Original Article

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 occur as a result of bacterial contamination. Those may cause a series of problems for human health such as allergic reactions, itching, breathing difficulties, fever and hypertension.

Objectives: This study aimed to isolate 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, preparation of garlic, ginger and onion nano-emulsions (60%) and investigating their cytotoxicity by sulforhodamine B (SRB) assay. Then, the antibacterial effect of the prepared nano-emulsions against the isolated bacteria was explored by determination of their minimum inhibitory concentrations (MICs) and measuring biogenic amines levels by high performance liquid chromatography (HPLC).

Results: The most common bacteria isolated from samples were *Salmonella* species "*Salmonella Typhimurium1*, 4{5},12: i: 1.2 and *Salmonella arizonae*", *Escherichia coli* "serotype O44: K74 and O125: K70", *Klebseilla pneumonia*, *Enterobacter* spp, *Staphylococcus aureus*, *Aeromonas hydrophilia*, *Proteus mirabilis*, *Pasteurella multocida* and *Lactobacillus* species. The biogenic amines detected on positive samples were putrescine, cadaverine, spermidine, spermine, putrescine, B-phenyl ethyl amine, histamine and tyramine. The sizes of the ginger oil nanoemulsion 60%, garlic oil nanoemulsion 60% and onion oil nanoemulsion 60% were (222.6±2.22 nm, 420.7±36.95 nm and 202.9±2.1 nm) respectively, indicating that they were safe and stable. The antibacterial effect of the used nano emulsions showed that *Salmonella* spp, *E. coli* and *S. aureus* were the most sensitive strains while *K. pneumonia* and *Enterobacter* spp. were the most resistant ones. The level of the detected biogenic amines were reduced greatly after addition the oil nanoemulsions 7.5% to examined samples.

Conclusion: Using of plant extract as ginger, garlic and onion nanoemulsions oils as antibacterial agents and for reduction of biogenic amines was more effective.

Keywords: Biogenic amines, Minced meat, High performance liquid chromatography (HPLC), Natural nano emulsions, Bacteria producing biogenic amines

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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 (Balamatsia et al., 2006). BAs are produced by the action of enzymes on free amino acids in meat during storage (Ruiz-Jiménez & Luque de Castro, 2006). The amount and proportion of these compounds reflect the quality and safety of the raw materials and the processing methods. Therefore, BAs can be used as indicators of the hygienic conditions of meat products for instance: Putrescine and cadaverine combination act as an index of acceptability in fresh meat and their quantities increase during microbial spoilage even during chilled storage (Triki et al., 2018; Algahtani et al., 2020).

Meat safety has been recently at the forefront of societal considerations. Also there is increased necessity to prevent or even 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 BAs formation through decrease the microbial growth and decarboxylase activity One of them is addition of natural preservatives and coatings (Saleh et al., 2017; A 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 and immune systems (Saad et al., 2019). Garlic significantly reduce the contents of putrescine, cadaverine, histamine, tyramine and spermidine ($P<0.05$) (Mah et al., 2009). 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). So, those help in the inhibition of the biogenic amine formation by their antibacterial activity. Also, ginger contains a higher amount of amine oxidases which help in reducing biogenic amine formation by inhibiting the growth of bacteria (Yeunyongsuwan & Kongkiattikajorn, 2005; Lu et al., 2015). This study aimed to explore the antibacterial effect of different nano plant extracts (garlic, onion and ginger) against some bacteria producing BAs and measuring the concentration of BAs before and after treatment with these nano-emulsions.

Materials and Methods

Sample collection

A total of 210 fresh minced meat samples collected from butcher shops at Al Qalyubia Governorate, Egypt. Weight 100 gram for each sample and placed it in sterile plastic bags then put it into icebox and transported as soon as possible to the laboratory. This research is excluded from ethical limitations, because the animals were not touched directly by the authors.

Bacteriological examination

Decarboxylase activity

Samples were prepared according to (Salfinger, 2001). Then one mL from each prepared sample was inoculated into nutrient broth and incubated at 37 °C for 24 h. A loopful from incubated nutrient broth was streaked over lysine iron agar in order to determine the ability of bacteria to form BAs due to its decarboxylase and deaminase activity. The agar was incubated at 37 °C for 24 h.

Bacteriological isolation and identification

According to the results of lysine agar, the suspected bacteria were inoculated on MacConkey agar, XLD agar, 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 ˚C for preservation and until further tests were done.

Serological identification of the isolated *Escherichia coli* and *Salmonella* species

This done by using the slide agglutination test technique (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. While for *Salmonella*, 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 & Weill 2007).

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 30 mL distilled deionized water which were mixed for half in heamogeneous blender 1500 watt and then add distilled deionized water slowly to the mixed oil phase. The droplet size, surface charge (zeta potential), size distribution (polydispersity indexes [PDI]) and electrical conductivity of the nanoemulsions was measured by Zetasizer Malvern Instrument (Malvern, UK). At fixed angle of 173˚ at 25 ˚C. Samples were analyzed in triplicate.

Cytotoxicity assay

Sulforhodamine B (SRB) assay was done to investigate the cytotoxicity of prepared nano-emulsions (Skehan et al., 1990). Different concentrations of nano-emulsion (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). The 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% CO_2 at 37 ˚C.

Antibacterial effects of (ginger, garlic and onion) nano-emulsions

This was done by using Minimum inhibitory concentration (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 column 1. 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 contained pepton water only, while positive control well was inoculated with the microbe in pepton water. The plate was incubated at 37 ºC for 24 h. 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 nano emulsion was determined as previously described with garlic nano emulsion.

BAs determination

Sampling

Different BAs (treptamine, B-phenyl ethyl amine, putrescine, cadaverine, histamine, serotonin, tyramine, spermidine and spermine) were detected in six selected samples; represented as (two samples were lysine positive, two samples were lysine positive with production of H_2S, one sample produced red lysine with H_2S and the last one was lysine negative). They were subjected to four treatment before measuring BAs by high performance liquid chromatography (HPLC), including:

First group: Free from any addition; second group: Treated with 7.5% of garlic nano-emulsions to samples; third group: Treated with 7.5% of onion nano-emulsions to samples; fourth group: Treated with 7.5% of ginger nano-emulsions to samples.

Extraction and formation of dansylamines

Treptamine, B-phenyl ethyl amine, putrescine, cadaverine, histamine, serotonin, tyramine, spermidine and spermine were extracted and determined according to Mietz and Karmas (1977), Ayesh, (2012), Sultan and Marrez, (2014) with some modifications.

Reagents

a) Dansyl chloride solution: 500 mg of dansyl chloride (5-Dimethylamino naphtalene-1-sulfonyl chloride) were dissolved in 100 mL acetone.

b) Standard solutions: Stock standard solutions of the tested amines: 25 mg of each standard were dissolved in 25 mL distilled water individually.

Extraction

Twenty five grams of homogenised sample were blended with 125 mL of 5% TCA for 3 min using a warning blender. Filtration was achieved using filter paper Watman No. 1. Ten millilitres of the extracts was transferred into a culture tube with 4 g NaCl and 1 mL of 50% NaOH then shacked and extracted three times by 5 mL n-butanol/chloroform (1: 1 v/v) stoppered and shacked vigorously for 3.0 min. Centrifugation for 5 min at 3000 rpm and the upper layer was transferred to 50 mL separating funnel using disposable Pasteur pipette. To the combined organic extracts (upper layer), 15 mL of n-heptane was added and extracted three times with 1 mL portions of 0.2 N HC1, the HCl layers were collected in a glass stoppered tube. Solution was evaporated just to dryness using water bath at 95 ˚C with aid of a gentle current of air.

Formation of dansylamines

One hundred µl of each stock standard solution were transferred to a vial 50 mL and dried. About 0.5 mL of saturated $NaHCO_3$ solution was added to the residue of the sample extract (or the standard). Stoppered and carefully mixed to prevent loss-due to spattering. Carefully, 1 mL dansyl chloride solution was added and mixed-thoroughly using vortex mixer. The reaction mixture was incubated at 55 ˚C for 45 min. About 10 mL of distilled water was added to the reaction mixture, stoppered and shacked vigorously using vortex mixer. The extraction of dansylated BAs was carried out using three times of 5 mL portions of diethylether, stoppered, shacked carefully for 1 min and the ether layers were collected in culture tube using disposable Pasteur pipette. The combined ether extracts were carefully evaporated at 35 ˚C in dry bath with aid of current air. The obtained dry film was dissolved in 1 mL methanol, then 10 µL was injected in HPLC.

Apparatus

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 4 um (4.6×150 mm) column was used for BAs separation. Data were integrated and recorded using Chromeleon Software, version 6.8.

Statical analysis

Statistical Minitab software, version 17 was used for statistical analyses. The significance level for statistical analyses was P≤0.05.

Results

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 results showed that (31.9%) of samples gave lysine positive. Their bacteriological analysis revealed isolation of (*E. coli*, *Klebsiella 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*). Moreover, (21.4%) of samples made deamination to lysine (represent by red color of indicator) with production of H_2S the bacteria isolated was (*Proteus mirabilis* and with red color only for *Pasteurella multocida*), Also, the negative results were detected by (19.04%) of samples and *Lactobacillus* species were isolated from them.

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

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

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.

The viability % of the rat cells (H_9C_2) using SRB assay using different concentrations of ginger oil, garlic oil and

Table 1. Isolated bacterial species and their decarboxylase activity (n=210)

Decarboxylase Activity	No. (%)	Isolated Bacteria
Lysine positive	67(31.9)	*E. coli* *K. pneumonia* *Enterobacter* spp *S. aureus*
Lysine positive with H_2S production	58(27.6)	*Salmonella* spp *A. hydrophila*
Red lysine with H_2S	45(21.4)	*P. mirabilis* *P. multocida*
Lysine negative	40(19.04)	*Lactobacillus* spp

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 IC_{50}>60% for onion, garlic and ginger oil nano-emulsions as shown in (Figures 1, 2 and 3).

Antibacterial activity of garlic, ginger and onion nano-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 which caused inhibition of bacterial growth. The results tabulated in Table 4 explained that the garlic nano-emulsion was greatly affected in *E. coli* and *Salmonella* at conc. 3.75%, while inhibited growth of *K. pneumonia*, *A. hydrophila*, *Enterobacter* species, *P. mirabilis* and *S. aureus* at conc. 7.5% and for *Lactobacillus* species at concentration 15%. Whereas onion nano-emulsion hinders growth of *E. coli*, *Salmonella*, *P. mirabilis* and *S. aureus* at conc. 7.5%, followed by inhibition to *A. hydrophila* and *Lactobacillus* at conc. 15%, and for *K. pneumonia* and *Enterobacter* species at conc. 30%. Moreover, ginger nano-emulsion reduced growth of *E. Coli*, *Salmonella*, *P. mirabilis* and *S. aureus* and *Lactobacillus* at conc. 7.5%, while for *A. hydrophila* inhibition occurs at conc. 30% and at 60% for *K. pneumonia* and *Enterobacter* species.

Table 2. Characterization of oil nano-emulsions (ginger, garlic and onion)

Variables	Nano-emulsion 60%		
	Ginger Oil	Garlic Oil	Onion Oil
Particle size (nm)	222.6±2.22	420.7±36.95	202.9±2.1
The polydispersity index (PDI)	0.338±0.012	0.432±0.023	0.28±0.016
Zeta potential (mv)	-14.4±0.75	-25.1±0.2	-15.8±0.35

Table 3. Viability of rat cells during using different concentrations of nano-emulsions

Concentration	Nano-emulsion		
	Ginger Oil	Garlic Oil	Onion Oil
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
Ic_{50} (%)	>60	>60	>60

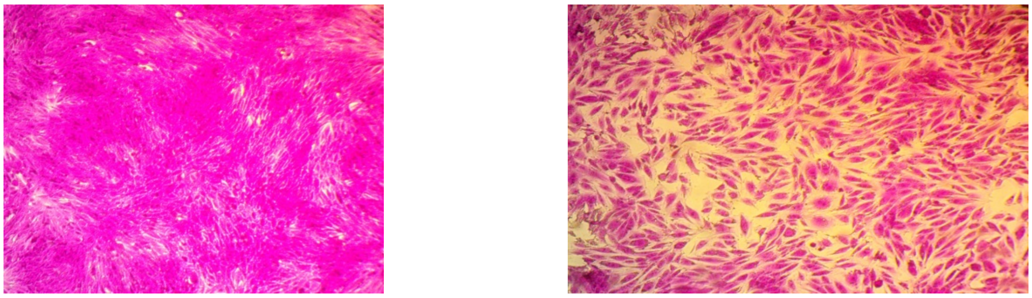

Figure 1. A) Effect of garlic oil nano-emulsion 0.006%, B) Effect of garlic oil nano-emulsion 60%

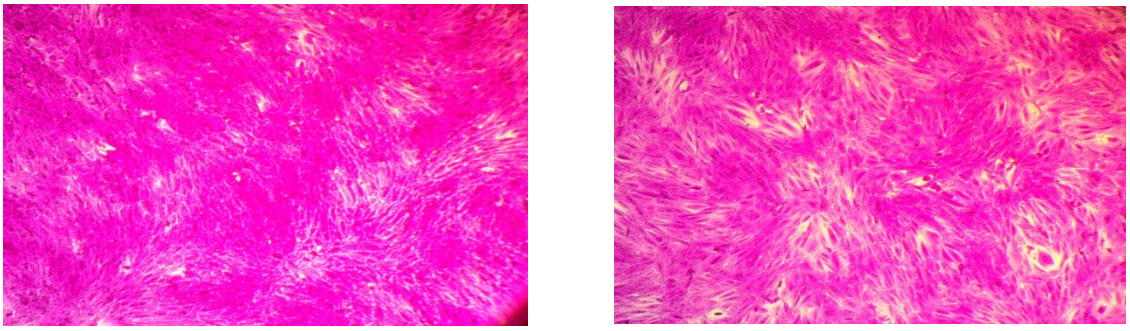

Figure 2. A) Effect of ginger oil nano-emulsion 0.006%, B) Effect of ginger oil nano-emulsion 60%

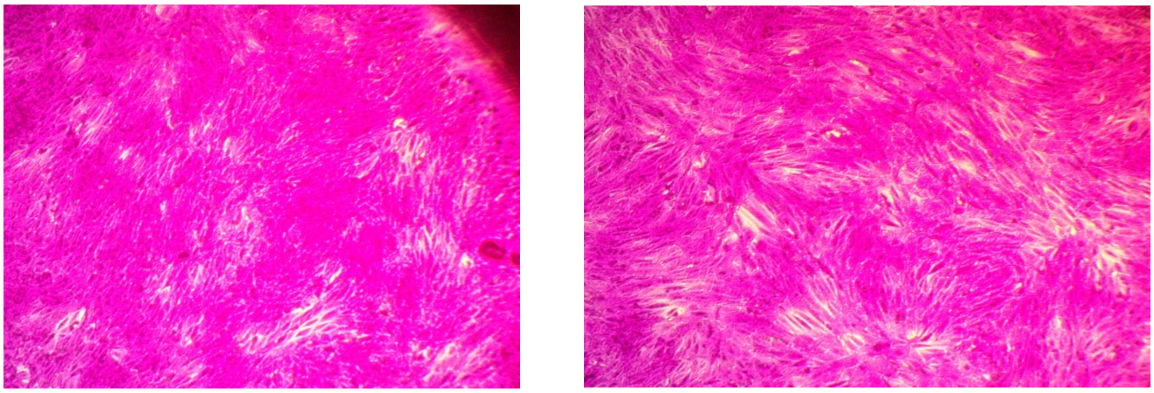

Figure 3. A) Effect of onion oil nano-emulsion 0.006%, B) Effect of onion oil nano-emulsion 60%

This indicated that the garlic nano-emulsion has a greatly antibacterial effect over a wide range of bacteria than onion and ginger nano-emulsions.

BAs detection by HPLC

According to the results reported in Tables 5, 6, 7 and 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. While varied to be 4.77, 10.26, 6.43, 3.25, 2.51 and 1.21 mg\kg for third group treated with onion nano-emulsion. For ginger nano-emulsion treated group it 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 group treated with onion nano-emulsion had 1.52, 1.12, 1.03, 0.8, 0.03 and 0.14 mg\kg. In ginger nano-emulsion treated group, 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 be 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 third group treated with onion nano-emulsion. But it was 1.002, 0.26, 2.92, 1.6, 0.75 and 1.49 mg\kg for ginger nano-emulsion treated group.

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 be 3.22, 0.73, 0.91, 0.91, 4.25 and 0.96 mg\kg for second group treated with garlic nano-emulsion. Although varied to be 2.93, 0.68, 0.87, 0.85, 3.97 and 0.90 mg\kg for third group treated with onion nano-emulsion, and 4.71, 1.07, 1.37, 1.33, 6.22 and 1.41 mg\kg for ginger nano-emulsion treated group.

But the level of Histamine and tyramine was not detected in all treated groups. 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 group.

Discussion

Presence of BAs in food act as indicator for bacterial decarboxylation of amino acids and their types and amount depend on the presence of different bacteria in foods (Ruiz-Capillas et al., 2007). For examples, species of many genera such as *Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Photobacterium* and the *lactic acid bacteria* (LAB) "*Lactobacillus*, *Pediococcus* and *Streptococcus*" are capable of decarboxylating one or more amino acid (Ekici & Omer, 2020). This can be detected by using simple method as using media contain pH indicator as bromcresol purple to determine the ability of microorganism to form BAs and to differentiate between bacteria (Kalhotka et al., 2012). In the present study, lysine iron agar was used to isolated bacteria producing decarboxylase enzyme. Many bacteria were isolated as (*E. coli*, *K. pneumonia*, *Enterobacter* spp, *S. aureus*, *Salmonella* spp, *A. hydrophila*, *P. marbalis*, *P. multocida* and *Lactobacillus* spp), as tabulated in Table 1. This came in accordance with 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) reported that several bacteria can produce BAs 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 BAs (cadaverine, histamine, putrescine and tyramine) in raw meat and fermented sausages and isolated bacteria were *Enterobacteriaceae* and *Lactobacillus* species. While Bermúdez et al. (2012) isolated a group of gram-positive bacteria as (LAB, *Staphylococcus* and *Bacillus*) from cheese and traditional sausage and found that they were formed BAs.

The importance of obtained safe food was increased globally and using plant-based products as additives for both raw and processed meat products have been investigated widely in order to avoid the development of aminogenic contaminant bacteria and in turn, to reduce BAs content as well (Lu et al., 2015). So in this study, the antibacterial effect on ginger, garlic and onion nano-emulsions was determined with conc. 60% against different aminogenic producing bacteria. These nanoemulsions characterization recorded that the mean diameter were (222.6±2.22 nm, 420.7±36.95 nm and 202.9±2.1 nm respectively) for (ginger oil 60%, garlic oil 60% and onion oil nano-emulsions 60% respectively) and their zeta potential were (-14.4±0.75 mv, -25.1±0.2 mv, -15.8±0.35 mv) respectively (Table 2). This came nearly to that reported by Hassan and Mujtaba (2019) for garlic oil nano-emulsion and with 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 >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 EOs to nanoforms 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 EO 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 & 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. Also, they have antibacterial effect on isolates until 7.5% concentration and reduction the BAs. 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 concentration 0.006% and

Table 4. In-vitro MIC of garlic, ginger and onion nano-emulsions

Isolates	MIC		
	Garlic	Onion	Ginger
E. coli (%)	3.75	7.5	7.5
Salmonella spp (%)	3.75	7.5	7.5
K. pneumonia (%)	7.5	30	60
A. hydrophila (%)	7.5	15	30
Enterobacter SPP (%)	7.5	30	60
P. mirabilis (%)	7.5	7.5	7.5
S. aureus (%)	7.5	7.5	7.5
Lactobacillus spp (%)	15	15	7.5

0.06% as recorded in Table 3, Figures 1, 2 and 3. Many authors recorded the effect of ginger nano emulsion 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 MIC of the used nano-emulsions (ginger oil 60%, garlic oil 60% and onion oil nano-emulsions 60%) were recorded in Table 4,and Figure 4 in which the MIC of garlic oil nano-emulsions mainly occur at 7.5% for most examined bacteria, this differ with Liu et al. (2022) who reported that the MIC of garlic oil nano-emulsion was 1.25% against MRSA and with Hassan and Mujtaba (2019) and Hassan et al. (2020) who determined that garlic oil nano-emulsion have greater effect toward gram-positive bacteria more than gram-negative ones. This finding came in accordance with Zheng et al. (2013) who found that garlic nano emulsion showed strong antibacterial activity against *S. aureus* at higher concentration.

The MIC for onion oil nano-emulsion in most bacteria appeared to be 7.5% while it may increase for 30% for other bacteria. The antibacterial effect of the onion was previously reported by Kabrah et al. (2016) who determined that onion extract is effective in vitro against many bacterial species including *Bacillus subtilis*, *Sal-*

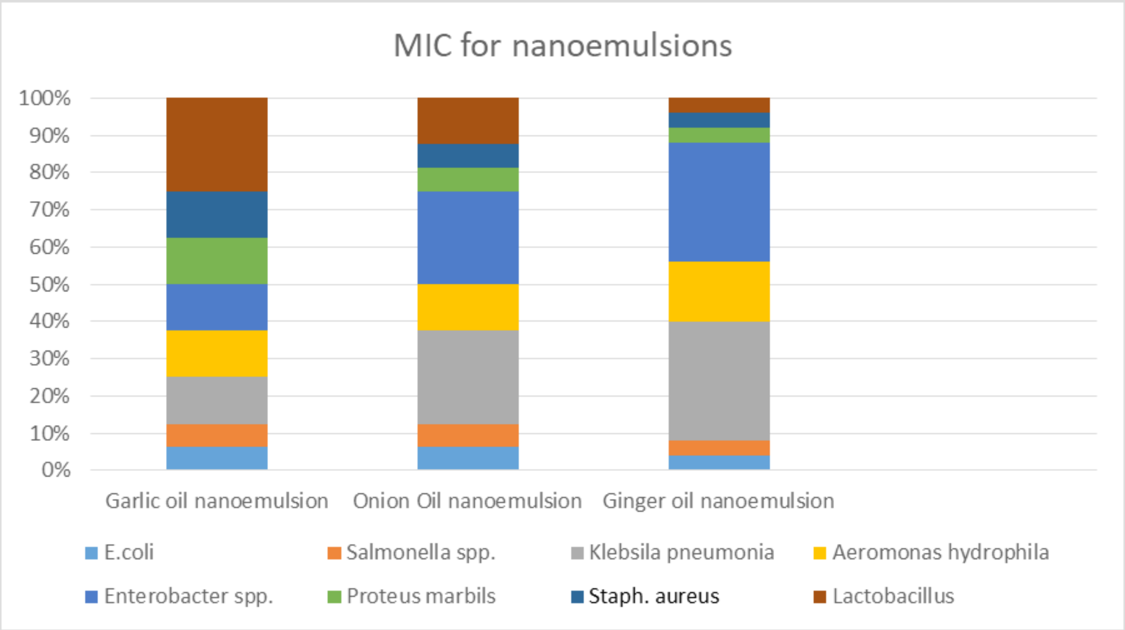

Figure 4. In-vitro MIC of garlic, ginger and onion nano-emulsions biogenic amines detection by HPLC

Table 5. Biogenic amines in 1st group (untreated group)

Sample Code	Treptamine (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	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

monella and *E. coli*. Similarly, this inhibiting effect was also noted on *S. aureus* and results showed a complete inhibition of all strains tested at a concentration of 6.5 mg/mL. It's noted that the partial size of nano-emulsion is pivotal in determining the antimicrobial ability of agents where reduced particle size of nano-emulsion, thus leading to increased exposure to microbial membrane and enhanced antibacterial activity Liu et al. (2022). So, the antibacterial effect of onion extract was enhanced by its transformation to nano form. In addition, the ginger oil nano-emulsion has the same concept; their conservation to nanoparticles enhanced 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. This came in accordance with Thakur et al. (2013) who reported that the ethanolic ginger extract showed more potent against *E. coli* and moderately inhibited the *P. aeruginosa* and *K. pneumonia*. The ginger extract contains many different bioactive compounds with antimicrobial activities that appear to be more sensitive to gram-positive bacteria than the gram-negative ones (Gurumayum, 2015).

The results tabulated in Table 5 and Figure 5 determined the level of BAs presented in six samples (two samples were lysine positive, two samples were lysine positive with production of H_2S, one sample produced red lysine with H_2S and the last one was lysine negative).

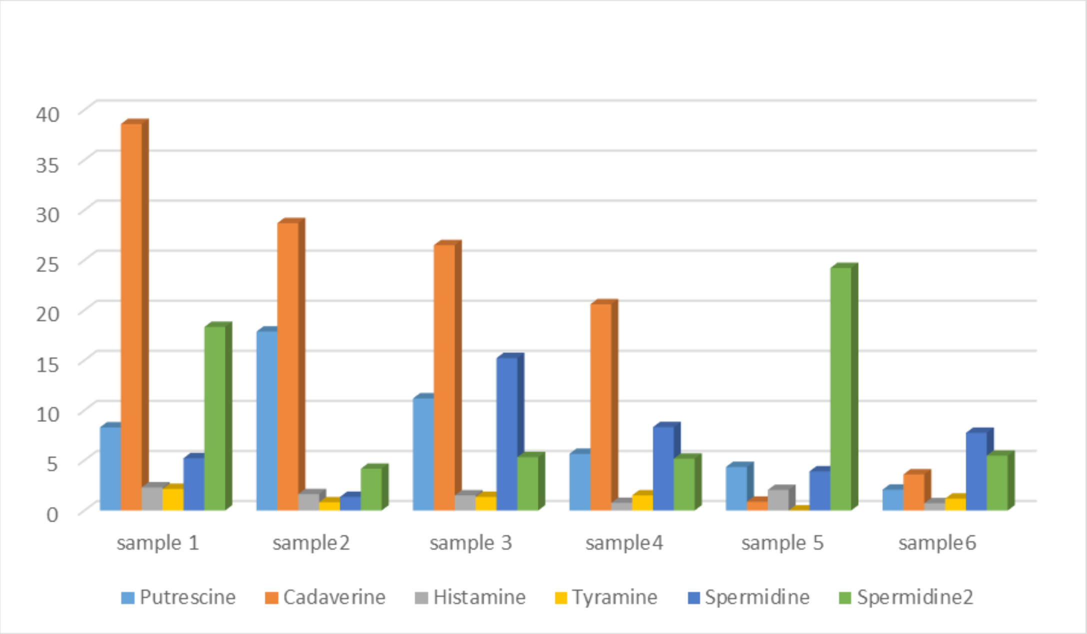

Figure 5. Biogenic amines in 1st group (untreated group)

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

Sample Code	Treptamine (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

The level of putrescine, cadaverine, tyramine and histamine were higher among the six samples, this mainly occur due to bacterial contamination of the samples or bad storage condition as recorded by Doeun et al. (2017). And this came in agreement with Stadnik and Dolatowski (2010) who mentioned that tyramine, cadaverine, putrescine and histamine were the dominant BAs in meat and meat products. Cadaverine represented the greatest amine present in meat due to presence of precursor lysine in high amount in meat (Vinci & Antonelli, 2002).

Meat represents a good source for BAs production, this occurred due to presence of great amount of protein that act as a start point for bacterial decarboxylation and subsequently BAs formation (Schirone et al., 2022). The presence of one or more BAs in meat samples act 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 act as index for microbial decayed and level of histamine and tyramine begin to elevate after several days of spoilage, there is no standards or guidelines are reported for 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 ac-

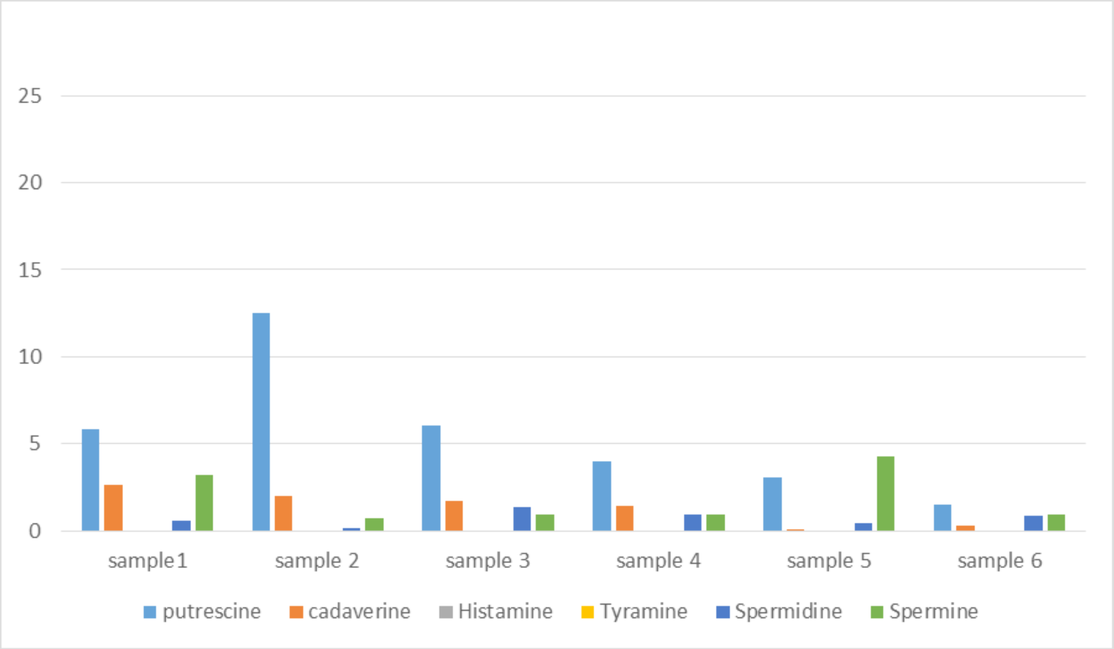

Figure 6. Biogenic amines in the 2nd group (treated with 7.5% garlic oil nano-emulsion)

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

Sample Code	Treptamine (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

cording to Hernandez-Jover et al. (1997) who mentioned that the BAI value of less than 5 mg\kg represents fresh meat and of good quality, while between 5 and 20 mg\kg it is still acceptable with some signs of deterioration. But, 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 and Figure 6 determined the level of BAs in minced meat samples after treatment with 7.5% from garlic oil nano-emulsion and as previously seen the level of BAs were decreased to low level and histamine and tyramine disappeared completely, this means effective treatment of samples with garlic oil nano-emulsion. This came in accordance with Zhou et al. (2016) that reported that garlic extract mainly reduced 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. Also, it assured the previous study of Mah et al. (2009) who reported that addition of 5% garlic during ripening of food reduced the biogenic amine level (putrescine, cadaverine, histamine, tyramine and spermidine) significantly by 8.7%. The results recorded in Table 7 and Figure 7 detected the level of BAs in minced meat samples after treatment with 7.5% with onion oil nano-emulsion, In which the level of biogenic amine markedly decrease in the treated samples than the untreated ones, similarly results detected by Majchrczyk and Surówka (2019), that addition of onion caused a

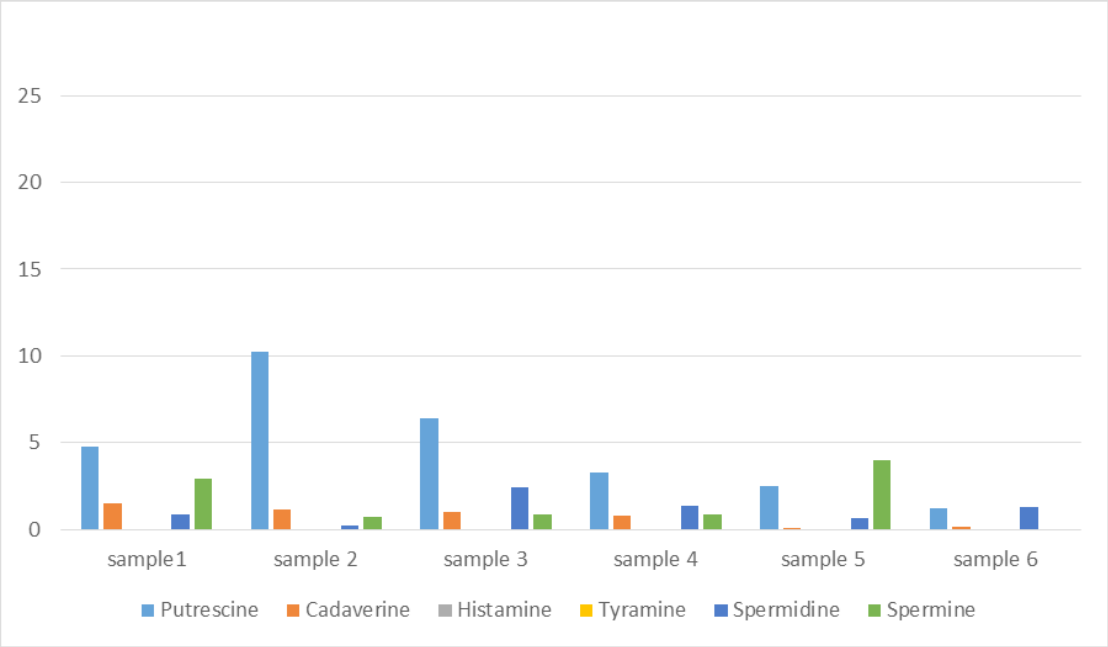

Figure 7. Biogenic amines in the 3rd group (treated with 7.5% onion oil nano-emulsion)

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

Sample Code	Treptamine (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	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

reduction in the total biogenic-amine content when compared with the control sample without an additive.

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

Conclusion

Using nano-emulsions of garlic, ginger and onion nano-emulsions leaded to significant reduction in the formation of undesired BAs in minced meat and control the bacterial growth in it.

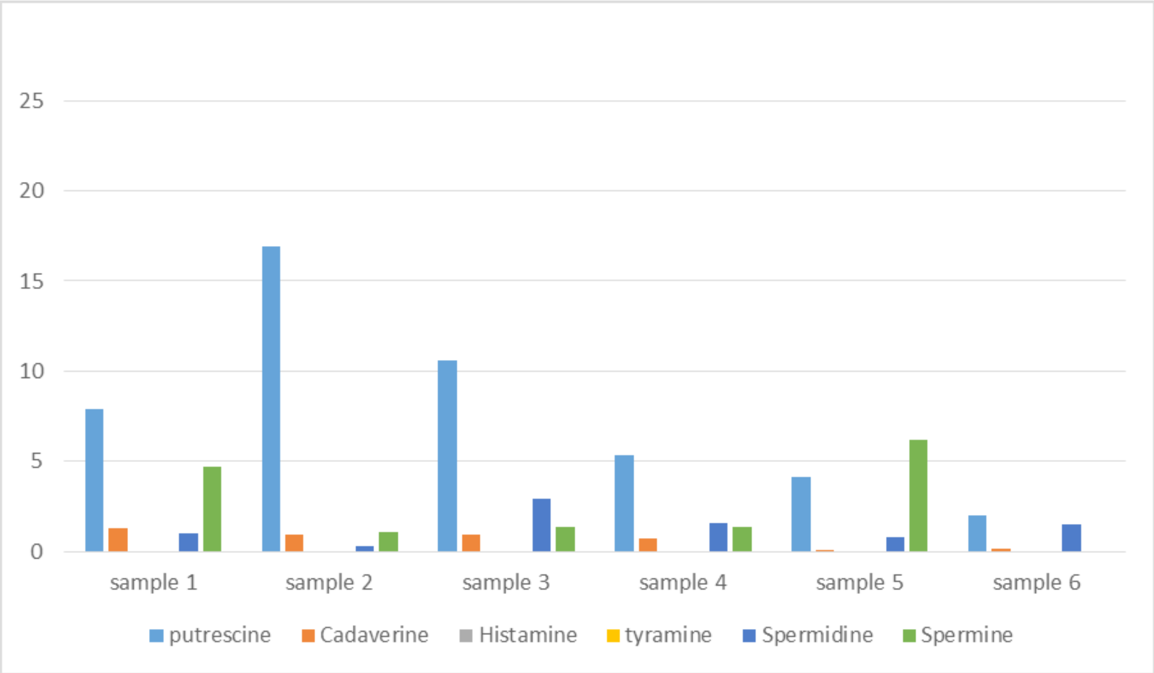

Figure 8. Biogenic amines in the 4th group (treated with 7.5% ginger oil nano-emulsion)

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Study design, experiments, data analysis and final approval: All authors; Writhing: Amany Omar Selim and Zeinab Abdelrahman Mahdy.

Conflict of interest

The authors declared no conflict of interest.

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