Global Scientific JOURNALS

GSJ: Volume 8, Issue 7, July 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

# Antimicrobial Activities and DNA Protections of 5-Methoxy-Isatin Thiosemicarbazone Derivatives combined with metals

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## ABSTRACT

Isatin (1H-indole-2,3-dione) and its derivatives represent an important class of heterocyclic compounds that can be used as precursors for drug synthesis. Since its discovery, a lot of research work has been done regarding synthesis, chemical properties, biological and industrial applications of Isatin. Herein, six isatin thiosemicarbazone derivative with 5-Methoxy-Isatin compounds were re-synthesized and evaluated for DNA protection studies using plasmid DNA (pUC19), All compounds were also utilized in vitro assay to assess the antimicrobial activity of compounds against different pathogenic bacterial strains. All isatin and thiosemicarbazone derivative compounds exhibited DNA protection activity which ranged from 24.5% to 50%. Among them, B(5-M-I)-4-P)-3-TSC) N(II) with concentrations of 0.0165 m had the greatest DNA protective activity. Besides, several derivatives of isatin thiosemicarbazone exhibited significant and selective antibacterial activity with low concentration. These compounds affected *E. coli*, *S. enteritidis* ATCC 13076, *S. aureus* ATCC 43300 and *S. lutea* ATCC 9341, the two compounds B(5-M-I)-4-(4-IP)-3-TSC) Z(II) and B(5-M-I)-4-P)-3-TSC) N(II) did not show any antibacterial effec, but they good DNA protections.

Keywords: Antimicrobial activity, DNA protection, Isatin, Thiosemicarbazone, 5Methoxy-isatin, Isatin derivatives, Metals.

## INTRODUCTION

Isatin with thiosemicarbazone and with some other chemical additions have a wide variety of biological activities such as antimicrobial, antiviral, and antitumor.[1] Isatin (2, 3-dioxindole) is an endogenous compound recognized in people. Its impact has been examining in some parts of the body. Antimicrobial medications are influential in the treatment of disease as a result of their particular harmfulness; that is, they can harm or execute an attacking microorganism without hurting the host. It was evident from the articles that isatin derivatives are known to be related to an expansive range of organic exercises like antibacterial and antifungal.[2]

The chemistry of transition metal complexes with ligands showed attracted attention that is because these metal ions can display several oxidation states, these have studied for their application in biological, clinical, analytical and pharmacological areas, the reduced Schiff base has recently gained considerable attention, because of the flexibility of Schiff base ligands can be improved by hydrogenation of their C=N bonds, they should thus co-ordinate metal ions move quickly .[3]

The indole nucleus observed to be the plain dynamic nucleus in the pharmacy field, as a few natural alkaloids having indole as their essential ring seen to be restoratively active operators. Isatin (indole-2, 3-dione) is an indole subordinate, an endogenous compound, broadly distributed in mammalian tissues and body fluids.[4,11]

Thiosemicarbazones compound also considerable interest because of their ability and potentially beneficial biological activities, such as antitumor, antibacterial. On study, the obtained results of the used compounds show enhanced activity compared to the ligand, which shows that the organized metal have an impact on the antimicrobial effects, Thiosemicarbazones are a class of compounds derived by the condensation of thiosemicarbazide with appropriate aldehydes or ketones. In most complexes, thiosemicarbazones can bind to metals through sulfur and hydrazonic nitrogen atoms.[5,19]

It seems that the battle of microbiologists with microbes will not end, because these microbes do not succumb due to their strong resistance to antibiotics. Encouraging these microbes and increasing their strength, not to mention their ability to make genetic changes, also the mistakes made by humans in the same right as the use of antibiotics and random use my fault for these antibiotics. Antibiotic resistance is a complicated thing caused by different factors include human behavior at many levels of society. Antibiotic resistance can affect anyone any age, and in

any country, this resistance can cause higher medical costs, more extended hospital stays and increased mortality for humans and animals.

Methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant Enterococcus (VRE) have caused an increased mortality rate, which means that antibiotic resistance is becoming a significant health issue, as of now, there are just a couple of clinical medications accessible for the treatment of MRSA, including vancomycin, daptomycin, linezolid, tigecycline, and ceftobiprole; nevertheless, these clinical medications have restriction themselves.[6]

The misuse of antibacterial drugs has contributed to being one of the world's most pressing public health problems today. The general public, doctors, and hospitals all play a role in ensuring the proper use of the drugs and minimizing the development of antibacterial resistance.[7] Thus, discovering new alternative antibacterial agents is very important for the future of public health.

Free radicals, for example, superoxide anion, hydroxyl radicals, and hydrogen peroxide are known to produce responsive oxygen species, which are created by physiological procedures and different outer variables, cause oxidative pressure and thereby initiate peroxidation of the lipid membrane, leading damage to the other biological molecules through a process that thought to implicated in the etiology of several diseases, including coronary artery diseases, stroke, rheumatoid arthritis, diabetes, and cancer [8, 9]

In vitro studies, isatin-3phenylhydrazone described to show more antimicrobial activity against Proteus vulgaris, Proteus aeruginosa, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus than the reference compounds amoxicillin and norfloxacin. [10] However, metal complexes of lanthanides reported increasing the antifungal potential of isatin bishydrazones by affecting various factors including lipophilicity of the molecule.[11]

DNA is under constant attack from many sources such as Radiation, ultraviolet light, and contaminants in our food, and our environment can all wreak havoc on our destruction on our hereditary material, conceivably prompting malignant growth and different infections. People are always presented to regular DNA harming, such as sunlight, dietary agents, and endogenously formed oxygen-free radicals.[12]

In our study, six compounds, 5-Methoxy-Isatin Thiosemicarbazone derivatives with metals, were synthesized and subjected to potential prevent DNA damage and screened for antibacterial activity against twenty-one gram-positive and gram-negative bacterial strains.

## MATERIAL AND METHODOLOGY

In this study, six isatin derivatives were re-synthesized (Table 1) at Kastamonu University Laboratories for evaluation of biological activities, including antimicrobial activity against different types of Gram-positive and Gram-negative bacteria and DNA protection. Twenty-one different strains of Gram-positive and Gram-negative bacteria were used to test the antibacterial activity of the six chemical compounds with metals. The Microbiology Department provided strains at the Gazi University Hospital and from the Department of Genetics and Bioengineering of Kastamonu University, out of the Twenty-one bacterial strains 11 (52.4%) of them were Gram-positive, and 10 (47.6%) were Gram-negative bacteria, as shown in the Table (2). The related information about the preparation of inoculum, statistical analysis are given in detail in supporting information of the current manuscript.

### Chemicals compounds

Table No. (1) 5-Methoxy-Isatin thiosemicarbazone and their complexes with metals

| CHEMICAL NAME | ABBREVIATIONS |
|---|---|
| Bis(5-mothoxyisatin)-4-(4-iodophenyl)-3-thiosemicarbazone) Zinc (II) , | B(5-M-I)-4-(4-IP)-3-TSC) Z(II) |
| Bis(5-mothoxyisatin)-4-(2,4 dichlorohenyl)-3-thiosemicarbazone) ZincI, | B(5-M-I)-4-(2,4 DCP)-3-TSC) Z(I) |
| Bis(5-Methoxyisatin) -4-(phenyl)-3-thiosemicarbazone) Nickel (II), | B(5-M-I)-4-P)-3-TSC) N(II) |
| Bis(5-Methoxyisatin)-4-(phenyl)-3-thiosemicarbazone) Zinc (II), | Is(5-M-I)-4-P)-3-TSC) Z(II) |
| Bis(5-Methoxyisatin)-4-( N-benzaldehyde l)-3-thiosemicarbazone) Nickel(II) | B(5-M-I)-4-(N-B 1)-3-TSC) N(II) |
| Bis(5-methoxy-isatin)-4-(3Methoxyphenyl)-3-thiosemicarbazone) Zinc (II). | B(5-M-I)-4-(3MXP)-3-TSC) Z(II) |

Table (2) Bacterial strains used

| BACTERIAL STRAINS | | ABBREVIATIONS | G. Stains |
|---|---|---|---|
| 1 | *Klebsiella pneumonia* | *K. pneumonia* | - |
| 2 | *Staphylococcus aureus* ATCC 25923 | *S. aureus* ATCC 25923 | + |
| 3 | *Staphylococcus aureus* | *S. aureus* | + |
| 4 | *Proteus vulgaris* | *P. vulgaris* | - |
| 5 | *Escherichia. Coli* | *E. coli* | - |
| 6 | *Serratia marcescens* | *S. marcescens* | - |
| 7 | *Staphylococcus epidermis* | *S. epidermis* | + |
| 8 | *alpha haemolytica Streptococcus* | *alpha h. Streptococcus* | + |
| 9 | *Enterococcus faecium* | *E. faecium* | + |
| 10 | *Pseudomonas aeruginosa* | *P. aeruginosa* | - |
| 11 | *Listeria monocytogenes* ATCC 7644 | *L. monocytogenes* ATCC 7644 | + |
| 12 | *Enterococcus durans* | *E. durans* | + |
| 13 | *Enterococcus aerogenes* ATCC 13048 | *E. aerogenes* ATCC 13048 | - |
| 14 | *Staphylococcus aureus* ATCC 43300 | *S. aureus* ATCC 43300 | + |
| 15 | *Salmonella enteritidis* ATCC 13076 | *S. enteritidis* ATCC 13076 | - |
| 16 | *Streptococcus pneumoniae* ATCC 10015 | *S. pneumoniae* ATCC 10015 | + |
| 17 | *Sarcina lutea* ATCC 9341 | *S. lutea* ATCC 9341 | + |
| 18 | *Salmonella typhimurium* NRRLE 4463 | *S. typhimurium* NRRLE 4463 | - |
| 19 | *Yersinia enterocolitica* ATCC 1501 | *Y. enterocolitica* ATCC 1501 | - |
| 20 | *Proteus mirabilis* ATCC 25933 | *P. mirabilis* ATCC 25933 | - |
| 21 | *Enterococcus faecalis* ATCC 25212 | *E. faecalis* ATCC 25212 | + |

**Preparation of stock solutions.**

Stock solutions of the six chemical compounds prepared according to their molecular weight, by dissolving the mixes in 1 ml of dimethyl sulfoxide (DMSO) in sterile test tubes to give final concentrations of 0.4 molars as shown in Table 3. The minimum inhibitory concentrations (MICs) of these chemical compounds providing positive results were diluted with DMSO to prepare a series of descending levels down. We found that 0.4 molar is more suitable as (MICs) for our chemicals. From each stock solutions, a drop of (~50 µl) was applied on a sterile 5 mm filter paper disks, the filter paper disks loaded with stock solutions and dried for 2–3 hours at 30 $^0$C under hygienic conditions to evaporate the solvent used and allow the temperature to equilibrate. DMSO alone was used as a negative control by applying it directly to see if there is any effect on these types of bacteria, by loaded filter papers discs with DMSO and kept to dry 2 - 3 hours at 30 $^0$C in sterile conditions to evaporate the solvent and left at room temperature with the same ways of the compounds.

Table 3. *Compounds with Molecular Wight*

| Codes/ NO. | Chemicals Names | M.W. gr /mol | WIT. gr/ml | CON./ molar |
|---|---|---|---|---|
| 19 | B(5-M-I)-4-(4-IP)-3-TSC) Z(II) | 967,9022 | 0.38716088 | 0.4 |
| 20 | B(5-M-I)-4-(2,4 DCP)-3-TSC) Z(I) | 853,9303 | 0.34157 | 0.4 |
| 22 | B(5-M-I)-4-P)-3-TSC) N(II) | 709.4234 | 0.28376936 | 0.4 |
| 23 | B(5-M-I)-4-P)-3-TSC) Z(II) | 716.11 | 0.2864440 | 0.4 |
| 24 | B(5-M-I)-4-(N-B 1)-3-TSC) N(II) | 707.4937 | 0.28299748 | 0.4 |
| 29 | B(5-M-I)-4-(3MXP)-3-TSC) Z(II) | 744.1632 | 0.29766528 | 0.4 |

Broad-spectrum antibiotics, Levofloxacin, and Zosyn were utilized as positive controls in this investigation to compare with the results of these compounds. Levofloxacin is one of the group of antibiotics called fluoroquinolones, Zosyn is an antibiotic of a combination (piperacillin and tazobactam); both of them are (broad-spectrum) antibiotics. Levofloxacin has a broad spectrum of antimicrobial activity and is effective against gram-positive and Gram-negative bacteria. It may be a better choice than ciprofloxacin because of its excellent proven clinical efficacy and lower incidence of adverse gastrointestinal reactions and used for the treatment of many bacterial infections.



**Inoculation procedures**

The six different chemical compounds, positive control Levofloxacin and Zosyn antibiotics and negative control DMSO, were tested against the 21 types of bacterial strains, using a disk-diffusion method [13]. Each bacterial strains wer inoculated into 5 ml of nutrient broth medium, and the inoculum was incubated at 37$^{o}$C for 18-24 hours. The standard sterile saline solution was prepared and sterilized in 5 ml test tubes. Each bacterial suspension was added to the one container of the sterile saline solution tube drop by drop by sterile dropper until the visible turbidity was equal to 0.5 McFarland standards having $10^8$ cell forming unit (CFU), the accuracy of the density of McFarland Standards were used by Spectrophotometer with a 1$^{-}$cm light path, a 0.5 McFarland Standard had an absorbance reading of (0.08 to 0.1 at 625-nm). Muller Hinton media agar was prepared and sterilized then poured into 100 mm sterile Petri dishes (4.0 - 0.5 mm thick), the prepared bacterial suspension was spread on Muller Hinton agar media petri dish by sterile cotton swabs. They left to dry for a few minutes in a safety cabin before applying the discs that loaded by chemical compounds suspension. The inoculated plates incubated at 37 $^{0}$C for 18-24 hours, the

results of the antibacterial activity estimated by measuring the inhibition zones, including disk paper against the tested bacteria.

**DNA Protection Test**

The DNA protection assay performed using California University Plasmid pUC 19 DNA (pDNA) isolated from *E. coli,* and the DNA concentration was confirmed spectrophotometrically. Fenton's reagent was prepared by using 30 mM $H_2O_2$, 50 mM ascorbic acid, and 80 mM $FeCI_3$, then adding distilled water up to the final be 10 ml.[14]

DNA fragments of defined sizes done by preparative agarose gel electrophoresis. The electrophoresis in a horizontal agarose gel and staining of the DNA with the fluorescent dye ethidium bromide, an agarose block containing the fragment of interest is cut out. At the same time, the gel illuminated with ultraviolet (UV) light.[15] Every compound was prepared in 1 mL of absolute ethanol at two concentrations 0.0165 molar and 0.102 molars and kept for 24 hours, then centrifuged at 600 ppm for 5 minutes, and the supernatant was collected.

The concentration of the DNA used was 230 ng/µl, the plasmid was prepared according to the purification protocols of the Gene JET Plasmid Miniprep Kit (Thermo Scientific, USA Company). Samples were prepared by adding 4 µl of pDNA, 3 µl Fenton's reagent 3 µl chemical and 10 µl of distilled water. The negative control contains only 4 µl of pDNA and 16 µl of distilled water, and Positive control was composed of 4 µl of pDNA, 3 µl of Fenton's reagent and 13 µl of distilled water, Samples were incubated for 30 min at 37°C, then 4 µl loading dye (Thermo Scientific, USA) was added. DNA was run on 1% agarose gels for 45 minutes and then visualized under ultraviolet light. All experiments were repeated three times, and band density was determined by gel image analysis software (Quantum, Vision-Capt., Vilber Lourmat SAS, and France). The DNA protection test was performed to show the protective ability of different compounds against radical hydroxyl damage generated by Fenton Reagents.

**Agar gel Argos preparations**

The gel was prepared by dissolving 0.8 grams of agarose powder in 100 ml boiling buffer solution (TAE) is Tris-Acetate-EDTA. The solution was then cooled to approximately 55°C, and 5µl of fluorescent dye was added mixed and poured into a casting tray which serves as a mold, a well-formed template (often called a comb) is placed across the end of the casting tray to form

wells when the gel solution solidifies. The gel was submerged in a buffer-filled electrophoresis chamber, which contains a positive electrode at one end and a negative electrode at the other. Samples were prepared for electrophoresis by mixing them with components; these samples are delivered to the sample wells with a micro-pipette.

## RESULTS

All our new synthesized compounds were tested in vitro for antibacterial activity by agar-plate diffusion method against the reference antibiotics Levofloxacin and Zosyn. The positive control, Levofloxacin, affected all bacteria used and gave different inhibitions zones except *K. pneumonia* and *E. faecium*, which showed resistance to Levofloxacin.

Zosyn also affected the bacteria used except for *P. vulgaris*, *E. coli*, *S. marrescens*, *P. aeruginosa*, *L. monocytogenes* ATCC 7644, *E. durans*, *E. aerogenes* ATCC 13048, *S. enteritidis* ATCC 13076, *S. pneumonia* ATCC 10015, *S. lutea* ATCC 9341, *P. mirabilis* ATCC 25933 as shown in Figure (1).

Dimethyl Sulfoxide (DMSO) alone used as a negative control because it did not show any effect on all strain of bacteria used on these article. In order to clarify any participating role of DMSO in the biological screening, separate studies carried out with the solutions alone of DMSO, and they showed no activity against any bacterial strains.[16]

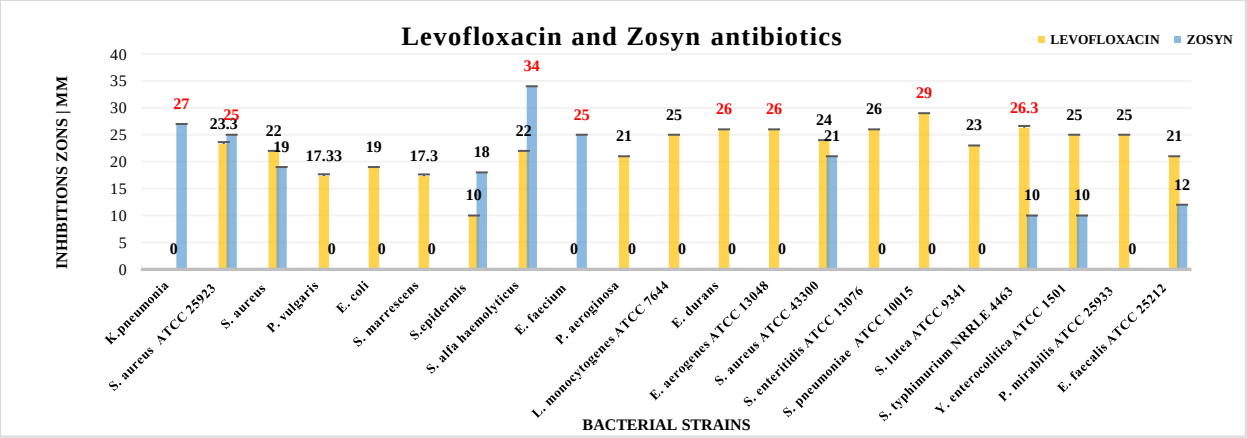


Figure 1. The Levofloxacin and Zosyn antimicrobial activity against the bacteria used.

## Antimicrobial activity

### *Bis(5-Mothoxyisatin)-4-(2,4 dichlorohenyl)-3-thiosemicarbazone) Zinc(I)

On this compound, out of 21 bacterial strains, only *S. enteritidis* ATCC 13076 was inhibited with mild inhibition zone 7mm compared with the two control antibiotics Levofloxacin and Zosyn, which gave 24 and 21 mm on diameter respectively, as shown in Figure 2. Although the compound was combined with zinc I, it affects only one type of bacteria. This is contrary to many articles, which indicate that the presence of some metals like zinc increases the effectiveness of compounds against bacteria.

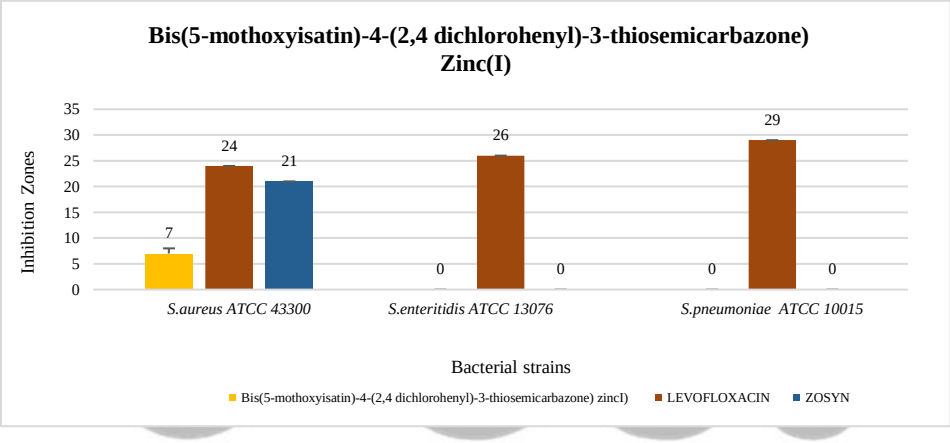


Figure 2. Antimicrobial activity of Bis(5-mothoxyisatin)-4-(2,4 dichlorohenyl)-3-thiosemicarbazone) zinc(I)

### *Bis(5-Methoxyisatin)-4-(phenyl)-3-thiosemicarbazone) Zinc (II)

Mild action was shown by this compound against *E.coli*, *S. aureus* ATCC 43300 and *S. lutea* ATCC 9341 with inhibition zones 6.3, 6.3 and 7 respectively, the presence of phenyl on this compound may inhinched the effect of the compound on these strains of bacteria, *the effect of the* Bis(5-Methoxyisatin)-4-(phenyl)-3-thiosemicarbazone) Zinc (II) *on E.coli and S. lutea* ATCC 9341 *is better than Zosyan antibiotic which showed no effect on these stearin's of bacteria* as showed in Fig. 3.

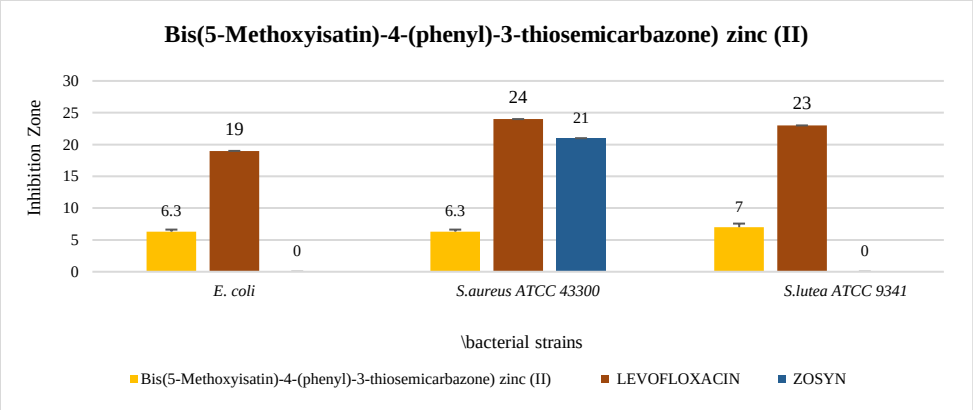


Figure 3. Antimicrobial activity of Bis(5-Methoxyisatin)-4-(phenyl)-3-thiosemicarbazone) Zinc (II)

## *Bis (5-Methoxyisatin)-4-( N-benzaldhyde l)-3-thiosemicarbazone) Nickel

On this compound there are only one gram-negative bacteria *E.coli* was affected by this compound with mild inhibition zone 6.3 mm on diameter, this is the only compound on this group was mixed with benzaldhyde and contains nickel metal, it is better than Zosyn antibiotic which did not gave an inhibition zone for the *E.coli*, as shown in Figure 4.

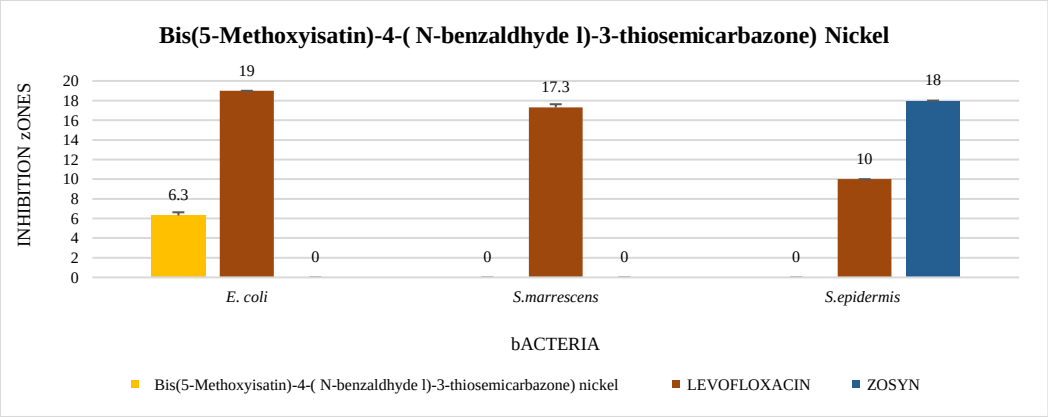


Figure 4. Antimicrobial activity of Bis(5-Methoxyisatin)-4-( N-benzaldhyde l)-3-thiosemicarbazone) Nicke

## *Bis(5-Methoxyisatin)-4-(3Methoxyphenyl)-3-thiosemicarbazone) Zinc (II)

Only one gram-positive bacteria *Enterococcus faecium* was affected by this compound with 8 mm diameter inhibition zone out of eleven gram-positive bacterial strains that had no effect by this compound. However, this compound had more impact than the Levofloxacin antibiotic and less effective than the Zosyn antibiotic, as control positive, her the Bis(5-methoxyisatin)-4-

(3Methoxyphenyl)-3-thiosemicarbazone) Zinc (II) the only compound effected the bacterial strain *Enterococcus faecium* as showen in Figure 5.

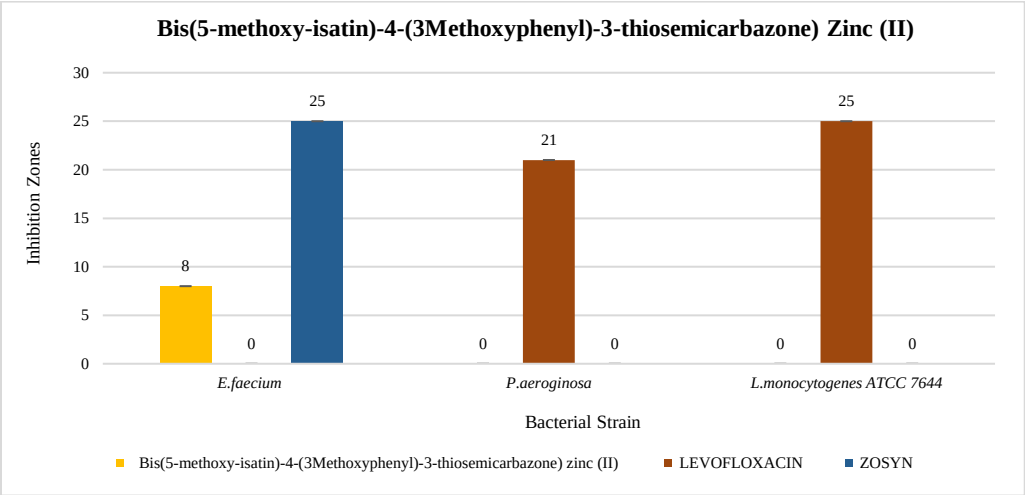


Figure 5. Antimicrobial activity of Bis(5-methoxy-isatin)-4-(3Methoxyphenyl)-3-thiosemicarbazone) Zinc (II)

## DNA protection

DNA protection of 5-Methoxy - Isatin thiosemicarbazone and its derivatives, on this group, the all six different compounds with different structures gave variation for the DNA protections, the compounds which they gave highest protections 50% and 47.59 for DNA protections that were B(5-M-I)-4-P)-3-TSC) N(II) with concentrations 0.0165 M and B(5-M-I)-4-(3MXP)-3-TSC) Z(II) 0.102 M. The lowest DNA protection was by compound B(5-M-I)-4-(N-B 1)-3-TSC) N(II) with concentrations 0.102 M, which gave 24 % of DNA protections, as sowed in Table 4 and Figure 5.

Experimental evidence showing that 100% of the extracts with variations exhibited considerable plasmid DNA protection against a high level of $H_2O_2$- driven oxidative damage suggests that treatment with these extracts might reduce oxidative damage to mitochondrial and genomic DNA.

| Sample No. | Chemicals Name | Mean | SEM |
|---|---|---|---|
| | N control | 100 | 0 |
| | P control | 0 | 0 |
| 19 | B(5-M-I)-4-(4-IP)-3-TSC) Z(II) 0.0165 m | 32.52 | 4.77 |
| | B(5-M-I)-4-(4-IP)-3-TSC) Z(II) 0.102 m | 40.15 | 1.66 |
| 20 | B(5-M-I)-4-(2,4 DCP)-3-TSC) Z(I) 0.0165 m | 39.55 | 0.615 |
| | B(5-M-I)-4-(2,4 DCP)-3-TSC) Z(I) 0.102 m | 35.06 | 4.79 |
| 22 | B(5-M-I)-4-P)-3-TSC) N(II) 0.0165 m | 50 | 13.4 |
| | B(5-M-I)-4-P)-3-TSC) N(II) 0.102 m | 35.33 | 7.41 |
| 23 | B(5-M-I)-4-P)-3-TSC) Z(II) 0.0165 m | 38.86 | 1.29 |
| | B(5-M-I)-4-P)-3-TSC) Z(II) 0.102 m | 37.4 | 0.4 |
| 24 | B(5-M-I)-4-(N-B 1)-3-TSC) N(II) 0.0165 m | 40.74 | 9.9 |
| | B(5-M-I)-4-(N-B 1)-3-TSC) N(II) 0.102 m | 24.43 | 0.43 |
| 29 | B(5-M-I)-4-(3MXP)-3-TSC) Z(II) 0.0165 m | 40.81 | 6.16 |
| | B(5-M-I)-4-(3MXP)-3-TSC) Z(II) 0.102 M | 47.59 | 3.39 |

Table (4) DNA protection of 5-Methoxy - Isatin thiosemicarbazone and it is derivative

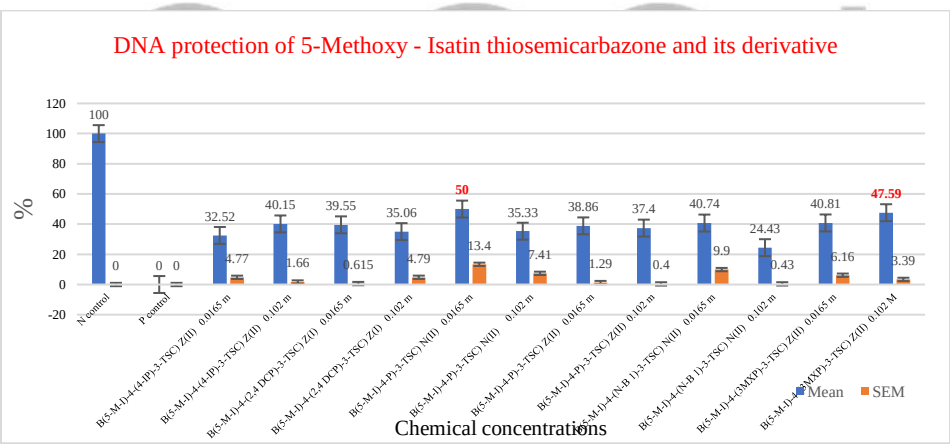


Figure (5) DNA protection of 5-Methoxy - Isatin thiosemicarbazone and its derivative

## DISCUSSIONS

The misuse of antibacterial drugs has contributed to one of the world's most pressing public health problems today. The general public, doctors, and hospitals all play a role in ensuring proper use of the drugs and minimizing the development of antibacterial resistance.[20] Thus, discovering new alternative antibacterial agents is very important for the future of public health. For the antimicrobial agents used in this study.

In our study, all compounds had different antibacterial activity in vitro against the tested Gram-positive and Gram-negative bacteria. The various activities of the compounds were correlated with the different combinations of chemical structures from Isatin and thiosemicarbazone.

On these newly synthesized compounds that composed of 5-Methoxy with Isatin thiosemicarbazone beside different chemicals and metal in a different position, most of them were provide mild antimicrobial activities compared to control positive antibiotics levofloxacın and zosyn . Four out of six compounds gave mild action against these bacteria, and the other two did not show any action against the bacterial tested. However, some compounds contain nickel and zinc, which did not show any different change in the result that may provide wider inhibition zones than the others, which were inconsistent with the articles of Varkey el al (2013) and Vaidya et al. (2017).

The DNA protection test was carried out to show the protective ability of the different chemicals compounds at various concentrations against hydroxyl radical damage generated by Fenton Reagent's. Two different concentrations were used for all the six compounds (0.0165 and 0.102 molar), and all of them showed a different percentage of DNA protection. the best compounds for the DNA protection on these group were, B(5-M-I)-4-P)-3-TSC) N(II) 0.0165 molar, and B(5-M-I)-4-(3MXP)-3-TSC) Z(II) 0.102 molar were 50%, and 48% respectively.

The presence of metals did not show any signs for the DNA protections; also, there was no relationship between there action on the bacteria and their DNA protections because some of those compounds were not affected bacteria but had proper protections to the DNA. It was not clear that the high concentration compounds 0.102 molars gave better DNA concentration than the lower 0.0165 molars, except in a few of them were contrary to this base with no explanation.

These compounds are new, so no DNA protection data is matching these compounds. However, Osman et al. (2015) showed that Juniper sapwood at 10 mg/ml gave the highest DNA protection activity (84 %), followed by Juniper 5 mg/mL (83 %), and approximately 71 % remained in a supercoiled form with Olive at 10 mg/ml. All wood extracts indicated had a DNA protective ability in their study. [14] Ganim *et al.* showed a survey of good results of DNA protections for different Isatin thiosemicarbazone compounds.[21]

In general, these synthesized compounds provide a good percentage of DNA protections.

## CONCLUSIONS:

Worldwide the high prevalence of diseases caused by bacterial infection increasing antibiotic resistance, is the reason for more urgent for medical research. In our study, we presented different biological properties of six compounds of chemicals combined with isatin-thiosemicarbazones with 5-Methoxy-Isatin and metals that were resynthesized. They were used to investigate antimicrobial activity against 21 different types of Gram-positive and Gram-negative bacteria and DNA protection. The chemical complexes showed different moderate inhibitions zones for bacteria used in this article, These compound showed different inhibitions zones, ranged between 6 to 8 mm inhibitions zones compared with the control antibiotics Levofloxacin and Zosyan, which gave inhibition zones between 0 to 34 mm. Broad-spectrum antibiotics Levofloxacin and Zosyn antibiotics were used as positive controls and exhibited some bacterial resistance. These test compounds may be beneficial for future treatment following toxicity studies. The different activities of the compounds against different types of bacteria could be correlated with the different chemicals structures when combined with Isatin and thiosemicarbazone. All compounds in this study were protective of DNA (24.5 to 50%). Two different concentrations were used for all the six compounds (0.0165 and 0.102 M), Among them, B(5-M-I)-4-P)-3-TSC) N(II) with concentrations of 0.0165 m had the greatest DNA protective activity.



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