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The Effect of Some Nano Plant Extract on Bacteria Producing Biogenic Amines Isolated From Minced Meat

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Abstract

Background: Biogenic amines are the end products of bacterial decarboxylation of amino acids which occurs as a result of bacterial contamination. Causing a series of problems for human health.

Objective: - This study aimed to isolating different bacteria that can produce decarboxylase enzymes and trail to control it by using Garlic, Onion and Ginger nano-emulsions.

Methods: Isolation and identification of some bacteria producing decarboxylase enzymes from minced meat, investigating the cytotoxicity of nanoparticles by SRB assay, then detect the antibacterial effect of Garlic, union and Ginger nano-emulsions 60% on isolated bacteria by MIC method and the effect on biogenic amines levels by HPLC.

Results: The (Salmonella species ("*Salmonella* Typhimurium1, 4{5}, 12: i: 1.2 and *S.* Arizonae"), E. coli "serotype O44:K74 and O125:K70", Klebsiella pneumonia, Enterobacter spp, *S. aureus, Aeromonas hydrophila, Proteus mirabilis, Pasteurella multocida* and Lactobacillus species) were the most isolated bacteria. The biogenic amines that were detected on positive samples were Putrescine, Cadaverine, Spermidine, Spermine, Putrescine , B-phenyl ethyl amine, Histamine and tyramine . The ginger oil emulsion 60% NPs, garlic oil emulsion 60% NPs and onion oil emulsion 60% NPs were safe and have sizes (222.6 ± 2.22 nm, 420.7 ± 36.95 nm and 202.9 ± 2.1 nm) respectively. Salmonella spp , *E.coli* and S.areus were the most sensitive to the three nanoparticles at 7.5%, while *Klebseilla pneumonia* and Enterobacter spp were the most resistant. The three nanoparticles at level 7.5% had a significant effect on the concentration of biogenic amines, as in histamine the untreated samples were 2.3mg/kg, while in treated samples it was zero also tyramine was 1.19mg/kg, while in treated samples was zero , Cadaverine, was 38.59mg/kg in untreated while in treated was 2.66 , 1.5 and 1.29 mg/kg (garlic , onion and ginger) ,Putrescine was 8.3mg/kg in untreated while in treated was 5.8 , 4.7 and 7.8 mg/kg (garlic , onion and ginger.

Conclusion: Using plant extract such as ginger, garlic and onion nano are emulsion oils as antibacterial agents more effective in their action as antibacterial and reduction of biogenic amine formation.
Keywords (Biogenic amines, gram negative and gram positive bacteria, HPLC, minced meat, natural Nano emulsions)

1- Introduction

Biogenic amines (BAs) are low molecular weight compounds with biological activity, produced as a result of the decarboxylation of amino acids or amination and transamination of aldehydes and ketones during the metabolic processes in living cells (**Jaguey-Hernández et al.,**

2021). It has multifunctional roles as physiological substances used for neurotransmission, regulation of growth and blood pressure, and other important roles in the intestinal immune system **(Erdag et al., 2019).** However, when they increased over the acceptable level, it leads to an adverse effect on nervous, respiratory, and cardiovascular systems and/or allergic reactions (**Visciano et al., 2020**). It may be polar or semi-polar compounds with an aliphatic (putrescine, cadaverine, spermine, and spermidine), aromatic (tyramine, phenylethylamine), or heterocyclic (histamine, pyrrolidine) structure (**Papageorgiou et al., 2018).**

These low-molecular-weight elements are formed mainly by enzymatic decarboxylation of different amino acids present in meat through microbial enzyme activity during storage (**Zhang et al., 2019**). Several groups of microorganisms were reported to produce decarboxylase enzymes like Enterobacteriaceae, Micrococcaceae, and Pseudomonadaceae (**Ekici and Omer, 2020** Meat and meat products are common sources of biogenic amines especially Fermented as meat products contain high amounts of amino acids that are easily broken down to hazardous biogenic amines, as (Histamine , tyramine which are the most biogenic amines found in meat products followed by putrescine and cadaverine Sausages had high levels of all biogenic amines except spermidine and spermine. (Simon Sarkadi and Livia 2019) .Thus, their ratio and amount of biogenic amines act as an index for the hygienic conditions of raw materials and manufacturing practices as Biogenic amine index (BAI) was calculated by summing tyramine, histamine, putrescine and cadaverine levels in the different meat types When one of the BA involved was not detected (ND), its value was considered as being 0. BAI < 5 mg/kg means good meat quality; between 5–20 mg/kg means acceptable meat quality; between 20–50 mg/kg means

poor meat quality; and BAI > 50 mg/kg means spoiled meat. **Triki et al.,(2018) and Algahtani et al.,(2020**).

Meat safety has been recently at the forefront of societal considerations. Also there is increased necessity to prevent and reduce the frequency and concentration of traditional and developing foodborne pathogens, **Brashears and Chaves,(2017**). So, many manufacturing techniques were developed to decrease or even prevent biogenic amines formation through decrease the microbial growth and decarboxylase activity One of them is addition of natural preservatives and coatings **Saleh et al., (2017; Eldaly et al., (2018); Mahmoud,(2019).**

Natural products, such as essential oils (EOs) represent complex mixtures of aromatic and volatile liquids frequently distilled from plant, and it has distinctive flavors, antioxidation, and antibacterial effects **Khan et al., (2019).** Garlic, Onion, and Ginger were the most used ingredients as a flavor enhancement in meat. Garlic has a wide spectrum of actions, not only antibacterial, antifungal , and antiprotozoal, but also it has beneficial effects on the cardiovascular , immune systems and also has a significant decrease in the level of putrescine, cadaverine, histamine, tyramine, and spermidine ($p < .05$) **Saad et al.,(2019).** Onion extract has been considered a natural preservative with antifungal and antibacterial effects against a wide variety of Gram-negative and Gram-positive bacteria **Kabrah et al.,(2016).** That helps in the inhibition of the biogenic amine formation by the antibacterial activity of plant extracts. Also, ginger contains a higher amount of amine oxidases which help reducing biogenic amine formation by inhibiting the growth of bacteria **Lu et al.,(2015**).

Extensive use of antibiotics in treating bacterial infections resulted in the development of multidrug-resistant bacterial strains. Providing effective and secure therapies for drug-resistant bacterial strains is an important global health challenge Thus, nanoparticles can be classified based on their chemical content. Mineral nanoparticles contain metallic oxides and nanoparticles like gold, titanium oxide, zinc oxide, silver, platinum, copper oxide, and iron oxide nanoparticles, while natural nanoparticles contain chitosan , carbon nanoparticles and nano plant extracts (garlic, onion, and ginger) using nanoparticles has replaced antibacterial agent that has demonstrated superior and effective bactericidal activity. As Nanoparticles carry out many antibacterial activities against gram-positive and gram-negative bacteria by destroying the cell membrane and producing reactive oxygen species (ROS)

Nanotechnology has greatly advanced because of the exceptional physical and chemical characteristics of nanoparticles. Nowadays, it has spread to about all scientific fields to create new alternatives and to solve bottlenecks related to various types of research topics as resolved antibacterial resistance, antibiofilm formation and Food packaging by using Polymer nanotechnology. There are many ways to synthesize nanoparticles, involving traditional techniques like physical and chemical approaches, and biological synthesis using plants, fungi, **Microalgae and Cyanobacteria** contribute to the green synthesis of pure nanoparticles Nanoparticles are produced in various sizes and shapes because of their new properties compared to their bulk peers. Nanoparticles act as a bridge between bulk matter and atomic or molecular structures The chemical, biochemical, and physicochemical characteristics of nanoscale substances are very different, mainly because of the high surface-to-volume ratio, which causes significant differences in their mechanical qualities, melting point, light absorption, thermal conductivity, biological, and catalytic activities **(Peidaei, F. et.al.(2021), Anvar, A. A., et.al.(2022),**

This study aimed to explore the antibacterial effect of different nano plant extracts (garlic, onion, and ginger) against on some bacteria producing biogenic amines and measuring the concentration of biogenic amines before and after treatment with these nano-emulsions.

2.-Material and methods

1- Sample collection:

A total of 210 fresh minced meat collected from butcher shops at Al Qalyubia Governorate Egypt and 100 gram for each sample and collected in sterile plastic bags then the samples were placed in icebox (4°C) and transported to laboratory

This research is excluded from ethical limitations, because the animals were not touched directly by the authors.

2- Bacteriological examination

Decarboxylase activity: Samples were prepared according to **(APHA, 2001).** Then one ml from each prepared sample was inoculated on nutrient broth and incubated at 37ºC for 24 hrs. A loopful from incubated nutrient broth was streaked over lysine iron agar in order to determine the ability of bacteria to form biogenic amines due to its decarboxylase and deaminase activity. The agar was incubated at 37ºC for 24 hrs.

3-Bacteriological isolation and identification according to Paul *et al.*, (2009) and Markey *et al.*,(2013;).

According to the results of lysine agar, the suspected bacteria were inoculated on MacConkey agar, XLD agar and 10% sheep blood agar, Baired parker agar and MRSA agar. Colonies were examined for their morphology, pigmentation and hemolytic ability. Then biochemical tests were performed. Finally, subculture the isolated strains into brain heart broth with 30% glycerin and kept in -18 ºC for preservation and until further tests were done.

4- Serological identification of the isolated *E. coli* and Salmonella species

By using the technique of slide agglutination test **(Markey *et al.*, (2013**).

Serotyping of *E. coli* isolates was performed using rapid diagnostic *E. coli* antisera sets (Anti-Coli, Sifin- Germany) obtained from the Animal Health Research Institute, Dokki, Egypt. Anti-Salmonella I (A-E+Vi) and anti-salmonella phase $H_1$and H_2 (SIFIN) obtained from the Animal Health Research Institute, Dokki, Egypt, were used . The serotyping of Salmonella was done according to the Kauffman-White scheme. **Grimont and Weill,(2007).**

5-Preparation, characterization and cytotoxicity assay of Garlic, Ginger and Onion nano-emulsions

Garlic, Ginger and Onion nano-emulsions (60%) were prepared in Nanomaterials Research and Synthesis Unit in Animal Health Research Institute, Dokki, Egypt. according to Rao and McClements (2011) Nano-emulsion oil parepared by adding 60 ml of each **Garlic, Ginger and Onion oil-emulsions** to 10 ml of tween 80 and 30ml distilled deionized water which were mixed for half in heamogeneous blender 1500watt and then add distilled deionized water slowly to the mixed oil phase

6- Zetasizer Malvern Instrument (Malvern, UK) was used to measure droplet size, surface charge (zeta potential), size distribution (polydispersity indexes [PDI]), and electrical conductivity of the nanoemulsions. At fixed angle of 173° at 25° C. Samples were analyzed in triplicate.

7- Cytotoxicity assay Sulforhodamine B (SRB) assay was done to investigate the cytotoxicity of prepared nano-emulsions **(Skehan et al., 1990)** Different concentrations (0.006, 0.06, 0.6, 6, and 60 %) were tested against rat heart/ myocardium cell line, obtained from Nawah

Scientific Inc. (Mokatam, Cairo, Egypt). Cells were maintained in DMEM media supplemented streptomycin (100 mg/mL), penicillin (100 units/mL), and 10% heat-inactivated fetal bovine serum and incubated in a humidified atmosphere containing 5% CO2 at 37°C.

8-Antibacterial effects of (Ginger, Garlic and Onion) nano-emulsions done by using (microdilution method (MIC)) according to (**Kowalska-Krochmal and Dudek-Wicher, 2021**).In 96 well-plates., 50 ul of peptone water broth was dispensed into each well of the column1.Then 50 ul of the garlic nano-emulsion was added in column "1". Double serial dilutions were performed using a multichannel pipette for transferring and mixing garlic nano-emulsion from column 1-6 in order to obtain different concentrations of the nano emulsion (60, 30, 15, 7.5, 3.75 and 1.875 %). Finally, 50 ul of each isolated bacteria inoculum (5×10^8 cfu\ ml) was inoculated in one row. Negative control well inoculated with pepton water only and positive control well inoculated with the bacteria . The plate was incubated at 37ºC for 24hrs. After incubation, A loopful from each concentration was inoculated on nutrient agar to determine MIC, which known as the lowest concentration that showed no bacterial growth. MIC for Onion and Ginger nanoemulsion was determined as previously described with Garlic nanoemulsion.

8- Biogenic amines determination.

Sampling:-

24 samples were been detected for present of biogenic amines (6 samples free from any nanoparticles (without treatment) , 6 sampleas with adding 7.5% of Garlic nano-emulsions , 6

samples with 7.5% of Onion nano-emulsions and another 6 samples with 7.5% of ginger nano-emulsions

Extraction and Formation of dansylamines

Treptamine,B-phenyl ethyl amine, Putrescine, Cadaverine, Histamine, Serotonin, Tyramine, Spermidine and Spermine were extracted from Twenty five grams of each homogenised sample and determined by using -High performance liquid chromatography (HPLC) used for dansylamines determination was an Agilent 1260 affinity system (Germany) equipped with auto sampler, pump, UV detector set at 254 nm wavelength. Agilent Poroshell 120 EC-C18 4um (4.6 mm × 150 mm) column was used for biogenic amines separation. Data were integrated and recorded using Chromeleon Software program **according to Mietz and Karmas (1977), Ayesh (2012), sultan and Marrez(2014)** with some modifications

Statistical analysis. 9-

Statistical software Minitab17. The significance level for statistical analyses was $P \leq 0.05$.

3.Results

1-Decarboxylase activity of samples and their bacterial isolation.

The results of lysine iron agar inoculation differ according to types of bacteria present in minced meat samples and its ability to make decarboxylation, or deamination and formation of hydrogen sulphide. **Table (1)** represented bacterial species isolated and their decarboxylase activity. The reported results showed that (31.9%) of samples give lysine positive . The bacteria isolate were (*E. coli, Klebseilla pneumonia, Enterobacter spp and S.. aureus)*, while (27.6%) of

samples yielded lysine positive with production of H_2S and the following bacteria was isolated (*Salmonella spp, Aeromonas hydrophila*), (21.4%) of samples make deamination to lysine (represent by red color of indicator) with production of H_2S the bacteria isolated was (*Proteus marbalis* and with red color only for *Pasteurella multocida),* and Also, the negative results were detected by 19.04% of samples and *Lactobacillus* species were isolated

Table (1) Isolated bacterial species and their decarboxylase activity

Decarboxylase activity	**Number of samples**	**%***	**Isolated bacteria**
Lysine positive	67	31.9%	***E. coli*** ***Klebsiella pneumonia*** ***Enterobacter spp*** ***S. aureus***
Lysine positive with H_2S production	58	27.6%	***Salmonella spp*** ***Aeromonas hydrophila***
Red lysine with H_2S	45	21.4%	***Proteus mirabilis***

			Pasteurella multocida
Lysine negative	40	19.04%	***lactobacillus spp***

*percentage related to total number of samples (n = 210)

The results of Salmonella and *E. coli* strains serotyping 2-

Salmonella strains were related to *Salmonella typhimurium* 1,4{5},12:i:1.2 and *S. arizonae*. While *E. coli strains* belonged to O44:K74 and O125:K70.

3-Characterization of Oil nano-emulsions (Ginger, Garlic and Onion).

The nano-emulsion was characterized by TEM nano-emulsion size, with a narrow size distribution indicating greater homogeneity in nanodroplet size (the homogeneous of nanoparticles, measured by PDI, the smaller the PDI the more homogeneous nanoparticles) and zeta potential indicates moderate stable suspensions, as in table (2)

Table (2) Characterization of Oil nano-emulsions (Ginger, Garlic and Onion).

	ginger oil emulsion 60% NPs	**garlic oil emulsion 60% NPs**	**onion oil emulsion 60% NPs**
Particle size	222.6 ± 2.22 nm	420.7 ± 36.95 nm	202.9 ± 2.1 nm
PDI	0.338 ± 0.012	0.432 ± 0.023	0.28 ± 0.016
Zeta potential	-14.4 ± 0.75 mv	-25.1 ± 0.2 mv	-15.8 ± 0.35 mv

The viability % of the rat cells (H9C2) using SRB assay using different concentrations of Ginger oil, Garlic oil, and Onion oil nano-emulsions (60, 6, 0.6, 0.06, and 0.006 %) after three days post inoculation showed the following results recorded in Table (3). In which the IC50 > 60% for onion, garlic, and ginger oil emulsions are as shown in (Figures 1, 2, and 3)

Table (3) **Viability of rat cells when using different concentrations of nano-emulsions.**

Concentration	**ginger oil nano emulsion**	**garlic oil nano emulsion**	**onion oil nano emulsion**
60	82.7832	52.9538	91.9771
6	98.4174	98.9923	97.0489
0.6	100.328	99.4962	99.8429
0.06	100.203	100.088	99.7756
0.006	99.8304	100.441	99.7083

Ic50	>60%	>60%	>60%

A B

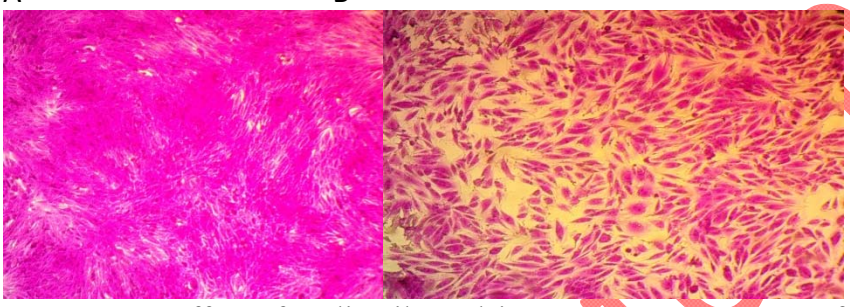

Figure **(1)** A effect of garlic oil emulsion 0.006% NPs B effect of garlic oil emulsion 60% NPs

A B

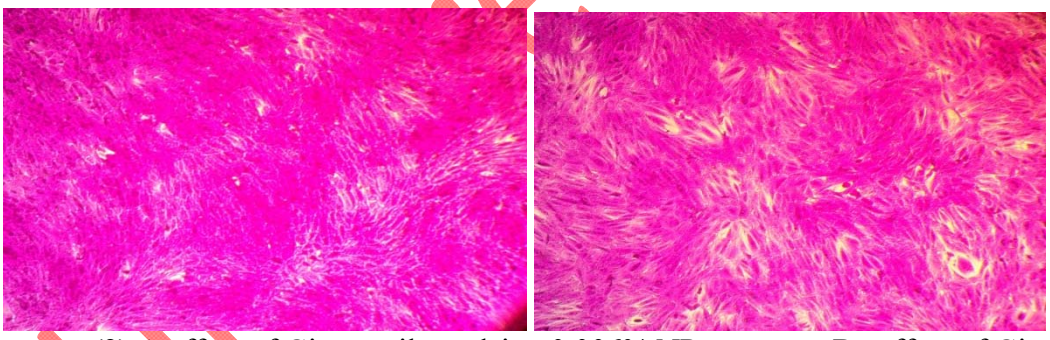

Figure **(2)** A effect of Ginger oil emulsion 0.006% NPs B effect of Ginger oil emulsion 60% NPs

A B

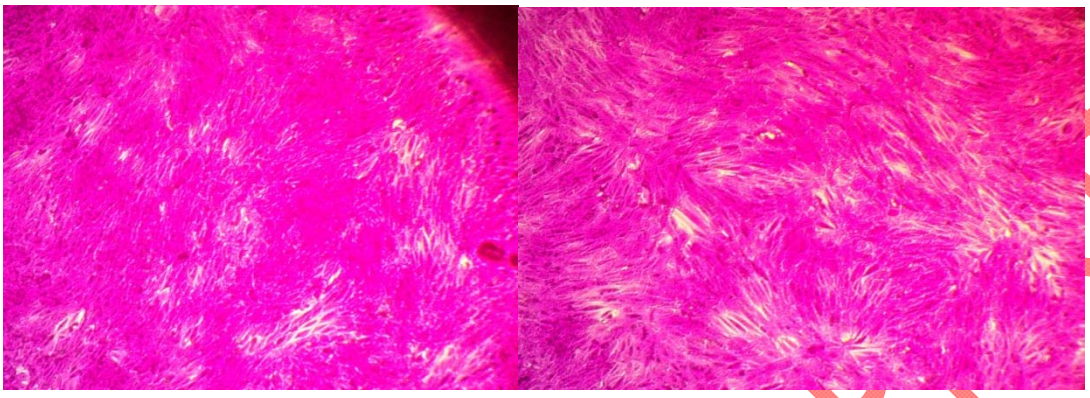

Figure **(3)** A: effect of Onion oil emulsion 0.006% NPs B: effect of Onion oil emulsion 60% NPs

4- Antibacterial activity of Garlic, Ginger, and Onion micro-emulsions (In-Vitro MIC)

The antimicrobial activity and microdilution susceptibility test of nano-emulsions used was determined using the MIC value as the lowest concentration of nano -emulsion was causes inhibition of bacterial growth. The results tabulated in Table (4) explained that the Garlic nano-emulsion was greated affected in *E. coli* and Salmonella at conc. 3.75%, while inhibiting the growth of *Klebsiella pneumonia, Aeromonas hydrophila*, Enterobacter species, *Proteus mirabilis*, and *S. aureus* at conc. 7.5%, and for lactobacillus species at concentration 15% . While Onion nano-emulsion hinders the growth of *E. coli*, Salmonella, *Proteus mirabilis*, and *S. aureus* at conc. 7.5%, followed by inhibition to *Aeromonas hydrophila* and Lactobacillus at conc. 15%, and for Klebsiella pneumonia and Enterobacter species at conc. 30%. Moreover, Ginger nano-emulsion reduced the growth of *E. coli*, Salmonella, *Proteus mirabilis*, and *Staph. aureus* and Lactobacillus at conc. 7.5%, while for *Aeromonas hydrophila* inhibition occurs at conc. 30%, and at 60% for *Klebsiella pneumonia* and Enterobacter species

That indicated the Garlic nano-emulsion has greater antibacterial effect more than onion and Ginger nano-emulsions.

Table (4) In- Vitro MIC of Garlic, Ginger, and Onion micro-emulsions.

Isolates	MIC of garlic	MIC of onion	MIC of ginger
E. coli	3.75%	7. 5%	7.5%
Salmonella spp	3.75%	7. 5%	7.5%
Klebsiella pneumonia	7. 5%	30%	60%
Aeromonas hydrophila	7. 5%	15%	30%
Entreobacter spp	7. 5%	30%	60%
Proteus mirabilis	7. 5%	7. 5%	7.5%
S. aureus	7.5%	7.5%	7.5%
Lactobacillus spp	15%	15%	7.5%

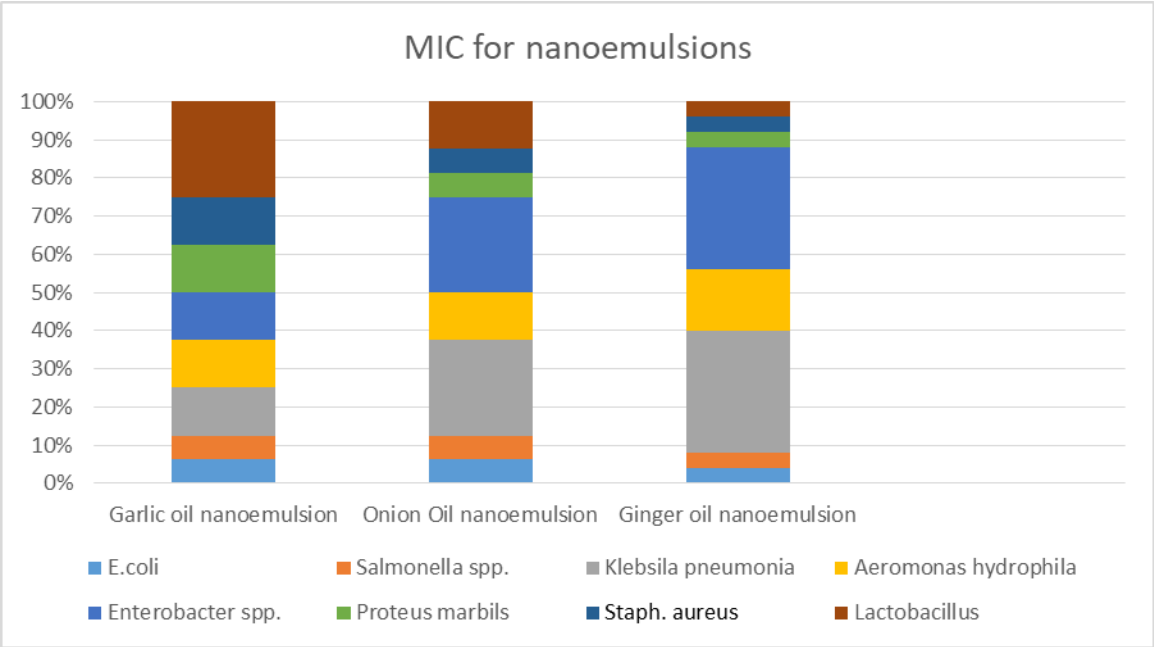

Figure **(4) In- Vitro MIC of Garlic, Ginger and Onion micro-emulsions.**

5-Biogenic amines detection by HPLC

According to the results reported in Table (5,6,7,8), the level of putrescine varied from 8.30, 17.87, 11.19, 5.66, 4.35, and 2.08 mg\kg in the first untreated group to be 5.82, 12.53, 6.07, 3.97, 3.05, and 1.46 mg\kg for second group treated with garlic nano-emulsion putrescine was varied from 4.77, 10.26, 6.43, 3.25, 2.51, and 1.21mg/kg for the third group treated with onion nano-emulsion while the ginger nano-emulsion treated groups putrescine was 7.86, 16.92, 10.60, 5.36, 4.12, and 1.97 mg/kg.

Moreover, the level of Cadaverine differed from 38.59, 28.70, 26.50, 20.60, 0.87, and 3.60 mg\kg in the first untreated group to be 2.66, 1.98, 1.72, 1.42, 0.06, and 0.25 mg\kg for second group treated with garlic nano-emulsion. The third groups treated with onion nano-emulsion had 1.52, 1.12, 1.03, 0.8, 0.03, and 0.14 mg/kg. While the ginger nano-emulsion treated groups, the level of Cadaverine was 1.29, 0.96, 0.89, 0.69, 0.03, and 0.12 mg/kg**.**

Furthermore, the level of spermidine differed from 5.22, 1.36, 15.22, 8.33, 3.91, and 7.76 mg\kg in the first untreated group to 0.58, 0.15, 1.38, 0.93, 0.44, and 0.87 mg\kg for second group treated with garlic nano-emulsion while varied to be 0.84, 0.22, 2.45, 1.34, 0.63, and 1.25 mg\kg for the third group treated with onion nano-emulsion and in ginger nano-emulsion 1.002, 0.26, 2.92, 1.6, 0.75, and 1.49 mg\kg**.**

Likewise, the level of spermine differed from 18.33, 4.17, 5.32, 5.18, 24.22, and 5.48 mg\kg in the first untreated group to levels 3.22, 0.73, 0.91, 0.91, 4.25, and 0.96 mg\kg for the second group treated with garlic nano-emulsion and group treated with onion nano-emulsion spermine was vary from 2.93, 0.68, 0.87, 0.85, 3.97, and 0.90 mg\kg. In ginger nano-emulsion treated groups spermine was vary from. 4.71, 1.07, 1.37, 1.33, 6.22 and 1.41 mg\kg **.**

But the level of histamine and tyramine was not detected in all treated groups, while it varied from 2.31, 1.63, 1.51, 0.76, 2.05, and 0.72 mg\kg for histamine, and 2.15, 0.82, 1.35, 1.51, ND, and 1.19 mg\kg for tyramine in the first groups**.**

Table (5) Biogenic amines in the first group (untreated groups):-

Sample code	Tryptamine mg/kg	B-phenylethylamine mg/kg	Putrescine mg/kg	Cadaverine mg/kg	Histamine mg/kg	Serotonin mg/kg	Tyramine mg/kg	Spermidine mg/kg	Spermine mg/kg
1	0.56	0.18	8.30	38.59	2.31	ND	2.15	5.22	18.33
2	ND	0.28	17.87	28.70	1.63	ND	0.82	1.36	4.17
3	ND	ND	11.19	26.50	1.51	ND	1.35	15.22	5.32
4	ND	ND	5.66	20.60	0.76	ND	1.51	8.33	5.18
5	ND	ND	4.35	0.87	2.05	ND	ND	3.91	24.22
6	ND	ND	2.08	3.60	0.72	ND	1.19	7.76	5.48

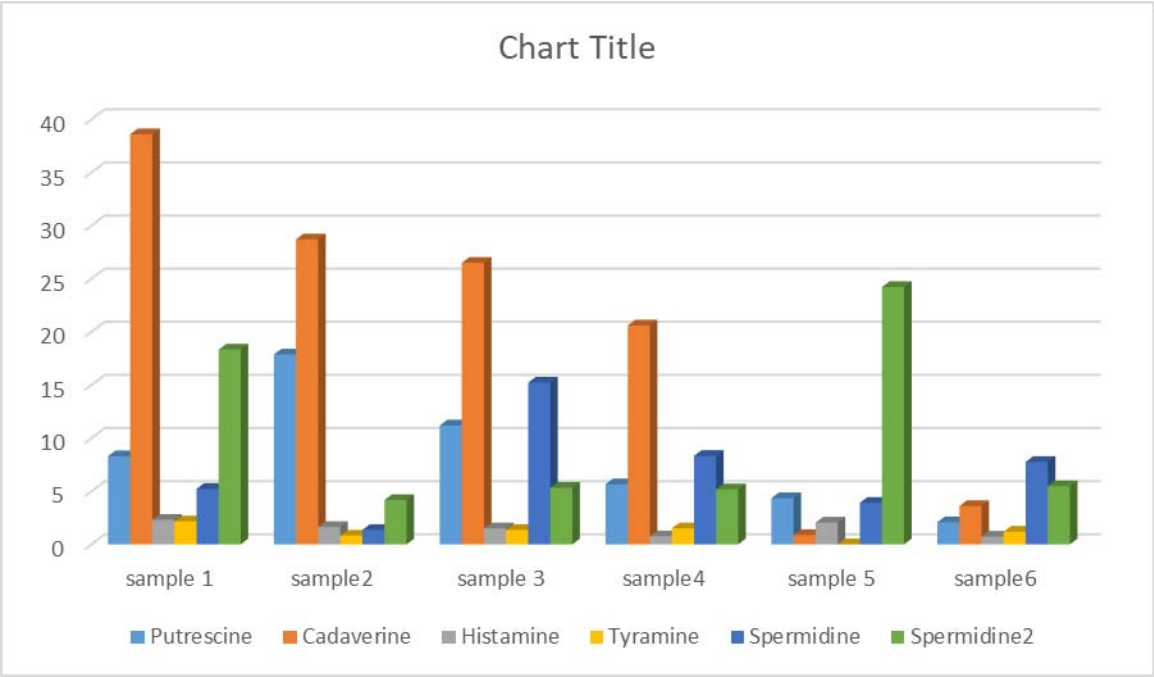

Figure **(5) Biogenic amines in first group (untreated group).**

Table (6) Biogenic amines in the second group (treated with 7.5% Garlic oil nano-emulsion).

Sample code	Tryptamine mg/kg	B-phenyl ethyl amine mg/kg	Putrescine mg/kg	Cadaverine mg/kg	Histamine mg/kg	Serotonin mg/kg	Tyramine mg/kg	Spermidine mg/kg	Spermine mg/kg
1	ND	ND	5.82	2.66	ND	ND	ND	0.58	3.22
2	ND	ND	12.53	1.98	ND	ND	ND	0.15	0.73
3	ND	ND	6.07	1.72	ND	ND	ND	1.38	0.91
4	ND	ND	3.97	1.42	ND	ND	ND	0.93	0.91
5	ND	ND	3.05	0.06	ND	ND	ND	0.44	4.25
6	ND	ND	1.46	0.25	ND	ND	ND	0.87	0.96

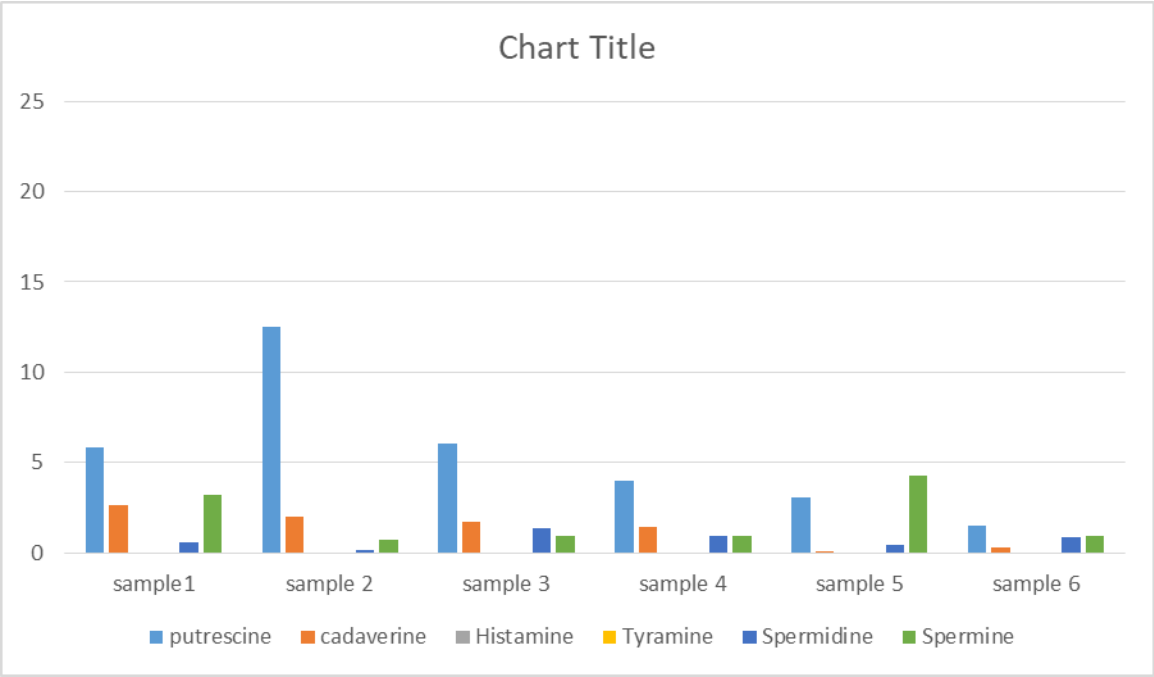

Figure **(6) Biogenic amines in the second group (treated with 7.5% Garlic oil nano-emulsion).**

Table (7) Biogenic amines in the third group (treated with 7.5% Onion oil nano-emulsion).

Sample code	Tryptamine mg/kg	B-phenyl ethyl amine mg/kg	Putrescine mg/kg	Cadaverine mg/kg	Histamine mg/kg	Serotonin mg/kg	Tyramine mg/kg	Spermidine mg/kg	Spermine mg/kg
1	ND	ND	4.77	1.52	ND	ND	ND	0.84	2.93
2	ND	ND	10.26	1.12	ND	ND	ND	0.22	0.68
3	ND	ND	6.43	1.03	ND	ND	ND	2.45	0.87
4	ND	ND	3.25	0.8	ND	ND	ND	1.34	0.85
5	ND	ND	2.51	0.03	ND	ND	ND	0.63	3.97
6	ND	ND	1.21	0.14	ND	ND	ND	1.25	0.90

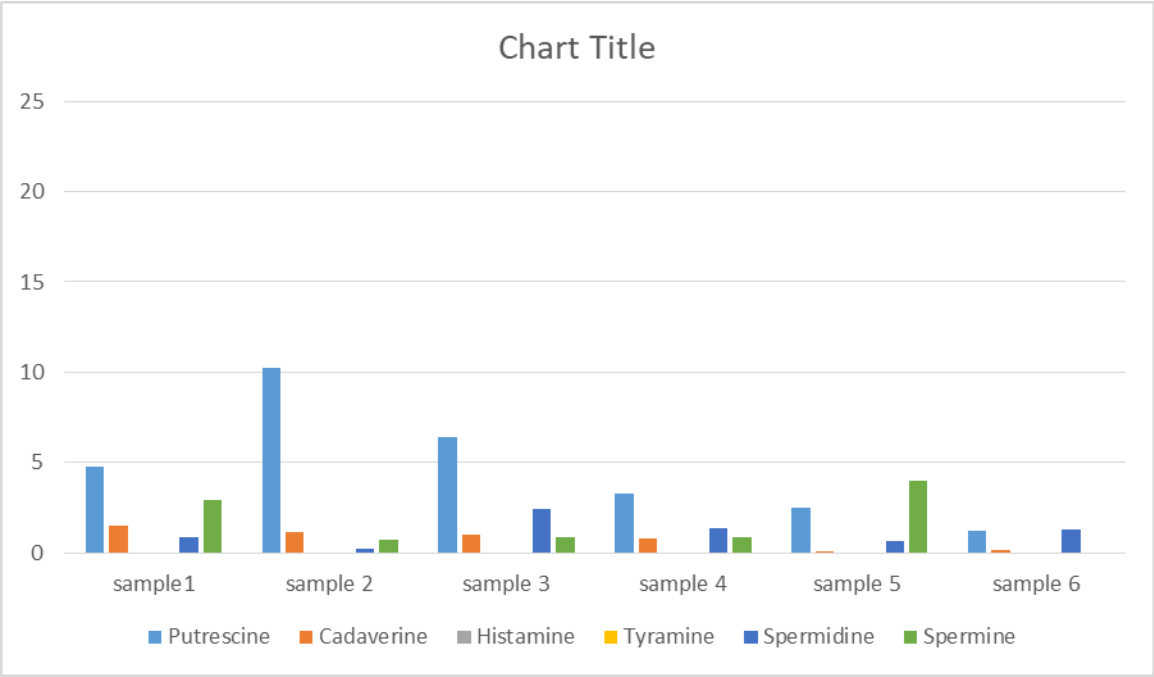

Figure **(7) Biogenic amines in the third group (treated with 7.5% Onion oil nano-emulsion).**

Table (8) Biogenic amines in the fourth group (treated with 7.5% Ginger oil nano-emulsion).

Sample code	Tryptamine mg/kg	B-phenylethylamine mg/kg	Putrescine mg/kg	Cadaverine mg/kg	Histamine mg/kg	Serotonin mg/kg	Tyramine mg/kg	Spermidine mg/kg	Spermine mg/kg
1	ND	ND	7.86	1.29	ND	ND	ND	1.002	4.71
2	ND	ND	16.92	0.96	ND	ND	ND	0.26	1.07
3	ND	ND	10.60	0.89	ND	ND	ND	2.92	1.37
4	ND	ND	5.36	0.69	ND	ND	ND	1.6	1.33
5	ND	ND	4.12	0.03	ND	ND	ND	0.75	6.22
6	ND	ND	1.97	0.12	ND	ND	ND	1.49	1.41

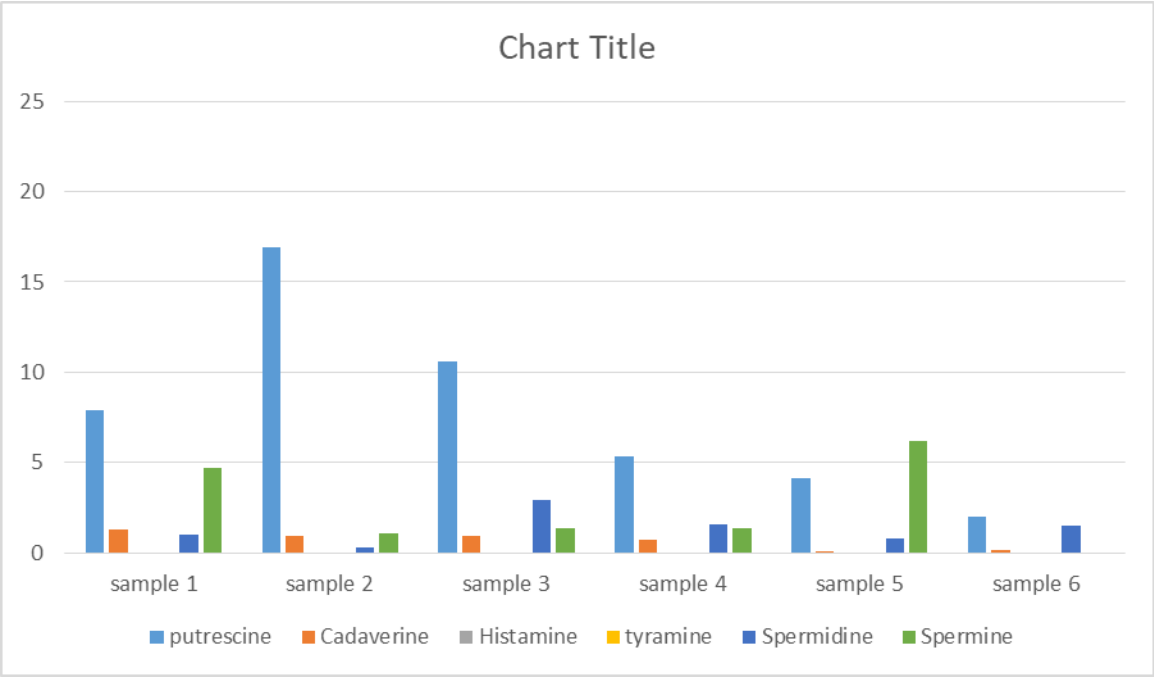

Figure **(8) Biogenic amines in the fourth group (treated with 7.5% Ginger oil nano-emulsion).**

4. Discussion

Presence of biogenic amines in food acts as an indicator for bacterial decarboxylation of amino acids. Their types and amounts depend on the presence of different bacteria in foods. **Ruiz-Capillas et al., (2007)**. For example, species of many genera such as Bacillus, Citrobacter, Clostridium, Klebsiella, Escherichia, Proteus, Pseudomonas, Salmonella, Shigella, Photobacterium and the lactic bacteria Lactobacillus, Pediococcus and Streptococcus are capable

of decarboxylating one or more amino acid **Ekici and Omer, (2020)**. The biogenic amines were detected using media containing pH indicators such as Bromcresol Purple to determine the ability of microorganisms to form biogenic amines and to differentiate between bacteria, **Kalhotka et al.,(2012).** The present study used lysine iron agar to isolate bacteria producing a decarboxylase enzyme. Some bacteria were isolated as (E. coli, Klebsiella pneumonia, Enterobacter spp, S. aureus, Salmonella spp, Aeromonas hydrophila, Proteus mirabilis, Pasteurella multocida, and Lactobacillus spp) as tabulated in Table (1). Which have many virulence factores not only decarboxylase activity but also causing diseases in the poultry industry and Infect a wide range of hosts, causes public health concerns. the widespread antimicrobial resistance especially among *S. aureus* **(Gholipour-Shoshod, A (2023) and Bagheri, S.,et.al.(2019)** Which coming by that mentioned by **Jairath et al., (2015)** who reported that decarboxylase activity in meat products is attributed mainly to Enterobacteriaceae, Pseudomonadaceae, Micrococcaceae, and lactic bacteria. Li et al., (2020) isolated several bacteria that produce biogenic amines like Enterobacteriaceae and pseudomonas, some strains belonging to the genera Staphylococcus and Bacillus, and LAB are isolated from meat and meat products. In addition, **Pircher et al., (2007)** detected the presence of different biogenic amines (cadaverine, histamine, putrescine, and tyramine) in raw meat and fermented sausages and isolated bacteria were Enterobacteriaceae and Lactobacillus species. And **Bermúdez et al., (2012)** isolated a group of gram-positive bacteria (lactic acid bacteria (LAB), Staphylococcus, and Bacillus) from cheese and traditional sausage and found that they formed biogenic amines

The importance of obtaining safe food has increased globally by using plant-based products as additives for raw and processed meat products to avoid the development of

aminogenic contaminant bacteria and, in turn, to reduce biogenic amine content Lu et al., (2015). This study determined the antibacterial effect of ginger NP, Garlic NP, and onion nano-emulsions with conc.60% against different aminogenic-producing bacteria. And as recorded in Table (2) the mean diameter of the nano-emulsion particles were (222.6 ± 2.22 nm, 420.7 ± 36.95 nm, and 202.9 ± 2.1 nm respectively) for (ginger oil NP 60%, Garlic oil NP 60%, and Onion oil nano-emulsions 60% respectively) and their zeta potential was ((-14.4 ± 0.75 mv, -25.1 ± 0.2 mv, -15.8 ± 0.35 mv) respectively. That is near to that reported by **Hassan and Mujtaba (2019)** for garlic oil nano-emulsion and by **Ningsih et al. (2020)** for ginger oil nano-emulsion.

PDI value is a parameter for determining the size distribution of droplets. Generally, a small PDI value indicates a narrow size distribution, while a value higher than 0.7 represents a broad size distribution **Gul et al., (2018**). The narrow size distribution indicates greater homogeneity in nanodroplet size (the smaller the PDI the more homogeneous nanoparticles). While Zeta potential represents the electrical charge of the particles and characterizes the colloidal system's behavior, which is vital for the stability of nano-emulsion **Pabast et al., (2018**). The transformation of crude essential oils to Nano forms helps in increase their distribution and their antibacterial activity as previously reported by **Ma et al., (2016) and Carpenter and Saharan, (2017**). Also, it was supposed that essential oil in nano-emulsions had an improved physicochemical stability and dispersibility in food matrices, leading to easier access to bacteria and consequently higher antibacterial activity **Donsì and Ferrari, (2016**). Cytotoxicity of the used nano-emulsions was tested against the rat cells (H_9C_2) using SRB assay

and found that they were safe to the cell until 60% concentration and have antibacterial effect on isolates until 7.5% concentration and reduction the biogenic amines. Also the ginger oil nano emulsion has repaired effect on cell at concentration 0.06% and 0 .6% while the garlic oil nano emulsion at concentration0 .006% and 0.06% as recorded in Table (3), Fig (1,2 &3). Many authors recorded the effect of ginger nano particale as anti-inflammatory and repairing the cells as **Zhang et al.** (2016), **Sung *et. al.***, (2019) and **Al-Badawi *et. al. (2022)***

The antibacterial activity and minimum inhibitory concentration (MIC) of the used nano emulsions (ginger oil NP 60%, Garlic oil NP 60%, and Onion oil nano emulsions 60%) recorded in Table (4), in which the MIC of garlic oil nano emulsions mainly occur at 7.5% for most examined Bacteria, **Liu et al., (2022)** who reported that the MIC of garlic oil nano emulsions was 1.25% against MRSA, and with **Hassan and Mujtaba, (2019)** and **Hassan et al., (2020)** who determined that Garlic oil nano emulsions have greated effect toward Gram-positive bacteria more than Gram-negative ones.. That came in accordance with **Zheng et al. (2013)** ,whose found that garlic nano emulsions showed stronged antibacterial activity against *S. aureus* at higher concentrations.

The MIC for onion oil nano emulsions in most bacteria appeared to be 7.5%, while it may increase to 30% for other bacteria. Kabrah et al. (2016),reported the antibacterial effect of the onion extract in vitro against many bacterial species included Bacillus subtilis, Salmonella, E. coli, and Staphylococcus aureus, the results showed a complete inhibition of all strains tested at a concentration of 6.5 mg/ml. It noted that the partial size of nano-emulsion is pivotal in determining the antimicrobial ability of agents that reduce the particle size of nano emulsion,

thus leading to increased exposure to the microbial membrane and enhanced antibacterial activity, **Liu et al.,(2022)**. So, the antibacterial effect of onion extract enhanced by its transformation to nano form. In addition, the Ginger oil nano emulsion has the same concept; its conservation of nanoparticles enhances their effect. The MIC of ginger oil nano-emulsion mainly appears at conc. 7.5%, while it may increase to 60% in the case of K. pneumonia and Enterobacter spp. That came in accordance with **Thakur et al. (2013)**, whose reported that the ethanolic ginger extract showed more potency against *E.coli* and moderately inhibited *P. aeruginosa* and *K. pneumonia*. The ginger extract contains many different bioactive compounds with antimicrobial activities that appear more sensitive to gram-positive bacteria than gram-negative ones **Gurumayum, (2015).** The results tabulated in Table (5), Fig (5) determined the level of biogenic amines presented in six samples (two samples were lysine positive, two were lysine positive with a production of H2S, one produced red lysine with H2S and the last one was lysine negative), the level of putrescine, cadaverine, tyramine and histamine were higher among the six samples, this mainly occurs due to bacterial contamination of the samples or bad storage condition as recorded by **Doeun et al.,(2017).** That came with agreement **Stadnik and Dolatowski (2010)** ,who mentioned that tyramine, cadaverine, putrescine, and histamine are the dominant biogenic amines in meat and meat products. Cadaverine represents the greatest amines present in meat due to precursor lysine in meat ,**Vinci and Antonelli,** 2002).(

Meat represents a good source for biogenic amine production, this was occurred due to the presence a lot of protein that acts as a starting point for bacterial decarboxylation and subsequently Biogenic amine formation **Schirone et al., (2022)**. The presence of one or more biogenic amines in meat samples acts as indicators of freshness, quality, and spoilage in meat

and meat products **Triki et al., (2018)**. The ratio between Spermine and Spermidine evaluates the quality of raw meat **Jastrzebska et al., (2015)**. While the sum of Cadaverine and Putrescine acts as an index for microbial decayed and the level of Histamine and Tyramine begin to elevate after several days of spoilage, there is no standards or guidelines have been reported for the presence of histamine in meat **Schirone et al., (2022)**

The biogenic amine index (BAI) consists of the total of putrescine, cadaverine, histamine, and tyramine, and according to **Hernandez-Jover et al., (1997)** mentioned that the range of BAI value is less than 5 mg/kg represents fresh meat and of good quality, between 5 and 20 mg/kg it is still acceptable with some signs of deterioration, between 20 and 50 mg/kg and above 50 mg/kg the meat is of low quality and spoiled.

The results tabulated in Table (6), Fig (6) determine the level of biogenic amines in minced meat samples after treatment with 7.5% from Garlic oil nano-emulsion and as previously seen the level of biogenic amines were decreased to a low level and Histamine and tyramine disappeared completely, this means effective treatment of samples with Garlic oil nano-emulsion. This came by **Zhou et al., (2016)** who reported that Garlic extract mainly reduces biogenic amine-producing bacteria and found that the level of Histamine and spermidine in the samples handled with garlic extract was reduced significantly than that of the control ones. And assures the previous study of **Mah et al., (2009)** that determined that the addition of 5% garlic during the ripening of food reduced the biogenic amine level (putrescine, cadaverine, histamine, tyramine, and spermidine) significantly by 8.7%. The results recorded in Table (7), Fig (7) detected the level of biogenic amines in minced meat samples after treatment with 7.5% Onion

oil nano-emulsion, In which the level of biogenic amine markedly decreased in the treated samples than the untreated ones, Similarly results detected by **Majcherczyk and Surówka (2019**) where addition of onion caused a reduction in the total biogenic-amine content when compared with the control sample without an additive.

While the results in Table (8), Fig (8) declared the level of biogenic amines in minced meat samples after the addition of 7.5% Ginger oil nano-emulsion, and as previously described with other additives the level of biogenic amines decreased markedly with this treatment. This came by **Kongkiattikajorn, (2015)**, who found the addition of ginger extract led to a reduction in total biogenic amines concentration by 64.7% in samples added with ginger extract, as compared to control samples. **Lu et al., (2015)** reported a marked reduction in biogenic amines by using plant extracts like (cinnamon, clove, and ginger) this occurs by inhibiting the growth of biogenic amine-producing bacteria. Many authors reported the effect of Garlic, Ginger, and Onion, but no previous research on the effect of their nano emulsions and the level of biogenic amines formation in food, so this work aimed to focus on this item .

Conclusion:

Using nano emulsions of Garlic, Ginger, and Onion led to a significant reduction in the formation of undesired biogenic amines in minced meat and controlled the bacterial growth in minced meat

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Declarations

a. Ethics approval and consent to participate
Ethical approval for animal research was not required as live animals were not used in this study as the samples was taken after slaughter at the abattoir samples were collected from minced meat as routine commercial food and fiber
b. Contest for publication: Not applicable

c.Availability of data and materials

The datasets generated and/or analysed during the current study are available in figure file and table file

d. . Competing interest:

The authors declare that they have no competing interests.

e. Funding:

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f. Authors&; contributions

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