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Effectiveness evaluation of *Chrysomya albiceps* (Diptera: Calliphoridae) and *Musca domestica* (Diptera: Muscidae) maggots extracts as antimicrobial and antiviral agent

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ABSTRACT

The present study aimed to evaluate the antimicrobial activity of *Chrysomya albiceps and Musca domestica* maggots' extracts produced from different solvents against some bacterial and fungal strains, beside the role of these extracts as anti-HSV therapeutic agents. Results obtained evoked a variable activity against both Gram-positive and Gram-negative bacteria depending on tested species and the solvent used in extraction. The highest antibacterial activity was attained by petroleum ether extract 24h post treatment for both Gram-positive and Gram-negative bacteria either by Microbial Growth Inhibition method or by Minimum Inhibitory Concentration method followed by hexane, ethyl acetate and acetone extract. Gram-positive bacterial strains were more sensitive than Gram-negative bacterial strains. Regarding the antifungal activity, tested extracts showed variable antifungal activity. In addition, petroleum ether extracts of tested species exhibited a vital role as potential anti-HSV agent due to their promising antiviral activity. In general, tested extracts induced remarkable effects on both antimicrobial and antiviral activities.

INTRODUCTION

The field of natural product discovery has undergone a tremendous development over the past few decades due to the consequence of several new and revolutionizing drug discovery and development techniques (Fouda *et al.*, 2013; Hassan *et al.*,2013; Hasaballah, 2015; Wohlleben *et al.*,2016). Insects are the largest group of the still existing organisms, their individual number account for as much as 80% of all known fauna and considered a large, unexplored and unexploited source of potentially useful compounds for modern medicine due to their mode of action of non-selective interaction with microbes' cell surface membranes (Leem *et al.*,1999; Pemberton, 1999; Hancock and Rozek, 2002; Zasloff, 2002; Boman, 2003; Bulet *et al.*,2004).

A great part of efforts have been achieved for the investigation and re-examination of insect sources to obtain compounds that may possess pharmacological activities. Many authors believe that, it is increasingly possible to use insect extracts as antimicrobial agents to ascertain phylogenetic patterns among insect species, for example (Esser *et al.*, 1979; Wachinger *et al.*,1998; Fenard *et al.*, 2001; Hou *et al.*,

2007; Slocinska *et al.*,2008). In the last few decades, five major groups of proteins act as antimicrobial agents 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). Drosomycin, metchinikowin, cecropin A&B and heliomicin as antifungal peptides/polypeptides isolated from insects (Fehlbaum *et al.*, 1994; Levashina *et al.*, 1995; Lamberty *et al.*, 1999).

Antimicrobial peptides/polypeptides are mainly synthesized in the fat body and released into the hemolymph where they play the crucial role in innate immune system and host defence mechanisms, with a broad-spectrum activity against both Gram-positive and Gram-negative bacteria and fungi (Hoffmann 1995; Hoffmann *et al.*1996; Januszanis *et al.*2012).

This study aimed to investigate the possible antimicrobial activity of *Chrysomya albiceps* (Diptera: Calliphoridae) and *Musca domestica* (Diptera: Muscidae) maggots petroleum ether, hexane, acetone and ethyl acetate extracts. In addition, to study the potential role of these extracts as anti-HSV therapeutic agents.

MATERIALS AND METHODS

Colonization of tested flies

The blowfly, *Chrysomya albiceps*

Chrysomya albiceps maggots were collected and transferred to Medical Entomology Insectary, Biology Department, Faculty of Science, Jazan University (KSA) and maintained for several generations under controlled conditions, at temperature of (27±2ºC), relative humidity (60±10%) and photoperiods (12h light: 12h dark). Adults were reared in mesh cages (30×30×30cm) with three sides of wire, maggots were feed on an artificial diet (liver), and the emerged flies were feed on milk powder and sucrose solution.

The house fly, *Musca domestica*

The housefly maggots were collected by hand trap and maintained for several generations under controlled conditions of temperature (27±2°C), relative humidity (70±5%) and photoperiods (12h light: 12h dark). The emerged flies were fed on dry diet (milk powder) and sucrose solution (cotton pads soaked in 10 % sucrose solution). Deposited eggs were collected from paper strips or cotton pads of feeding. Larvae were reared on an artificial diet (wheat bran, milk, powder yeast; 200:100:5gm) per 200 ml distilled water according the method described by Busvine, (1962).

Preparation of maggots' extracts

The extraction was performed according to the methods of Ahn *et al.*(2000) and Meylears *et al.*(2002). The extraction was carried out using petroleum ether, hexane, acetone and ethyl acetate solvents.

Antimicrobial bioassay

Antibacterial activity of tested extracts

Six pathogenic bacterial strains were used for the antibacterial assay; *Staphylococcus aureus* (ATCC25923), *Staphylococcus* pyogenes (ATCC12344) and *Bacillus subtilis* (ATCC6051) as Gram-positive bacterial strains; whereas, *Escherichia coli* (ATCC25922DQ), *Klebsiella pneumoniae* (ATCC11296) and *Pseudomonas aeruginosa* (ATCC10145) were used as Gram-negative bacterial strains. Microbial growth inhibition was tested using agar well diffusion method (Valgas *et al.*,2007; Hasaballah & Elnaggar, 2017). 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).

Antifungal activity of tested extracts

The fungi *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Geotricum candidum* and *Penicillium* sp. strains were used. All tested microorganisms were supplied by the Microbiology Department, Faculty of Science, Jazan University, KSA. Sucrose-Nitrate agar medium gm/L consisted of: Sucrose, $NaNO_3$, K_2HPO_4, 1.0 $mgSO_4$, $7H_2O$ and distilled water, 1000 ml was used in this test. The pH value was adjusted to 7-7.3 before sterilization (Tadashi, 1975). The detection of inhibitory clear zone around the paper disks is an indication of the antagonistic properties of the extracts under evaluation.

Antiviral assay

Determination of samples cytotoxicity on VERO cell

Petroleum ether extract was used to determine the anti-HSV activity. Growth medium was decanted from 96 well micro titer plates after confluent sheet of VERO cell formed. Plate was incubated at 37°C and examined frequently for up to 2days. Cells were checked for any physical signs of toxicity. A 20µl of MTT solution was added to each well. Incubation for 1-5h was done to allow the MTT metabolism. The media dumped off. Plate dried on paper towels to remove residue. Formazan (MTT metabolic product) re-suspend in 200µl DMSO, Placed on a shaking table, 150 rpm for 5 minutes, to thoroughly mix formazan into the solvent. The optical density was read at 560nm and subtracts background at 620nm. The maximum non-toxic concentration of each extract was determined and was used for further biological studies.

Antiviral assay (MTT Assay Protocol)

Equal volume (1:1 v/v) of non-lethal dilution of tested extracts was incubated and the virus suspended for 1h. A 100µl from viral/ sample suspension was added, placed on a shaking table, 150rpm for 5 minutes. Incubation at (37°C & 5% CO_2) was done for 1day to allow the virus to take effect. A 2ml of MTT solution per 96 well plates was prepared at 5mg/ml in PBS. A 20µl MTT solution was added to each well, placed on a shaking table, 150rpm for 5 minutes, to thoroughly mix the MTT into the media. The media dumped off. Plate dried on paper towels to remove residue. Formazan (MTT metabolic product) re-suspend in 200µl DMSO, placed on a shaking table, 150rpm for 5 minutes, to thoroughly mix Formazan into the solvent. The optical density was read at 560nm and subtracts background at 620nm. Optical density should be directly correlated with cell quantity.

Statistical analysis

The statistical analysis of the data obtained was done according to Armitage, (1974) and Lentner *et al.*(1982) and the analysis was revised by Quattro-pro for windows program version 2.0 Microsoft, windows version 7.0, graphics were drawn using Harvard Graphics program version 4.0. The obtained data were assessed by calculation of mean (M), standard deviation (SD) and student t-test.

RESULTS

The blowfly, *Chrysomya albiceps*

Antimicrobial activity using well diffusion method

Antibacterial activity

Data given in table (1) and figures (1A-1C) represent the effect of petroleum ether, hexane, acetone and ethyl acetate of *C. albiceps* maggots' whole body extracts against different Gram-positive bacterial strains.

Table 1: Antibacterial activity (indicated by growth-inhibition zone) of *Chrysomya albiceps* maggots' extracts against different strains of Gram-positive bacteria.

Bacteria	Gram +/-	Growth-inhibition zone in mm caused by extracts				Standard (Ampicillin)
		Petroleum ether	Hexane	Acetone	Ethyl acetate	
Staphylococcus aureus	+ve	18.5±0.45^{d}	17.0±0.50^{d}	15.8±0.68^{d}	16.0±0.52^{d}	27.6±0.22^{a}
Staphylococcus pyogenes	+ve	17.5±0.40^{d}	16.4±0.40^{d}	NA	NA	25.8±0.14^{a}
Bacillus subtilis	+ve	18.2±0.25^{d}	16.9±0.44^{d}	16.0±0.49^{d}	17.6±0.40^{d}	28.2±0.33^{a}

All data represented as Mean ± SD; NA: No Activity; Means followed by the same letters aren't statistically significant (P>0.05).

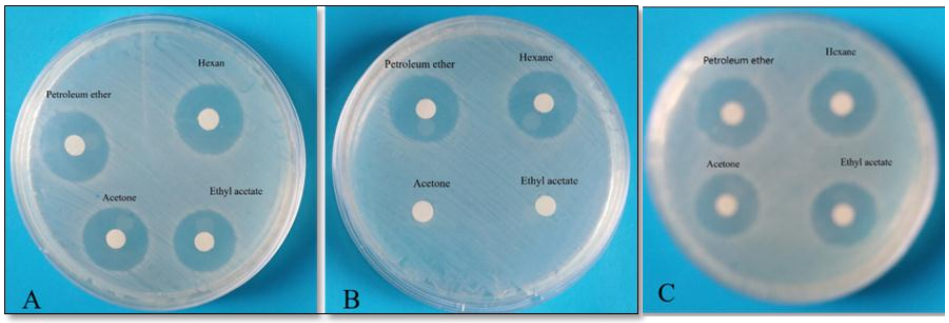

Fig. 1: Antibacterial activity indicated by growth-inhibition zone of *Chrysomya albiceps* maggots' different crude extracts against Gram-positive bacteria. (A: *Staphylococcus aureus*; B: *Staphylococcus pyogenes*; C: *Bacillus subtilis*)

Results show that, the highest antibacterial activity was recorded by petroleum ether against *S. aureus* with growth-inhibition zone of (18.5±0.45mm), vs. (27.6±0.22mm) for the standard antibiotic (Ampicillin). Meanwhile, petroleum ether and hexane extracts recorded antibacterial activity against *S. pyogenes* with growth-inhibition zones of (17.5±0.40mm and 16.4±0.40mm); respectively, compared with (25.8±0.14mm) for the standard antibiotic (Ampicillin).

Table 2: Antibacterial activity of *Chrysomya albiceps* maggots' extracts against different strains of Gram-negative bacteria.

Bacteria	Gram +/-	Growth-inhibition zone in mm caused by extracts				Standard *(Gentamycin)*
		Petroleum ether	Hexane	Acetone	Ethyl acetate	
Escherichia coli	-ve	17.3±0.40^{d}	16.8±0.40^{d}	NA	16.5±0.50^{d}	27.6±0.10^{a}
Klebsiella pneumoniae	-ve	16.8±0.25^{d}	16.0±0.57^{d}	NA	NA	25.2±0.12^{a}
Pseudomonas aeruginosa	-ve	NA	NA	NA	NA	22.3±0.16^{a}

See foot note of Table 1

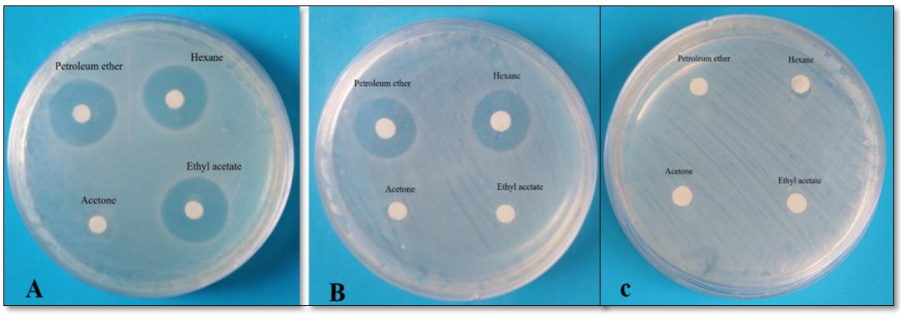

Fig. 2: Antibacterial activity indicated by growth-inhibition zone of *Chrysomya albiceps* maggots' different crude extracts against Gram-negative bacteria. (A: *Escherichia coli*; B: *Klebsiella pneumoniae*; C: *Pseudomonas aeruginosa*).

On the other hand, data given in Table (2) and illustrated in Figures (2A-2C) show that petroleum ether, hexane, and ethyl acetate extracts induced potent antibacterial activity against *E. coli* with growth-inhibition zones of (17.3±0.40, 16.8±0.40 and 16.5±0.50mm); respectively, compared with (27.6±0.10mm) for the standard antibiotic (Gentamycin). In addition, petroleum ether and hexane extracts exhibited antibacterial activity against *k. pneumoniae* equal to (16.8±0.25 and 16.0±0.57mm); respectively, compared with (25.2±0.12mm). All tested extracts recorded no activity against *P. aeruginosa*.

Antifungal activity

Regarding the antifungal activity, data given in Table (3) and illustrated in Figures (3A-3E) exhibit that petroleum ether, hexane, acetone extracts of *C. albiceps* maggots' whole body showed growth-inhibitory effect against *A. flavus* and *G. candidum* (17.6±0.46, 17.0±0.58, 16.4±0.60 and 19.7±0.43, 18.0±0.70, 14.6±0.51 mm); respectively, compared with (Amphotericin B).

Table 3: Antifungal activity of *Chrysomya albiceps* maggots' 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	17.6±0.46 [d]	17.0±0.58[d]	16.4±0.60[d]	NA	24.6±0.29[a]
Aspergillus fumigatus	16.5±0.44[d]	15.8±0.52[d]	NA	15.0±0.44[d]	25.8±0.17[a]
Candida albicans	17.7±0.73[d]	16.8±0.36[d]	NA	NA	21.6±0.14[a]
Geotricum candidum	19.7±0.43[d]	18.0±0.70[d]	14.6±0.51[d]	NA	23.0±0.10[a]
***Penicillium* sp.**	NA	NA	NA	NA	24.0±0.20[a]

See foot note of Table 1

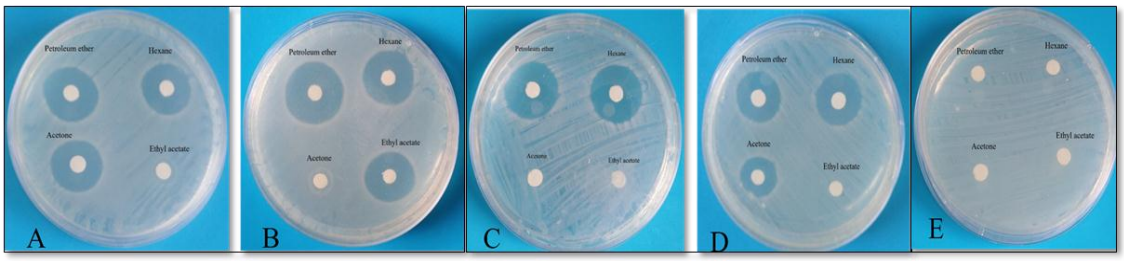

Fig. 3: Antifungal activity indicated by growth-inhibition zone of *Chrysomya albiceps* maggots' different crude extracts against fungi strains. (A: *Aspergillus flavus*; B: *Aspergillus fumigatus*; C: *Candida albicans*; D: *Geotricum candidum*; E: *Penicillium* sp.).

Whereas, petroleum ether, hexane and ethyl acetate extracts recorded growth-inhibition zones of (16.5±0.44, 15.8±0.52 and 15.0±0.44mm) for *A. fumigatus*, compared with 25.8±0.17mm for the standard (Amphotericin B). All tested extracts showed no activity against *Penicillium* sp. Generally, petroleum ether extract showed much activity against all fungal strains tested than those of hexane, acetone and ethyl acetate.

Determination of Minimum Inhibitory Concentration (MIC) by Microdilution Method

The antibacterial activity of *C. albiceps* maggots' crude extracts showed that tested extracts induced the growth of Gram-positive bacteria tested except acetone and ethyl acetate extracts against *S. pyogenes* (Table 4).

Table 4: Antibacterial activity of *Chrysomya albiceps* maggots' extracts as indicated by Microdilution plate at 480nm.

	Bacterial strains	Conc. (mg/ml)	*Chrysomya albiceps* maggots' different extracts			
			Petroleum ether	Hexane	Acetone	Ethyl acetate
G+ve	***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	1.5±0.1[d]	1.7±0.2[d]	1.6±0.5[d]	1.7±0.5[d]
		25.0	1.5±0.4[d]	1.5±0.1[d]	1.7±0.2[d]	1.7±0.4[d]
		12.5	1.4±0.2[d]	1.5±0.3[d]	1.5±0.5[d]	1.6±0.2[d]
	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	1.9±0.1[d]	1.9±0.4[d]	NA	NA
		25.0	1.9±0.2[d]	1.7±0.3[d]	NA	NA
		12.5	1.7±0.4[d]	1.7±0.4[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	2.2±0.6[d]	2.2±0.1[d]	2.3±0.1[c]	2.5±0.1[d]
		25.0	2.2±0.1[d]	2.3±0.2[d]	2.3±0.4[c]	2.5±0.4[d]
		12.5	2.0 ±0.3[d]	2.3±0.5[d]	2.2±0.7[c]	2.3±0.1[d]
G-ve	***Escherichia coli***	**Control**	4.1±0.7[a]	3.9±0.5[a]	3.7±0.6[a]	4.2±0.4[a]
		50.0	2.4±0.1[d]	2.4±0.2[d]	NA	2.5±0.1[d]
		25.0	2.2±0.2[d]	2.4±0.1[d]	NA	2.3±0.4[d]
		12.5	2.2±0.2[d]	2.3±0.4[d]	NA	2.4±0.2[d]
	Klebsiella pneumoniae	**Control**	4.1±0.7[a]	3.9±0.5[a]	3.7±0.6[a]	4.2±0.4[a]
		50.0	2.5±0.4[d]	2.6±0.2[d]	NA	NA
		25.0	2.4±0.4[d]	2.5±0.1[d]	NA	NA
		12.5	2.3±0.3[d]	2.5±0.3[d]	NA	NA
	Pseudomonas aeruginosa	**Control**	4.1±0.7[a]	3.9±0.5[a]	3.7±0.6[a]	4.2±0.4[a]
		50.0	NA	NA	NA	NA
		25.0	NA	NA	NA	NA
		12.5	NA	NA	NA	NA

See foot note of Table 1

The lowest MIC value (12.5mg/ml) was recorded with petroleum ether extract against *B. subtilis*. Concerning the antibacterial activity against Gram-negative bacteria, acetone extract recorded no activity at all. Ethyl acetate extract has no activity against *K. pneumoniae* and *P. aeruginosa*. Also, all tested extracts have no activity against *P. aeruginosa* (table 4). The lowest MIC value was recorded by petroleum ether extract against *E. coli* at 25.0mg/ml.

Antiviral Assay

The antiviral activity of *C. albiceps* maggots' petroleum ether extracts against Herpes simplex virus (HSV-1) was tested and the maximum non-toxic concentration (MNTC) was determined. The MNTC of tested *C. albiceps* extract recorded 39.06µg/ml. The obtained results revealed that, the tested extract was effective as anti-HSV (Table 5) and Figure (4).

Table 5: Antiviral activity of tested maggots' petroleum ether extracts against Herpes simplex virus (HSV-1) using methyl thiazolyltetrazolium (MTT) assay protocol.

Test	Conc. (µg/ml)	O.D			Mean O.D	Viability	Toxicity	Viral activity %	Anti-viral effect %
Control Vero	--	0.272	0.295	0.282	0.283	100	0	-	-
HSV 1	--	0.112	0.124	0.123	0.119667	42.28504	57.71496	100	**0**
C. albiceps	39.06	0.278	0.284	0.264	0.275333	97.29093	2.709069	4.693874	**95.3061258**
M. domestica	2.44	0.154	0.152	0.166	0.157333	55.59482	44.40518	76.93872	**23.06127945**

Fig. 4: Activity of maggots' petroleum ether extracts against HSV-1 at 39.06µg/ml. (A) *Chrysomya albiceps* maggots' extract; (B) *M. domestica* maggots' extract, and (C) Control.

The house fly, *Musca domestica*

Antimicrobial activity using well diffusion method

Antibacterial activity

Antibacterial activity results of *M. domestica* maggots' whole body extracts showed that the highest activity against *S. aureus* was recorded by petroleum ether extract (13.6±0.36mm). Petroleum ether and hexane extracts showed growth-inhibition zones of (14.0±0.52, 15.5±0.47mm) against *B. subtilis*; respectively, compared with (28.2±0.33mm) for the standard (Ampicillin), whereas all extracts exhibited no activity against *S. pyogenes* (Table 6) and figures (5A-5C).

Table 6: Antibacterial activity as indicated by growth-inhibition zone of *Musca domestica* maggots' extracts against different strains of Gram-positive bacteria.

Bacteria	Gram +/-	Growth-inhibition zone in mm caused by extracts				Standard *(Ampicillin)*
		Petroleum ether	Hexane	Acetone	Ethyl acetate	
Staphylococcus aureus	+ve	13.6 ±0.36 [d]	12.2±0.50[d]	11.0±0.22[d]	12.7±0.47[d]	27.6±0.22[a]
Staphylococcus pyogenes	+ve	NA	NA	NA	NA	25.8±0.14[a]
Bacillus subtilis	+ve	14.0±0.52[d]	15.5±0.47[d]	NA	NA	28.2±0.33[a]

See foot note of Table 1

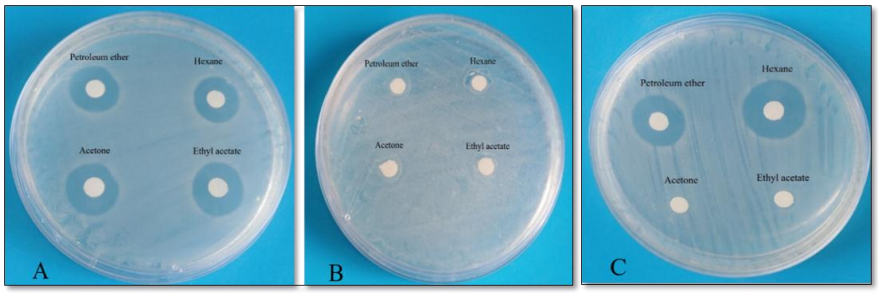

Fig. 5: Antibacterial activity indicated by growth-inhibition zone of *Musca domestica* maggots' different crude extracts against Gram-positive bacteria. (A: *Staphylococcus aureus*; B: *Staphylococcus pyogenes*; C: *Bacillus subtilis*).

On the other hand, tested extracts showed antibacterial activity against *E. coli* equal to (14.3±0.57, 13.0±0.51 and 12.0±0.36mm) for petroleum ether, hexane and ethyl acetate extracts; respectively. While, petroleum ether extract recorded activity against *K. pneumonia* with growth-inhibition zone of (14.8±0.44mm), compared with (25.2±0.12mm) for the standard antibiotic (Gentamycin). Tested extracts exhibit no activity against *P. aeruginosa* tested, (Table 7) and figures (6A-6C).

Table 7: Antibacterial activity as indicated by growth-inhibition zone of *Musca domestica* maggots' extracts against different strains of Gram-negative bacteria.

Bacteria	Gram +/-	Growth-inhibition zone in mm caused by extracts				Standard *Ampicillin*
		Petroleum ether	Hexane	Acetone	Ethyl acetate	
Escherichia coli	-ve	14.3±0.57[d]	13.0±0.51[d]	NA	12.0±0.36[d]	27.6±0.10[a]
Klebsiella pneumoniae	-ve	14.8±0.44[d]	NA	NA	NA	25.2±0.12[a]
Pseudomonas aeruginosa	-ve	NA	NA	NA	NA	22.3±0.16[a]

See foot note of Table 1

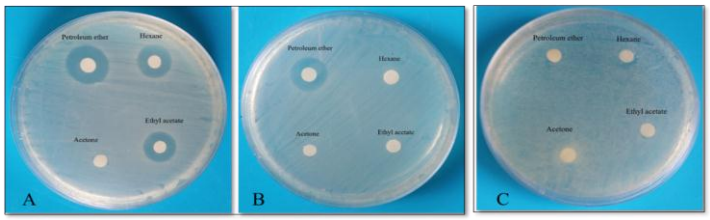

Fig. 6: Antibacterial activity indicated by growth-inhibition zone of *Musca domestica* maggots' different crude extracts against Gram-negative bacteria. (A: *Escherichia coli*; B: *Klebsiella pneumoniae*; C: *Pseudomonas aeruginosa*).

Antifungal activity

As shown from the results given in Table (8) and illustrated in Figures (7A-7E), both petroleum ether and hexane extracts of *M. domestica* maggots' exhibited growth-inhibition zones against *A. flavus* of (12.7±0.24, and 12.0±0.58mm) respectively, compared with (24.6±0.29mm) for the standard (Amphotericin B).

Table 8: Antifungal activity as indicated by growth-inhibition zone of *Musca domestica* maggots' extracts against different strains of fungi.

Fungi	Growth-inhibition zone in mm caused by extracts				Standard
	Petroleum ether	Hexane	Acetone	Ethyl acetate	*(Amphotericin B)*
Aspergillus flavus	**12.7±0.24^{d}**	**12.0±0.58^{d}**	**NA**	**NA**	**24.6±0.29^{a}**
Aspergillus fumigatus	**11.4±0.36^{d}**	**10.7±0.70^{d}**	**9.4±0.34^{d}**	**NA**	**25.8±0.17^{a}**
Candida albicans	**12.1±0.35^{d}**	**NA**	**NA**	**NA**	**21.6±0.14^{a}**
Geotricum candidum	**13.2±0.50^{d}**	**12.±0.40^{d}**	**NA**	**NA**	**23.0±0.10^{a}**
***Penicillium* sp.**	**NA**	**NA**	**NA**	**NA**	**24.0±0.20 ^{a}**

See foot note of Table 1

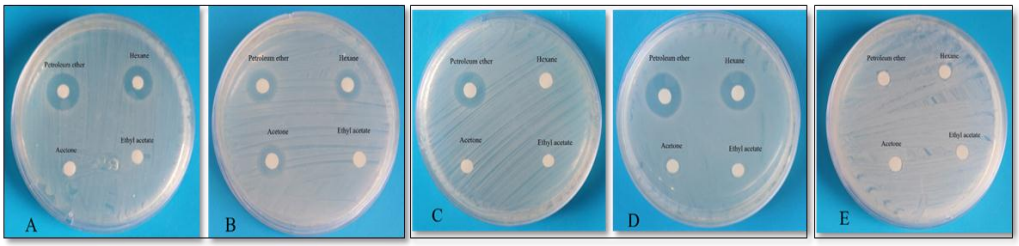

Fig. 7: Antifungal activity indicated by growth-inhibition zone of *Musca domestica* maggots' different crude extracts against fungi strains. (A: *Aspergillus flavus*; B: *Aspergillus fumigatus*; C: *Candida albicans*; D: *Geotricum candidum*; E: *Penicillium* sp.).

The highest growth-inhibition zone against *A. fumigatus* (11.4±0.36mm) was recorded by petroleum ether. On the other hand, ethyl acetate extract didn't show any activity against all tested fungi species.

Determination of Minimum Inhibitory Concentration (MIC) by Microdilution Method

None of the tested extracts recorded antibacterial activity against *S. pyogenes*. Acetone and ethyl acetate extracts of *M. domestica* maggots' showed no activity against *B. subtilis*. The lowest MIC value 50.0mg/ml was recorded by petroleum ether, hexane, and ethyl acetate extracts against *S. aureus*, while the same value was obtained for petroleum ether and hexane extracts against *B. subtilis*. On the other hand, petroleum ether extract showed activity against *E. coli* and *K. pneumoniae*, while all tested extracts recorded no activity against *P. aeruginosa*. The lowest MIC value 50.0mg/ml was recorded by petroleum ether and hexane against *E. coli* and petroleum ether against *K. pneumoniae* (Table 9).

Table 9: Antibacterial activity of *Musca domestica* maggots' extracts as indicated by Microdilution plate at 480nm.

Bacterial strains		Conc. (mg/ml)	*Musca domestica* maggots' different extracts			
			Petroleum ether	Hexane	Acetone	Ethyl acetate
G+ve	***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	3.2±0.1[d]	3.2±0.4[b]	3.3±0.2[a]	3.3±0.5[a]
		25.0	3.2±0.2[d]	3.1±0.1[b]	3.2±0.6[a]	3.3±0.2[a]
		12.5	2.9±0.2[d]	3.1±0.4[b]	3.0±0.2[b]	3.2±0.1[a]
	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	NA	NA	NA	NA
		25.0	NA	NA	NA	NA
		12.5	NA	NA	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	3.2±0.1[c]	3.4±0.2[b]	NA	NA
		25.0	3.2±0.6[c]	3.1±0.1[b]	NA	NA
		12.5	3.1±0.2[c]	3.1±0.5[b]	NA	NA
G-ve	***Escherichia coli***	**Control**	4.1±0.7[a]	3.9±0.5[a]	3.7±0.6[a]	4.2±0.4[a]
		50.0	3.3±0.4[b]	3.2±0.2[c]	NA	3.6±0.1[a]
		25.0	3.3±0.6[b]	3.4±0.1[c]	NA	3.4±0.3[b]
		12.5	3.2±0.4[b]	3.2±0.1[c]	NA	3.4±0.1[b]
	Klebsiella pneumoniae	**Control**	4.1±0.7[a]	3.9±0.5[a]	3.7±0.6[a]	4.2±0.4[a]
		50.0	3.5±0.1[b]	NA	NA	NA
		25.0	3.4±0.4[b]	NA	NA	NA
		12.5	3.4±0.2[b]	NA	NA	NA
	Pseudomonas aeruginosa	**Control**	4.1±0.7[a]	3.9±0.5[a]	3.7±0.6[a]	4.2±0.4[a]
		50.0	NA	NA	NA	NA
		25.0	NA	NA	NA	NA
		12.5	NA	NA	NA	NA

See foot note of Table 1

Antiviral Assay

The antiviral activity of *M. domestica* maggots' petroleum ether extracts against Herpes simplex virus (HSV-1) was tested and the maximum non-toxic concentration (MNTC) was determined. The MNTC of tested *M. domestica* extract recorded 2.44µg/ml. The obtained results revealed that, the tested extract was effective as anti-HSV (Table 5 and Fig. 4).

DISCUSSION

There is already a long history of use of insects in Folk Medicine (Ratcliffe *et al.*2014). The use of insects in Folk Medicine encourage the scientists to develop potential new medicines for treating serious diseases such as viral infections and problems associated with the newly emerging antibiotic-resistance. The present study aimed at evaluate the antimicrobial activity of *C. albiceps* and *M. domestica* maggots' whole body extracts using Well diffusion and Microdilution methods, and to determine the activity of these maggots' petroleum ether extract as anti-HSV-1.

Insects are known to possess well developed immune system that forms a potent defense against any invading bacteria (Gotz and Boman, 1985; kimbrell, 1991; Hasaballah 2018). 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).

In this study, the antibacterial activity results showed that, tested extracts evoked a variable activity against both Gram-positive bacteria and Gram-negative bacteria depending on the solvent used in extraction. Generally, petroleum ether was the most effective against different bacteria species followed by hexane, ethyl acetate and acetone extract. Also, Gram-positive bacterial strains were more sensitive to the tested maggots' extracts than Gram-negative bacterial strains. Similar results were

observed by Leem *et al.* (1999) using, *Acantholyda parki* isolates as a broad antibacterial spectrum against both Gram-negative and Gram-positive bacteria.

Insects body produce combinations of antibacterial peptides in response to natural infection leading to a broad spectrum activity against micro-organisms (Yamauchi, 2001). 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 this study, the antibacterial activity of *C. albiceps* maggots' extract was more effective than those of *M. domestica,* in the same context Thomas *et al.* (1999), concluded that antibacterial effect of different extracts was arranged as the following: *Lucilia sericata* maggots' extracts was more effective than *C. albiceps; S. carnaria* and *M. domestica,* where these extracts were able to decrease the total bacterial count of *S. aureus* in vitro and to combat clinical infections in a variety of wound types including these caused by antibiotic-resistant strains. Such findings may be due to the presence of antibacterial agents in either their body or their secretion/excretion (Amer *et al.* 2019).

On the other hand, tested extracts showed a variable antifungal activity against *A. flavus, A. fumigatus, C. albicans* and *G. candidum* fungal strains with no activity against *Penicillium*. In general, petroleum ether was more effective than those of hexane, ethyl acetate and acetone. However, the present study has shown that the bacterial strains tested were more sensitive to tested extracts used than the fungal strains. In agreement with these results, Meylaers *et al.* (2004) recorded antibacterial activity of *M. domestica* methanolic whole body extract against *Saccharomyces cerevisiae*. Hou *et al.* (2007) stated that, the housefly larvae have higher activity against Gram-positive bacteria than Gram-negative bacteria without any antifungal activity. Similar results was also recorded by Yamada and Natori, (1994) with, *S. peregrine*; Rees *et al* .(1997) using, *B. pascuorum*, Leem *et al.* (1999) using, *A. parki*; Vizioli *et al.* (2001) using the mosquito vector, *Anpheles gambiae;* Cytrynska *et al.* (2007) using, *Galleria mellonella*.

Petroleum ether of tested extracts showed promising activities as antiHSV-1. In agreement, Esser *et al.* (1979) treated murine virus capsid with melittin; Wachinger *et al.* (1998) reduced the viral infectivity with an intracellular action of the peptide taken up into the cells; Baier *et al.*(2000) who found that, nasal application of lipopeptide increased protection against the lethal infection of influenza; Fenard *et al..*, (2001) who found that, the honeybee venom inhibits the replication of T-lymphotropic HIV-1 isolates; Chernysh *et al.* (2002) who tested the activity of alloferon against HSV-1 and Hepatitis B&C infection; Slocinska *et al.* (2008) who stated that, peptides extracted from insects able to cause antiviral action against HSV; Ai *et al.* (2008) who stated that, protein fractions extracted and purified from the housefly larvae possess antiviral activity. Moreover, Amer *et al.*(2019) reported that, insects extracts possess antiviral activity against Hepatitis A Virus (HAV).

CONCLUSION

From the results demonstrated previously it could be concluded that, *C. albiceps and M. domestica* tested maggots' extracts evoked a variable activity against both Gram-positive bacteria and Gram-negative bacteria depending on the solvent used in extraction. Petroleum ether was the most effective against different bacteria species followed by hexane, ethyl acetate and acetone extract. Gram-positive bacterial strains were more sensitive than Gram-negative bacterial strains. Tested extracts also showed a variable antifungal activity. In addition, petroleum ether

extract may play a role as a potential anti-HSV agent due to their promising antiviral activity.

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