and functional studies. The presence of cytolytic molecules and their biological activity in insects was detected during 1980s. Numerous reports were available subsequently on these molecules in insects. Till date, nearly 30 species of insects belonging to various orders have been screened for the detection and characterization of these molecules.

Review of literature (Review Tables I to IV) was carried out by screening peer reviewed research articles published from 1980 to till date on works related to naturally occurring cytolytic molecules with their functional properties. The survey of literature on cytolytic activity in insects revealed the fact that it was detected in some representative species of major insect orders such as Lepidoptera, Coleoptera, Hemiptera, Hymenoptera, Diptera, Mantodea, Homoptera and Odonata (Review Table I). These reviews on the occurrence of cytolytic molecules from various orders of insects suggest that they play an integral part on every stage of life cycle in the development and physiology of insects. Review Table II exhibit literature pertaining to various methods and strategies involved in the detection and purification of cytolytic molecules in different species of insects.
Review Table III showed the important characteristic features and the functional role of some cytolytic molecules as well as their application in the field of biomedical sciences namely ‘anoplin’, ‘hinnavin’, ‘cecropin’, ‘melittin’, ‘attachin’, ‘trialysin’, ‘sarcotoxin’ etc, in various species of insects. The literature documented the typical involvement of these molecules in lytic activities. There was not substantial evidence of indication of these cytolytic molecules with carbohydrate binding properties. Reports showed that there were hemolytic and bacteriolytic properties of the cytolytic molecules identified in different species of insects. In those attempts only few erythrocyte types and naturally occurring or environmental bacterial isolates were used to elucidate the cytolytic properties of the isolated molecules.

Overall, the review of literature related to these cytolytic molecules for the past few decades clearly documented studies limited only to a smaller number of species of insects with deficiency in complete characteristic assessment of these molecules. Nevertheless, few studies were reported in hemolymph and secretion from larvae and pupae of C. megacephala with antibacterial activity as revealed in Review Table IV and no attempt was so far made in this insect on isolation, purification and characterization of lytic molecules.
## Review Table I: Cytolytic molecules / factors detected in the hemolymph / serum / whole body extracts of various species of insects
(Insects are arranged alphabetically under their respective insect order)

<table>
<thead>
<tr>
<th>Insect ORDER</th>
<th>Genus and species</th>
<th>Cytolytic activity</th>
<th>Author(s), year and country</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RBC</td>
<td>Bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gram-positive</td>
</tr>
<tr>
<td>LEPIDOPTERA</td>
<td>Artogeia rapae</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Bombyx mori</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Galleria mellonella</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Galleria mellonella</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Helicoverpa armigera</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Heliothis virescens</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Hyalophora cecropia</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hyalophora cecropia</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hyphantria cunea</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lonomia obliqua</td>
<td>+</td>
<td>ND</td>
</tr>
</tbody>
</table>

(+) = presence of cytolytic activity, (-) = absence of cytolytic activity and ND = not determined
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</tr>
<tr>
<td></td>
<td></td>
<td>Gram-positive</td>
<td>Gram-negative</td>
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<tr>
<td>COLEOPTERA</td>
<td>Acalolepta luxuriosa</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Paederus dermatitis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Zophob urutus</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>HEMIPTERA</td>
<td>Rhodnius prolixus</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Triatoma infestans</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>HYMENOPTERA</td>
<td>Anoplius samariensis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Anterhynchium flavomarginatum micado</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Apis mellifera</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Apis mellifera</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Eumenes fraterculus</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Eumenes rubrofemoratus</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>Halictus sexcinctus</td>
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<td></td>
<td></td>
<td>RBC</td>
<td>Bacteria Gram-positive Gram-negative</td>
</tr>
<tr>
<td>HYMENOPTERA</td>
<td>Lasioglossum laticeps</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Megabombus pennsylvanicus</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Myrmecia pilosula</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Oreumenes decoratus</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Orancistrocerus drewnseni</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Pachycondyla goeldii</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Pogonomyrmex barbatus</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Protopolybia exigua</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Vespa magnifica</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Vespula crabro</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DIPTERA</td>
<td>Aedes aegypti</td>
<td>ND</td>
<td>+</td>
</tr>
</tbody>
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<td>DIPTERA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Aedes aegypti</em></td>
<td>ND</td>
<td>Lowenberger <em>et al</em>., 1999, Germany.</td>
</tr>
<tr>
<td></td>
<td><em>Armigeres subalbatus</em></td>
<td>+</td>
<td>Sasaki <em>et al</em>., 2010, Japan.</td>
</tr>
<tr>
<td></td>
<td><em>Bactrocera dorsalis</em></td>
<td>+</td>
<td>Dang <em>et al</em>., 2006; Dang <em>et al</em>., 2009, China.</td>
</tr>
<tr>
<td></td>
<td><em>Ceratitis capitata</em></td>
<td>+</td>
<td>Marchini <em>et al</em>., 1993, Italy; Bessin <em>et al</em>., 2004, France.</td>
</tr>
<tr>
<td></td>
<td><em>Glossina morsitans morsitans</em></td>
<td>+</td>
<td>Gooding, 1977, Canada; Ingram and Molyneux, 1988, UK.</td>
</tr>
<tr>
<td></td>
<td><em>Phormia terranovae</em></td>
<td>ND</td>
<td>Dimarcq <em>et al</em>., 1988, France.</td>
</tr>
<tr>
<td></td>
<td><em>Phormia terranovae</em></td>
<td>+</td>
<td>Lambert <em>et al</em>., 1989, France.</td>
</tr>
<tr>
<td></td>
<td><em>Sarcophaga peregrina</em></td>
<td>ND</td>
<td>Okada and Natori, 1983; Okada and Natori, 1984; Okada and Natori, 1985a; Okada and Natori, 1985b, Japan.</td>
</tr>
<tr>
<td></td>
<td><em>Sarcophaga peregrina</em></td>
<td>ND</td>
<td>Ando <em>et al</em>., 1987, Japan.</td>
</tr>
<tr>
<td></td>
<td><em>Sarcophaga peregrina</em></td>
<td>ND</td>
<td>Baba <em>et al</em>., 1987, Japan.</td>
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<tbody>
<tr>
<td></td>
<td>Simulium bannaense</td>
<td>+</td>
<td>Wei <em>et al.</em>, 2015, China.</td>
</tr>
<tr>
<td></td>
<td>Simulium bannaense</td>
<td>+</td>
<td>Wu <em>et al.</em>, 2015, China.</td>
</tr>
<tr>
<td></td>
<td>Stomoxys calcitrans</td>
<td>+</td>
<td>Spates and Deloach, 1980; Spates 1981, U.S.A.</td>
</tr>
<tr>
<td>MANTODEA</td>
<td>Sphodromantis viridis</td>
<td>+</td>
<td>Zare-Zardini <em>et al.</em>, 2015, Iran.</td>
</tr>
<tr>
<td>HOMOPTERA</td>
<td>Cryptotympana dubia</td>
<td>+</td>
<td>Park <em>et al.</em>, 2007, Korea.</td>
</tr>
<tr>
<td>ODONATA</td>
<td>Aeschna cyanea</td>
<td>ND</td>
<td>Bulet <em>et al.</em>, 1992, France.</td>
</tr>
</tbody>
</table>

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Review Table II: Strategies adopted for isolation of cytolytic molecule from various species of the insects

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<tr>
<th>Insect ORDER</th>
<th>Genus &amp; Species</th>
<th>Source of cytolytic molecule</th>
<th>Method of isolation</th>
<th>Chromatographic columns /matrix used</th>
<th>Elution strategy</th>
<th>Author(s), year and country</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEPIDOPTERA:</td>
<td>Artogeia rapae</td>
<td>Larval hemolymph</td>
<td>IEC</td>
<td>CM-Sepharose</td>
<td>Linear gradient of 0.1-1.0 M ammonium acetate, pH 6.0</td>
<td>Yoe et al., 2006, South Korea.</td>
</tr>
<tr>
<td></td>
<td>Bombyx mori</td>
<td>Larval hemolymph</td>
<td>IEC►GF►RP - HPLC</td>
<td>CM-Toyopearl 650M; Toyopearl HW-60; CLC-ODS</td>
<td>Linear gradient of 0.3-1.0 M ammonium acetate, pH 7.0; 0.1 M ammonium acetate, pH 7.0; Linear gradient 25-30 % acetonitrile with 0.1 % TFA</td>
<td>Morishima et al., 1990, Japan; Yamano et al., 1994, Japan.</td>
</tr>
<tr>
<td></td>
<td>Galleria mellonella</td>
<td>Larval hemolymph</td>
<td>GF►IEC►IEC</td>
<td>Sephadex G-200; DEAE-cellulose and CM Sephadex</td>
<td>0.02M Phosphate buffer pH 6.0; Linear gradient of NaCl (0 to 1M).</td>
<td>Phipps et al., 1989; Phipps et al., 1994, Canada.</td>
</tr>
<tr>
<td></td>
<td>Galleria mellonella</td>
<td>Larval hemolymph</td>
<td>GF►RP - HPLC</td>
<td>Sephadex G-50; Vydac 218TP54 C18</td>
<td>5 % acetic acid; Linear gradient 20-40 % acetonitrile with 0.1 % TFA</td>
<td>Kim et al., 2004, Korea.</td>
</tr>
<tr>
<td></td>
<td>Heliothis virescens</td>
<td>Larval hemolymph</td>
<td>Immunoaffinity chromatography</td>
<td>Anti-cecropin-IgG-Sepharose 4B</td>
<td>0.05 M ammonium acetate (pH 2.8)</td>
<td>Lackey and Ourth, 1993; Lockey and Ourth 1996, USA.</td>
</tr>
<tr>
<td></td>
<td>Hyalophora cecropia</td>
<td>Pupal hemolymph</td>
<td>GF►IEC►HIC►IEC</td>
<td>Sephadex G-100; CM-Sepharose; phenyl-Sepharose; CM-Sepharose</td>
<td>0.15 M ammonium acetate, pH 5; Linear gradient of 0.05-1.0 M ammonium acetate, pH 5.0; 2 M ammonium formate, pH 6.6; 2 M ammonium formate, pH 6.6</td>
<td>Hultmark et al., 1982, Sweden.</td>
</tr>
<tr>
<td></td>
<td>Hyalophora cecropia</td>
<td>Pupal hemolymph</td>
<td>GF►IEC</td>
<td>Sephadex G-100; CM-Sepharose</td>
<td>0.15 M ammonium acetate, pH 5; Linear gradient of 0.03-0.5 M ammonium acetate, pH 5.0</td>
<td>Hultmark et al., 1983, Sweden; Engstrom et al., 1984, Sweden; Carlsson et al., 1998, Sweden.</td>
</tr>
<tr>
<td></td>
<td>Hyphantria cunea</td>
<td>Larval hemolymph</td>
<td>IEC►RP - HPLC</td>
<td>CM-Sepharose; CCCF-100, YMC-PACK ODS-18</td>
<td>Linear gradient NaCl (0 to 1M); Linear gradient 0-60 % acetonitrile with 0.05 % TFA</td>
<td>Park et al., 1997, Korea.</td>
</tr>
</tbody>
</table>

IEC: ion exchange chromatography, GF: gel filtration chromatography, RP-HPLC: reverse phase high performance liquid chromatography, HIC: hydrophobic interaction chromatography, TFA: trifluoroacetic acid
## Review Table II continued

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<thead>
<tr>
<th>Insect ORDER</th>
<th>Genus &amp; Species</th>
<th>Source of cytolytic molecule</th>
<th>Method of isolation</th>
<th>Chromatographic columns /matrix used</th>
<th>Elution strategy</th>
<th>Author(s), year and country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COLEOPTERA:</strong></td>
<td>Acalolepta luxuriosa</td>
<td>Larval hemolymph</td>
<td>RP - HPLC</td>
<td>TSKgel ODS 120T</td>
<td>Linear gradient 20–40 % acetonitrile with 0.05 % TFA</td>
<td>Saito et al., 2005, Japan.</td>
</tr>
<tr>
<td></td>
<td>Paederus dermatitis</td>
<td>Adult extract</td>
<td>GF ► RP - HPLC</td>
<td>Sephadex G-50; semipreparative C18</td>
<td>Phosphate buffer (0.1 M, pH 6); Linear gradient 5-65 % acetonitrile with 0.098 % TFA</td>
<td>Memarpoor - Yazdi et al., 2013, Iran.</td>
</tr>
<tr>
<td></td>
<td>Zophob utratus</td>
<td>hemolymph</td>
<td>HPLC ► RP- HPLC ►</td>
<td>Sep-Pak CIS; Aquapore RP 300 Ca; Aquapore RP 300 Ca; Protein-Pak 125 column; Aquapore RP 300 Ca</td>
<td>Step gradients of acetonitrile with 0.05 % TFA upto 60 %; Linear gradient 10-60 % of acetonitrile with 0.1 % TFA; Mild gradient elution of 18-38 % acetonitrile in acidified water; 30 % acetonitrile in acidified water; Linear gradient 20-40 % of acetonitrile with 0.1 % TFA</td>
<td>Bulet et al., 1991, France.</td>
</tr>
<tr>
<td><strong>HEMIPTERA:</strong></td>
<td>Rhodnius prolixus</td>
<td>Larval crop content and crop extract</td>
<td>GF ► GF</td>
<td>Bio-Gel P-6; SP-Sephadex</td>
<td>0.1 M buffered saline pH 6.0.</td>
<td>Azambuja et al., 1983, Brazil.</td>
</tr>
<tr>
<td></td>
<td>Triatoma infestans</td>
<td>Male adult saliva</td>
<td>IEC ► HIC ► IEC</td>
<td>Hitrap Q, phenyl-Superose and Mono S</td>
<td>Linear gradient Tris-HCl (0 to 20mM ); 3.4 M (NH₄)₂SO₄; Linear gradient Tris-HCl (0 to 1mM )</td>
<td>Amino et al., 2002, Brazil.</td>
</tr>
<tr>
<td><strong>HYMENOPTERA:</strong></td>
<td>Anoplius samariensis</td>
<td>Venom sacs extract</td>
<td>RP - HPLC</td>
<td>CAPCELL PAK C18</td>
<td>Linear gradient 5-95 % of acetonitrile with 0.1 % TFA</td>
<td>Konno et al., 2001, Brazil.</td>
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<td>HYMENOPTERA:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anterhynchium flavomarginatum micado</strong></td>
<td>Venom sacs extract</td>
<td>RP - HPLC</td>
<td>CAPCELL PAK C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>Linear gradient 5-95 % of acetonitrile with 0.1 % TFA</td>
<td>Konno <em>et al.</em>, 2000, Brazil; Cabrera <em>et al.</em>, 2004, Brazil.</td>
</tr>
<tr>
<td><strong>Apis mellifera</strong></td>
<td>Venom</td>
<td>HPLC</td>
<td>Tecnokroma RP C18</td>
<td>Stepwise gradient 50-80 % of acetonitrile with 0.1 % TFA</td>
<td>DeGrado <em>et al.</em>, 1982, U.S.A; Alia <em>et al.</em>, 2013, Syria.</td>
</tr>
<tr>
<td><strong>Apis mellifera</strong></td>
<td>Royal jelly</td>
<td>RP - HPLC</td>
<td>C-18 TOSOH-ODS</td>
<td>Linear gradient 5-60 % of acetonitrile with 0.04 % TFA</td>
<td>Fontana <em>et al.</em>, 2004, Brazil.</td>
</tr>
<tr>
<td><strong>Eumenes fraterculus</strong></td>
<td>Venom sacs extract</td>
<td>RP - HPLC</td>
<td>CAPCELL PAK C&lt;sub&gt;18&lt;/sub&gt;,</td>
<td>Linear gradient 5-70 % of acetonitrile with 0.1 % TFA</td>
<td>Rangel <em>et al.</em>, 2011, Brazil.</td>
</tr>
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<td><strong>Eumenes rubrofemoratus</strong></td>
<td>Venom sacs extract</td>
<td>RP - HPLC</td>
<td>Vydac C-18</td>
<td>Linear gradient 5-95 % of acetonitrile with 0.1 % TFA</td>
<td>Monincová <em>et al.</em>, 2010, Czech Republic.</td>
</tr>
<tr>
<td><strong>Halictus sexcinctus</strong></td>
<td>Venom</td>
<td>RP - HPLC</td>
<td>Vydac C-18 column</td>
<td>Linear gradient 2-70 % of acetonitrile with 0.1 % TFA</td>
<td>Monincová <em>et al.</em>, 2012, Czech Republic.</td>
</tr>
<tr>
<td><strong>Lasioglossum laticeps</strong></td>
<td>Venom</td>
<td>RP - HPLC</td>
<td>µBondapak C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>Linear gradient 20-100 % of acetonitrile with 0.1 % TFA</td>
<td>Argiolas and Pisano, 1985, Maryland.</td>
</tr>
<tr>
<td><strong>Megabombus pennsylvanicus</strong></td>
<td>Venom</td>
<td>RP - HPLC</td>
<td>Zorbax 300SB-C3 (5 µm particle size)</td>
<td>Linear gradient 10-95 % of acetonitrile with 0.05 % TFA</td>
<td>Wu <em>et al.</em>, 1998, Australia; Davies <em>et al.</em>, 2004, Australia; Zelezetsky <em>et al.</em>, 2005, Italy.</td>
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<tr>
<td><strong>Oreumenes decoratus</strong></td>
<td>Venom sacs extract</td>
<td>RP - HPLC</td>
<td>CAPCELL PAK C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>Linear gradient 5-65 % of acetonitrile with 0.1 % TFA</td>
<td>Konno <em>et al.</em>, 2007, Brazil.</td>
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<td>Orancistrocerus</td>
<td>Venom sacs extract</td>
<td>HPLC</td>
<td>Tosoh TSKgel ODS 120T</td>
<td>Linear gradient 1 - 65 % of acetonitrile with 0.1 % TFA</td>
<td>Murata et al., 2009, Japan.</td>
</tr>
<tr>
<td></td>
<td>drewseni</td>
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<td></td>
<td>Pachycondyla</td>
<td>Venom</td>
<td>RP - HPLC</td>
<td>C18- reverse-phase (5 µm particle size)</td>
<td>Linear gradient 50-55 % of acetonitrile with 0.1 % TFA</td>
<td>Orivel et al., 2001, France.</td>
</tr>
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<td></td>
<td>goeldii</td>
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<td></td>
<td>Pogonomyrmex</td>
<td>Venom</td>
<td>GF</td>
<td>Sephacyr I S-200</td>
<td>0.1 M KCl buffered with sodium borate at pH 8.2</td>
<td>Bernheimer et al., 1980, U.S.A.</td>
</tr>
<tr>
<td></td>
<td>barbatus</td>
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<tr>
<td></td>
<td>Protopolybia</td>
<td>Venom</td>
<td>RP - HPLC</td>
<td>SHISEIDO Nucleosil C-18 (ODS)</td>
<td>Linear gradient 5-60 % of acetonitrile with 0.1 % TFA; Isocratic elution with 40% acetonitrile (containing 0.1% TFA)</td>
<td>Mendes et al., 2005, Brazil.</td>
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<tr>
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<td>exigua</td>
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<td></td>
<td>Vespa magnifica</td>
<td>Venom</td>
<td>GF ◄ IEC ◄ RP - HPLC</td>
<td>Sephadex G-50; CM-Sephadex ; C-25, Hypersil BDS C_{18}</td>
<td>0.1 M PBS (pH 6.0); NaCl (0 to 0.8M); Linear gradient 20-80 % of acetonitrile with 0.1 % TFA</td>
<td>Xu et al., 2006, China.</td>
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<tr>
<td></td>
<td>Vespa crabro</td>
<td>Venom sac extract</td>
<td>HPLC ◄ HPLC</td>
<td>Sep-Pak C_{18}; µBondapak C_{18}</td>
<td>Stepwise gradients of 20, 40, 60, 80 and 100 % of aqueous methanol with 0.05% TFA; Linear gradient 50-55 % of acetonitrile with 0.05 % TFA</td>
<td>Argiolas and Pisano, 1984, Maryland; Krishnakumari and Nagaraj, 1997, India.</td>
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</tbody>
</table>

IEC: ion exchange chromatography, GF: gel filtration chromatography, RP-HPLC: reverse phase high performance liquid chromatography, HIC: hydrophobic interaction chromatography, TFA: trifluoroacetic acid
**Review Table II continued**

<table>
<thead>
<tr>
<th>Insect ORDER</th>
<th>Genus &amp; Species</th>
<th>Source of cytolytic molecule</th>
<th>Method of isolation</th>
<th>Chromatographic columns /matrix used</th>
<th>Elution strategy</th>
<th>Author(s), year and country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DIPTERA:</strong></td>
<td><strong>Aedes aegypti</strong></td>
<td>Adult hemolymph</td>
<td>RP - HPLC</td>
<td>Aquapore RP300 C₈</td>
<td>Linear gradient 10 - 60 % of acetonitrile with 0.1 % TFA</td>
<td>Lowenberger et al., 1995, U.S.A.</td>
</tr>
<tr>
<td></td>
<td><strong>Aedes aegypti</strong></td>
<td>Adult hemolymph</td>
<td>RP - HPLC</td>
<td>Sep-Pak C₁₈; Delta-Pak C₁₈</td>
<td>Step gradients of 2, 40 and 80 % of acetonitrile with 0.05% TFA; Linear gradient 50-55 % of acetonitrile with 0.05 % TFA</td>
<td>Lowenberger et al., 1999, Germany.</td>
</tr>
<tr>
<td></td>
<td><strong>Armigeres subalbatus</strong></td>
<td>Pupa hemolymph</td>
<td>Batch adsorption method</td>
<td>Fomaldehyde-fixed human red blood cells (FHRBC)</td>
<td>0.2 M N-acetylneuraminic acid in PBS-Ca</td>
<td>Sasaki et al., 2010, Japan.</td>
</tr>
<tr>
<td></td>
<td><strong>Bactrocera dorsalis</strong></td>
<td>Pupal extract</td>
<td>IEC ➤ GF ➤ RP - HPLC</td>
<td>CM-Sepharose; HiPrep 16/60 Sephacryl S-100; Hypersil ODS column</td>
<td>Segment gradient 0% - 50%, 50% - 100% of 1M ammonium acetate buffer pH 5.0; 50 mM ammonium acetate buffer pH 5</td>
<td>Dang et al., 2006; Dang et al., 2009, China.</td>
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<td></td>
<td><strong>Ceratitis capitata</strong></td>
<td>Adult female reproductive accessory gland secretion fluid (AGF)</td>
<td>GF ➤ IEC ➤ IEC ➤ GF ➤ RP - HPLC</td>
<td>Superose 12HR 10/30; CM-Sepharose; CM-Sepharose; Superose 12; Reverse-phase C-8 column</td>
<td>100 mM ammonium acetate buffer pH 6.6; Discontinuous gradient of 0.01M - 1M ammonium acetate pH 6.6; Linear gradient of 0.01 - 0.3 M ammonium acetate, pH 6.6; 154mM NaCl and 10mM Na-phosphate buffer, pH 6.7; Linear gradient 20-80 % of acetonitrile with 0.125 % TFA</td>
<td>Marchini et al., 1993, Italy; Bessin et al., 2004, France.</td>
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IEC: ion exchange chromatography, GF: gel filtration chromatography, RP-HPLC: reverse phase high performance liquid chromatography
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<td><strong>DIPTERA:</strong></td>
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<tr>
<td><em>Phormia terranovae</em> Larval hemolymph</td>
<td>IEC ➔ GF ➔ RP - HPLC ➔ RP - HPLC</td>
<td>CM-Trisacryl; Ultrogel (IBF/LKB) AcA 202; Sep-Pak catridge (C&lt;sub&gt;18&lt;/sub&gt;, Waters); Bakerbond C&lt;sub&gt;18&lt;/sub&gt;-WP</td>
<td>Linear gradient of 40 - 500 mM ammonium acetate, pH 6.8; 100 mM ammonium acetate, pH 6.8; Stepwise elution increasing acetonitrile in 0.1 % TFA; linear gradient of 25 - 35% acetonitrile in water</td>
<td>Dimarcq et al., 1988, France.</td>
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<tr>
<td><em>Phormia terranovae</em> Larval hemolymph</td>
<td>IEC ➔ RP - HPLC ➔ RP-HPLC</td>
<td>CM-Trisacryl (IBF/LKB); Sep-Pak catridge (C&lt;sub&gt;18&lt;/sub&gt;, Waters); Bakerbond C&lt;sub&gt;18&lt;/sub&gt;-WP</td>
<td>Linear gradient of 40 -500 mM ammonium acetate, pH 6.8; Stepwise elution increasing acetonitrile in 0.1 % TFA; linear gradient of 22 - 28% acetonitrile in water</td>
<td>Lambert et al., 1989, France.</td>
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<tr>
<td><em>Sarcophaga peregrina</em> Larval hemolymph</td>
<td>IEC ➔ UF ➔ GF ➔ IEC ➔ HIC ➔ RP - HPLC</td>
<td>CM-cellulose ; Membrane filter (UF disc type A, Spectrum); Sephadex G-50; column of hydroxyapatite; Synchropak RP-P (C&lt;sub&gt;18&lt;/sub&gt;)</td>
<td>10 mM PBS (pH 6.0) containing 250 mM NaCl; 10 mM PBS (pH 6.0) containing 130 mM NaCl; Stepwise elution-130, 260 mM NaCl; 10 mM PBS (pH 6.0); Linear gradient 0-50 % of acetonitrile with 0.05 % TFA</td>
<td>Okada and Natori, 1983; Okada and Natori, 1984; Okada and Natori, 1985a; Okada and Natori, 1985b, Japan.</td>
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<tr>
<td><em>Sarcophaga peregrina</em> Larval hemolymph</td>
<td>IEC ➔ HIC ➔ RP - HPLC</td>
<td>DEAE-cellulose; column of hydroxyapatite; Synchropak RP-P (C&lt;sub&gt;18&lt;/sub&gt;)</td>
<td>Linear gradient of 10-200 mM phosphate buffer, pH 6.0; 10 mM PBS (pH 6.0); Linear gradient 15-45 % of acetonitrile with 0.05 % TFA</td>
<td>Ando et al., 1987, Japan.</td>
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<tr>
<td><strong>Sarcophaga peregrina</strong></td>
<td>Larval hemolymph</td>
<td>IEC ➔ RP-HPLC</td>
<td>CM-Sepharose; Synchropak RP-P (C&lt;sub&gt;18&lt;/sub&gt;)</td>
<td>0.1 to 0.5 M ammonium formate solution; Linear gradient 15-50 % of acetonitrile with 0.05 % TFA</td>
<td>Baba et al., 1987, Japan.</td>
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</tr>
<tr>
<td><strong>Sarcophaga peregrina</strong></td>
<td>Embryonic cell line-NIH- Sape-4</td>
<td>HPLC</td>
<td>Synchropak RP-P (C&lt;sub&gt;18&lt;/sub&gt;)</td>
<td>Linear gradient 15-35 % of acetonitrile with 0.05 % TFA</td>
<td>Matsuyama and Natori, 1988; Yamada and Natori, 1993; Yamada and Natori 1994, Japan.</td>
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</tr>
<tr>
<td><strong>Simulium bannaense</strong></td>
<td>Salivary gland homogenate</td>
<td>RP-HPLC</td>
<td>Inertsil C4</td>
<td>Linear gradient 0-70 % of acetonitrile with 0.1 % TFA</td>
<td>Wei et al., 2015, China.</td>
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<tr>
<td><strong>Simulium bannaense</strong></td>
<td>Salivary gland homogenate</td>
<td>RP-HPLC</td>
<td>Wondasil C18</td>
<td>Linear gradient 0-60 % of acetonitrile with 0.1 % TFA</td>
<td>Wu et al., 2015, China.</td>
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<tr>
<td><strong>MANTODEA:</strong></td>
<td>Sphodromantis viridis</td>
<td>Adult extract</td>
<td>RP - HPLC</td>
<td>C18 semi-preparative RP-HPLC</td>
<td>Linear gradient 5-65 % of acetonitrile with 0.098 % TFA</td>
<td>Zare - Zardini et al., 2015, Iran.</td>
</tr>
<tr>
<td><strong>HOMOPTERA:</strong></td>
<td>Cryptotympana dubia</td>
<td>Adult homogenate</td>
<td>HPLC ➔ RP - HPLC ➔ HPLC</td>
<td>C18 column; YMC-Pack ODS-A; Hydrosphere C18</td>
<td>Step gradients of 10, 50 and 100 % of acetonitrile with 0.05% TFA; Linear gradient 15-45 % of acetonitrile with 0.1 % TFA; Linear gradient of 20-35 % of acetonitrile with 0.1 % TFA</td>
<td>Park et al., 2007, Korea.</td>
</tr>
<tr>
<td><strong>ODONATA:</strong></td>
<td>Aeschna cyanea</td>
<td>Larval hemolymph</td>
<td>HPLC ➔ RP - HPLC</td>
<td>Sep-Pak CIS; Aquapore RP 300 C8</td>
<td>Step gradients of 10, 30, 60 and 100 % of acetonitrile with 0.05% TFA; Linear gradient 10-60 % of acetonitrile with 0.1 % TFA</td>
<td>Bulet et al., 1992, France.</td>
</tr>
</tbody>
</table>

IEC: ion exchange chromatography, GF: gel filtration chromatography, RP-HPLC: reverse phase high performance liquid chromatography, TFA: trifluoroacetic acid
Review Table III: A review of literature on the characteristic features and functional analysis of cytolytic molecules detected in various species of insects (Insects are arranged alphabetically under their respective insect order)

<table>
<thead>
<tr>
<th>Insect ORDER</th>
<th>Genus &amp; Species</th>
<th>Isolated protein/peptide</th>
<th>Molecular weight (kDa)</th>
<th>Functional analysis</th>
<th>Author(s), year and country</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gram-positive</td>
<td>Gram-negative</td>
</tr>
<tr>
<td>LEPIDOPTERA:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artogeia rapae</td>
<td>Hinnavin II</td>
<td>4.14</td>
<td>ND</td>
<td>B. megaterium</td>
<td>E. coli</td>
</tr>
<tr>
<td>Bombyx mori</td>
<td>Cecropin A, B</td>
<td>6.76 and 6.83</td>
<td>ND</td>
<td>B. subtilis*</td>
<td>E. coli*</td>
</tr>
<tr>
<td>Galleria mellonella</td>
<td>Gai lysin-1</td>
<td>75</td>
<td>Sheep, human, guinea pig and rabbit</td>
<td>B. cereus</td>
<td>E. coli, P. aeruginosa, P. mirabilis</td>
</tr>
<tr>
<td>Galleria mellonella</td>
<td>Gm cecropin</td>
<td>4.16</td>
<td>ND</td>
<td>B. subtilis</td>
<td>E. coli</td>
</tr>
<tr>
<td>Heliothis virescens</td>
<td>Hiliothis cecropin</td>
<td>7.5</td>
<td>ND</td>
<td>S. aureus</td>
<td>P. aeruginosa</td>
</tr>
</tbody>
</table>

ND - Not determined
* - Most sensitive bacterial strain
# - Molecular weight determined by acidic native PAGE
@ - Molecular weight determined by SDS-PAGE
$ - Molecular weight determined by MALDI-MS analysis
## Review Table III continued

<table>
<thead>
<tr>
<th>Insect ORDER Genus &amp; Species</th>
<th>Isolated protein/peptide</th>
<th>Molecular weight (kDa)</th>
<th>Hemolytic activity</th>
<th>Bacterial species screened for antibacterial/lytic activity</th>
<th>Functional analysis</th>
<th>Miscellaneous functions</th>
<th>Author(s), year and country</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEPIDOPTERA: Hyphantria cunea</td>
<td>Hyphantria cecropin A</td>
<td>~ 3.9&lt;sup&gt;$\dagger$&lt;/sup&gt;</td>
<td>ND</td>
<td>B. megaterium</td>
<td>E. coli</td>
<td>Antifungal - Candida tropicalis</td>
<td>Park et al., 1997, Korea.</td>
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<td></td>
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<td></td>
<td>M. luteus</td>
<td>E. cloaca, P. aeruginosa, S. typhymurium, S. sonnei</td>
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</tr>
<tr>
<td>COLEOPTERA: Acalolepta luxuriosa</td>
<td>Cecropin</td>
<td>3.90&lt;sup&gt;$\S$&lt;/sup&gt;</td>
<td>ND</td>
<td>M. luteus</td>
<td>E. coli</td>
<td>ND</td>
<td>Saito et al., 2005, Japan.</td>
</tr>
<tr>
<td>Paederus dermatitis</td>
<td>Sarcotoxin Pd</td>
<td>3.61&lt;sup&gt;$\S$&lt;/sup&gt;</td>
<td>Human</td>
<td>(B. subtilis)</td>
<td>(E. coli)</td>
<td>Antifungal- A. niger, A. fumigates and C. albicans</td>
<td>Memarpoor- Yazdi et al., 2013, Iran.</td>
</tr>
<tr>
<td>Zophob utratus</td>
<td>Antibacterial peptide -A</td>
<td>8.11&lt;sup&gt;$\S$&lt;/sup&gt;</td>
<td>ND</td>
<td>M. luteus</td>
<td>E. coli</td>
<td>ND</td>
<td>Bulet et al., 1991, France.</td>
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<td></td>
<td>(coleoptericin)</td>
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<td>P. naltophilia</td>
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<tr>
<td>HEMIPTERA: Rhodnius prolixus</td>
<td>Hemolytic factor</td>
<td>ND</td>
<td>Horse, Rabbit, human, Sheep</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Azambuja et al., 1983, Brazil.</td>
</tr>
<tr>
<td>Triatoma infestans</td>
<td>Trialysin</td>
<td>22*</td>
<td>Human</td>
<td>ND</td>
<td>E. coli</td>
<td>Parasite lysis- T. cruzi</td>
<td>Amino et al., 2002, Brazil.</td>
</tr>
</tbody>
</table>

ND - Not determined
$\dagger$ - Molecular weight determined by acidic native-PAGE
$\S$ - Molecular weight determined by MALDI-MS analysis
$\dagger$ - Molecular weight determined by Tricine SDS-PAGE
$\S$ - Molecular weight determined by acidic native-PAGE
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<td><strong>HYMENOPTERA:</strong></td>
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<td>Anoplius samariensis</td>
<td>Anoplín</td>
<td>1.15&lt;sup&gt;§&lt;/sup&gt;</td>
<td>Human</td>
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<td>Hemolytic activity</td>
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<td>Gram negative</td>
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<td>S. aureus</td>
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<td>S. saprophyticus</td>
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<td>B. subtilis</td>
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<td>B. thuringiensis</td>
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<td>E. coli</td>
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<td>E. cloacae</td>
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<td>P. aeruginosa</td>
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<td>P. mirabilis</td>
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<td>P. aeruginosa</td>
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<td>Antifungal-C. albicans, and stimulating degranulation – rat peritoneal mast cell</td>
<td>Konno et al., 2001, Brazil.</td>
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<td>Stimulating degranulation - rat peritoneal mast cell and RBL-2H3 Cells</td>
<td>Konno et al., 2000, Brazil; Cabrera et al., 2004, Brazil.</td>
</tr>
<tr>
<td>Anterhynchium flavomarginatum micado</td>
<td>Eumenine mastoparan - AF (EMP-AF),</td>
<td>1.15&lt;sup&gt;§&lt;/sup&gt;</td>
<td>Human</td>
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<td>S. aureus</td>
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<td>S. epidermidis</td>
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<td>S. saprophyticus</td>
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<td>S. subtilis</td>
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<tr>
<td>Apis mellifera</td>
<td>Melittin</td>
<td>~3.5&lt;sup&gt;¶&lt;/sup&gt;</td>
<td>Human</td>
<td></td>
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<td>Moderate mast cell degranulation PT18 cells and RBL-2H3 Cells; Leishmanicidal-Leishmania major</td>
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<td>Halictus sexcinctus</td>
<td>Halictines1.2 (HAL I &amp; II)</td>
<td>1.4 and 1.45&lt;sup&gt;§&lt;/sup&gt;</td>
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ND - Not determined
¶ - Molecular weight determined by Tricine SDS-PAGE
§ - Molecular weight determined by MALDI-MS analysis
**Review Table III continued**

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<tr>
<th>Insect ORDER Genus &amp; Species</th>
<th>Isolated protein/peptide</th>
<th>Molecular weight (kDa)</th>
<th>Functional analysis</th>
<th>Bacterial species screened for antibacterial/lytic activity</th>
<th>Miscellaneous functions</th>
<th>Author(s), year and country</th>
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<tr>
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<td><em>Lasioglossum laticeps</em></td>
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<td>2.89&lt;sup&gt;$\ddagger$&lt;/sup&gt;</td>
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<td>Antifungal-</td>
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<td><em>B. subtilis</em></td>
<td><em>P. aeruginosa</em></td>
<td><em>C. albicans,</em></td>
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<tr>
<td><em>Megabombus pennsylvanicus</em></td>
<td>Bombolitin I, II, III, IV,V</td>
<td>1.9&lt;sup&gt;$\ddagger$&lt;/sup&gt;</td>
<td>Guinea pig</td>
<td>ND</td>
<td>ND</td>
<td>Stimulation of histamine release from rat peritoneal mast cells</td>
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<td><em>Myrmecia pilosula</em></td>
<td>Pilosulin 1</td>
<td>6.05&lt;sup&gt;$\ddagger$&lt;/sup&gt;</td>
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<td><em>S. aureus</em></td>
<td><em>E. coli</em></td>
<td>Cytotoxic effect on EBV B-cells</td>
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<td><em>Oreumenes decoratus</em></td>
<td>Decoralin</td>
<td>2&lt;sup&gt;$\ddagger$&lt;/sup&gt;</td>
<td>Mouse</td>
<td><em>S. aureus</em></td>
<td><em>E. coli</em></td>
<td>Degranulation activity in rat peritoneal mast cells; leishmanicidal activities</td>
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<td><em>Orancistrocerus drewnseni drewnseni</em></td>
<td>Eumenine Mastoparan - OD and Orancis - Protonectin</td>
<td>1.26 and 1.55&lt;sup&gt;$\ddagger$&lt;/sup&gt;</td>
<td>Sheep</td>
<td>ND</td>
<td>ND</td>
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<td><em>Pachycondyla goeldii</em></td>
<td>Ponericins</td>
<td>3&lt;sup&gt;$\ddagger$&lt;/sup&gt;</td>
<td>Sheep and horse</td>
<td><em>(B. stearothermophilus</em></td>
<td><em>(L. lactis sp.</em></td>
<td>Insecticidal -</td>
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<td><em>B. subtilis</em></td>
<td><em>P. aeruginosa)</em></td>
<td><em>A. domesticus,</em></td>
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<td><em>B. megaterium)</em></td>
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<td><em>P. goeldii</em></td>
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<td>*Anti-yeast -</td>
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<td><em>S. cerevisiae</em></td>
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ND - Not determined

<sup>$\ddagger$</sup> - Most sensitive bacterial strain

<sup>$\ddagger$</sup> - Molecular weight determined by MALDI-MS analysis

<sup>$\ddagger$</sup> - Molecular weight determined by HPLC size exclusion chromatography with TSK 3000 SW column
## Review Table III continued

<table>
<thead>
<tr>
<th>Insect ORDER</th>
<th>Genus &amp; Species</th>
<th>Isolated protein/peptide</th>
<th>Molecular weight (kDa)</th>
<th>Functional analysis</th>
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<td>Protopolybia exigua</td>
<td>Protopolybia MPII</td>
<td>1.58, 1.56 and 1.55&lt;sup&gt;‡&lt;/sup&gt;</td>
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<td>Protopolybia MPIII</td>
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<td>Protopolybia MPIII</td>
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<td>Pogonomyrmex barbatus</td>
<td>Barbatolysin</td>
<td>3&lt;sup&gt;¶&lt;/sup&gt;</td>
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<td>Vespa magnifica</td>
<td>Vespid chemotactic peptide 5e, 5f, 5g</td>
<td>~1.3&lt;sup&gt;§&lt;/sup&gt;</td>
<td>Rabbit</td>
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<td>Vespa crabro</td>
<td>Mastoparan C and Crabrolin</td>
<td>1.5 and 1.49&lt;sup&gt;§&lt;/sup&gt;</td>
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<td>Aedes defensin A and B</td>
<td>4.07 and 4.07&lt;sup&gt;§&lt;/sup&gt;</td>
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<sup>§</sup> - Molecular weight determined by MALDI-MS analysis  
<sup>¶</sup> - Molecular weight determined by Tricine SDS-PAGE  
<sup>®</sup> - Most sensitive RBC type
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<td>Armigeres subalbatus</td>
<td>Sialic acid-specific lectin</td>
<td>Four protein bands: 9.8, 12.5, 14.0 and 15.4</td>
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<td>4.79</td>
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<td>Low cytotoxicity to mammalian cells; Neutralize LPS; Anti inflammatory activity</td>
<td>Wei et al., 2015, China.</td>
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<td>Wu et al., 2015, China.</td>
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<td>Zare-Zardini et al., 2015, Iran.</td>
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<td>Cryptotympana dubia</td>
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<td>2.7</td>
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<td>S. aureus, B. subtilis, M. luteus, E. coli</td>
<td>Anti-fungal, C. albicans; C. tropicals</td>
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<td>Park et al., 2007, Korea.</td>
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**ND** - Not determined  
**S** - Molecular weight determined by MALDI-MS analysis
**Review Table IV: A review of literature on antibacterial activity of various crude preparations from *Chrysomya megacephala***

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<td>1</td>
<td>Larval and pupal hemolymph</td>
<td><em>B. subtilis</em> and <em>S. aureus</em></td>
<td>Sahalan <em>et al.</em>, 2007, Malaysia.</td>
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<td>Faraldo <em>et al.</em>, 2008, Brazil.</td>
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<td>Excretory/secretory products and larval extract</td>
<td><em>B. subtilis</em> and <em>S. aureus</em></td>
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<td>Fractions of larval secretion/excretion</td>
<td><em>B. subtilis</em></td>
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<td>Excretory/secretory products of larvae</td>
<td><em>S. aureus</em></td>
<td>Ratcliffe <em>et al.</em>, 2015, Brazil.</td>
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<td>Larval secretion/excretion</td>
<td><em>S. aureus</em> and <em>P. aeruginosa</em></td>
<td>Chaiwong <em>et al.</em>, 2016, Thailand.</td>
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ND - Not determined