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Synthesis, characterization, and some pharmacological evaluation of 5-Thiocyanatouracil compound

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***Abstract*---**5-Thiocyanotouracil (5-TCU) was prepared in convenient method involved direct reaction of Potassium thiocyanate with 5-Iodouracil in refluxing aqueous solvent to give white crystalline in very good yield and high purity. This compound is important because it is similar in structure to 5-Flourouracil, which is of great importance as anti-cancer drug that is still used today. FT-IR, 1H- NMR, 13C- NMR, and CHN elemental analyses were used to describe the product. Median lethal dose LD50, in vitro antiradical activity and antibacterial effects were done. The results indicated that 5-TCU has relatively high LD50, non-significate antioxidant effect compare with ascorbic acid, and some antibacterial activity.

***Keywords*---**5-Iodouracil, Thiocyanato uracil, Potassium thiocyanate, DPPH, antibacterial, LD50.

Introduction

The Sulfur atom, which is attached to the carbon atom of the organic moiety, and on the other side to a nitrile group called thiocyanates. These compounds are used as intermediates in the preparation of many organic compounds due to their high stability [1]. These compounds were interesting types of organic thiocyanate

because they occur naturally in the extracellular fluids of mammal and play an important role in the defense system [2,3]. There are many reviews about the cyanates in organic chemistry [4,5]. These compounds have gained very great importance due to their distinctive properties in the field of cancer preventive chemotherapy [6]. Heterocyclic organic compounds have uses in many fields' agrochemical, polymer science, medicinal chemistry and industries. Moreover, it possesses chemical structures in which its structural unit is available in many natural products such as vitamins, hormones and antibiotics, in addition to many prepared compounds such as barbituric acid and veronal that are used as hypnotic agents [7, 8]. Uracil is one of the most important heterocyclic organic compounds that are prevalent in many natural products [9]. Since uracil and also its derivatives have considerable pharmacological applications are of tremendous significance in the field of drug discovery, as anti-inflammatory, antioxidants, anti-viruses, anti-fungi, anti-bacteria, and anti-cancer medications, etc. [11]. In this study, 5-thiocyanatouracil was prepared easily with good yield from the reaction of KSCN with 5-Iodouracil in aqueous medium. The resulting compound was characterized using spectroscopic methods which confirm the proposed structure of the product.

The term toxicity refers to a change, induced by a substance in the normality that may be irreversible. This definition emphasizes of the importance of dependable experimental measurements to distinguish between normality and adverse change. The development of organic chemistry has modified the recent chemical science and aid to find many advance contributions to pharmaceutical, materials science and a number of other areas. Extensive use of chemicals highlighted on the importance of toxicity problems and decrease harmful effects on living organisms as well as on environmental [12]. The estimated human toxicity is dependent on the results of tests in rats, mice and other drugs animal models by in vitro evaluation. In general, chemicals toxicity is illustrated by the median lethal dose (LD50), where LD means lethal dose and 50 means acute dose kills 50% of the animals on which the chemical is used any method such as oral, dermal, inhaled, and intravenous and intraperitoneally under controlled laboratory conditions. Determination of this trial examined the relationship between dosage and most extremely dead reaction means that the LD50 of any chemical is dose-response-death ratio. LD50 is shown in milligrams of substance per kilogram of animal's body weight (mg/kg body weight). It provides information about possible health risks short and long term exposure [13]

One of the main goals of this work was to use DPPH radical scavenging photometric assays to assess the antioxidant activity of 5-TCU.When reduced by an antioxidant, the chemical compound DPPH, which is made up of stabilized free radical molecules, appears as a deep purple powder. Once oxidants exceed the activity of antioxidant enzymes, a state is known as oxidative stress, which can cause lipid peroxidation, and damage of DNA, Free radicals (reactive oxygen and nitrogen species) and antioxidants like superoxide dismutase, glutathione peroxidase, and catalase as well as the non-enzymatic antioxidants like glutathione, vitamin C, E, and D cause an imbalance that leads to an oxidative stress state. The primary ROS is superoxide (O2), which is created by NADPH oxidase during the mitochondrial respiratory electron transport chain when oxygen (O2) molecules are reduced. Superoxide dismutase, an enzyme, produces

ROS like hydrogen peroxide (H_2O_2) during this process. Excess H_2O_2 under oxidative stress catalyzes the formation of potentially toxic ROS such hydroxyl ions (HO^-) when there are the reduced metals as Cu, Fe, and Ni. Under these conditions, reactive chemicals have been shown to target proteins, lipids, and nucleic acids, modifying cellular structures and functions. Reactive nitrogen species (RNS), a different class of reactive species, are equally important to ROS [14].

Nitric oxide (NO) free radical considered the largest member of this group. It has the power to influence cells and tissues either directly or indirectly. As it has the ability to directly alter itself, while indirect effects, are mediated by the interaction of NO with oxygen or superoxide radicals (O_2•) (O_2). The majority of its physiological actions are directly mediated by cyclic guanosine 3′,5'-monophosphate (cGMP). Additionally, it may interact with proteins that contain iron and zinc, as well as nitrosylate proteins to produce S-nitrosothiols. The organism's many antioxidants protect it against ROS and RNS damage. An oxidation-sensitive substrate can be protected from peroxidative damage via antioxidants, which are chemicals that are present in much lower quantities than the substrate [15].

Microbes are extremely varied, and their genetic character is constantly changing in reaction to the environment. The developing resistance of microorganisms to the available antibacterial, arsenal has demanded the discovery of new pharmacological medicines. Numerous bacterial genes that code for new drug gable proteins that may one day be used as antibacterial targets have been discovered because to developments in molecular microbiology and genomics. Promising anticancer and antibacterial treatments are being produced using new synthetic chemicals and natural plant materials [16]. Blocking uracil-DNA glycosylase with a specific inhibitor may have therapeutic benefits by increasing the anti-cancer activities of current chemotherapeutic medicines, such as pemetrexed PEM, which induce uracil incorporation through inhibition of thymidylate synthase. Similar to how floxuridine inhibits thymidylate synthase, it also causes 5-Fluorodeoxyuridine triphosphate to be metabolized, which is then combined with uracil as a result of inhibiting thymidylate synthase [17].

Methods

Experimental:

All chemicals used in this study were obtained from commercial sources and in purest available grades used when necessary. All reactions were carried out in distilled water, the melting point were measured by electro thermal melting point apparatus which is heated electrically and are uncorrected. Thin layer chromatography (TLC) was used for monitoring the reaction. The spectra of ^{1}H-NME and ^{13}C-NMR were recorded on Bruker AVANCE NEO- 400 run at 400MHz at college of education (pure sciences)/Department of Chemistry/Basrah University with TMS ($SiMe_4$) as internal standard in δ units at 295K. The chemical shifts were expressed in δ- scale (ppm). A Carlo Erba EA 1108-Elemental Analyzer apparatus was used to do microanalysis on carbon, hydrogen, and nitrogen. On the Jasco FTIR spectrophotometer, the IR spectra were captured as discs of potassium bromide.

Preparation of 5-Thiocyanatouracil:

In 250 mL. round bottom flask with tow neck equipped with condenser and charged with (1,46gm, 0.015mole) KSCN dissolved in 25 mL of distilled water and magnetic bar was stirred until KSCN was dissolved, to this solution, (3.57gm, 0.015 mole) from 5-Iodouracil dissolved in distilled water (40 mL) was added. The mixture for 5 hr was heated under reflux. then left at room temperature overnight with continuous stirring, the reaction mixture was monitored by TLC. White crystal was formed which filtered off, recrystallization from distilled water afforded white needles of titled compound (2.06 gm, Yield = 81%), R_f value= 0.5 Using ethanol/Benzene (1:9) with MP = 259-261 C°, Anal. Calc. for $C_5H_3N_3O_2S$: C: 35.50; H: 1.79; N: 24.84; Found: C: 35.43; H: 1.76; N: 24.99. IR(KBr) ($\upsilon_{max}.cm^{-1}$): 3286, 3136, 3030, 1870, 1745, 1647, 1606, 1465, 1431, 1215, 1138, 732, 549; ^{1}H-NMR: δ:7.89 (1H-H4), 11.38 (2H br. NH-1, NH-3); ^{13}C-NMR: δ:93.01 (CN), 110.50 (C-5), 146.37 (C-6), 150.63 (C-2), 160.89 (C-4).

Median Lethal Dose (LD50)

Eight-week-old male albino rats weighing 130-140 g were used (Rats were obtained from the University of Basrah's College of Veterinary Medicine in Iraq.) The tap water was available, and a standard serving of a dry pellet diet was delivered. For a week, rats were housed collectively in a quarantine facility. All of the rats were kept in an animal house at the College of Pharmacy at the University of Basrah in Iraq under controlled illumination (12:12 light to dark cycles) and temperature controls (20 2°C). The College of Pharmacy at the University of Basrah in Iraq conducted its experiments in accordance with the guidelines outlined in its "Instructions for the Care and Use of Laboratory Animals" document. In this study, 35 rats in total were employed. Each treatment involved seven animals, who were watched for 48 hours. To establish the poisonous LD 50, Probit analysis was used to examine the observation of mortality (lethal dose per 50 rats). 5-TCU was given orally after being dissolved in sterile distilled water [13].

Antiradical activity of 5-TCU using DPPH method

DPPH is a **1,1-Diphenyl-2-picrylhydrazyl** Obtained from Sigma (Shanghai, China), to obtain 0.2mM DPPH solution 7.89 mg of DPPH dissolved into 100mL of MDSO, which was then incubated in the dark and at room temperature (23–25 C°) for two hours with the use of aluminum foil wrapped over test tubes and flasks. Control solution was prepared by adding 1mL of DPPH into tube containing 3 mL of DMSO. Several concentrations of 5-TCU were prepared first concentration was 100µg/mL of DMSO, other concentrations include 150, 200, 250 µg/mL of both 5-TCU and Ascorbic acid AA as a positive control. The main indicator of 5-SeU and AA antioxidant capability in spectrophotometric analysis is the absorbance of DPPH radicals in the reduction process. 1 ml was added of 0.2mM DPPH to each tube containing 5-SeU or AA. Stirred and left to stand at room temperature for ten minutes. At 517 nm, the absorbance was measured using a spectrophotometer. The absorbance of the control reaction is called blank (containing all reagents except the test compound).

A percent inhibition versus concentration curve was used to determine the sample concentration required for 50% inhibition, which was then represented as the IC50 value for each of the test solutions.Since AA was used as a reference material, the equation was used to calculate the suppression of absorbance and DPPH scavenging activity.

$$(A^0 - A1/A^0) * 100 = \text{percent inhibition}$$

A^0 is the absorbance of the control reaction, while A1 is the absorbance of either 5-SeU or AA is. The concentration and inhibiting percentage curve were utilized to determine the IC50 rate for the test sample. The test samples' IC50 was calculated using the concentration and inhibiting percentage curve [12].

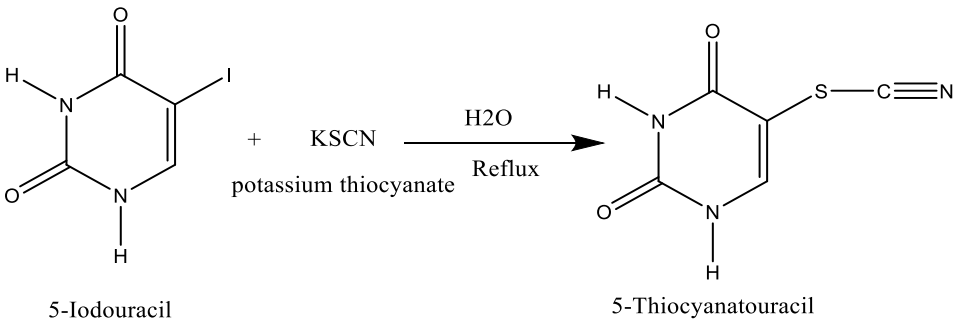
Antibacterial activity

Gram positive bacteria include Staphylococcus aureus (S. aurous) and Streptococcus pyogens (S. pyogens), while Gram negative bacteria; were Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), and K. pneumoniae were collected from the University of Basrah's central laboratory /College of Pharmacy. At 37 °C for 24 hours, all bacterial strains were grown on Mueller Hinton agar (MHB) from Merck in Germany. The Kirby Bauer, Disk Diffusion Test technique was used to examine the antibacterial properties of 5-SeU on the examined pathogens. The bacterial strains were spread out on Mueller-Hinton agar (MHA) using a sterile cotton swab (Merck, Germany). An antimicrobial susceptibility disk that had been sterilized was used as the test's "blank." 5-TCU was placed onto disks at doses of 50, 100, 150, 200 and 250 µg/mL of DMSO. The disks were then put on the agar plate and incubated for around 24 hours at 37°C. The inhibitory zone was measured after incubation. Three cultures are conducted on each concentration. [18].

Results and Discussion

5-Thiocyanatouracil synthesis

5-Thiocyanatouracil was first synthesized from reaction of bis [5-uracildisulfide] with sodium cyanide in aqueous buffered solution in the presence of BrCN used as thiocyanating agent and to prevent the formation of disulfide again [16]. In this study 5-Iodouracil was react with KSCN in equimolar ratio in aqueous solvent to give white crystals in very good yield % ca~ 81%. As show in Scheme (1):



Scheme (1): Synthesis of 5-Thiocyanatouracil.

The IR spectrum of prepared compound was showed medium band at 3286 and 3136cm^{-1} refer to ט (N-H1, N-H3) respectively [19]. Weak band at 3030 cm^{-1} refer to C_4 H; Fig. (1). Medium band appeared clearly at 1870 cm^{-1} be in –CN group [18,19]. Tow strong bands at 1745 and the second at 1653 cm^{-1} attributed to carbonyl groups at C_2O and C_6O respectively [20]. Strong band at 1606 cm^{-1} belong to N-H bending. Very potent tow bands at 1465, 1431 cm^{-1} ascribed to symmetrical and irregular C_4=C_5. Tow strong bands appeared at 1215 and 1138 cm^{-1} may attributed to vibrational stretching of C_2-N_1 and C_4-N_3 respectively. Strong band at 732 cm^{-1} may recognized to N-H deformation. Fig: (1).

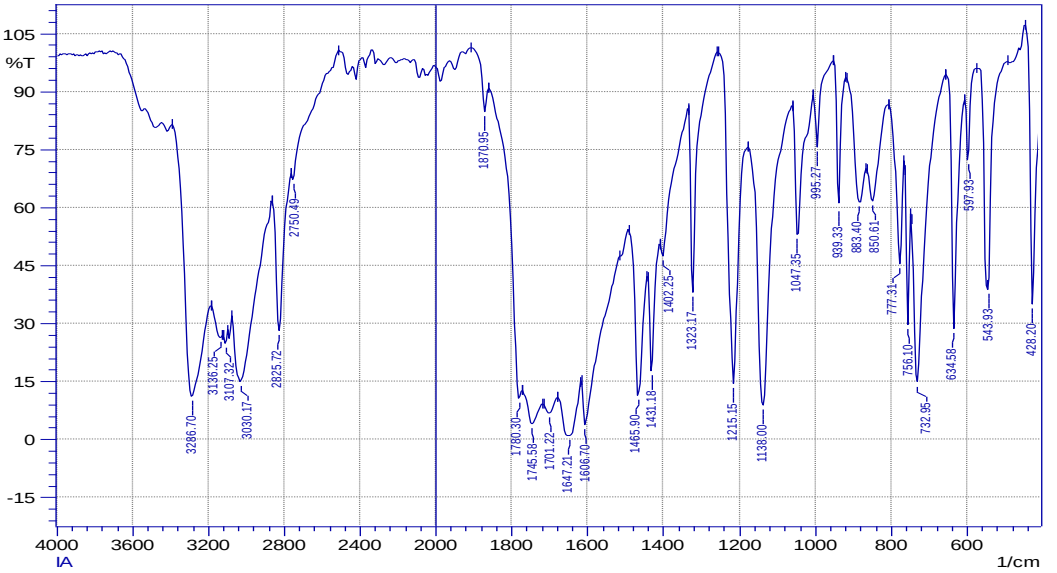


Fig. (1): IR specrtum for 5-Thiocyanatouracil

The ^{1}H-NMR spectrum of the product compound shows interesting singlet signals at δ= 7.87 attributed to one proton (1H-C_4) and broad singlet signal at δ=11.38 belong to (2H, br, NH-1, NH-3) [19]. Fig: (2).

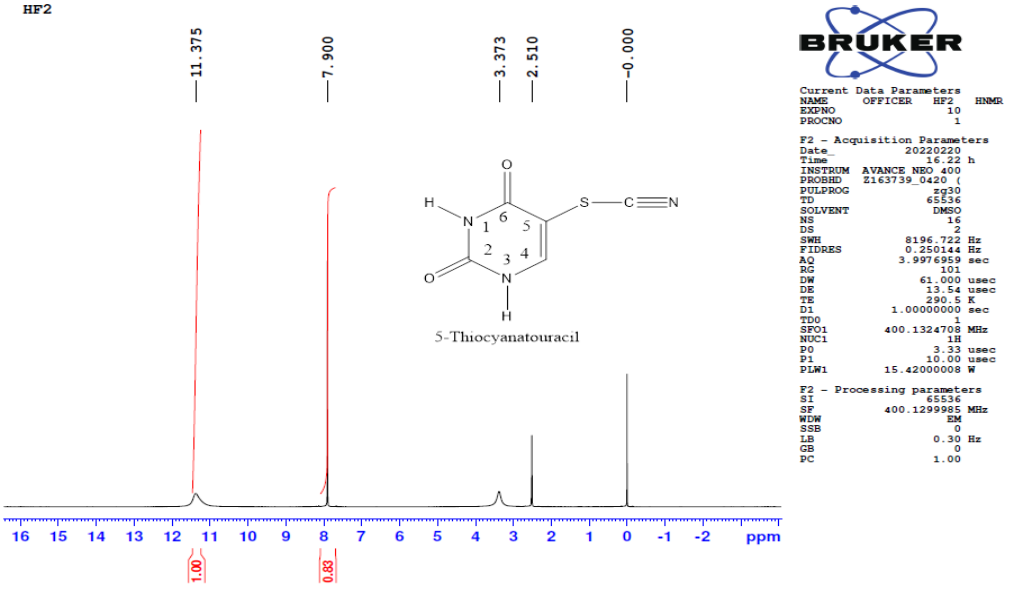


Fig:(2) ^{1}H-NMR spectrum for 5-Thiocyanatouracil in DMSO-d_6

The ^{13}C-NMR spectrum for 5-thiocyanatouracil was represented in Fig: (3), there are five resonances signals in this spectrum belong to four carbon atoms of uracil ring the last one belong to carbon of cyanide group. Thus the five signal in the spectrum of 5-thiocyanatouracil at 110.50, 146.37, 150.63 and 160.89 ppm could be assigned to C5, C6, C2 an C4, respectively [19]. The signal at 93.01 ppm due to SCN [21].

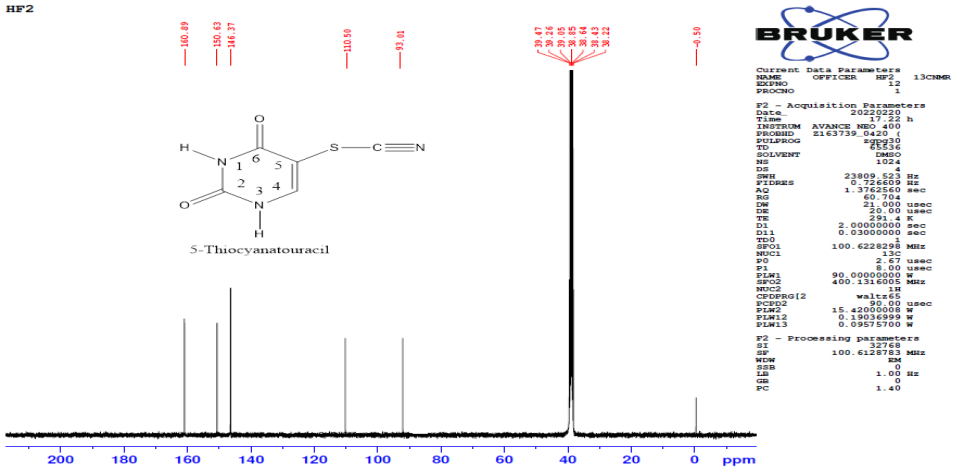


Fig (3) ^{13}C-NMR spectrum for 5-Thiocyanatouracil in DMSO-d_6

Median Lethal Dose

Single oral dose of 500, 1000, 1500, 2000, and 2500 mg/kg BW of the 5-TCU substance were administered by oral gavage at concentrations. After 48 hours, the mortality percentage estimated the results showed that the LD50 of 5-TC affected male rats is 2058.824 mg/kg BW; this dose was determined via the slope equation of the dose- response curve; the findings are reported at table (1) and fig. (4). There was no mortality seen in the group received 500 mg/kg B.W. However, the response increased with dosage, going from 14.285714 % at 1000 mg/kg to 71.428% at 2500 mg/kg. According to the findings, there is a 50% mortality rate between 2000 and 2500 mg/kg. The graphical representation of the mortality % vs. concentration was used to calculate the LD50 using a straight-line data equation. The LD50 dosage was estimated using the equation y = 0.0343x - 20, which depends on the slope of the line connecting dose to percent fatality. The equations calculated LD50, as seen in figure (4) and table (1)
The resultant response curve is a straight line.
The linear regression equation:

y = 0.0343x - 20, was used to derive the LD50 (LD_{50}=2058.824mg/kg BW) [22].

Table (1): showed the correlation of various concentrations of 5-TCU with rat's mortality rate

NO. group	Dose (TCU)mg/kg (n=7)	Dead rats	Mortality%
1	500	0	0
2	1000	1	14.285714
3	1500	2	28.571429
4	2000	3	42.857143
5	2500	5	71.428571

n= total number of rats /group

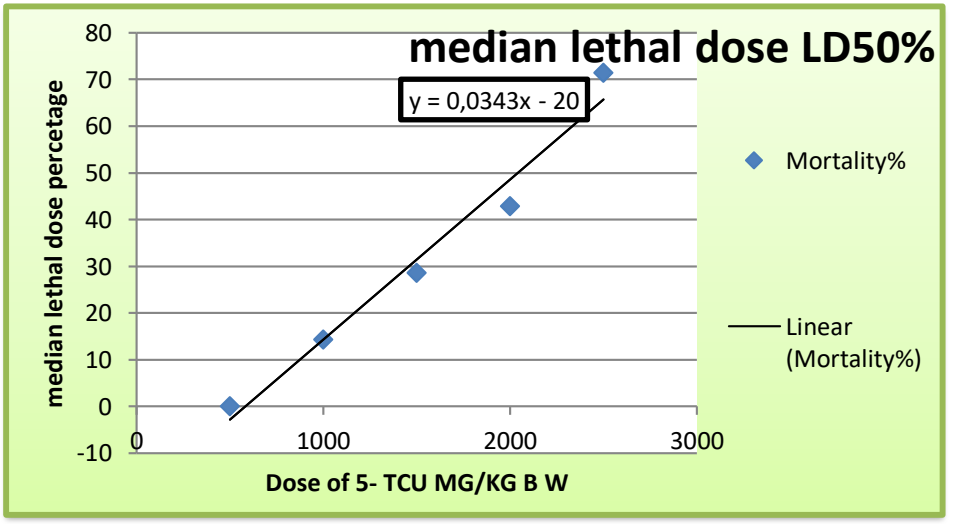


Fig. (4): presented the dose of 5-TCU related to mortality%

Degree to which the analytical value is near to the real value is the description of accuracy in analytical chemistry. Since its real value is unknown, it is difficult to calculate the LD50 with precision. The LD50, believed to remain the real value or identical to the true assessment, is derived using the mortality statistics acquired from a research. The LD50 has certain restrictions even though it is a valuable tool for categorizing compounds according to their level of toxicity. Since it varies, it cannot be regarded as a biological constant. The test incorporates biochemical and histological analyses, but gives little information besides physiological functions. The LD50 is inadequate for demonstrating information on specific concerns for newborn and baby humans. The LD50 test only provides semi quantitative, and frequently misleading, results for the evaluation of pharmacokinetic behavior and bioavailability [23].

Antioxidant activity using DPPH

Table 2 illustrates the antioxidant activity results. The antioxidant properties of both test substances (5-TCU and AA), at various doses, were assessed. The significant differences between 5-TCU (40.97893.131) and AA (56.4594.835) at a concentration of 150 µg/mL. while at high scavenging effects were seen for (5-TCU) compound up to (83.9074.116) and for standard antiradical AA up to (81.79642.449), and also at low doses of 100 g/mL, there were no significant differences between (5-TCU) 27.772.852 and AA 32.7682.4168. As concentration increased, the antioxidant properties become more potent. (Y = aX + b) was used to create regression line for 5-TCU and AA. IC50: Median antiradical inhibition rate IC50 of AA (y = 15.903x + 20.099) is 48.73 and IC50 of 5-TCU (y = 19.285x + 6.3064) is 49.67 as represented at fig.5

Table (2): 5-TCU Antiradical effects enhanced, with increased concentrations compare with standard antiradical AA.

Test compounds	Inhibition%			
	100(µg/ml)	150(µg/ml)	200(µg/ml)	250(µg/ml)
AA	32.768±2.4168	56.459±4.835	68.603±3.723	81.7964±2.449
5-TCU	27.77±2.852	40.9789±3.131*	65.417±5.4642	83.907±4.116
P value	NS	< 0.05	NS	NS

*Significant differences, NS non-significant. Results stated as mean± SD mean of the six replicates.

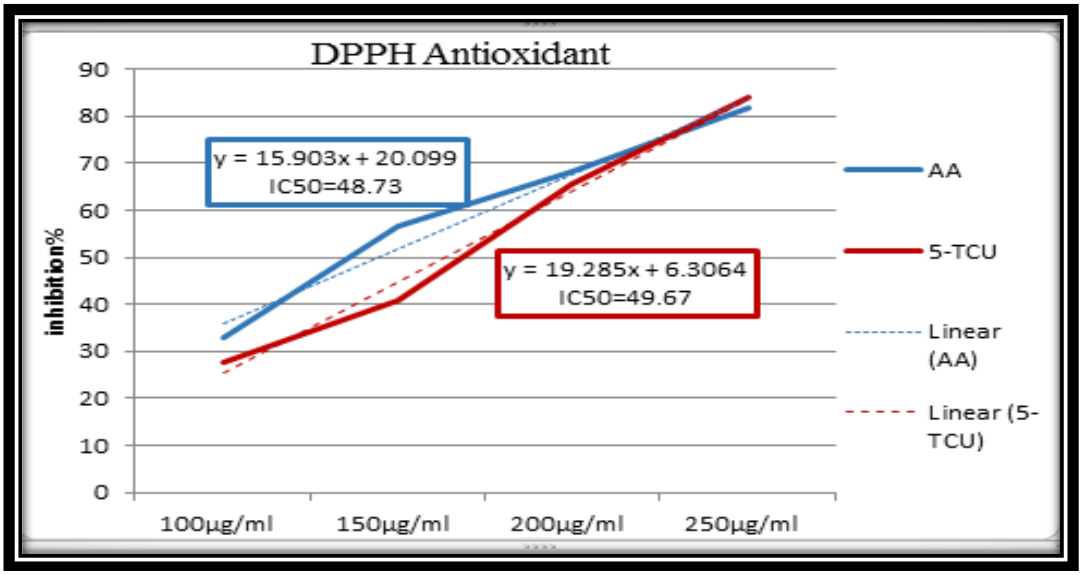


Fig. (5): IC50 considered for both 5-TCU and ascorbic acid (AA).
N=numbers of examinations, and P≤0.05.

The sample concentration sufficient to inhibit a radical by 50% was calculated as the IC50 value. Lower IC50 values correspond to substances with stronger antioxidant activity. Because NO_2 is the preferable site of free radical assault, compound had the highest antioxidant activity and the highest IC50. The IC50 value for ascorbic acid in the DPPH test is 48.73, making it a standard [24]. According to published study classified the severity of any substance toxicity according to IC50; mildly active at 100 to 250 µg mL^{-1}; moderately active at 50 to 100 µg mL^{-1}; highly active at 10 to 50 mg mL^{-1}; extremely active at 10 µg mL^{-1}. [12].

When evaluating the antioxidant activity of antioxidants, free radical scavengers, lipid peroxidation inhibition, chelating agents, reducing power, and synergists are the antioxidant mechanisms that are most commonly discussed. The scavenging activity of the stable 2,2'-diphenyl-1, picrylhydrazil (DPPH) radical allows for a rapid assessment of the antioxidant activities. The DPPH radical is soluble in polar solvents like DMSO and has both stable and soluble.. When compared to other antioxidant in vitro techniques, the DPPH in vitro methodology is thought to be the most practical. Since it just requires a UV-V spectrophotometer and is characterized by fewer stages, reagents, and costs than other in vitro antioxidant techniques, the DPPH approach is thought to be the easiest method. It is therefore often employed for in vitro antioxidant evaluation [25].

In response to a wide range of stressors, cells have developed a number of mechanisms to encourage elevated intracellular levels of thiols like GSH and thioredoxin. In cell cultures, animal models, and humans, exogenous thiols have been utilized to successfully raise the thiol levels in cells and tissues. In all of these systems, higher levels of GSH and other thiols have been correlated with greater resistance to oxidative stress, and in some circumstances, to the prevention or treatment of illness in humans. For these goals, a wide range of

thiol-related substances have been used. Among these are thiols like GSH and its byproducts, cysteine, and NAC; dithiols like lipoic acid, which is internally reduced to the thiol form; and "prothiol" substances like OTC, which are enzymatically transformed into free thiols inside the cell. The cumulative consequences must be taken into account when selecting a thiol for a particular function, such as protecting organs against ischemia reperfusion damage or protecting the lungs from oxidant exposure, e.g., significant increases in the level of free thiols in the blood are linked to harmful consequences. Any transport or cell signaling activities that depend on the production and dissolution of disulfide bonds in membrane proteins might likewise be negatively impacted by changes in the thiol redox gradient across cells. It has been demonstrated that therapeutic thiol administration has a lot of potential, and its effectiveness should be enhanced by choosing substances and delivery techniques that will limit changes in the thiol status of areas outside of the targeted areas. [26]

Antibacterial activity

The antibacterial activity of 5-TCU was altered with different types of bacteria. The results showed that 50, 100, 150 µg/mL concentrations of 5-TCU had no antibacterial activity against all test bacteria. At 200 and 250 µg/mL concentrations showed antibacterial activity in dose- dependent manner against: S. aureus, E. coli, and Pseudo aeruginosa. The detailed shown at table (3) and fig. (6)

Table (3): antibacterial effect of 5-TCU represented as zone of inhibition (mm)

	Zone of inhibition		
	S.aureus	E coli	Pseudo. aeruginosa
200µg/ml	7.95±1.443	9±1.372	13.025±2.553
250µg/ml	15.7±1.358	14.125±1.80	18.0±1.41

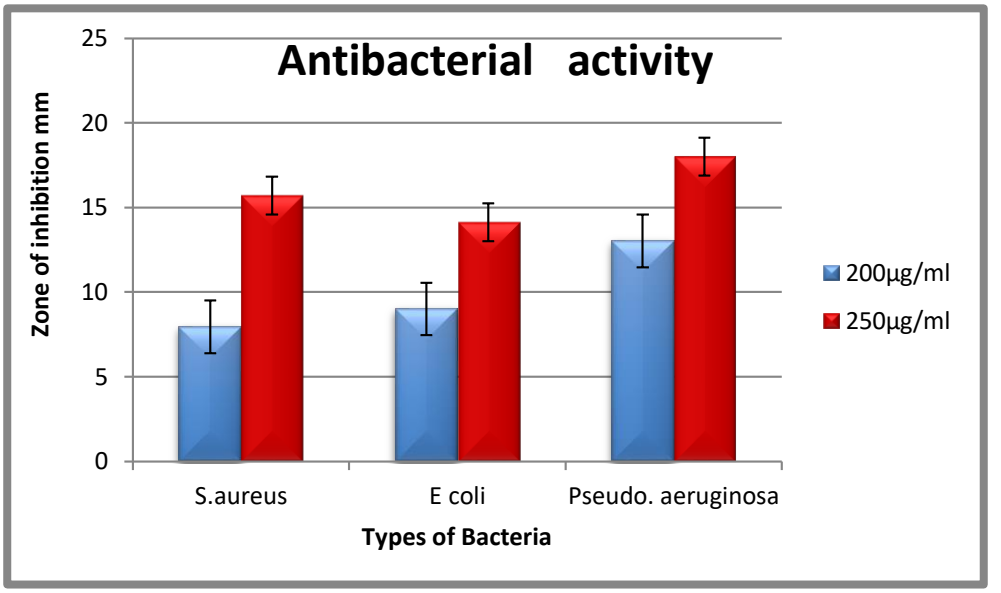


Fig. (6): illustrates the dose-dependent growth inhibition of 5-TCU

The developing resistance of microorganisms to the available antibacterial, arsenal has demanded the discovery of new pharmacological medicines. Numerous bacterial genes that code for new drug gable proteins that may one day be used as antibacterial targets have been discovered because to developments in molecular microbiology and genomics. Recently, regulatory proteins have drawn a lot of interest as one such class of prospective targets. Promising anticancer and antibacterial treatments are being produced using new synthetic chemicals and natural plant materials [16].

Because bacteria have diverse cell wall topologies and varied defense barriers, they have variable sensitivities. 5-TCU has some antibacterial properties; thiol group may combine with important enzyme or other proteins that have sulfenyl groups to limit glycolysis. Na^+, amino acids, and peptides are lost as a result of the structural harm caused to the microbial cytoplasmic membrane by the oxidation of SH-groups. Additionally, the cell's ability to absorb glucose, amino acids, purines, and pyrimidines as well as to synthesize nucleic acids and proteins is impeded. [27]

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Conflicts Of Interest: There are no conflicts of interest.

References

1. Shaaban, S., Arafat, M. A., & Hamama, W. S. (2014). Vistas in the domain of organoselenocyanates. *ARKIVOC, 2014*(1), 470-505. doi: 10.3998/ark.5550190.p008.763
2. Chandler, J. D., & Day, B. J. (2012). THIOCYANATE: A potentially useful therapeutic agent with host defense and antioxidant properties. *Biochemical*

Pharmacology, 84(11), 1381-1387. doi: https://doi.org/10.1016/j.bcp.2012.07.029
3. King, L., Huang, Y., Li, T., Wang, Q., Li, W., Shan, Z., . . . Liu, L. (2022). Associations of urinary perchlorate, nitrate and thiocyanate with central sensitivity to thyroid hormones: A US population-based cross-sectional study. *Environment International, 164*, 107249. doi: https://doi.org/10.1016/j.envint.2022.107249
4. Knoke, D., Kottke, K., & Pohloudek-Fabini, R. (1973). [Synthesis of organic thiocyanates. No. 45. 1. Aliphatic thiocyanates]. *Pharmazie, 28*(9), 574-584.
5. Todorović, U., Klose, I., & Maulide, N. (2021). Straightforward Access to Thiocyanates via Dealkylative Cyanation of Sulfoxides. *Organic Letters, 23*(7), 2510-2513. doi: 10.1021/acs.orglett.1c00460
6. Nguyen, N., Sharma, A., Nguyen, N., Sharma, A. K., Desai, D., Huh, S. J., . . . Robertson, G. P. (2011). Melanoma Chemoprevention in Skin Reconstructs and Mouse Xenografts Using Isoselenocyanate-4. *Cancer Prevention Research, 4*(2), 248-258. doi: 10.1158/1940-6207.CAPR-10-0106
7. Sharma, V., Chitranshi, N., & Agarwal, A. K. (2014). Significance and biological importance of pyrimidine in the microbial world. *Int J Med Chem, 2014*, 202784. doi: 10.1155/2014/202784
8. Abdellattif, M. H., Shahbaaz, M., Arief, M. M. H., & Hussien, M. A. (2021). Oxazinethione Derivatives as a Precursor to Pyrazolone and Pyrimidine Derivatives: Synthesis, Biological Activities, Molecular Modeling, ADME, and Molecular Dynamics Studies. Molecules, 26(18). doi: 10.3390/molecules26185482
9. Lu, G. Q., Li, X. Y., Mohamed, O. K., Wang, D., & Meng, F. H. (2019). Design, synthesis and biological evaluation of novel uracil derivatives bearing 1, 2, 3-triazole moiety as thymidylate synthase (TS) inhibitors and as potential antitumor drugs. Eur J Med Chem, 171, 282-296. doi: 10.1016/j.ejmech.2019.03.047
10. Shaker, R. M., Abd Elrady, M., & Sadek, K. U. (2016). Synthesis, reactivity, and biological activity of 5-aminouracil and its derivatives. Mol Divers, 20(1), 153-183. doi: 10.1007/s11030-015-9595-1
11. Elwahy, A., Diab, H., Salem, M., & Abdelhamid, I. (2021). Aminouracil and aminothiouracil as versatile precursors for a variety of heterocyclic systems. ARKIVOC, 2021. doi: 10.24820/ark. 5550190.p011.474.
12. Egorova, K. S., & Ananikov, V. P. (2017). Toxicity of Metal Compounds: Knowledge and Myths. Organometallics, 36(21), 4071-4090. doi: 10.1021/acs.organomet.7b00605
13. Siva Kumar, T., Shobha Rani, A., Sujatha, K., Purushotham, B., & Neeraja, P. (2017). Toxicity evaluation of ammonium sulfate to albino rat. Asian Journal of Pharmaceutical and Clinical Research, 10(1). doi: 10.22159/ajpcr. 2017.v10i1.15355
14. More, G. K., & Makola, R. T. (2020). In-vitro analysis of free radical scavenging activities and suppression of LPS-induced ROS production in macrophage cells by Solanum sisymbriifolium extracts. Sci Rep, 10(1), 6493. doi: 10.1038/s41598-020-63491-w
15. Kükürt, A., Gelen, V., Başer, Ö. F., Deveci, H. A., & Karapehlivan, M. (2021). Thiols: Role in Oxidative Stress-Related Disorders. In (Ed.), Accenting Lipid Peroxidation. IntechOpen. https://doi.org/10.5772/intechopen.96682

16. Shinu, P., Mouslem, A. K. A., Nair, A. B., Venugopala, K. N., Attimarad, M., Singh, V. A., . . . Deb, P. K. (2022). Progress Report: Antimicrobial Drug Discovery in the Resistance Era. Pharmaceuticals, 15(4), 413.
17. 16. Nguyen, M. T., Moiani, D., Ahmed, Z., Arvai, A. S., Namjoshi, S., Shin, D. S., . . . Gerson, S. L. (2021). An effective human uracil-DNA glycosylase inhibitor targets the open pre-catalytic active site conformation. Prog Biophys Mol Biol, 163, 143-159. doi: 10.1016/j.pbiomolbio.2021.02.004
18. Hriouech, S., Akhmouch, A. A., Mzabi, A., Chefchaou, H., Tanghort, M., Oumokhtar, B., . . . Remmal, A. (2020). The Antistaphylococcal Activity of Amoxicillin/Clavulanic Acid, Gentamicin, and 1,8-Cineole Alone or in Combination and Their Efficacy through a Rabbit Model of Methicillin-Resistant Staphylococcus Aureus Osteomyelitis. Evidence-based complementary and alternative medicine: eCAM, 2020, 4271017. doi: 10.1155/2020/4271017
19. European Patent Application, EP 3 351 535 A1. Bulletin 2018. /30
20. Singh, S., Mosslemin, M. H., & Hassanabadi, A. (2018). Synthesis of 5-Aryl-1,3-Dimethyl-7-Selenoxopyrimidino[4,5-d] Pyrimidine-2,4(1H,3H)-Dione. Journal of Chemical Research, 42(5), 264-266. doi: 10.3184/174751918X15265512038216
21. Pillai, S. K., Kobayashi, K., Michael, M., Mathai, T., Sivakumar, B., & Sadasivan, P. (2021). John William Trevan's concept of Median Lethal Dose (LD50/LC50) – more misused than used. *Journal of Pre-Clinical and Clinical Research, 15*(3), 137-141. doi: 10.26444/jpccr/139588
22. Abonia, R., Gutierrez, L. F., Zwarycz, A. T., Correa Smits, S., & Laali, K. K. (2019). An Efficient Selectfluor-Mediated Oxidative Thio- and Selenocyanation of Diversely Substituted Indoles and Carbazoles. Heteroatom Chemistry, 2019, 1459681. doi: 10.1155/2019/1459681
23. Abdulhafiz, F., Farhan Hanif Reduan, M., Hamzah, Z., Abdul Kari, Z., Dawood, M. A. O., & Mohammed, A. (2022). Acute oral toxicity assessment and anti-hyperuricemic activity of Alocasia longiloba extracts on Sprague-Dawley rats. Saudi Journal of Biological Sciences, 29(5), 3184-3193. doi: https://doi.org/10.1016/j.sjbs.2022.01.050
24. Al-Haidari, A. S., & Al-Tamimi, E. O. (2021). Synthesis of new derivatives of 1,3,4-thiadiazole and 1,3,4-oxadiazole on cyclic imides and studying their antioxidant. Eurasian Chemical Communications, 3(7), 508-517. doi:10.22034/ecc.2021.289788.1185
25. Permatasari, L., & Rohman, A. (2016). 2, 2'-diphenil-1-picrylhydrazil (DPPH) radical scavenging activity of extracts and fractions of Rambutan (Nephelium lappaceum L.) peel. Research Journal of Phytochemistry, 10(2), 75-80.
26. Ulrich, K., & Jakob, U. (2019). The role of thiols in antioxidant systems. Free Radic Biol Med, 140, 14-27. doi: 10.1016/j.freeradbiomed.2019.05.035
27. Welk, A., Patjek, S., Gärtner, M., Baguhl, R., Schwahn, C., & Below, H. (2021). Antibacterial and antiplaque efficacy of a lactoperoxidase-thiocyanate-hydrogen-peroxide-system-containing lozenge. BMC Microbiology, 21(1), 302. doi: 10.1186/s12866-021-02333-9