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Research Article

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Semi-Synthesis of Nano-Antibacterial Heterocyclic Compounds from the *Streptomyces Candidus* Secondary Metabolites as Food Additives

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Abstract The actinobacterial strain *Streptomyces candidus* proved as a promising source for the biosynthesis of new secondary metabolites. The seventh fermentation day was selected for the purification by using the column chromatography*;* mixture of ethyl acetate and n-butanol in different ratios were used to collect eleven fractions (FI-FXI). Thirty metabolites were detected in only four fractions by using the GC-MS. The seven aliphatic compounds of FI were successfully chemically modified to seven heterocyclic compounds by the one pot reaction. The semi-synthesized compounds (148 to 208 nm) converted to the nano-size (1.92 to < 20 nm); then evaluated for their MIC and cytotoxic effect. They more sensitive against the *S. aureus* and *L. Monocytogenes* (6.25µL); while less sensitive against *E. coli* and *S. typhimurium* (50µL) for their MIC. The compounds found non-toxic by using the *Artemia salina* nauplii assay development; the $LC_{50}$was equal to the 67.6 µL (< 1000 µg\ml).

Keywords *Streptomyces*, fermentation, MIC, cytotoxicity, nano

Introduction

The world facing what is known as the "antibiotic crises", due to the increased emergence of microbial resistance to many antimicrobials. In 2001, the Union of Concerned Scientists announced that 55% of drugs used for growth promotion and disease prevention in animals are also used for humans [1]. This poses a significant risk, leading to the launching of the first global strategy to combat this serious problem by the World Health Organization (WHO) in September 2001 [2]. Then in 2012, the World Health Organization (WHO) announced that antimicrobial resistance is one of the greatest health threats facing the world [3].

On the other hand in the field of food industries, several studies linked many bacterial resistances to the antimicrobial food preservatives with the consumption of food products of animal origin [4-6]. This problem resulted from the misuse of antibiotics in veterinary medicine and their residues in the food products of animal origin [7-10]. Those antibiotic resistant microorganisms, including food borne pathogens, became also more tolerant to several food processing and preservation methods [11]. These scenarios motivate the search for new bioactive antimicrobial food preservatives, to overcome this problem in food manufacturing [12-13].

Considerable numbers of bacterial species have been found to possess important biological activities and producing a significant number of antimicrobial agents; that can be use as food additives. There are used as there are or chemically modified to improve their potency; particularly in the modern drug discovery process in the pharmaceutical area [14]. *Actinomycetes* in general are known as the most economically and biotechnologically valuable microorganism until now. They are well known for their ability to produce a wealth of secondary metabolites; with structural complexity and diverse biological activities [15].

Currently, the synthesis and chemical modification of many organic compounds are already applied in current drug syntheses; to improve their potency [14, 16]. In the last few years, many papers described new strategies to the synthesis and chemical modification of many natural occurring chemical compounds to be used as antimicrobial agents [17]. This type of chemical reactions is in agreement with the green chemistry principles [18]. For example; the synthesis of new semi-synthetic molecules from the essential oils and their constituents has been explored [19-20].

The chemical modifications of the organic compounds usually use in the modern drug discovery process in the pharmaceutical area [21]. It gave a great opportunity to improve the biological activities of the unmodified natural molecules; the one pot reaction gained a significant interest in the last few years in the organic synthesis and semi-synthesis of new compounds. This is due to its efficacy for the generation of different heterocyclic compounds in one synthetic step [22-23]. In the area of food industry, studies on the use of semi-synthetic compounds as food preservative are rare [17]. The semi-synthetic compounds can be used as an alternative to the, largely used, synthetic food a preservatives [24]. To extend the shelf life, by protect the food products from the pathogenic microorganisms and ensure the food safety. Hence, the applications of nanotechnology in food are rapidly emerging which including all areas of food chain starting from the agricultural applications to food processing and packaging [25-27].

The aim of this work is to apply the nano-sized semi-synthesized products as antibacterial food additives.

Materials and Methods

Materials

Streptomyces candidus (NRRL ISP-5141) was purchased from the Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University (ASU), Egypt. Cultures of the tested food pathogenic bacteria were purchased from the Central Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute (AHRI), *Agricultural Research Center (ARC), Egypt. Escherichia coli* (ATCC 25922) was tested on nutrient agar prepared from nutrient broth purchased from Oxoid LTD Company, Basing stoke, Hampshire, England; and agar No.1 Bacteriological, Lab M Limited, 1 Quest Park, Moss Hall Road, Bury, Lancashire, BL9 7JJ, UK. *Salmonella typhimurium* (ATCC 14028) was tested on *Salmonella-Shigella* agar and *Staphylococcus aureus* (ATCC 25923) was tested on *Staphylococci* 110; both were purchase from Biolife Italiana S.r.l. Viale Monza, Milan, Italy.*Listeria monocytogenes* (NCINB 50007) was tested on *Listeria* selective agar base, purchased from Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK.

Experimental Methods

Preparation of the precursors

Fermentation of *Streptomyces candidus*

According to the ATCC Bacterial Culture Guide [28], the lyophilized *Streptomyces candidus* revived on DSM medium 65 used for the fermentation step. By using a modified method to that described by Vanama et al. (2014); 5 –days old broth culture of the strain used for the fermentation step. In 250 ml Erlenmeyer flasks, 50 ml of the GYM-broth medium was inoculated with pre-prepared spore suspension; then incubated on a rotary shaker at 125 rpm and 30±1 °C in dark for seven days. The culture centrifuged at 5.000 rpm for 15 minutes at 4 ^{o}C, then the filtrate dried by freeze drier (Alpha 1-4 LSC plus, Christ).

Fractionation by column chromatography (CC)

By using a modified method to that used by Alma *et al.* (2012) [29];column (30 x 2.5 cm) was packed with silica gel 60 column (0.063 – 0.200 mm – Merck); followed by elution of the column with a non-polar solvent to make sure nice packing. Then, left for an hour until the complete settling of the silica; non-polar solvent eluted from the column and the powdered crude extract applied uniformly onto the top of the silica. Elution of the column was carried out with mixtures of n-butanol and ethyl acetate with a gradient of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 % n-butanol: ethyl acetate. Total of 11 fractions (FI to FXI) of 10 ml each were collected in glass petri plates for evaporation of the solvents, weighed and stored in refrigerator until use.

Structural identification by GC-MS

Structural identification of the purified fractions (FI to FXI) by column chromatography was carried out on a Gas Chromatography (Varian cp-3800) equipped and coupled to a mass detector Mass Spectroscopy (320 – MS Varian) and a FV5MS Column. Complete identification for the compounds in each fraction presented in **(Figures 1-4) and (Table 1)**.

Chemical modification of the FI compounds

Modification of the compounds by the one pot reaction

Following the method that described by Mohareb *et al.* [30]; the reaction mixture consisted of 0.25 g of prepared cyanoacetic acid hydrazide prepared according to Kabirifard *et al.*, [31], 6 mg of the FI compounds and appropriate amount of 1, 4-Dioxan. The mixture heated under reflux for 2 hours then poured onto a beaker containing an ice/water. The formed solid white product was collected by centrifugation at 10.000 rpm and 4°C for 10 minutes; then, weighed and stored in refrigerator until use. Structural identification of the semi-synthesized compounds was carried out by GC-MS. Complete identification for the compounds presented in (Figure 5) and (Figure 6 a - g).

Conversion of the modified compounds to the nanoparticles

Conversion of the semi-synthesized heterocyclic compounds to the nanoparticles by using modified method to that described by Mohammad pour *et al*. [32]. They were prepared via the ionotropic gelation with Sodium tripolyphosphate (STPP) anions. The semi-synthesized compounds dissolved in sterile distilled water (SDW) in the ratio of 1:1 then shake well by Vortex; while the STPP dissolved in SDW in the ratio of 1:1, then shake well by Vortex. Then the compounds suspension added to STPP solution in the ratio of 1:2, and then the mixture remained under magnetic stirring 3000 rpm for 30 minutes. The percentage of the STPP was gradually increased every 30 min until reach the final ratio1:8 of the compounds suspension to STPP solution respectively. The morphological characteristics of nanoparticles were investigated by Jeol-2100 high resolution transmission electron microscope (TEM). Particle size was investigated and the results shown in (Figure 7 a-b).

Bio-evaluations for the nano- sized compounds

Determination of the minimum inhibitory concentration (MIC)

By using modified method that described by Oskay [33], the microtitre broth dilution technique was used to determine the minimum inhibitory concentrations of the semi-synthesized nanoparticles. *E. coli*, *S. typhimurium*, *S. aureus* and *L. monocytogenes* were used as test bacteria, while 1% 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) used as an indicator for the bacterial activity. Any color change from purple to pink, which showed the growth of the test bacteria, was assessed visually and the MIC values determined; the results presented in Table (2).

Cytotoxic activity

The test was performed using the *Artemia salina* nauplii assay development; by using modified method that described by Gopal *et al*. [34] and Morshed *et al*. [35]. Cultivation of *Artemia salina* nauplii was carried out by using a modified method that described by Prashith *et al*. [36]. *Artemia salina* cysts were allowed to hatch and mature as nauplii in a container filled with a liter of filtered air-bubbled artificial sea water. The nauplii were collected by Pasteur pipette for bioassay after 48 hours. By using glass test tubes each one containing 1000 µL SDW, serial dilutions of the nano compounds were prepared starting by using 1000 µL of the nano compounds. The concentrations ranged from 1000 µL to 15.625µL; then all the test tubes total volume raised to 5 ml by using salty SDW. A tube containing 5ml salty SDW was used as a control.10 shrimp nauplii were transferred to each of all experimental test tubes under identical conditions and maintained throughout the experiment period. After 24 hours of incubation the numbers of dead nauplii were observed, and the mortality percentage was calculated as illustrated in Table (3). The 50% mortality (LC_{50}) of brine shrimp nauplii was obtained by extrapolation as shown in (Figure 8). The assay performed in duplicate and the results were calculated as an average of two determinations.

Results

Identification of the seventh day crude ingredients

Thirty compounds from only four fractions (FI, FII, FVII and FVIII) contained purified compounds as shown in **Figures 1, 2, 3** and **4** respectively. Almost all the detected compounds are aliphatic hydrocarbons. According to the GC-MS library; all the detected compounds were identified based on the CAS number, retention time, peak area percentage, the molecular formula and the molecular weight as presented in **Table 1**.

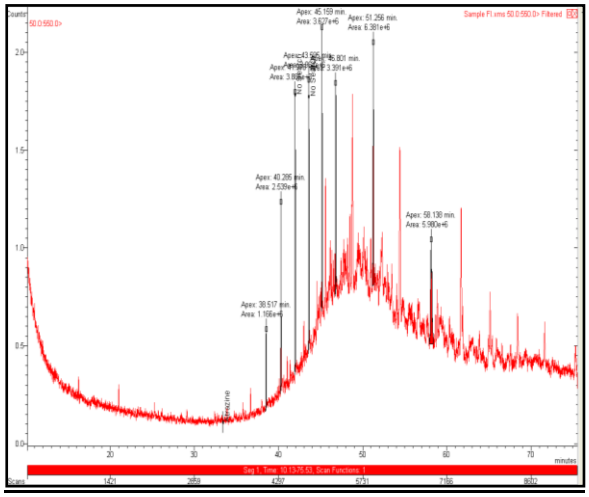


Figure 1: The GC-MS chromatogram for the FI compounds from the seventh day crude extract purified by column chromatography

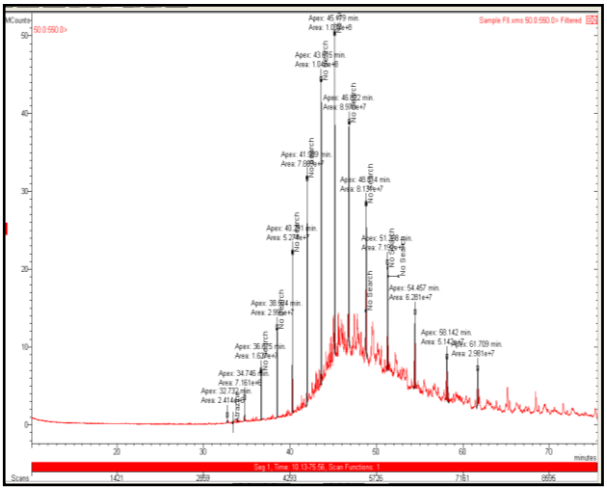


Figure 2: The GC-MS chromatogram for the FII compounds from the seventh day crude extract purified by column chromatography

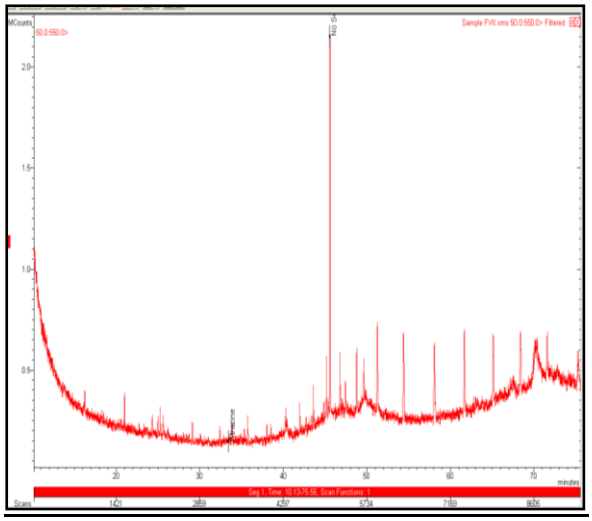


Figure 3: The GC-MS chromatogram for the VII compounds from the seven thday crude extract purified by column chromatography.

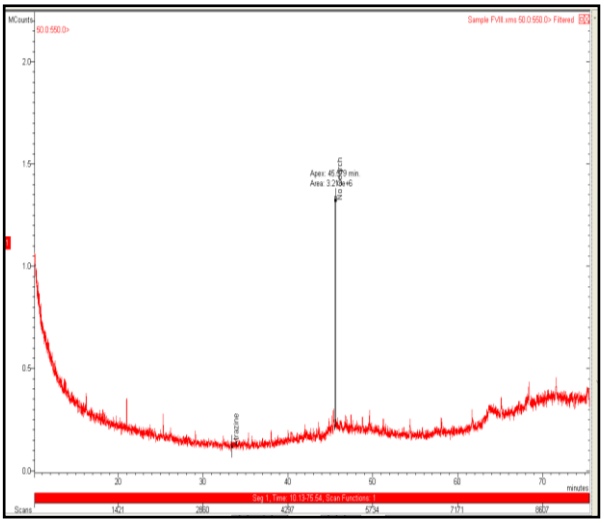
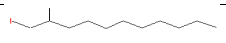
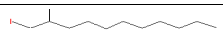
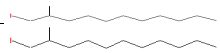
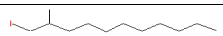
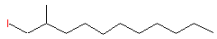
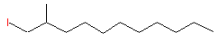
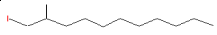
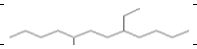
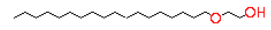
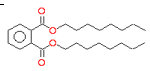
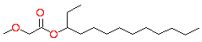
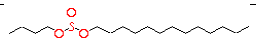
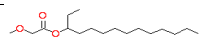
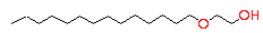
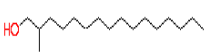
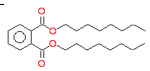


Figure 4: The GC-MS chromatogram for the VIII compounds from the seventh day crude extract purified by column chromatography

Table 1: The detected compounds in FI, FII, VII and VIII from the seventhday crudes

F*	Compound name	CAS Number	RT (min)	Area %	Molecular formula	Molecular Weight g/mol	Structure
FI	1-Iodo-2-methyl	73105-	38.715	1.166 e^{+6}	$C_{12}H_{25}I$	296	
	Nonadecane	629-92-5	40.258	2.53e^{+6}	$C_{19}H_{40}$	268	

	Eicosane	112-95-8	41.978	3.806e^{+6}	$C_{20}H_{42}$	282	
			46.801	3.391e^{+6}			
	Hexadecane	544-76-3	4.595	3.984e^{+6}	$C_{16}H_{34}$	226	
	Nonadecane	629-92-5	45.159	3.627e^{+6}	$C_{19}H_{40}$	268	
	Octadecane, 1-	930-02-9	51.256	6.381e^{+6}	$C_{20}H_{40}O$	296	
	Hydroxylamine, O-	29812-	58.138	5.890e^{+6}	$C_{10}H_{23}NO$	173	
FII	Eicosane	112-95-8	37.732	2.414e^{+6}	$C_{20}H_{42}$	282	
			36.675	1.62e+7			
	1-Iodo-2-	73105-	34.746	7.161e+6	$C_{12}H_{25}I$	296	
	Nonadecane	629-92-5	2.905	2.905	$C_{19}H_{40}$	268	
	Nonadecane	629-92-5	40.29	5.27e+7	$C_{19}H_{40}$	268	
	Nonadecane	629-92-5	41.989	7.839e+7	$C_{19}H_{40}$	268	
	Nonadecane	629-92-5	43.615	1.048e+7	$C_{19}H_{40}$	268	
	Nonadecane	629-92-5	45.179	1.039e+7	$C_{19}H_{40}$	268	
	Nonadecane	629-92-5	46.822	8.97e+6	$C_{19}H_{40}$	268	
	Nonadecane	629-92-5	48.814	8.13e+7	$C_{19}H_{40}$	268	
	Nonadecane	629-92-5	51.288	7.122e+7	$C_{19}H_{40}$	268	
	Nonadecane	629-92-5	54.457	6.28e+7	$C_{19}H_{40}$	268	
	Nonadecane	629-92-5	58.142	4.142e+7	$C_{19}H_{40}$	268	
	Nonadecane	629-92-5	61.709	2.98e+7	$C_{19}H_{40}$	268	
VII	Dodecane, 5,8-diethyl-	24251-	56.128	2.429e+6	$C_{16}H_{34}$	226	
	Ethanol, 2- (octadecyloxy)-	2136-72-3	45.156	626525	$C_{20}H_{42}O_2$	314	
	Di-n-octyl phthalate	117-84-0	45.578	4.96e+6	$C_{24}H_{38}O_4$	390	
	Methoxyacetic acid, 3-tridecyl ester	-	51.253	2.325e+6	$C_{16}H_{32}O_3$	272	
	Sulfurous acid, butyl tridecyl ester	-	54.377	2.746e+6	$C_{17}H_{36}O_3S$	320	
	Methoxyacetic acid, 3-tetradecyl ester	-	58.078	2.791e+6	$C_{17}H_{34}O_3$	286	
	Nonadecane	629-92-5	61.665	2.880e+6	$C_{19}H_{40}$	268	
	Ethanol, 2- (tetradecyloxy)-	2136-70-1	68.633	1.915e+6	$C_{16}H_{34}O_2$	258	
	1-Hexadecanol, 2-methyl-	2490-48-4	71.605	1.426e+6	$C_{17}H_{36}O$	256	
VII I	Di-n-octyl phthalate	117-84-0	45.579	3.216e+6	$C_{24}H_{38}O_4$	390	

***F: Fraction**

The one pot reaction

Total of 17 mg dried white precipitate was resulted from the reaction. The chromatogram obtained from the GC-MS analysis (Figure 5) confirmed the presence of seven different heterocyclic compounds, the spectrum for each compound presented in Figure 6 (a - g).

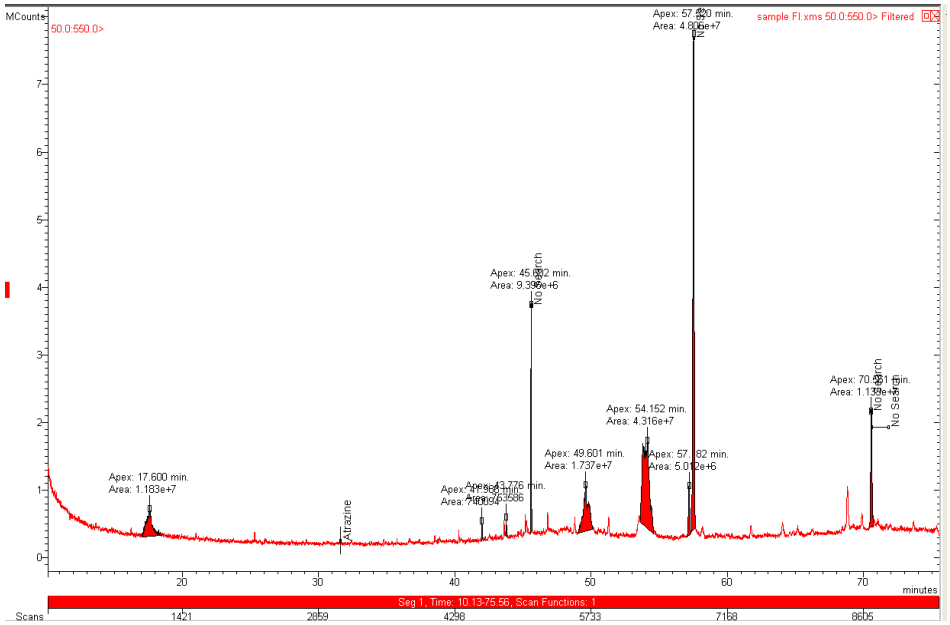
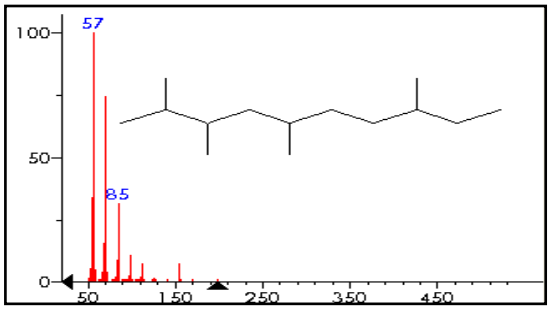
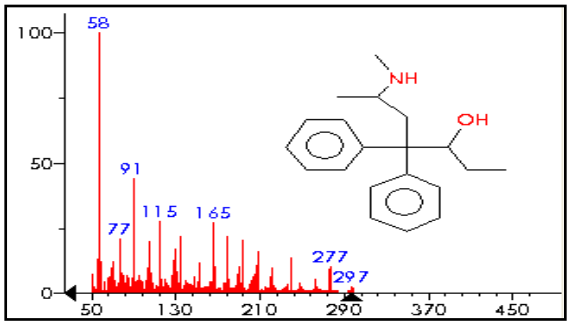


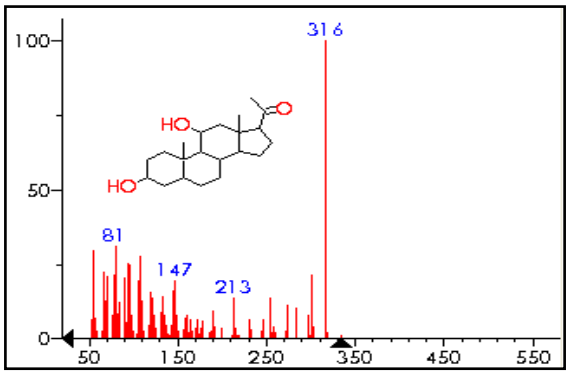
Figure 5: The GC-MS chromatogram for the prepared heterocyclic compounds from the purified FI aliphatic hydrocarbons.



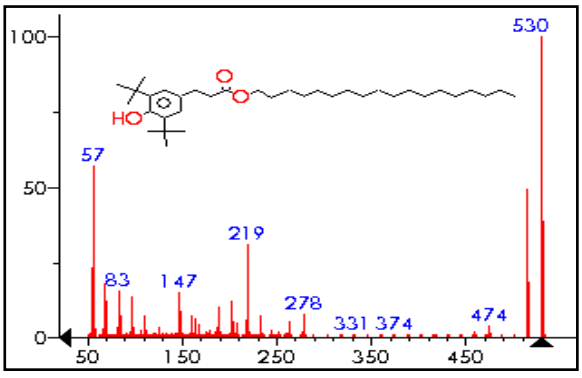
a) Name: Decane, 2,3,5,8-tetramethyl- RT(min): 41.986 Area%: 740094 Molecular formula: $C_{14}H_{30}$ CAS Number : 192823-15-7 Molecular Weight g/mol: 198



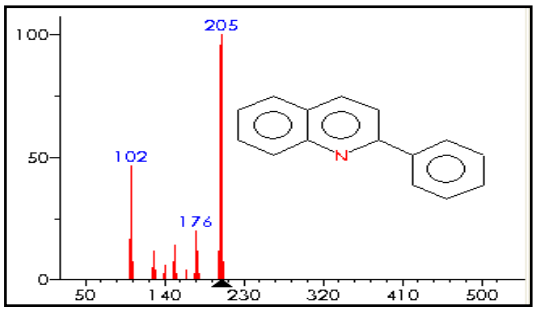
(b)Name: α-N-Normethadol RT(min): 43.776 Area%: 753586 Molecular formula: $C_{20}H_{27}NO$ CAS Number: 38455-85-5 Molecular Weight g/mol: 297



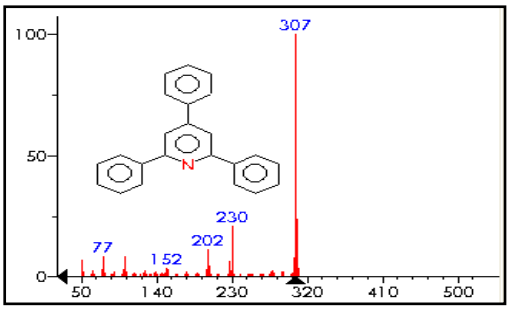
(c) Name: Pregnan-20-one, 3,11-dihydroxy- , (3β,5α,11β)- RT(min): 49.601 Area%: $1.373e^{+7}$ Molecular formula: $C_{21}H_{34}O_3$ CAS Number : 565-89-9 Molecular Weight g/mol: 334



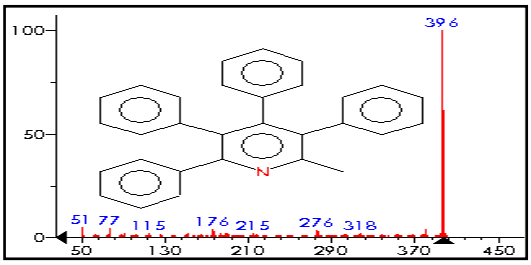
(d) Name: Benzenepropanoic acid, 3,5-bis 1,1-dimethylethyl)-4-hydroxy-,octadecyl RT(min): 53.895 Area%: $4.316e^{+7}$ Molecular formula: $C_{35}H_{62}O_3$ CAS Number: 2082-79-3 Molecular Weight g/mol: 530



(e) Name: Quinoline, 2-phenyl- RT(min): 57.182 Area%: 5.012e^{+6} Molecular formula: $C_{15}H_{11}N$ CAS Number: 612-96-4 Molecular Weight g/mol: 205



(f)Name: Pyridine, 2,4,6-triphenyl- RT(min): 57.520 Area%: 4.8e^{+7} Molecular formula: $C_{23}H_{17}N$ CAS Number: 580-35-8 Molecular Weight g/mol: 307

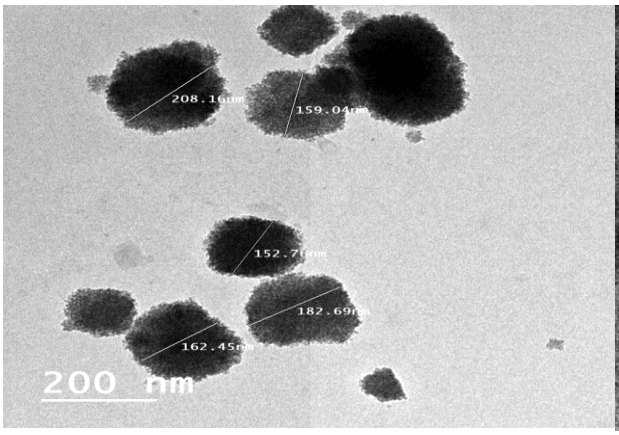


(g) Name: Pyridine, 2-methyl-3,4,5,6-tetraphenyl- RT(min): 70.561 Area%: 1.133e^{+7} Molecular formula: $C_{30}H_{23}N$ CAS Number : 41728-97-6 Molecular Weight g/mol: 397

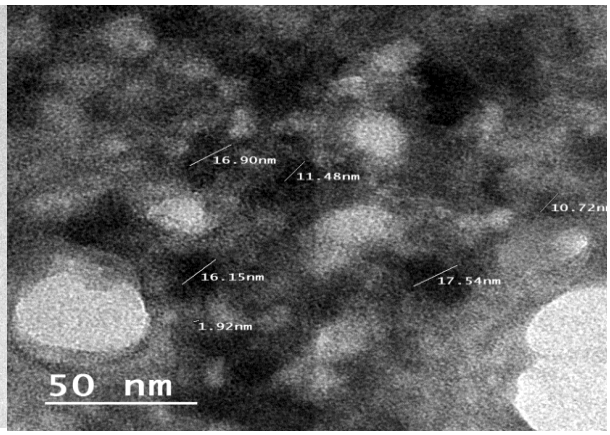
Figure 6: Mass spectrum and chemical structures of the detected semi- synthesized heterocyclic compounds.

The conversion to the nanoparticles

The original particle size of the semi-synthesized heterocyclic compounds crystals illustrated in the scale of 1µm (Figure 7-a). The sizes ranged between 148 to 208 nm (> 100 nm). The resulted nano-sized compounds illustrated in the scale of 50 nm (Figure 7-b). The sizes ranged between 1.92 to < 20 nm. By comparing the morphology of the compounds crystals before and after their conversion, the micro-size crystals are appeared amorphous while the nano particles appeared spherical.



(a) Crystals of the heterocyclic compounds in micro-size(scale of 200nm)



(b)Crystals of the heterocyclic compounds in nano-size (50 nm)

Figure 7: TEM image of the semi- synthesized heterocyclic compounds

The minimum inhibitory concentrations

MIC as illustrated in **Table (2)**, for both *E. coli* and *S. typhimurium* was the same; which is 50µL. Both *S. aureus* and *L. monocytogenes* showed greater sensitivity towards the compounds; the MIC for both of them equal to 6.25µL.

Table 2: Minimum inhibitory concentration (MIC) of the Nanoparticles against *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and *Listeria monocytogenes*

Concentration (µL)	Growth observation against*			
	Escherichia coli	***Salmonella typhimurium***	***Staphylococcus aureus***	***Listeria monocytogenes***
100	-	-	-	-
50	-	-	-	-
25	+++	+++	-	-
12.5	+++	+++	-	-
6.25	+++	+++	-	-
3.125	+++	+++	+	+
1.56	+++	+++	++	++

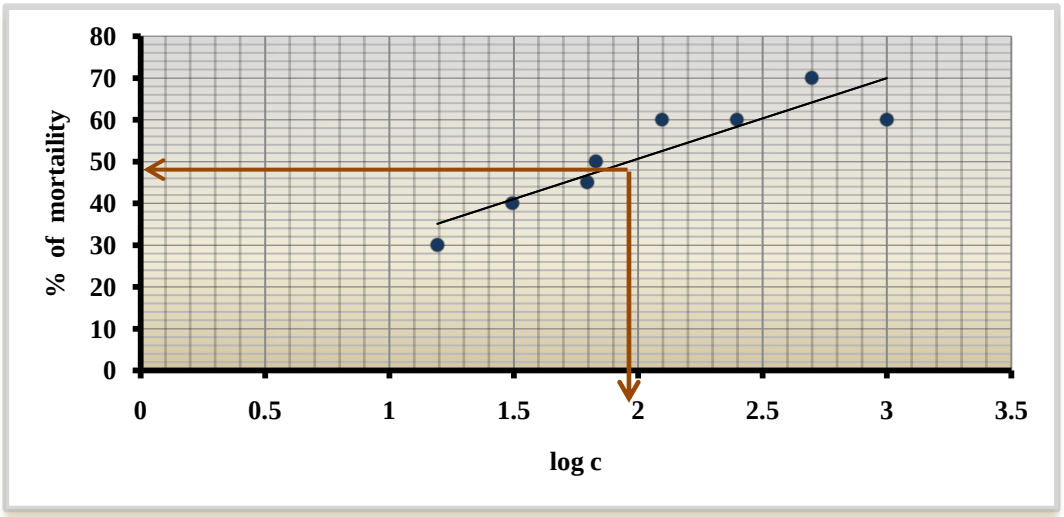
*****The results determined according to the color change from purple to pink: (-): colorless, i.e. inhibition of bacterial growth. (+): faint pink color, i.e. weak bacterial growth. (++): moderate pink color, i.e. moderate bacterial growth. (+++): purple color, i.e. ineffective antibacterial.

Cytotoxic activity

The nauplii showed different mortality rate at different concentrations as illustrated in **Table (3)**. The LC_{50} value for the compounds was obtained from the graphs shown in **Figure (8)**. Plot of percent of mortality versus log concentration on the graph paper produced an approximate linear correlation between them. From the graph, the concentration at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred at 1.83; which equal to the 67.6 µL of the nano compounds.

Table 3: Lethality Bio-assay of the nanoparticles administered to brine shrimp nauplii

Concentration (µL)	Log C	Number of nauplii	Number of live	% of mortality
1000	3	10	4	60
500	2.698	10	3	70
250	2.397	10	4	60
125	2.096	10	4	60
62.5	1.795	10	5.5	45
31.25	1.494	10	6	40
15.62	1.193	10	7	30



LC_{50}: in which 50% mortality (LC50) of brine shrimp nauplii occurred.
Figure 8: Determination of the (LC_{50}) of brine shrimp nauplii

Discussion

In the last few years, many researchers investigated different strategies to the chemical modification of many natural occurring chemical compounds to be used as antimicrobial agents [17]. By using the compounds of Fraction I (Table 1), different heterocyclic compound were prepared as presented in (Figure 5) and (Figure 6 a - g). This reaction is known as one-pot multi-component coupling reactions (MCRs) [22-23]; in which several organic moieties are coupled in one step, for carbon-carbon and carbon-heteroatom bond formation. It is an attractive synthetic strategy for the synthesis of small-molecule libraries with several degrees of structural diversities [37].

Pyridine ring systems, particularly 2,4,6-triarylpyridines, represent an important class of heterocyclic compounds because of their unique position in medicinal chemistry [38-40]. In addition, the excellent thermal stabilities of these pyridines gained a growing interest for their incorporation into polymers as monomeric building blocks in thin films and organometallic polymers [41]. Since, Krohnke's original report on the synthesis of 2,4,6- triarylpyridines [42], there has been a plethora of research targeting their syntheses [23, 43-46]. Alkaloids are very useful and important models in the design and development of antimicrobial agents [47]. It is well-known that the quinoline nucleus and its derivatives play a vital role in the search on wide antibacterial activity spectrum; many of which possess interesting physiological and biological properties [48-49]. Structure-activity relationship studies revealed that the antimicrobial activity in this heterocyclic class of quinoline molecules depends on the nature of the peripheral substituents and their spatial relationship within the quinoline skeleton [50-51].

Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl is a saturated fatty acid [52], has the trade name Hostanox 010 P (Irganox 1010) [53]. It has antifungal and antioxidant activity [54]; it is used as a stabilizer in food-contact polymers like plastics [55]. Steroid structures derive from the carbon atom numbers in the C17-borne side chain; pregnane contain two carbon atoms in the side chain [56]. For pregnan-20-one, 3, 11-dihydroxy- , (3β, 5α, 11β) -, we did not found any research work for this structure. Negligible amounts from α-N-Normethadol and Decane, 2,3,5,8-tetramethyl, were detected in the compounds mixture. According to WIPO/PCT, (2016) [57], α-N-Normethadol (6-(Methylamino)-4,4-diphenyl-3-heptanol) considered as an enantiomer of methadone; whereas there is no specific research papers specifically for this enantiomer. Generally, methadone possesses one asymmetric carbon atom and is thus capable of existing in dextro, levo and racemic form. It is also well known that one of the optical isomers may have properties rendering it many times more useful than the other optical isomers [57]. Decane, 2,3,5,8-tetramethyl-,primary it is a natural compound that identified in *Curculigo orchioide*s rhizome extract [58]; while it was identified by the GC/MS chromatogram of polypropylene waste plastic [59].

The common problems and key challenges in the discovery of new compounds are the evaluation of their biological activity. Because of it well determine the value of this mixture in its application in the field of food industry after that. The nano sized compounds mixture was evaluated for both its MIC against the selected four food pathogenic bacterial strains and its toxicological effects. The MIC defined as the lowest inhibitory concentration of the antimicrobial agent contained in the microtitre well in which the absence of visual color change (colorless) first observed. For visualization of microbial growth, tetrazolium salts are used; which become some of the most widely used tools in cell biology for measuring the metabolic activity of cells ranging from mammalian to microbial origin [60]. These salts are converted by the dehydrogenases of living microorganisms to intensely colored formazen [61-60]. The color changes from purple to pink which showed the organism growth and the intensity of the color assessed visually; while the MIC does not exhibit reduction of TTC into formazan. The results as shown in Table (2) revealed that the compounds mixture are active against Gram-positive than Gram negative bacteria. This might be attributed to the morphological differences between those two types of microorganisms. Gram - negative bacteria have an outer lipopolysaccharide membrane; hence their cell wall is impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes. Gram- positive bacteria have only peptidoglycan layer, not an effective permeability barrier for the antibacterial agents [62].

At the same line, the goal of toxicological evaluation is identifying the biological effects and effectively predicts the *in-vivo* toxicity of the compounds of interest; while the toxicity of nanoparticles is dose- and size-dependent

[63-64]. For the safety assessment of the nano-sized compounds for use as food additive and because of animal experimentation has become an ethical and emotional issue [65-66]; *in-vitro* techniques are increasingly used for the analysis of cytotoxicity of nanoparticles including cell culture, the WST-1 assay [67], XTT assay, MTT assay [68], LDH assay, BrdU assay and fluorescence microscopy [69]. *Artemia salina* becomes a widely used model organism in toxicity assessment especially for nanoparticles [70].

Cytotoxicity studies on brine shrimps are considered to be a useful tool for preliminary assessment of *in-vitro* toxicity; while it is an efficient, inexpensive and relative rapid way to detect toxic compounds in low amounts [71]. As shown in (Table 3) and (Figure 8), the nano-sized compounds showed LC_{50} value of 67.6 µL. This means that the volume of 67.6 µL contained 0.1352 mg of the nano-sized compounds in respect to the original concentration of the nano particles; so the LC_{50} value less than 1000 µg\ml. According to the chemical labeling and classification of acute systemic toxicity based on oral LD_{50} values, which recommended by the Organization for Economic Co-operation and Development (OECD) for a substance to be labeled as non-toxic [72]. The semi-synthesized nano-sized compound non-toxic and can be used safely as antibacterial food additive.

Conclusion

In the present study, the thirty biosynthesized secondary metabolites that identified from the seventh day crude extract of *Streptomyces candidus* culture were not identified before from this strain.

We succeeded to use natural, biosynthesized aliphatic hydrocarbons as a precursor to the semi-synthesis of different nano-sized bioactive-antibacterial heterocyclic compounds; through the simple, quick, convenient and environmentally friendly procedures.

Those semi-synthesized nano-sized compounds are recommended to be used as antibacterial food preservatives; especially for the food products of animal origin.

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Ethical Responsibilities of Authors

- No funding was received
- Conflict of interest: - Author 1 declares that she has no conflict of interest.
  Author 2 declares that she has no conflict of interest.
  Author 3 declares that he has no conflict of interest.
- This article does not contain any studies with human participants or animals performed by any of the authors

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