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Original article

Antimicrobial and anticancer activities of *Periplaneta americana* tissue lysate: An *in vitro* study

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ABSTRACT

Objective: Cancer has emerged as a life-threatening disease over the past few decades, accounting for a higher rate of mortality. The advancement in cancer therapy has improved the quality of life of patients, however, the chemotherapy/ radiotherapy resistance conferred by the cancer cells emerged as a new threat. It demands the need for novel anticancer molecules from natural products. Animal product-based anticancer therapeutics are emerging as novel drug candidates and they are also highly nutritional. **Methods:** In this study, the cyclohexane extract of the American cockroach, *Periplaneta americana* L., has been evaluated for its inhibitory activities against fungal and bacterial human pathogens; *Candida albicans* and MRSA and its effect as a cancer therapeutic agent.

Results: Results showed that inhibition zones were (8, 9 mm) with minimum inhibitory concentration (MIC) of 62.5 µg/ml and 0.9 µg/ml, for both fungal and bacterial cultures, respectively. The ultrastructural changes of *Candida albicans* and MRSA, after treatment, were observed using a transmission electron microscope (TEM). Cockroach extracted lysate had no cytotoxic effect on normal MRC-5 human-lung cell line with CC₅₀ value = 118 ± 3.4 µg/ml. However, the antitumor activity of Cockroach lysate showed an inhibitory effect against MCF-7 (breast human carcinoma) cell line with IC₅₀ values = 30.2 ± 1.62 µg/ml. Chemical analysis of cockroach extract using the HPLC technique coupled with an ESI mass spectrometer proved that this extract contained high biologically active compounds.

Conclusion: Overall, the study proves the potential of *P. americana* lysate as a potential therapeutic agent against microbial infections and cancer cells.

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1. Introduction

Cancer incidence and mortality show a rapid increase worldwide, making cancer the major cause of death in most regions, it has become one of the leading causes of death in many geographic areas such as Europe, China, and the United States (Mathur et al.,

2020; Torre et al., 2016). Management of cancers is done with the help of a combination of radiotherapy, chemotherapy, and modern immunotherapy. However, these methods could not effectively change the causal interaction of individual factors related to the pathological process (Bower, 2014). In addition, the increased chemotherapy/ radiotherapy resistance conferred by the neoplastic cells often raises concern about the current cancer-treatment regimen (Narayanankutty, 2020; Zhang et al., 2016). Drug resistance is also a complex process involving multiple factors such as the glutathione system of the body; this enables the cancer cells to escape from the therapeutic regimen and thereby reducing the treatment efficacy and increasing the recurrence of the tumor. Thus, it has become essential to have novel strategies and improved therapeutic compounds to manage the multi-drug resistant cancer cells (Zhao et al., 2017).

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Phytochemicals have been widely utilized as the anticancer agents and primary target in drug discovery; however, these compounds are facing serious flaws in terms of bioavailability and half-life in the body. As an alternative, natural secretions from insects and animals have demonstrated potential as alternative medicine therapies (Alves and Alves, 2011; Ratcliffe et al., 2011). Ethanolic extract of the Pieridae family of butterflies led to more than 70% inhibition of tumor growth in albino rats bearing Walker 256 carcinomas (Wang et al., 2011). Similar reports of arthropod extracts as anticancer agents emerged regarding the Asian Rhinoceros beetle, *Allomyrina dichotomus* (Dossey, 2010). Five new phenolic compounds were extracted from the medicinal insect *Blapsyrhynchopetera Fairmaire*, the cytotoxicity of these five compounds was tested against ten common human tumor cell lines and the results showed that these compounds exhibited antitumor activities against tumor cell lines (Xiao et al., 2017). *P. americana* extract showed significant anti-tumor activity that might be related to enhancing immune function in vivo (Zhao et al., 2017). *Periplaneta americana* L., constituents also have favorable tissue-repairing, antibacterial, antitumor, and immune-enhancing activities. This insect had been used in traditional Chinese medicine (Yang and Lee, 2015; Zhao et al., 2017). In addition, *Periplaneta americana* L. was shown to protect the liver, promote blood vessel growth, aid in tissue repair, improve microcirculation, and enhance immunity (Tingshun et al., 2012). This study aimed at exploring *Periplaneta americana* L. full-body extracts for their antimicrobial and anticancer activities against human Breast cancer cell lines.

2. Materials and methods

2.1. Collection and identification of the American cockroaches

Adult cockroaches (n = 50) were collected from sewage pipes, (Giza), Egypt, using bait trapping and active collection methods. The collected samples were classified and identified as *Periplaneta americana* L. using a dichotomous key.

2.2. Organ lysate of cockroach

Collected cockroaches were immobilized after exposure to 4 °C for 15 min., Legs and wings were removed. Extraction was carried out by homogenizing the cockroaches using a tissue grinder (mortar) with two types of solvents (ethanol 95%, and cyclohexane), (10 gm of cockroaches: 100 ml. solvent). The samples were collected and filtered. Finally, lysates were stored at –20 °C for further studies.

2.3. Antimicrobial assay

The antimicrobial activity of the whole lysate was measured against human pathogenic yeast and bacteria; (*Candida albicans* (RCMB005003(1) ATCC[®] 10231[™]) and MRSA (clinical isolate), obtained from the Regional Center for Mycology and Biotechnology Antimicrobial Unit Test Organism, Al-Azhar University, Nasr City, Egypt. respectively and expressed as the diameter of inhibition zones using agar well diffusion method according to the methods of (Narayanankutty et al., 2021b). Briefly, about 20 ml of the medium (using nutrient agar media for testing bacteria and malt extract agar media for testing yeast) was poured into sterile plates (9 cm) and allowed to solidify. 5 mm diameter holes were cut in the agar using a sterile cork borer. Plates were inoculated with 0.5 ml of fixed inoculum of (MRSA and *Candida albicans*). Plates were dried for 30 min. Holes were filled with 100 µl of the prepared lysate dissolved in DMSO. Negative control wells were loaded with DMSO; as it was used as negative control; plates were

left in a cooled refrigerator at 4 °C for one hour for diffusion; then the plates were incubated for 24 h at 37 °C for testing human pathogenic bacteria and 28 °C for testing human pathogenic yeast. At the end of the incubation period, the inhibition zones were measured at three points along the diameter of the plate and the mean was calculated (Narayanankutty et al., 2021b).

2.4. Determination of the MIC

The minimum inhibitory concentrations (MIC) of cockroach extract. After testing against *Candida albicans* and MRSA were measured using the dilution method (Narayanankutty et al., 2021a,b) using two-fold serial dilution starting with 1000 µg/ml. After incubation, the inhibition zones were observed on all of the plates, and the diameter of these zones was measured in millimeters. MIC was determined as the lowest concentration of the lysate giving visible inhibition.

2.5. Evaluation of the cytotoxic effects of insect lysate against human cancer cells

2.5.1. Cell lines and culture medium

Mammalian breast cancer cell line MCF-7 (passage number 25) was obtained from the American Type Culture Collection, (ATCC, Rockville, MD). Chemicals used were Dimethyl sulfoxide (DMSO), MTT, and trypan blue dye purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin, and 0.25% Trypsin-EDTA which were purchased from Lonza (Belgium). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO₂ and were sub-cultured two to three times a week.

2.5.2. Cytotoxic assay

The tumor cell lines were suspended in the medium at a concentration 5x10⁴ cell/well in Corning[®] 96-well tissue culture plates and then incubated for 24 h. The cockroach lysate was then added to 96-well plates (three replicates) to achieve twelve concentrations. Six vehicle controls with media or 0.5% DMSO were prepared for every 96 well plates. After incubating for 24 h, the numbers of viable cells were determined including the untreated controls. All well plates were then incubated at 37 °C and 5% CO₂ for 4 h. Eighty-five µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette, and incubated at 37 °C for 10 min. Then, the optical density of aliquots was measured at 590 nm using the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/Odc)]x100% where Odt is the mean optical density of wells treated with the tested sample and Odc is the mean optical density of untreated cells by using standard MTT assay (Koottasseri et al., 2021; Roy et al., 2016).

The media was removed from the well plate and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 ml of PBS) was added to each well.

The relation between the survived cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the lysate. IC₅₀ required to cause toxic effects against 50% of intact cells, was estimated from graphic plots of the dose–response curve for each concentration using Graph pad Prism software (San Diego, CA. USA).

2.5.3. Microscopic observation of the tumor cells treated with cockroach lysate

After the end of the treatment, the plates were inverted to remove the medium, the wells were washed three times with 100 μ l of phosphate-buffered saline (pH 7.2) and then the cells were fixed to the plate with 10% formalin for 15 min. at room temperature. The fixed cells were stained with crystal violet for 20 min. The cellular morphology was observed using an inverted microscope (CKX41; Olympus, Japan) equipped with a digital microscopy camera to capture the images representing the morphological changes compared to control cells.

2.6. Transmission electron microscopy preparation

Cells; *Candida albicans*, MRSA, and MCF-7 (human carcinoma) were centrifuged at 4000 rpm for 10 min from 24 hrs old cultures grown on nutrient broth media and washed with distilled water; then residual cells were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.0) for 2 hrs at room temperature, rinsed in the same buffer, and postfixed in 1% osmium tetroxide for 2 h at room temperature. Samples were dehydrated in an ethanol series ranging from 10% to 90% for 15 min in each alcohol dilution and finally with absolute ethanol for 30 min. Through a graded sequence of epoxy resin and acetone infiltrations, samples were eventually penetrated in pure resin. On copper grids, ultrathin sections were collected. The sections were then dyed twice with uranyl acetate and lead citrate, at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Egypt. Stained slides were examined at 80 kV using a JEOL-JEM 1010 transmission electron microscope (Amin, 2016; Amin et al., 2020).

2.7. Liquid Chromatography-Mass Spectrometry (LC-MS), separation and analysis

HPLC analysis coupled with an ESI mass spectrometer as a detector allows the simultaneous isolation of the compounds of cockroach lysate with the determination of the molecular weight of the isolated peaks.

Cyclohexane extracted lysates of the American cockroaches were subjected to LC-ESI-MS analysis (Center of Drug Discovery and Development Research, Faculty of Pharmacy, Ain Shams University) for the identification of homologous compounds through Agilent Mass Hunter software, while keeping in view compensation needed for charges in positive ESI MS as well as electron fragmentations, to ensure searches for the correct parent mass, to obtain the chromatograms and the prospective mass spectra of every separated fraction of the mixture of compounds.

2.8. Identification of the separated compounds

Cyclohexane-extracted lysates of cockroaches were subjected to LC-ESI-MS analysis as described above, to obtain the chromatograms and the prospective mass spectra of every separated fraction of the mixture of compounds. The MS spectra for the compounds present in cyclohexane extracted lysates were run against the NIST Mass Spectral Search Program-2009 version 2.0f for the identification of homologous compounds through Agilent Mass Hunter software, while keeping in view compensation needed for charges in positive ESI MS as well as electron fragmentations, to ensure searches for the correct parent mass.

3. Results

3.1. Antibacterial potential of cockroach extracts

The cyclohexane and ethanol extracts of the whole cockroach are found to have significant antibacterial properties. Among these, the cyclohexane extract had significantly higher potential against *S. mutans*, *E. cloacae*, and MRSA. Ethanol extract showed antibacterial activity against MRSA alone. None of these extracts were effective against *S. enterica* (Table 1).

3.2. Antifungal activity

The antifungal activity of the cockroach extracts was analyzed against *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *C. albicans*, and *P. italicum*. Cyclohexane extract was most effective one with highest activity against *C. albicans*; it was also effective against all other fungi, except for *A. niger*. On contrary, the ethanol extract was effective against *A. niger* and *A. fumigatus* (Table 2).

3.3. Determination of MIC against microbial strains

The MIC values of cockroach lysate were estimated against *C. albicans* and MRSA strains. The activity was higher against MRSA compared to the *C. albicans* (Table 3; Fig. 3).

3.4. Cytotoxic activity on breast cancer cells

As shown in Fig. 4, there observed a dose-dependent cytotoxic activity for the *P. Americana* extract with an IC_{50} value of 30.2 ± 1.62 μ g/ml. On contrary, the toxicity against normal human lung fibroblast cells was significantly lower (IC_{50} value = 118 ± 3.4 μ g/ml).

3.5. Pathological changes after treatment of MCF-7 cells with cockroach extract

Morphological alteration of MCF-7 cells line upon exposure using *Periplaneta americana* L. extract was observed under an inverted microscope. The crystal-stained cells indicated the number of dead cells which increased correspondingly with the concentration of the lysate. The presence of apoptotic bodies and extensive vacuolation in the cell cytoplasm indicated an autophagy-like mechanism of cell death which was seen in the treated cells. Cell elongation shrinkage and signs of detachment from the surface of the wells denoting cell death (Fig. 5)

3.6. Transmission electron microscopy analysis

The integrated MCF-7 cells in control group are interconnected with many plasma cell membrane extensions with many microvilli, as demonstrated by electron microscopy (Fig. 6-A). The separation of apoptotic bodies from apoptotic cells, the disrupted cell membrane, and the contracted nucleus were all signs of apoptosis in the treated group. Finally, all of the injured cells were lysed (Fig. 6-B).

3.7. Ultrastructural changes in MRSA and *Candida albicans*

In *Candida albicans*, the untreated cells (control) represented a typical morphology of rounded cells with an intact regular cell wall (CW), cell membrane (CM), mitochondria (M), nucleus (N), and vacuole (V) with a uniform central cytoplasmic density (Fig. 7A), while, the affected cells lost their integrity, aggregation of the cytoplasmic contents and large detachment of cell membrane from cell

Table 1

Inhibitory effects of cockroach extracts (using cyclohexane and ethanol) against Gram-positive and Gram-negative bacteria.

Extract	<i>Streptococcus mutans</i>	<i>Enterobacter cloacae</i>	MRSA	<i>Salmonella enterica</i>
Cyclohexane extract	15 ± 0.4 mm	10 ± 0.2 mm	28 ± 0.3 mm	–ve
Ethanol extract	–ve	–ve	20 ± 0.1 mm	–ve

*Calculated mean is for triplicate measurements ± SD.

Table 2

Inhibitory effects of cockroach extracts (using cyclohexane and ethanol) against human pathogenic fungi.

Extract	<i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus flavus</i>	<i>Candida albicans</i>	<i>Penicillium italicum</i>
Cyclohexane extract	–ve	18 ± 0.2 mm	13 ± 0.4 mm	27 ± 0.2 mm	15 ± 0.3 mm
Ethyl alcohol 95% extract	11 ± 0.2 mm	15 ± 0.1 mm	–ve	–ve	–ve

In all experiments, diameters were calculated by mm, without subtracting the diameter of the well-diameter (5 mm), the calculated mean is for triplicate measurements ± SD.

Table 3Determination of minimal inhibitory concentration (MIC) of organ lysate of *Periplaneta americana* L.

MIC (µg/ml) inhibition zones (mm)			
Test organisms			
No.	Conc. (µg/ml)	<i>Candida albicans</i>	MRSA
1	1000	27	28
2	500	23	26
3	250	17	26
4	125	12	23
5	62.5	9	18
6	31.25	00	15
7	15.6	00	14
8	7.8	–	11
9	3.9	–	10
10	1.9	–	9
11	0.9	–	8
12	0.45	–	00
13	0.22	–	00

wall that had increasing thickness and irregularity were observed (Fig. 7B). Untreated control of *Staphylococcus aureus* (MRSA) cells showed uniform cytoplasmic density, a spherical shape, and a rigid surface with intact cell membranes (Fig. 7C), however, cells treated with pure compound showed detachment from the cell wall, translucent cytoplasm, shrunken, misshapen cells and completely deformation of the cells (Fig. 7D).

3.8. Fractionation and identification of compounds using LC-MS

Body extract of the American cockroaches was prepared using cyclohexane and subjected to LC-MS, for qualitative analyses. thirty compounds were separated based on the *m/z* ratio and retention time in the column. The identified compounds are listed in Table 4.

4. Discussion

Natural products, especially plant extract has been the major source of bioactive compounds with pharmacological and biological potentials (Shweta and Arunaksharan, 2017; Vinayak et al., 2019). Apart from these, the utility of animal-derived compounds is also emerging in the past few decades (Abd El-Sattar et al., 2021). *Periplaneta americana* has been extensively studied for its uncommon ability to resist environmental threats, which has contributed to its evolutionary presence for more than 300 million years. Insect extracts are traditionally used in many diseases' treatments, including the treatment of arthritis and diabetics (Srivastava et al., 2009). Insect larval extracts have been also used in folklore medicine in different parts of the world (Hosni et al.,

Table 4

The main components identified from cyclohexane cockroach extract using LC-MS analysis.

No.	Compounds	Formula
1	1-(<i>S,S</i>)-1,1-Bis(ethoxycarbonyl)-2-bis-(<i>p</i> -tolylsulfanyl)-1-ethanol	C ₂₂ H ₂₆ O ₇ S ₂
2	1-Allyl(2-nitro-1-phenylethyl)sulfane	C ₁₁ H ₁₃ NO ₂ S
3	2-(2-Ethyl-3- <i>tert</i> -butoxyaminoquinazolin-4(3 <i>H</i>)-one	C ₁₄ H ₁₉ N ₃ O ₂
4	7-(Isopropoxy)-2,2,5-trimethylchromene	C ₁₅ H ₂₀ O ₂
5	5-Ethyl-6,7-dihydro-5 <i>H</i> cyclopentapyrazine	C ₈ H ₁₂ N ₂
6	Di-isopropyl 2-phenyl ethenyl phosphonate	C ₁₄ H ₂₁ O ₃ P
7	(<i>E</i>)-3-Benzylidene-5 methyl-dihydrofuran-2(3 <i>H</i>)-one	C ₁₂ H ₁₂ O ₂
8	2,5-Dihydroxy-4'-methoxy-flavanone	C ₁₆ H ₁₄ O ₅
9	4,4-Dimethyl-3-oxacholest-5-en-7-one	C ₂₈ H ₄₆ O ₂
10	5-Methyl-2-(thiophen-2-yl) pyridine	C ₁₀ H ₉ NS
11	(2 <i>S</i> ,5 <i>R</i> , <i>R</i> _s)-2-Allyl-5-methyl-2-(<i>p</i> -tolylsulfanyl)methyl tetrahydro-pyran	C ₁₇ H ₂₂ O ₂ S
12	(<i>E</i>)-3-Benzylidene-5 methyl-dihydrofuran-2(3 <i>H</i>)-one	C ₁₆ H ₁₆ N ₂ S
13	2-Hydroxy-2-methylhexanoic acid	C ₇ H ₁₄ O ₃
14	2,7,12,17-Tetraethyl-3,8,13,18-tetramethyl-21 <i>H</i> ,23 <i>H</i> -porphyrin copper	C ₃₂ H ₃₆ Nu
15	2-Methyl-6-(3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1 <i>H</i> cyclopenta[<i>a</i>]phenanthren-17-yl) heptanoic acid	C ₂₇ H ₄₆ O
16	2-Ethyl-3- <i>tert</i> -butoxyaminoquinazolin-4(3 <i>H</i>)-one	C ₁₄ H ₁₉ N ₃ O ₂
17	Dimethyl alpha-(<i>N</i> acetylamino)benzyl phosphonate	C ₁₁ H ₁₆ NO ₄ P
18	2-(Allylthio)-1-nitro-2-phenylethane $\text{S} \text{S} [1-(allylsulfanyl)-2-nitroethyl] \text{ benzene}$	C ₁₁ H ₁₃ NO ₂ S
19	Methyl 5-formyl-4-methoxycarbonylmethyl-2-methylpyrrole-3-propionate	C ₁₃ H ₁₇ NO ₅
20	1-Oxa-2-oxo-5,5,8-trimethyl-8-phenylbicyclo [3.5.0 (5 <i>a</i> ,8 <i>a</i>)] dec-5(4 <i>a</i>)-ene	C ₁₈ H ₂₂ O ₂
21	(<i>E</i>)-6,8-Dimethyl-1-phenyl-3-styryl-1 <i>H</i> -[1,2,4] triazol [3,4- <i>f</i>] purine-5,7(6 <i>H</i> ,8 <i>H</i>) dione	C ₂₂ H ₁₈ N ₆ O ₂
22	2-(3,6-Dihydro-pyridin-2-yl)-5,6,7,8-tetrahydro Spiro [benzo [d][1,3]thiazine 4,1'-cyclohexane]	C ₁₈ H ₂₂ N ₂ S
23	2-Methoxy-2-methyl octanoic acid	C ₁₀ H ₂₀ O ₃
24	4,4 - (Iminomethylene)bis (<i>N</i> , <i>N</i> -dimethylaniline)	C ₁₇ H ₂₁ N ₃
25	4-Phenyl-3-(phenylamino)-1 <i>H</i> -1,2,4-triazole-5(4 <i>H</i>)-thione	C ₁₄ H ₁₂ N ₄ S
26	Thiomorpholine	C ₄ H ₉ NS
27	6 <i>a</i> -Hydroxy-4-methoxy-6 <i>a</i> ,6 <i>b</i> ,7,8,9,10,10 <i>a</i> ,10 <i>b</i> -octahydro-6 <i>H</i> benzo[3,4]cyclobuta[1,2- <i>c</i>] chromen-6-one	C ₁₆ H ₁₈ O ₄
28	3-(2'-Phenylethyl)-2,3-dihydro-6-phenyl-2,4-dioxo-4 <i>H</i> -1,3-thiazine	C ₁₈ H ₁₅ NO ₂ S
29	3,9-Dimethoxy-12,13-dihydro-5 <i>H</i> indolo [2,3- <i>a</i>] pyrrolo [3,4- <i>c</i>]carbazole-6-benzyl	C ₂₉ H ₂₁ N ₃ O ₄
30	2-Isopropoxy-5-phenyl-1,3,4-oxadiazole	C ₁₁ H ₁₂ N ₂ O ₂

2022; Talbot et al., 2006). Insects can rapidly clear microbial infections by producing varieties of immune-induced molecules including antibacterial and/or antifungal types. With emerging of antimicrobial-resistant pathogens and the passage of the resistance via resistant genes (R-factor) to susceptible microbial individuals, commonly simple infections may become serious leading to death

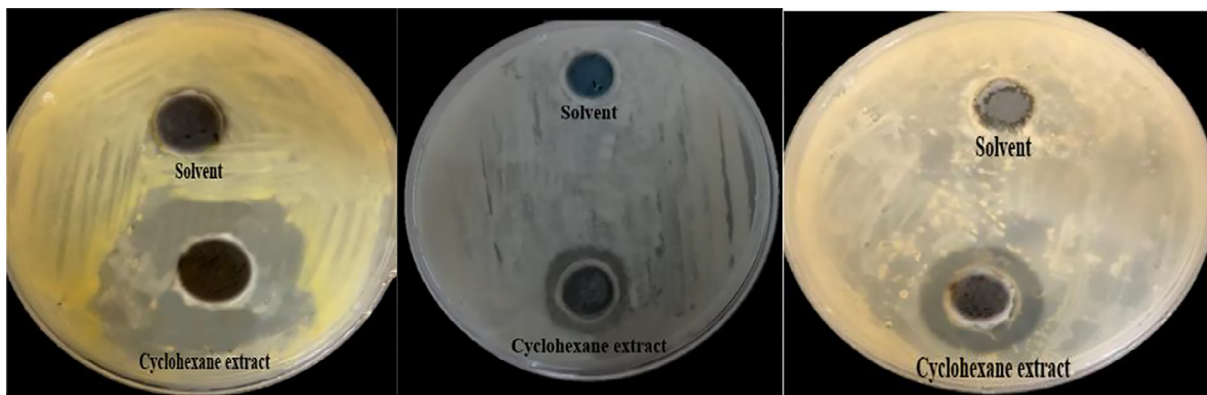


Fig. 1. The inhibitory effect of cyclohexane extract against *Streptococcus mutans*, *Enterobacter cloacae* and Methicillin-Resistant *Staphylococcus aureus* respectively.

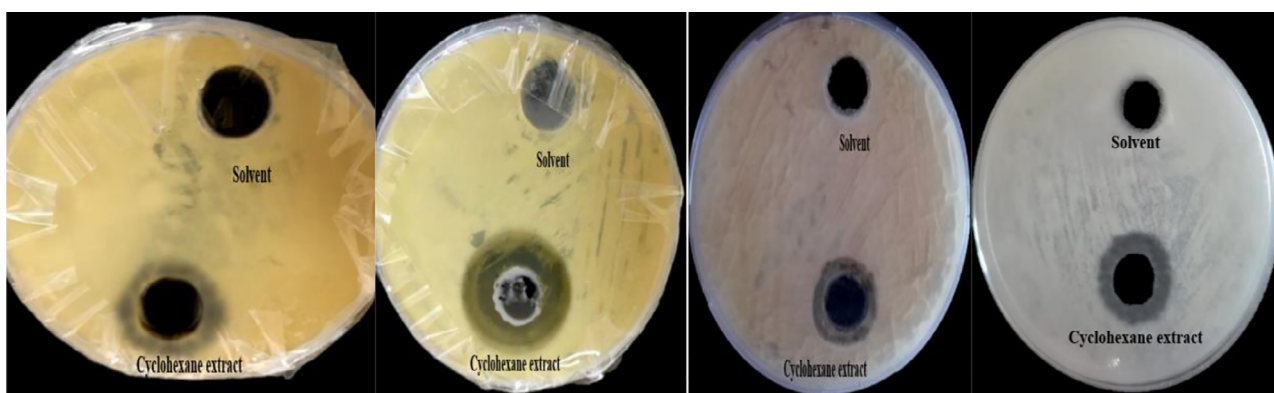


Fig. 2. The inhibitory effect of cyclohexane extract against *Aspergillus fumigatus*, *Aspergillus flavus*, *Candida albicans* and *Penicillium italicum* respectively.

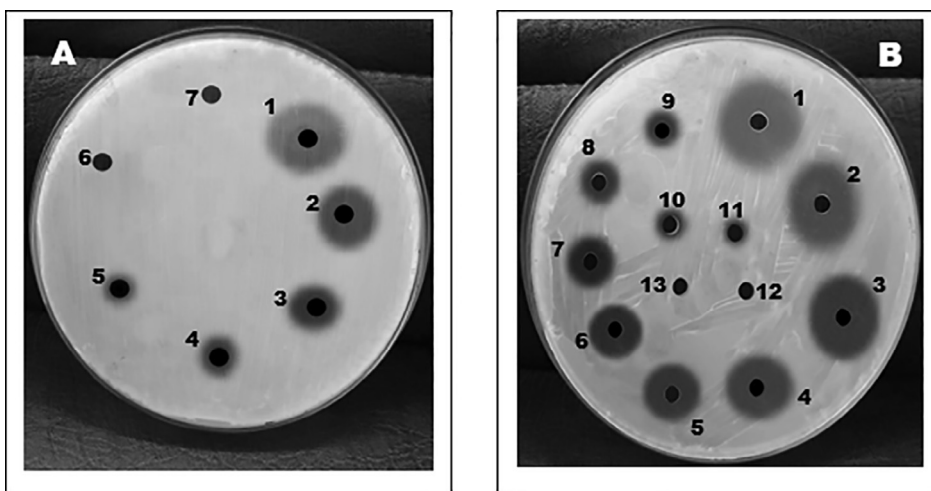


Fig. 3. The minimum inhibitory concentrations (MIC) of pure compound extracted from *Periplaneta americana* L. against *Candida albicans*; (A): 1;1000 µg/ml, 2; 500 µg/ml, 3; 250 µg/ml, 4; 125 µg/ml, 5; 62.5 µg/ml and methicillin resistant *Staphylococcus aureus* (MRSA); (B): 1; 1000 µg/ml, 2; 500 µg/ml, 3; 250 µg/ml, 4; 125 µg/ml, 5; 62.5 µg/ml, 6; 31.25 µg/ml, 7; 15.6 µg/ml, 8; 7.8 µg/ml, 9; 3.9 µg/ml, 10; 1.9 µg/ml and 11; 0.9 µg/ml.

(Nicasio et al., 2008; Nordmann et al., 2007). Antibiotic-resistant microbes continue to elevate at an alarming rate as a health problem facing humanity (Devasahayam et al., 2010; Salzet, 2001). Cockroach extract proved its inhibition activity against Gram-positive and Gram-negative resistant pathogenic bacteria (Table 1, Fig. 1) Such insect-antimicrobial activity was previously docu-

mented by Gordya et al. (2017). Different insect peptides described as defensin, dipterocins, cecropins, and proline proved their activity against bacterial infection (Hedayati et al., 2007). Our results proved the antifungal activity of cockroach extract against different pathogenic fungi and yeast (Fig. 2) as *Aspergillus flavus* which produces aflatoxin B1, the most toxic and potent hepatocarcinogenic

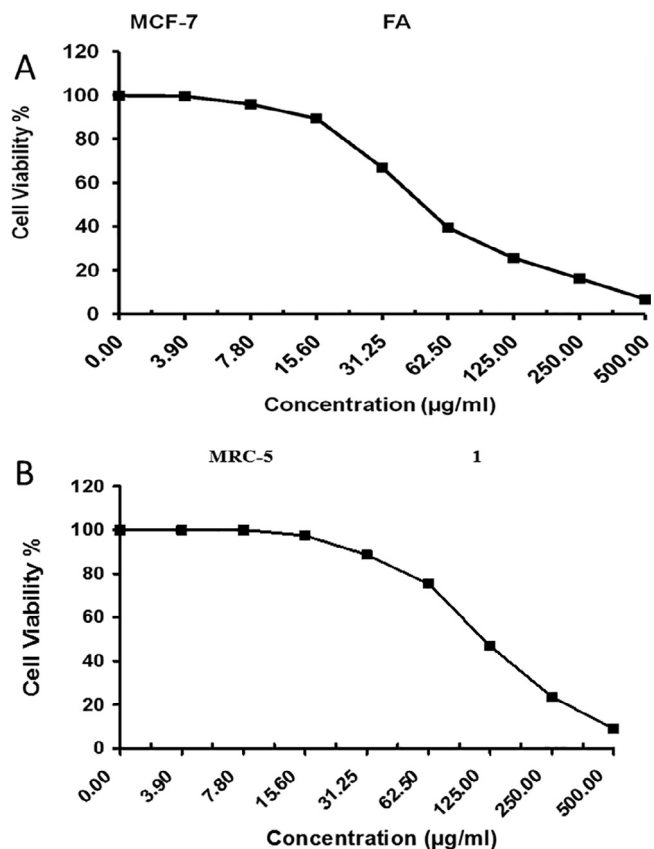


Fig. 4. *In vitro* antitumor activity of different concentrations of cockroach extract against MCF-7 tumor cell lines and MRC-5 cells (normal fibroblast).

natural compound ever characterize (Walsh et al., 2008), *A. fumigatus* which incriminated as the causative pathogen of chronic pulmonary aspergillosis, *A. niger* which produce ochratoxins and the pathogenic *Candida albicans* initiate a wide range of diseases such as chronic disseminated candidiasis, endocarditis, vaginitis, meningitis, and endophthalmitis- and *Penicillium italicum* -attributed to the pathogenesis of pneumonia, hypersensitivity, allergic alveoli, skin sensitivity and emphysema; these pathogenic fungi resist wide range of anti-fungal drugs. The before mentioned results are in agreement with Chernysh et al. (2002), who proved the fungicidal activity of grasshopper gut bacteria against the

yeast-like fungus *Candida*. Treatment of human breast cancer cell line with cockroach lysate recording obvious cell inhibition at IC50 concentrations = $30.2 \pm 1.62 \mu\text{g/ml}$ res which are less than that needed to produce cytotoxicity for normal cells (CC50 = $118 \pm 3.4 \mu\text{g/ml}$). The safety of this lysate and its cheap supply recommend it for therapeutic manufacturing. The lysate can inhibit the growth of tumor cells which lowered the cell adhesion capacity and cell viability in a concentration-dependent manner. *Calliphora* maggots extract at 0.28 mg/ml could inhibit human cancer cells as mentioned by Oliver et al. (1994). The most available antitumor agents are derived from plants, microbes, and animal secondary metabolites (Anusmitha et al., 2021; Illam et al., 2017; Kim et al., 2022). Few studies are available concerning bioactive molecules derived from insects which are considered a cheap and abundant source of therapeutics; arthropod extracts as anticancer agents were evidenced regarding the Asian rhinoceros beetle, *Allomyrina dichotomus*, the Texas lubber grasshopper, *Brachystola magna*.2 and the North American yellow jacket wasp, *Vespula pensylvanica* (Wang et al., 2011). Results concerning tumor cell apoptosis (Fig. 6) after treatments with cockroach extract hypothesized that the lysate may inhibit the synthesis of DNA, RNA, and proteins, and block the energy metabolism of tumor cells. Previous studies proved the cytotoxicity of cockroach lysate against different human cancer lines (Hu et al., 2011). Apoptosis might also be one of the mechanisms of the inhibitory activity of *Periplaneta americana* extract against cancer cells. Apoptosis, to as a process of programmed cell death, has a vital role in cancer development and therapies (Narayanankutty, 2019, 2020; Roy et al., 2018). Multiple genes are involved in apoptosis in cancer cells, as the gene coding proteins, pro-apoptotic and anti-apoptotic types. *Periplaneta americana* extract is suggested to inhibit the proliferation of human hepatoma cells by inducing apoptosis and reducing the mitochondrial membrane potential, up-regulating apoptotic protein expression. In addition, a previous study revealed that *Periplaneta americana* extract induced apoptosis in human hepatocellular carcinoma cells via the mitochondrial pathway (Zhao et al., 2017). Thirty compounds were separated from cockroach lysate (Table 4) through fractionation using liquid chromatography-mass spectrometry. It is expected that the compounds can act together to produce their biological activities; previous studies in plant extract have reported such cumulative and additive effects (Parathodi Illam et al., 2019). These compounds possess characteristics in their structure like the arrangement of specific functional groups and the presence of active components, which make them biologically significant for their potential therapeutic value against various infectious and non-infectious diseases.

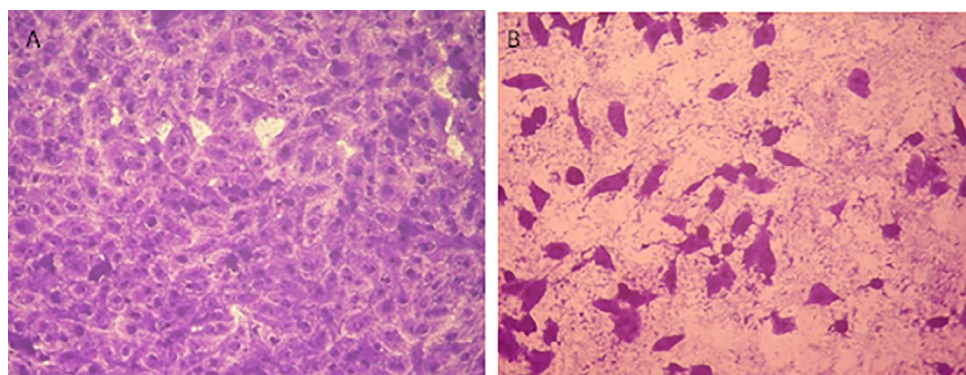


Fig. 5. Morphological characteristics of human breast MCF-7 cells treated with and without the lysate observed under an inverted microscope after 24hr. The tumor cells stained with crystal violet stain. (A); Control human breast MCF-7 carcinoma cells, (B); treated with pure compound.

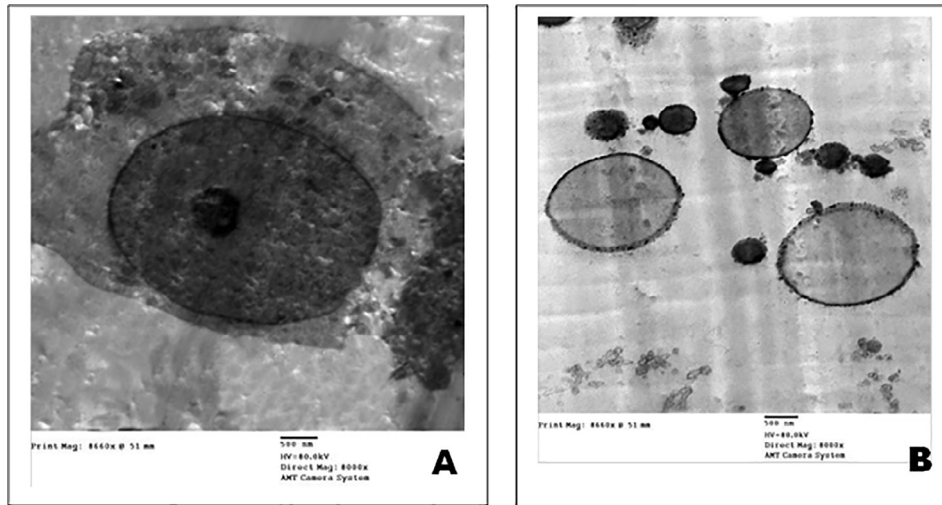


Fig. 6. TEM micrographs of the ultrastructural characteristics of MCF-7 cells treated with and without cockroach extract. (A) Control MCF-7 cells. (B) MCF-7 cells treated with cockroach extract. (Scale bar = 500 nm).

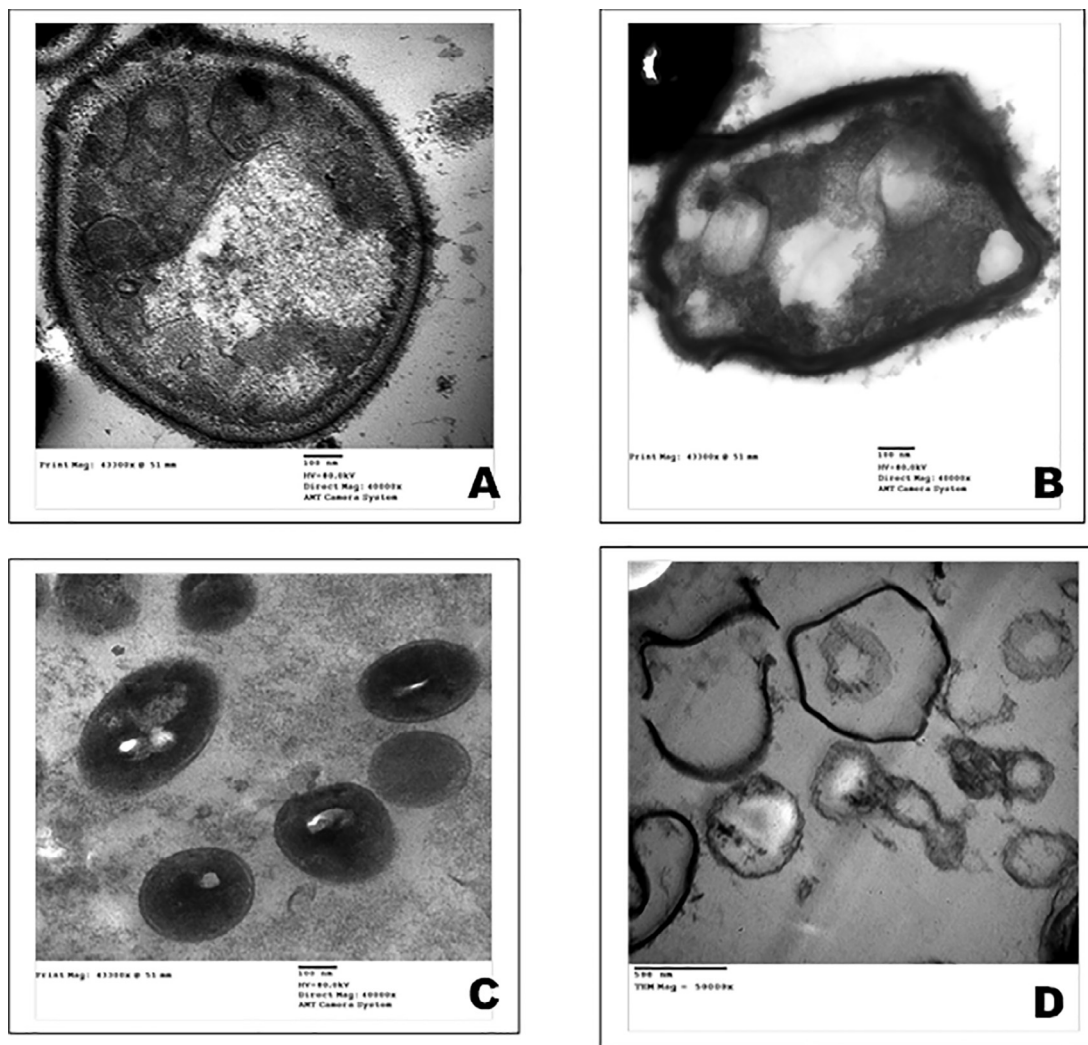


Fig. 7. TEM micrographs of *Candida albicans*; A: control and B: treated with organ lysate C: control of *Staphylococcus aureus* (MRSA); and E: treated with organ lysate.

5. Conclusion

Traditionally the phytochemicals were utilized as the source of drug candidates against infectious diseases and cancers; however, the present study identifies the potential of insect-based drug discovery against these diseases. The cyclohexane extract of the cockroach has shown promising antibacterial and antifungal activities against drug-resistant pathogens; besides, the anticancer properties of the same are also found to be promising. In conclusion, these findings suggest that compounds present in cockroach body extract are of potential therapeutic value. Further identification, characterization, and functional studies *in vitro* and *in vivo* using individual compounds can act as a potential alternative in developing novel therapeutics against various pathogens.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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