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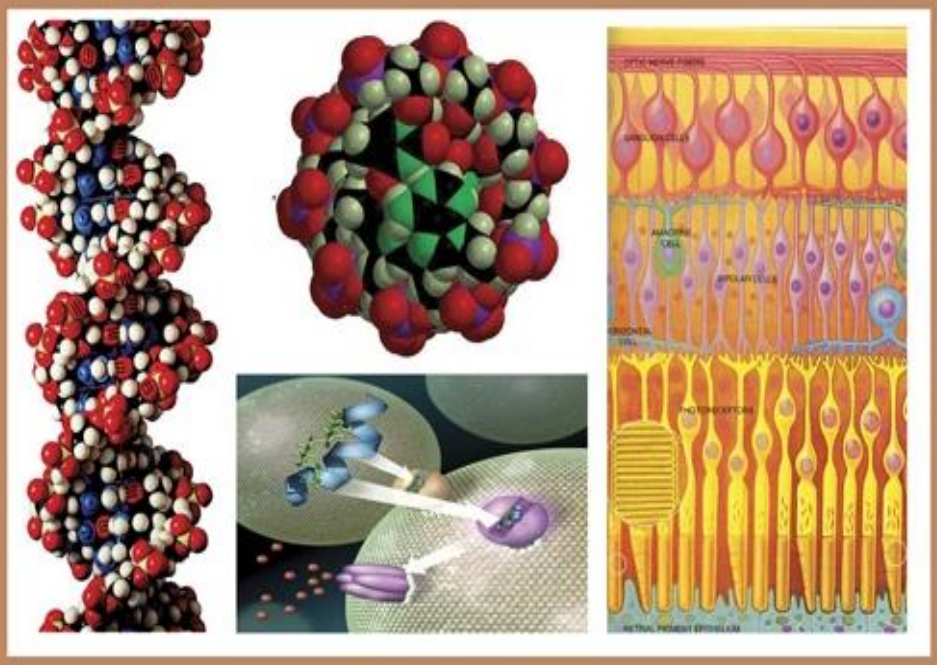

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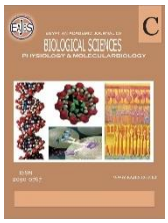

Gc-Ms analysis and Antimicrobial Effect of Ootheca of The Egyptian Pygmy Mantis, *Miomantis paykullii* (Order: Mantodea)

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ABSTRACT

The current study focuses at Mantid's ootheca's antibacterial activity against gram-negative bacteria (*Escherichia coli* ATCC 25922, *Proteus vulgaris* RCMB 004 (1) ATCC 13315), gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* RCMB 015 (1) NRRL B-543), and antifungal activity (*Aspergillus fumigatus*). Antibacterial activity was found in the ootheca of mantis against gram negative bacteria (*Escherichia coli* ATCC 25922, Proteus vulgaris RCMB 004 (1) ATCC 13315), followed by antibacterial activity against gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* RCMB 015 (1) NRRL B-543). There was no antifungal activity against *Aspergillus fumigatus* (RCMB 002008), but there was some activity against *Candida albicans* (RCMB 005003 (1) ATCC 10231). The minimum inhibitory concentration (MIC) was antifungal activity of 78 µg/mL with the resulting Escherichia coli ATCC 25922, 156.3 µg/ml with the following: *Proteus vulgaris* RCMB 015(1) ATCC 13315, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* RCMB 015(1) NRRL B-543(gram positive bacterias), as well as anti-microbial with the minimum inhibitory level (MIC). *Bacillus subtilis* B-543(gram positive bacteria) (Fungi).The present study also revealed the presence of 48 compounds in ootheca of mantid using Gas-separation technique GC-MS Analysis.

INTRODUCTION

A small genus of mantis from Africa is the Egyptian Pygmy Mantis. They are found in Burkina Faso, Egypt, Ghana, Cameroon, Kenya, Nigeria, Mozambique, Uganda, Senegal, Zimbabwe and the rest of Europe. The country is located in the following countries: The men and women are about the same height, and the women are bulkier and more burdensome than the men. The color of this mantis species can range from light green to beige, light brown to dark brown. There are no special markers or color designs on their bodies in the Egyptian Pygmy Mantises. This Mantis species is very relaxed. A prey that doesn't seem to concern this mantis, occasionally disabled from targeting. This mantis can sprint or fly away when threatened by the humans or predators. It consumes virtually all prey insect species of the right size. Fruitflies are the favorites for Egyptian pygmies Mantis of all sizes and ages, as well as small crickets, moths, green bottle flies and other insect species, about half of their length.

The woman settled around 10 little beige oothecal after she had matted. 15 nymphs are hatched of any ootheca around.

The study's aim is to demonstrate the mantid ootheca's antimicrobial properties and to learn more about its various compounds. Gas chromatography–mass spectrometry (GC–MS), which isolates and analyzes compounds in one phase using a mass detector and easily accessible chemicals, is one of the best methods for identifying these compounds. GC–MS databases (Gomathi *et al.,* 2015).

MATERIALS AND METHODS

Collection of Insects:

Both male and female mantis was collected from Assuit and ootheca was found near the female.

Antimicrobial Activity Assay*:*

The antimicrobial activity was investigated on the oothecal against microorganisms. All microbial strains were provided from the culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

The antimicrobial profile was tested against two Gram-positive bacterial species (*Bacillus subtilis, Staphylococcus aureus*), two Gram-negative bacterial species (*Escherichia coli, Proteus vulgaris*) and two fungi (*Aspergillus fumigatus* and *Candida albicans*) using a modified well diffusion method. Briefly, 100 µl of the test bacteria/or fungi were grown in 10 mL of fresh media until they reached a count of approximately 10^8 cells/ml for bacteria or 10^5 cells/mL for fungi (Abdelrahman et al., 2017&Ibrahim et al., 2014) One hundred µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained and tested for susceptibility by well diffusion method on Mueller-Hinton and Sabaroud agar (Clinical and Laboratory Standards Institute, 2012.) One hundred µL of each sample (at 10 mg/ml) was added to each well (10 mm diameter holes cut in the agar gel). The plates were incubated for 24-48 h at 37 °C (for bacteria and yeast) and for 48 h at 28 °C (for filamentous fungi). After incubation, the microorganism's growth was observed. The resulting inhibition zone diameters were measured in millimeters and used as a criterion for antimicrobial activity. If an organism is placed on the agar, it will not grow in the area around the well if it is susceptible to the chemical. This area of no growth around the disc is known as a "Zone of inhibition" or "Clear zone". The size of the clear zone is proportional to the inhibitory action of the compound under investigation. Solvent controls (DMSO) were included in every experiment as negative controls. DMSO was used for dissolving the tested compounds and showed no inhibition zones, confirming that it has no influence on growth of the tested microorganisms. Gentamycin and ketoconazole (Sigma Aldrich, USA) were used as standard antibacterial and antifungal drugs at 30 and 50ug/ml, respectively.

MIC Determination:

The tested extract was screened *in vitro* for their antibacterial and antifungal activities at a different concentration to determine the lowest concentration inhibiting the growth of the organism that recorded as the MIC (Ibrahim et al., 2014; Ahmed Hany et al., 2014). All measurements of MIC values were repeated in triplicate.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis:

Ootheca was collected and, 200 milligrams were homogenized in 1ml methanol centrifuged at 4500rpm for 10 minutes, the supernatant was taken to GC-MS. The chemical composition of samples was performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25

mm x 0.25 µm film thickness). The column oven temperature was initially held at 50 C and then increased by 5℃ /min to 230℃ for 2 min. increased to the final temperature 290℃ by 30℃ /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250, 260℃ respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 ℃. The components were identified by comparison of their retention times and mass spectra with those of the WILEY 09 and NIST 11 mass spectral databases.

RESULTS

In this study, the antibacterial effectiveness of the Mantid ootheca against Gram-V bacteria (Escherichia coli, *Bacillus subtilis*), against Gram+V bacteria (*Staphylococcus aureus, Bacillus subtilis)* as well as antifungal activity (*Aspergillus fumigatus*, *Candida albicans*) is investigated. Antibacterial activity against gram negative bacteria was first, followed by antibacterial activity against gram positive bacteria (*Escherichia coli*, *Proteus vulgaris*), (*Staphylococcus aureus*, bacilli-free subtilis). There slight activity for *Candida albicans* and no antifungal activity for *Aspergillus fumigatus* (Table 1). Antimicrobial activity was measured at a minimum inhibitory concentration (MIC) of 78 ug to ml against E. coli, 156.3 µg/ml against (gram negative bacteria) *Proteus vulgaris*, and 156.3 ug/ ml against *Staphylococcus aureus, Bacillus subtilis* (gram positive bacteria). For *Candida albicans*, the minimum inhibitory level (MIC) of antimicrobial activity was 1250 µg/ml, and no *Aspergillus fumigatus* activity was observed (Table 2).

The present research also found that 48 compounds were present in mantid oothecal using GC-MS analysis gas separation technique. Molecular weights of different bioactive compounds and names are listed with the retention period, percentage peak (Table 3). N-Methyl-à-aminoisobutyric acid Dimethyl-cyano-phosphine, (R)-(T)-2,2-dimethylbutane-3 -d, 2-butanamine, 3-methyl, leu-gly, 5,5,6-trimethyl-4,7,9-trioxa bicyclo[4.2.1]nonane, 4-d1-heptane, 1,2-cyclooctanediol, 1-dodecanol, 1-decanol, 1,2-epoxynonane, 3-hexadecyloxycarbony l-5-(2-hydroxyethyl)-4-met hylimidazolium ion, 2,4,6,8-tetramethyl-1-undecene, eicosyltrichlorosilane, 1-dodecanol, 1,10-decane-1,1,10,10-d4-diol, 2-decenal, (z)-, 2-nonenal, (e)-, z-10-tetradecen-1-ol acetate, 1,10-decane-1,1,10,10-d4-diol, l,4l-dihydroxyeicosane, 2-tridecenal, (z)-, e-2-tetradecen-1-ol, 2-dodecenal, 1-tetradecanol, 8-hexadecenal, 14-methyl-, (z)-, 3-chloropropionic acid, heptadecyl ester, (e)-tetradec-2-enal, (e)-hexadec-2-enal, 2-tridecenal, (z)-, palmitaldehyde, diallyl acetal, 1-tetradecanol, 3-trifluoroacetoxypentadecane, oxacyclotetradecan-2-on, e, 13-methyl2(3h)-furanone,5-heptyldihydro, 1-pentadecene, 2-methyl-,9-octadecen-1-ol, (z)-, 1-hexadecanol, e-2-tetradecen-1-ol, 2-piperidinone, n-[4-bromo-n-butyl]-7-hexadecenal, (z)-, palmitaldehyde, diallyl acetal, myristoyl chloride, dodecanoyl chloride, 3-hexadecyloxycarbony l-5-(2-hydroxyethyl)-4-met hylimidazolium ion,2-hexadecen-1-ol,3,7,11,15-tetramethyl-,[r-[r*,r*-(e)]]-,, octadecane,1-(ethenyloxy, z-(13,14-epoxy) tetradec-11-en-1-o l acetate.

Table 1: Mean inhibition area in mm developed on a variety of pathogens. The results are shown in the table below: The research was performed with the technique of diffusion agar, diameter of a well: 6.0 mm (100 µl was tested), *NA: No activity. Concentrated sample

Sample code / Tested microorganisms	Egg	Control
FUNGI		***Ketoconazole***
Aspergillus fumigatus (RCMB 002008)	NA	18
Candida albicans RCMB 005003 (1) ATCC 10231	11	21
Gram Positive Bacteria:		*Gentamycin*
Staphylococcus aureus ATCC 25923	23	25
Bacillus subtilis RCMB 015 (1) NRRL B-543	20	27
Gram Negatvie Bacteria:		*Gentamycin*
Escherichia coli ATCC 25922	28	31
Proteus vulgaris RCMB 004 (1) ATCC 13315	21	26

Table 2: Minimum inhibitor concentration antifungal action (MIC) in the g/ml of the microorganisms examined The results are shown in the table below: The research was performed with the technique of diffusion agar, diameter of a well: 6.0 mm (100 µl was tested), *NA: No activity

Sample code / Tested microorganisms	Egg
FUNGI	
Aspergillus fumigatus (RCMB 002008)	NA
Candida albicans RCMB 005003 (1) ATCC 10231	1250
Gram Positive Bacteria:	
Staphylococcus aureus ATCC 25923	156.3
Bacillus subtilis RCMB 015 (1) NRRL B-543	156.3
Gram Negatvie Bacteria:	
Escherichia coli ATCC 25922	78.15
Proteus vulgaris RCMB 004 (1) ATCC 13315	156.3

Table 3: Show compounds in ootheca separated by GC-MS

Compound Name	**Molecular Formula**	**Molecular Weight**	RT	Area
N-Methyl-à-aminoisobutyric acid	C5H11NO2	117	4.12	0.41
Dimethyl-cyano-phosphine	C3H6NP	87	4.12	0.41
(R)-(T)-2,2-Dimethylbutane-3-d	C6H13D	87	4.12	0.41
2-Butanamine, 3-methyl-	C5H13N	87	4.35	37.77
Leu-Gly	C8H16N2O3	188	4.35	37.77
5,5,6-Trimethyl-4,7,9-trioxa bicyclo [4.2.1] nonane	C9H16O3	172	4.35	37.77
4-D1-heptane	C7H15D	101	4.35	37.77
1,2-Cyclooctanediol	C8H16O2	144	42.89	0.87
1-Dodecanol	C12H26O	186	42.89	0.87
1-Decanol	C10H22O	158	42.97	0.72
1,2-Epoxynonane	C9H18O	142	42.97	0.72
3-Hexadecyloxycarbony l-5-(2-hydroxyethyl)-4-met hylimidazolium ion	C24H45N2O3	409	43.23	0.54
2,4,6,8-Tetramethyl-1-undecene	C15H30	210	43.23	0.54
Eicosyltrichlorosilane	C20H41Cl3Si	414	43.23	0.54
1-Dodecanol	C12H26O	186	43.23	0.54
1,10-Decane-1,1,10,10-d4-diol	C10H18D4O2	178	43.33	0.86
2-Decenal, (Z)-	C10H18O	154	43.33	0.86
2-Nonenal, (E)-	C9H16O	140	43.33	0.86
Z-10-Tetradecen-1-ol acetate	C16H30O2	254	43.59	1.56
1,10-Decane-1,1,10,10-d4-diol	C10H18D4O2	178	43.59	1.56
L,4L-Dihydroxyeicosane	C20H42O2	314	43.59	1.56
2-TridecenaL, (Z)-	C13H24O	196	43.59	1.56
E-2-Tetradecen-1-ol	C14H28O	212	43.94	11.65
2-Dodecenal	C12H22O	182	43.94	11.65
1-Tetradecanol	C14H30O	214	43.94	11.65
8-Hexadecenal, 14-methyl-, (Z)-	C17H32O	252	43.94	11.65
3-Chloropropionic acid, heptadecyl ester	C20H39ClO2	346	43.94	11.65
(E)-Tetradec-2-enal	C14H26O	210	44.22	11.63
(E)-Hexadec-2-enal	C16H30O	238	44.22	11.63
2-Tridecenal, (Z)-	C13H24O	196	44.22	11.63
Palmitaldehyde, diallyl acetal	C22H42O2	338	44.22	11.63
1-Tetradecanol	C14H30O	214	44.22	11.63
3-Trifluoroacetoxypentadecane	C17H31F3O2	324	44.33	1.31
Oxacyclotetradecan-2-on e, 13-methyl-	C14H26O2	226	44.33	1.31
2(3H)-furanone,5-heptyldihydro-	C11H20O2	184	44.33	1.31
1-Pentadecene, 2-methyl-	C16H32	224	44.33	1.31
9-Octadecen-1-ol, (z)-	C18H36O	268	44.49	11.50
1-Hexadecanol	C16H34O	242	44.49	11.50
E-2-Tetradecen-1-ol	C14H28O	212	44.49	11.50
2-Piperidinone, N-[4-bromo-n-butyl]-,	C9H16BrNO	233	44.49	11.50
7-hexadecenal, (Z)-	C16H30O	238	44.49	11.50
Palmitaldehyde, diallyl acetal	C22H42O2	338	44.71	1.90
Myristoyl chloride	C14H27ClO	246	44.71	1.90
Dodecanoyl chloride	C12H23ClO	218	44.71	1.90
3-Hexadecyloxycarbony l-5-(2-hydroxyethyl)-4 methylimidazolium ion	C24H45N2O3	409	45.01	13.58
2-Hexadecen-1-ol,3,7,11,15-tetramethyl-, [r-[r*,r*-(e)]]-	C20H40O	296	45.01	13.58
Octadecane,1-(ethenyloxy)-	C20H40O	296	45.01	13.58
Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C16H28O3	268	45.01	13.58

DISCUSSION

Mantid antibacterial oothecal action against gram-ve (*Escherichia coli, Proteus vulgaris)* and anti-infungal activity (*Aspergillus fumigatus*; *Candida albicans* are currently being investigated. The ootheca of mantids had antibacterial action against gram negative bacteria, followed by antibacterial activities against the gram of the healthy bacteria (*Escherichia coli, Proteus vulgaris*), (Staphylococcus aureus ATCC 25923, bacilli-free subtilis RCMB 015(1) NRRLB-543). No antifungal activity was observed against *Aspergillus fumigatus* while slight activity was observed for *Candida albicans*.

Different compounds were observed in ootheca of mantids. Fatty alcohol with a long chain, 1- Dodecanol, registered for *Staphylococcus aureus* with the highest antibacterial activity (Togashi etal.,2007). 2- Decenal, (Z)- is an aldehyde present in animal food (in the amount of trace), and is part of the coriander essential oil. 2-decenal is sometimes used as a fragrance, and it's known for its Nematicidal activity (*Ramya et al.,2015).*3-Trifluoroacetoxypentadecane is a Fluro compound known as anti-nephrotoxic and antioxidant activities also organic pollutants obtained from water sample tap and deionized water (Subramani Palani 2011; William Boadi & Kerry Parchman 2015;). 8-Hexadecenal, 14-methyl-, is a phermone detected in *Trogoderma granarium Everts* Khapra beetle (the10th International Conference of Working on Protection of Stored Product 2010) also identified in methanolic extract of *Dodonaea angustifolia* leaves (GC-MS study) (Revathi and Dhanaraj 2019). Palmitaldehyde, diallyl acetal identified in methanolic fractions of *Sansevieria roxburghiana* leaves showing potent antimicrobial activity using (GC-MS analysis) (*Kaleena et al., 2011).* More long chain hydrocarbons were included in the GC-MS spectrum. If the molecule increases in the number of carbon atoms, hydrophilicity is decreased and lipophilicity increases. Increased lipophilicity of a pharmaceuticals reduces transmission through the gut cells (Wils *et al.,*1994; Parasuraman *et al.,* 2009). 3-Trifluoroacetoxypentadecane compound identified in methanolic extract of *Adiantum capillus-veneris as* anti-nephrotoxic and antioxidant activities (Haider *et.al.,*2016). 2-Piperidinone, N-[4-bromo-n-butyl]- is a bio-active component identified in the *Sida cordata* whole plant by GC–MS analysis possess antimicrobial activity (Mani Ganesh & Murugan Mohankumar 2017), it is an *Alkaloid obtained from* GC-MS analysis of ethanol extract of the stem *Hugonia mystax*L. (Linaceae)(Vimalavady and Kadavul 2013). N-[4-bromo-n-butyl]- 7-Hexadecenal, (Z)- is an aliphatic aldehydes and 2-Dodecanol is a fatty alcohol,both obtained in GC-MS analysis of extracting chloroform of the stem *Hugonia mystax*L. (Linaceae) (Vimalavady *et al., 2013).* 2-Hexadecanol identified in GC/MS analysis of *Paecilomyces lilacinus* acetone extract showed antimicrobial and antibacterial activity (Marwa T. A. Abdel-Wareth *et al*.,2019). Myristoyl chloride as antibacterial in three monosaccharide monomyristate Derivatives (Jumina Jumina 2019). N-Methyl-à-aminoisobutyric acid Alpha-(methylamino) isobutyric acid is a non-proteinogenic alpha-amino acid that is isobutyric acid in which the alpha-hydrogen has been replaced by a methylamino group. It has a role as a human urinary metabolite. It is an alanine derivative, a non-proteinogenic alpha-amino acid and a secondary amino compound. It derives from an isobutyric acid. (https://pubchem.ncbi.nlm.nih.gov/).2-Butanamine, 3-Methyl and 1-Hexadecanol were-detected in *Pocillopora verrucosa* crude extraction using ethyl acetate by gas

chromatography, 1-hexadecanol showed antibacterial and antimicrobial activity. (Moaz Hamed and Hussein Hussein 2020). N-[4-bromo-n-butyl]- 7-Hexadecenal, (Z)- is an aliphatic aldehydes GC-MS analysis of chloroform extract of the stem. 1-Tetradecanol was detected in seeds of *Scirpus articulates* by GC/MS (Aastha Bhardwaj 2014). 1-Tetradecanol, is a straight chain saturated fatty alcohol And other fatty alcohols, 1-tetradecanol is used for its emollient properties in cosmetics, for example cold creams. In the chemical synthesis of other products including surfactants, it is often used as intermediary. (Kreutzer 1984). 3-hexadecyloxycarbony L-5-(2-hydroxyethyl)-4-met hylimidazolium ion is am amino compound and showed antimicrobial effect,also it was identified in in the ethanolic leaves extract of *Blepharis maderaspatensis (*Suriyavathana *et al.*,2014). *Locusta migratoria migratoryiodas* (Oraha and Lockey 1990), *Heliodis Virescens* (Buckner *et al.,* 1996), and *Musca domestica* are the principal areas of alcohol (Golaobiowski *et al.,* 2012b). There is a lot of alcohol in *Locusta migratoria migratoria migratorios.* Alcohols were discovered to have antimicrobial activity (Gobiowski *et al.,* 2012b). *Bactrocera dorsalis* eggs are *Bactrocera dorsalis* larvae (Qinge *et al.,* 2016), *Ostrinia nubilalis* also contains fatty acids, alcohols, and alkanes (Frenoy *et al.,* 1992). These chemicals can serve as a chemical attractant for parasitoids, according to research on these species. *Lucilia sericata* (Gobiowski *et al.*, 2012c), *Leptinotarsa decemlineata* (Nelson *et al.,* 2003), and *Stenocara gracilipes* are among the insect species that use alkana (Lockey 1988). Alkanes can help distinguish organisms by acting as a chemical signal (Lockey 1988). Parents transfer antimicrobial substances to their offspring's food sources with the aid of certain insect species and parental care to avoid extinction and thus limit contact between offspring and microbes (Trumbo 2012). Egg attendance is a common type of parental care among insects (Trumbo 2012; Wong and Kolliker 2012; see Royle *et al.,* 2012). Though it is thought that parental egg consumption protects eggs from intraspecific or interspecific predation (Cocroft 1999; Zink 2003; Wong and Kolliker 2012; Trumbo 2012; Miller and Zink 2012), little is known about egg protection against fungal and bacterial infections (Costa 2006; Trumbo 2012). Fungi and bacteria can be successful environmental selection agents for the presence of parental eggs in insects since many animals develop their eggs in organic substrates or in the soil, where they are constantly in contact with spores, fungi, and mold. Costa (2006), Cremer and Sixt (2009), Trumbo (2012), and Reber and Chapuisat (2012) are just a few examples. Both of these bacteria are in danger of destroying food sources, as well as infectious eggs and/or influencing embryo growth and survival.

Conclusion

Future studies are needed to purify the compounds with antimicrobial activity and investigate their antitumor effect against different cell lines.

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