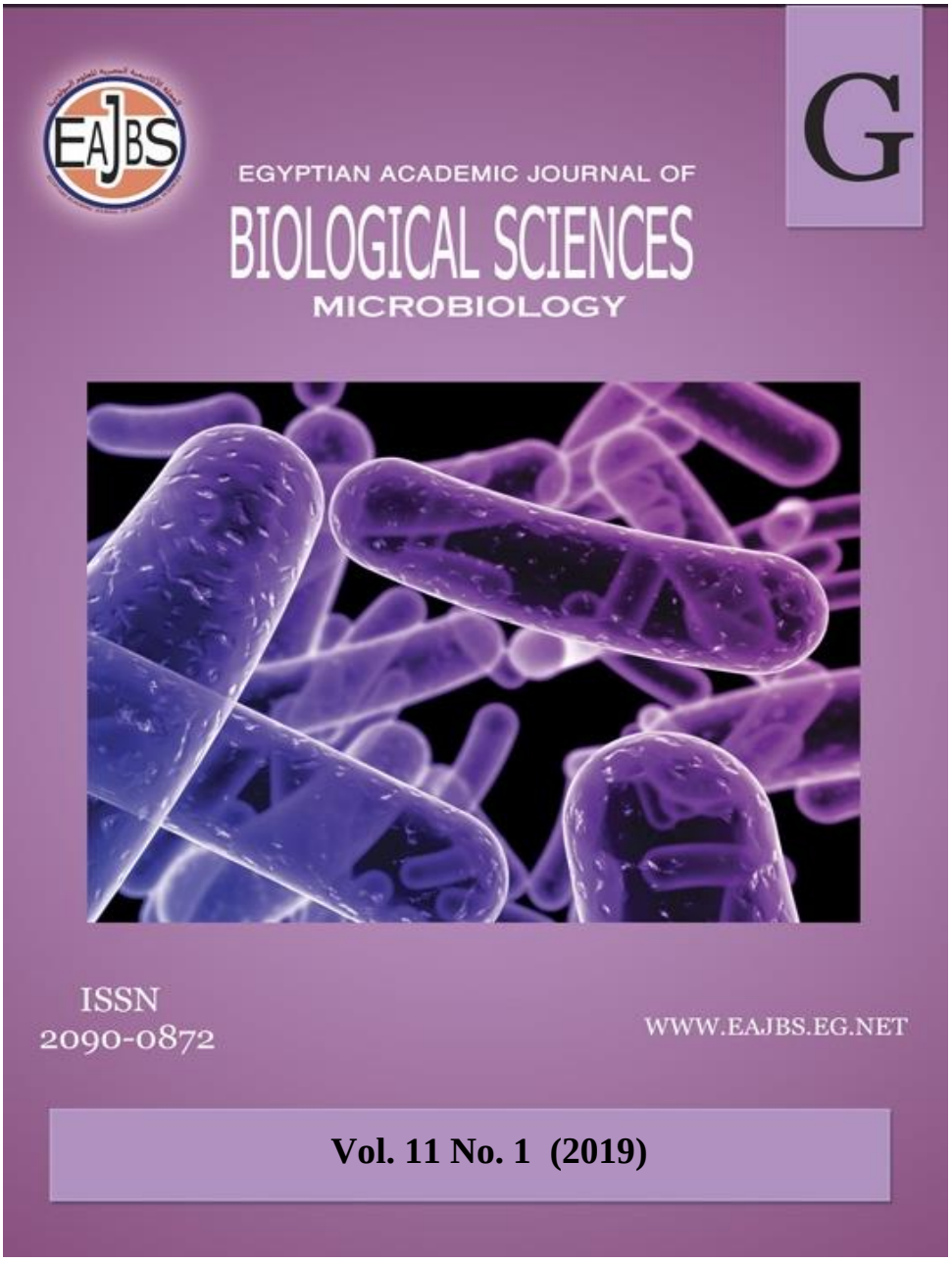

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Antimicrobial Activity of *Sarcophaga carnaria* (Diptera: Sarcophagidae) Maggots' Body Extracts

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ABSTRACT

The present study evaluated the antibacterial and antifungal activities of *Sarcophaga carnaria* maggots' body different extracts. The extraction was carried out using petroleum ether, hexane, acetone and ethyl acetate. The obtained results revealed that all *S. carnaria* maggots' tested extracts evoked a variable activity against both Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus pyogenes* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) depending on the solvent used in extraction. The highest antibacterial activity (growth- inhibition zone) was 17.0±0.55 and 16.4±0.61mm obtained by petroleum ether extract against *S .aureus* and *S. pyogenes* (Gram-positive bacteria). Meanwhile, petroleum ether, acetone and ethyl acetate extracts showed growth-inhibition zone of 16.0±0.46, 15.4±0.50 and 16.2±0.45mm against *B. subtilis*, respectively, compared with 28.2±0.33mm for the standard *(Ampicillin).* Also, acetone extract of *S. carnaria* maggots' whole body exhibited no activity against all tested Gram- negative bacterial strains tested. Generally, petroleum ether extraction of *S. carnaria* maggots' whole body was the most effective against different bacteria species followed by hexane, ethyl acetate and acetone extractions. Also, Gram-positive bacterial strains were more sensitive to *S. carnaria* maggots' extracts than Gram-negative bacterial strains. In addition, petroleum ether and hexane extracts of *S. carnaria* maggots' whole body showed a variable antifungal activity against *A. flavus, A. fumigatus, C. albicans* and *G. candidum* fungal strains, whereas both acetone and ethyl acetate extracts from *S. carnaria* maggots' whole body showed no activity against all tested fungi species.

INTRODUCTION

Antimicrobial peptides considered as important factors of the innate immunity several organisms, as they are small, usually cationic and amphipathic molecules. Based on, amino acid sequence and biochemical properties, antimicrobial peptides are divided into three classes; linear peptides without cysteine, peptides with stabilized structure by disulfide bridges and peptides with an overrepresentation of one amino acid (Januszanis *et al*, 2012).

In insects, antimicrobial peptides/ polypeptides are synthesized mainly in a fat body (functional analogue of mammalian liver) and are released into hemolymph where they play a crucial role in innate immune systems and host defense mechanisms, and having a broad spectrum of activity against both Gram- positive and Gram- negative bacteria and against fungi (Hoffmann, 1995; Hoffmann *et al*, 1996; Januszanis *et al*, 2012). In 2007, Hou *et al.* reported that it is increasingly possible to use the antimicrobial activity of insect body extracts to ascertain phylogenetic patterns among insect species. Five major groups of antibacterial proteins have been isolated from different species of insects: cecropins, insect defensins, attacin-like (glycine- rich) proteins, proline- rich peptides and lysozymes (Hultmark, 1993; Cociancich *et al*, 1994). In addition, drosomycin, metchinikowin, cecropin A&B and heliomicin are antifungal peptides/ polypeptides isolated from insects (Fehlbaum *et al*, 1994; Levashina *et al*, 1995; Lamberty *et al*, 1999). In addition, the usage of insect extracts in Folk Medicine encouraged scientists to develop potential new medicines for treating serious diseases such as viral infections and the problems associated with the newly emerging antibiotic-resistant bacteria. There is already a long history of the use of these insect extracts in Folk Medicine (Ratcliffe *et al*, 2014).

The present study aimed at evaluating the activity of *Sarcophaga carnaria* maggots' body petroleum ether, hexane, acetone and ethyl acetate extracts against different Gram-positive and Gram- negative bacterial strains. In addition, the activity of *S. carnaria* maggots' body tested extracts against different fungi strains (*Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Geotricum candidum* and *Penicillium sp.*) was also investigated.

MATERIALS AND METHODS

Tested flies: *Sarcophaga carnaria* maggots were collected and reared for several generations in Medical Entomology Insectary, Biology Department, Faculty of Science, Jazan University, SKA under controlled conditions of temperature (27±3°C), relative humidity (60±15%) and photoperiods (12h light: 12h dark). Adults were reared in mesh cages (30×30×30cm) with three sides of the wire. Maggots were feed on an artificial diet (liver). The emerged flies were feed on dry diet (milk powder) and sucrose solution (cotton pads soaked in 10% sucrose solution) (Amer *et al*, 2019).

Preparation of maggots' extracts:

The extraction was performed according to the method of Hassan *et al*, (2018). The extraction was carried out using petroleum ether, hexane, acetone and ethyl acetate.

Antimicrobial bioassay*:*

Six pathogenic bacterial strains were used for the antibacterial assay; *Staphylococcus aureus, Staphylococcus pyogenes* and *Bacillus subtilis* as Gram-positive bacterial strains whereas, *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were used as Gram-negative bacterial strains**.** In addition, *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Geotricum candidum* and *Penicillium sp.* strains were used for in vitro evaluation of the antimicrobial activity. All tested microorganisms were supplied by the Microbiology Department, Faculty of Science, Jazan University, SKA.

Antibacterial activity of tested extracts:

Microbial growth inhibition was tested using agar well diffusion method (Magaldi *et al*, 2004; Valgas *et al*, 2007). Also, Minimum Inhibitory Concentration (MIC) was determined based on the microdilution method by broth microdilution method using 96-well micro-plates (Irith *et al*, 2008).

Statistical analysis: The statistical analysis of the data obtained was done according to Armitage, (1974) and Lentner *et al*, (1982) and Quattropro revised the analysis for windows program version 2. Microsoft, windows version 7 and graphic were drawn using Harvard Graphics program version 4. The obtained data were assessed

by calculation of mean (M), standard deviation (SD) and student t-test.

RESULTS

An Antibacterial Activity using Well Diffusion Method*:*

Data given in table (1) and illustrated in figures (1A-C) evoked that petroleum ether and hexane extracts of *Sarcophaga carnaria* maggot' whole body showed antibacterial activity (growth- inhibitory zone) against both *Staphylococcus aureus* and *S. pyogenes* bacterial strains tested. The highest antibacterial activity (mean growth-inhibition zone) was 17.0±0.55 and 16.4±0.61 mm obtained by petroleum ether extract against *S .aureus* and *S. pyogenes*. Meanwhile, petroleum ether, acetone and ethyl acetate extracts showed growth-inhibition zone of 16.0±0.46, 15.4±0.50 and 16.2±0.45mm against *Bacillus subtilis*, respectively, compared with 28.2±0.33 mm for the standard *(Ampicillin).*

On the other hand, tested *S. carnaria* maggots' crude body extracts showed antibacterial activity against all Gram-negative bacterial strains tested except *Pseudomonas aeruginosa* (Table 2 & Figure 2A-C). The highest antibacterial activity attained by petroleum ether extract against *Escherichia coli* and *Klebsiella pneumoniae*, as the growth- inhibition zone recorded 16.1±0.43 and 16.0±0.36mm, compared with 27.6±0.10 and 25.2±0.12mm for the standard antibiotic (*Gentamycin*). Also, acetone extract of *S. carnaria* maggots' whole body exhibited no activity against all tested Gram-negative bacterial strains tested.

Also, from the results given in table (3) and Figure (3 A-E), both acetone and ethyl acetate extracts from *S. carnaria* maggots' whole body showed no activity against all tested fungi species. Petroleum ether extract recorded 16.7±0.61, 15.0±0.44, 16.5±0.35 and 17.7±0.63 mm growth- inhibition zones against *Aspergillus flavus, A. fumigatus, Candida albicans* and *Geotricum candidum,* respectively, compared with 24.6±0.29, 25.8±0.17, 21.6±0.14 and 23.0±0.10 mm for the standard (*Amphotericin B*). Also, all tested extracts had no activity against *Penicillium sp.*

Determination of Minimum Inhibitory Concentration (MIC) by Microdilution Method:

The antibacterial activity of *S. carnaria* maggots' different crude extracts against tested Gram-positive bacteria showed that acetone and ethyl acetate of *S. carnaria* maggots' didn't show any activity against both *S. aureus* and *S. pyogenes* (Gram-positive bacterial strains). Meanwhile, *S. carnaria* maggots' hexane extract had no activity against *B. subtilis* (Table 4). The MIC value of 25.0 mg/ml was recorded by petroleum ether and hexane extracts of *S. carnaria* maggots against *S. aureus* and *S. pyogenes*, respectively (Table 5).

On the other hand, against Gram-negative bacteria, only *S. carnaria* maggots' acetone extract recorded no activity against all tested strains. In addition, all tested extracts had no activity against *P. aeruginosa* (Table 6).

Also, petroleum ether extract of *S. carnaria* maggots recorded MIC against *E. coli* and *K. pneumoniae* equal to 25.0 mg/ml, respectively (Table 7).

Table 1: Antibacterial activity as indicated by growth-inhibition zone of *Sarcophaga carnaria* maggots' different crude extracts against different strains of Gram-positive bacteria.

Bacteria	Growth-inhibition zone in mm caused by extracts				Standard *(Ampicillin)*
	Petroleum ether	Hexane	Acetone	Ethyl acetate	
Staphylococcus aureus	17.0 ±0.55^{d}	16.3±0.51^{d}	*NA*	*NA*	27.6±0.22^{a}
Staphylococcus pyogenes	16.4±0.61^{d}	16.0±0.52^{d}	*NA*	*NA*	25.8±0.14^{a}
Bacillus subtilis	16.0±0.46^{d}	*NA*	15.4±0.50^{d}	16.2±0.45^{d}	28.2±0.33^{a}

Means followed by the same letter are not significantly different (p>0.05).

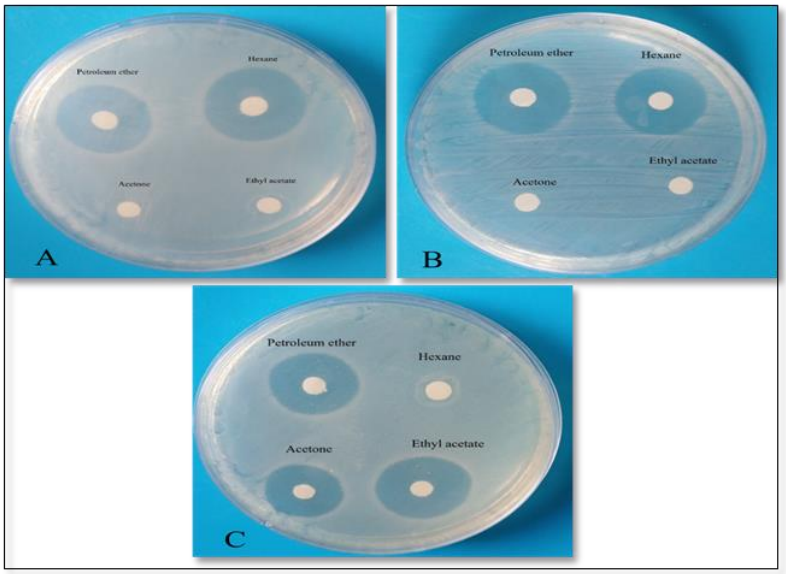

Fig.1: Antibacterial activity indicated by growth-inhibition zone of *Sarcophaga carnaria* maggots' different crude extracts against Gram-positive bacteria. (A: *Staphylococcus aureus*; B: *Staphylococcus pyogenes*; C: *Bacillus subtilis*).

Table 2: Antibacterial activity as indicated by growth-inhibition zone of *Sarcophaga carnaria* maggots' different crude extracts against different strains of Gram-negative bacteria.

Bacteria	Growth-inhibition zone in mm caused by extracts				Standard *(Ampicillin)*
	Petroleum ether	Hexane	Acetone	Ethyl acetate	
Escherichia coli	16.1±0.43[d]	15.3±0.51[d]	*NA*	15.2±0.46[d]	27.6±0.10[a]
Klebsiella pneumoniae	16.0±0.36[d]	15.7±0.69[d]	*NA*	*NA*	25.2±0.12[a]
Pseudomonas aeruginosa	*NA*	*NA*	*NA*	*NA*	22.3±0.16[a]

See footnote of table 1.

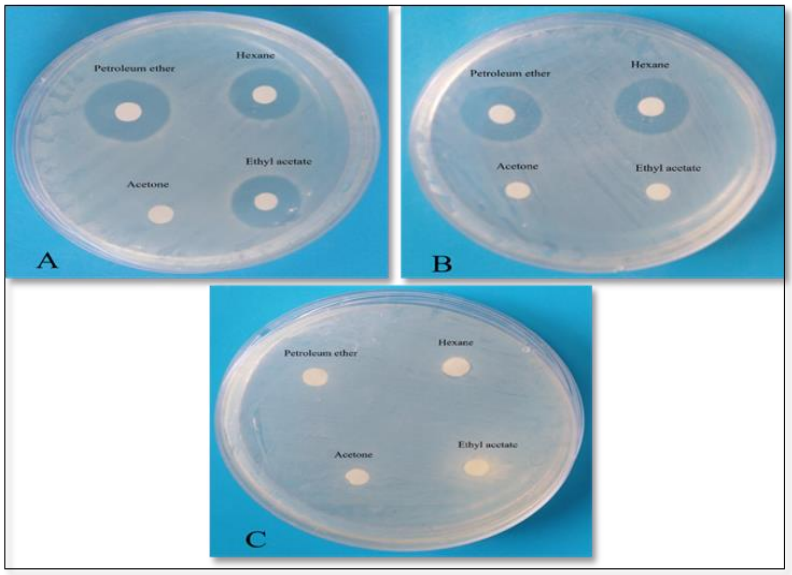

Fig.2: Antibacterial activity indicated by growth-inhibition zone of *Sarcophaga carnaria* maggots' different crude extracts against Gram-negative bacteria. (A: *Escherichia coli*; B: *Klebsiella pneumoniae*; C: *Pseudomonas aeruginosa*).

Table 3: Antifungal activity as indicated by growth-inhibition zone of *Sarcophaga carnaria* maggots' different crude extracts against different strains of fungi.

Fungi	Growth-inhibition zone in mm caused by extracts				Standard *(Amphotericin B)*
	Petroleum ether	Hexane	Acetone	Ethyl acetate	
Aspergillus flavus	16.7±0.61^{d}	15.0±0.48^{d}	*NA*	*NA*	24.6±0.29^{a}
Aspergillus fumigatus	15.0±0.44^{d}	15.4±0.40^{d}	*NA*	*NA*	25.8±0.17^{a}
Candida albicans	16.5±0.35^{d}	*NA*	*NA*	*NA*	21.6±0.14^{a}
Geotricum candidum	17.7±0.63^{d}	16.0±0.42^{d}	*NA*	*NA*	23.0±0.10^{a}
Penicillium sp.	*NA*	*NA*	*NA*	*NA*	24.0±0.20^{a}

See footnote of table 1.

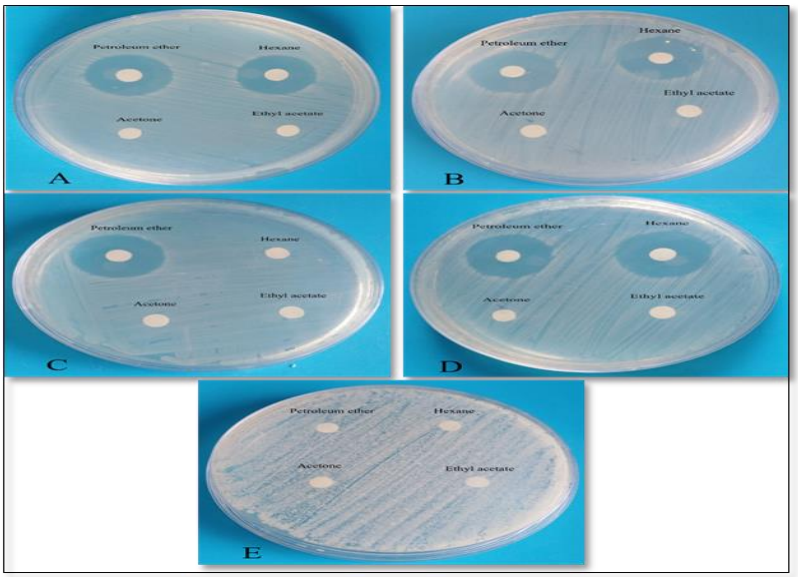

Fig.3: Antifungal activity indicated by growth-inhibition zone of *Sarcophaga carnaria* maggots' different crude extracts against fungi strains. (A: *Aspergillus flavus*; B: *Aspergillus fumigatus*; C: *Candida albicans*; D: *Geotricum candidum*; E: *Penicillium sp.*).

Table 4: Antibacterial activity of *Sarcophaga carnaria* maggots' different crude extracts against different strains of Gram-positive bacteria as indicated by Microdilution plate at 480nm.

Bacterial strains	Conc. (mg/ml)	*Sarcophaga carnaria* maggots' different extracts			
		Petroleum ether	Hexane	Acetone	Ethyl acetate
Staphylococcus aureus	Control	4.7±0.3^{a}	4.4±0.5^{a}	4.3±0.6^{a}	4.0±0.3^{a}
	50.0	2.6±0.7^{d}	2.8±0.4^{c}	*NA*	*NA*
	25.0	2.6±0.2^{d}	2.8±0.1^{c}	*NA*	*NA*
	12.5	2.5±0.1^{d}	2.7±0.4^{c}	*NA*	*NA*
Staphylococcus pyogenes	Control	4.7±0.3^{a}	4.4±0.5^{a}	4.3±0.6^{a}	4.0±0.3^{a}
	50.0	2.6±0.5^{d}	2.9±0.2^{c}	*NA*	*NA*
	25.0	2.5±0.5^{d}	2.7±0.1^{d}	*NA*	*NA*
	12.5	2.5±0.2^{d}	2.7±0.2^{d}	*NA*	*NA*
Bacillus subtilis	Control	4.7±0.3^{a}	4.4±0.5^{a}	4.3±0.6^{a}	4.0±0.3^{a}
	50.0	2.9±0.1^{c}	*NA*	2.8±0.1^{b}	2.9±0.1^{c}
	25.0	2.8±0.8^{c}	*NA*	2.9±0.4^{b}	2.9±0.3^{c}
	12.5	2.8±0.2^{c}	*NA*	2.8±0.7^{b}	3.0±0.3^{c}

See footnote of table 1.

Table 5: Minimal Inhibitory Concentrations (MIC) of *Sarcophaga carnaria* maggots' different crude extracts against different strains of Gram-positive bacteria

Bacterial strains	*Sarcophaga carnaria* maggots' different extracts			
	Petroleum ether	Hexane	Acetone	Ethyl acetate
Staphylococcus aureus	25.0	25.0	*NA*	*NA*
Staphylococcus pyogenes	25.0	25.0	*NA*	*NA*
Bacillus subtilis	50.0	*NA*	50.0	50.0

Table 6: Antibacterial activity of *Sarcophaga carnaria* maggots' different crude extracts against different strains of Gram-negative bacteria as indicated by Microdilution plate at 480nm.

Bacterial strains	Conc. (mg/ml)	*Sarcophaga carnaria* maggots' different extracts			
		Petroleum ether	Hexane	Acetone	Ethyl acetate
Escherichia coli	Control	4.7±0.3^{a}	4.4±0.5^{a}	4.3±0.6^{a}	4.0±0.3^{a}
	50.0	3.0±0.5^{c}	3.0±0.4^{c}	*NA*	3.1±0.4^{b}
	25.0	3.0±0.2^{d}	3.1±0.1^{b}	*NA*	3.1±0.2^{b}
	12.5	2.9±0.1^{d}	2.9±0.4^{c}	*NA*	3.1±0.1^{b}
Klebsiella pneumoniae	Control	4.7±0.3^{a}	4.4±0.5^{a}	4.3±0.6^{a}	4.0±0.3^{a}
	50.0	3.1±0.4^{b}	3.1±0.3^{c}	*NA*	*NA*
	25.0	3.0±0.7^{c}	3.1±0.1^{c}	*NA*	*NA*
	12.5	2.9±0.4^{c}	3.0±0.3^{c}	*NA*	*NA*
Pseudomonas aeruginosa	Control	4.7±0.3^{a}	4.4±0.5^{a}	4.3±0.6^{a}	4.0±0.3^{a}
	50.0	*NA*	*NA*	*NA*	*NA*
	25.0	*NA*	*NA*	*NA*	*NA*
	12.5	*NA*	*NA*	*NA*	*NA*

See footnote of table 1.

Table 7: Minimal Inhibitory Concentrations (MIC) of *Sarcophaga carnaria* maggots' different crude extracts against different strains of Gram-negative bacteria.

Bacterial strains	*Sarcophaga carnaria* maggots' different extracts			
	Petroleum ether	Hexane	Acetone	Ethyl acetate
Escherichia coli	25.0	50.0	*NA*	50.0
Klebsiella pneumoniae	25.0	50.0	*NA*	*NA*
Pseudomonas aeruginosa	*NA*	*NA*	*NA*	*NA*

DISCUSSION

Antimicrobial agents in insects are peptides that synthesized as effector molecules in the epithelial and midgut tissues (Brey *et al*, 1993; Lehane *et al*, 1997; Ferrandon *et al*, 1998) and released into the haemolymph. Kuhn-Nentwig, (2003) detected the presence of polar compounds on the epicuticular layer of arthropods, including social insects (Hölldobler and Wilson, 1990; Turillazzi *et al*, 2006) and these compounds proved antimicrobial activity against several bacterial species. Also, the epidermis may produce antibacterial and antifungal peptides in response to local infections (Brey *et al*, 1993; Ferrandon *et al*, 1998). Also, insects are known to have both cellular and humoral immune systems which together form a potent defense against invading bacteria

(Gotz and Boman, 1985; Dunn, 1986 & kimbrell, 1991). In cellular immunity, mechanisms such as phagocytosis and encapsulation are operative (Boman and Hultmark, 1987) while humoral responses mainly involve the production of a variety of antibacterial and antifungal proteins that are induced or increased in response to infection (Abraham *et al*, 1995).

The obtained results showed that all *S. carnaria* maggots' tested extracts evoked a variable activity against both Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus pyogenes* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) depending on the solvent used in extraction by two tested methods.

Generally, petroleum ether extraction of *S. carnaria* maggots' whole body was the most effective against different bacteria species followed by hexane, ethyl acetate and acetone extractions. Also, Gram-positive bacterial strains were more sensitive to *S. carnaria* maggots' extracts than Gram-negative bacterial strains. Similar results concerning with antibacterial activity of different insect extracts were also observed by Leem *et al*, (1999) where the sawfly, *Acantholyda parki* extract recorded a broad antibacterial spectrum against both Gram-negative and Gram-positive bacteria and Yamauchi, (2001) who suggested that insect bodies produce combinations of antibacterial peptides in response to natural infection leading to a broad spectrum of activity against micro-organisms. In spite of such a response, the susceptible insects within the host range of a given pathogen are successfully killed by the pathogen and in contrast, the insects resistant against the pathogen appear to be out of the host range.

In agreement with the present results, the antibacterial activity of *S. carnaria* maggots' whole body extracts was comparable to that of Zeariya, (2009) where, the darkling beetle, *Ocnera hispida* different whole body extracts recorded antibacterial activity against different Gram-positive and Gram-negative bacterial strains. Also, different insect body extracts showed antibacterial activity against both Gram-positive and Gram-negative bacteria, it suffices to look on the next few examples; Hara and Yamakawa, (1995) using, the silkworm, *Bombyx mori*, the European bumblebee extracts, the antibacterial activity of *Bombus pascuorum* and *Tenebrio molitor* larvae was recorded by Rees *et al*, (1997) and Lee *et al*, (1998). In addition, Lowenberger *et al*, (1995), Lauth *et al*, (1998) and Vizioli *et al*, (2001) recorded the antibacterial activity of *Aedes aegypti*, *Chironomus plumosus* and *Anopheles gambiae* extracts against only Gram-positive bacteria.

On the other hand, petroleum ether and hexane extracts of *S. carnaria* maggots' whole body showed a variable antifungal activity against *A. flavus, A. fumigatus, C. albicans* and *G. candidum* fungal strains, whereas both acetone and ethyl acetate extracts from *S. carnaria* maggots' whole body showed no activity against all tested fungi species. Overall, petroleum ether extracts of *S. carnaria* maggots' whole body were more effective against fungal strains than those of hexane. However, the present study has shown that the bacterial strains tested were more sensitive to the different maggots' whole body extracts used than the fungal strains tested. In agreement with these results, Meylaers *et al*, (2004) observed that methanolic whole body extract of uninfected last instar larvae of the housefly, *M. domestica* displayed antifungal activity against *Saccharomyces cerevisiae* beside the antibacterial activity. Hou *et al*, (2007) reported that the extract of the housefly larvae showed higher activity against Gram-positive bacteria than Gram- negative bacteria and did not show any antifungal activity. In consistent with the present results, Zeariya, (2009) showed that the different whole body extracts from *H. ephippiger* (nymph), *O. hispida* (adult) and *M. domestica* (3^{rd} instar larvae and adult) induced antibacterial activity more than antifungal activity.

Conclusion*:*

In conclusion, petroleum ether, hexane, acetone and ethyl acetate of *S. carnaria* maggots' whole body recorded variable antibacterial activity against both Gram-positive and Gram-negative bacterial strains. Also, petroleum ether and hexane extracts of *S. carnaria* maggots' whole body showed antifungal activity against *A. flavus*, *A. fumigatus*, *C. albicans* and *G. candidum* fungal strains. Thus, the tested extracts of *S. carnaria* maggots' whole body used can be considered as new antimicrobial agents; however more studies are needed to reach the bioactive compounds in these extracts.

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ARABIC SUMMARY

النشاط ضد ميكروبى لمستخلصات أجسام يرقات *ساركوفاجا كارينارية* (ثنائية الأجنحة: ساركوفاجيدى)

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قيمت الدراسة الحالية نشاط المستخلصات المختلفة لأجسام يرقات *ساركوفاجا كارينارية* ضد البكتيريا والفطريات. تم الإستخلاص بإستخدام الإثير البترولى، الهكسان، الأسيتون وأسيتات الإيثيل. النتائج المُتحصل عليها بينت أن جميع المستخلصات المُختبرة أظهرت نشاط متباين ضد كلاً من البكتيريا موجبة الجرام (*ستافيلوكوكس ايوريس، ستافيلوكوكس بيوجينس و باسيليس سابتليس*) والبكتيريا سالبة الجرام (*ايشيريشيا كولاى، كليبسيلا نيمونيا و سيدموناس ارجينوزا*) إعتماداً على المُذيب المُستخدم فى الإستخلاص. أعلى نشاط ضد بكتيرى هو 17.0±0.55 و 16.4±0.16 مم تم الحصول عليه بواسطة مستخلص الإثير البترولى ضد *ستافيلوكوكس ايوريس و ستافيلوكوكس بيوجينس* (بكتيريا موجبة الجرام). بينما مستخلصات الإثير البترولى، أسيتون وأسيتات الإيثيل سجلت 16.0±0.46، 15.4±0.50 و 16.2±0.45 مم ضد بكتيريا *باسيليس سابتليس* تقريباً مقارنةً مع 28.0±0.33 مم المُضاد القياسى (*أمبيسيللين*). أيضاً، مستخلص الأسيتون لأجسام يرقات *ساركوفاجا كارينارية* لم يُسجل أى نشاط ضد جميع أنواع البكتيريا سالبة الجرام المُختبرة. بشكل عام، مستخلص الإثير البترولى لأجسام يرقات *ساركوفاجا كارينارية* هو الأعلى تأثيراً على أنواع البكتيريا يليه مستخلصات الهكسان، أسيتات الإيثيل والأسيتون. أيضاً، السلالات البكتيرية موجبة الجرام كانت أكثر حساسية لمستخلصات أجسام يرقات *ساركوفاجا كارينارية* من السلالات البكتيرية سالبة الجرام. بالإضافة إلى أن مستخلصات الإثير البترولى والهكسان لأجسام يرقات *ساركوفاجا كارينارية* أظهرت نشاطاً متبايناً ضد سلالات *اسبرجلس فلافس، اسبرجيلس فاميجيتوس، كانديدا البيكانز و جيوتريكم كانديدم* الفطرية. بينما مستخلصات الأسيتون وأسيتات الإيثيل لأجسام يرقات *ساركوفاجا كارينارية* لم تُسجل أى نشاط ضد جميع السلالات الفطرية المُختبرة.