MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

UNIVERSITY OF BASRAH

COLLEGE OF PHARMACY

CHARACTERIZATION AND EVALUATION OF THE ANTIOXIDANT ACTIVITY OF NEW TRIAZOLES DERIVATIVES

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﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا

الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

(سورة المجادلة، آية: 11)

راء

أهدي ثمرة جهدي هذا إلى التي رفدتني بدعواتها، ومدتني بكل وسائل العون المادية و المعنوية غاليتي أمي.

والى والدي الذي مد لي يد العون .

ومن ثم أهديه إلى نفسي التي مخرت عباب بحر الصعوبات طوال مسيرة الدرس العصيبة.

اتقدم بالشكر والعرفان الى استاذتي

د.رؤى سلمان

د.هبة ناجح

Aim

The aim of this study is to evaluate the antioxidant activity of new triazole derivatives .

This has been determined by DDPH free radical scavenging activity .

Ascorbic acid was used as a standard

1.1.Mefenamic Acid

Mefenamic acid $(C_{15}H_{15}NO_2)$ is a member of the anthranilic acid derivatives (or fenamate) class of nonsteroidal anti-inflammatory drugs (NSAIDs), and is used to treat mild to moderate pain. (1) (figure 1)

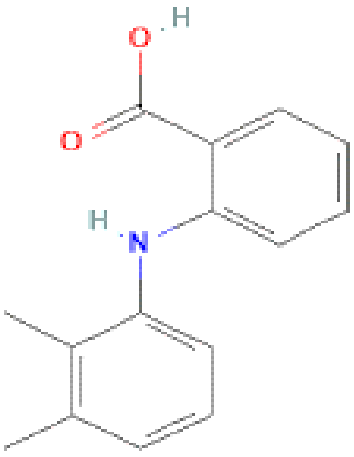


Figure1

1.2.Pharmacological activities

1-There is evidence that supports the use of mefenamic acid for perimenstrual migraine headache prophylaxis, with treatment starting two days prior to the onset of flow or one day prior to the expected onset of the headache and continuing for the duration of menstruation . (1)

2-Mefenamic acid is a potent inhibitor of cyclooxygenase. It has a central as well as peripheral analgesic action. The drug is commonly used in patients with injuries, osteoarthritis, rheumatoid arthritis and dysmenorrhea . (2)

3- as anti-inflammatory medicine, of having some role as an anti- viral medicine also. It can be used along with different anti-viral drugs being tried for the treatment of COVID-19. (2)

1.3.Antioxidant activity of mefenamic acid

1-Zinc complexes with the non-steroidal anti-inflammatory drug mefenamic acid show significant antioxidant activity . (3) (figure 2)

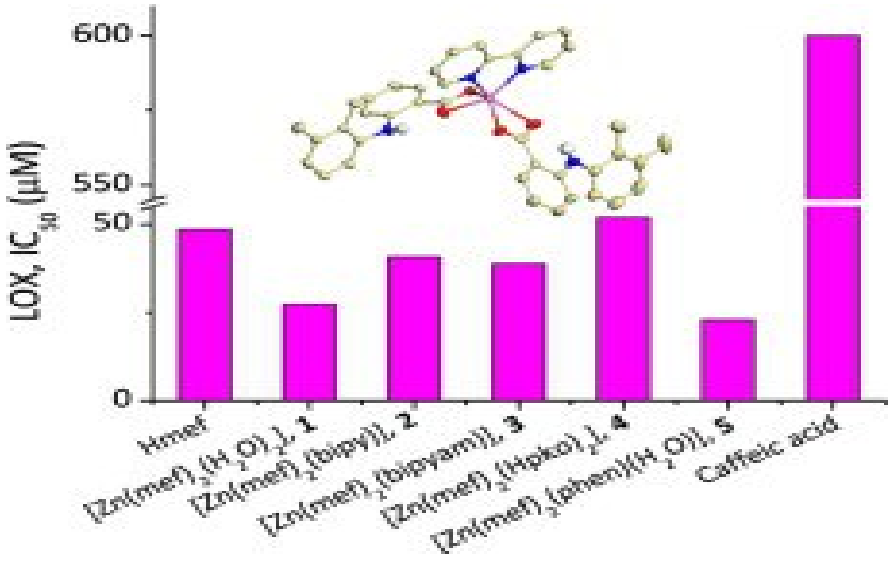


Figure 2

2_Some new complexes of mefenamic acid with potentially interesting biological activity are described.

The anti-oxidant properties of the complexes were evaluated using DPPH assay and compared with those of the free drug and vitamin C.

The complex [Mn(mef)2(H2O)2] exhibits the highest antioxidant activity(4) (figure 3)

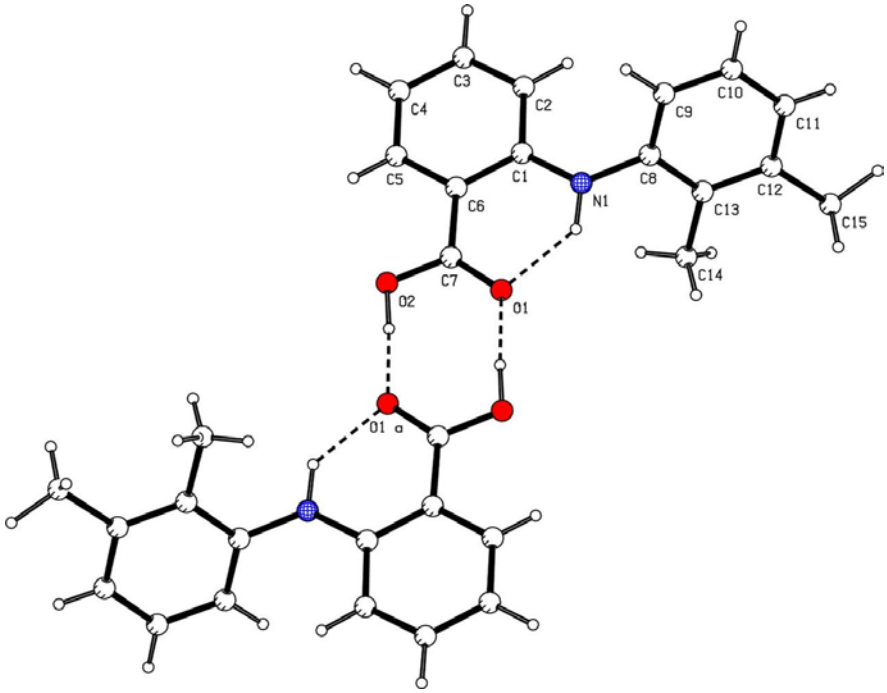


Figure 3

2.1.Antioxidants

■Antioxidants play an important role in food preservation by inhibiting oxidation processes and contributing to health promotion rendered by many dietary supplements, nutraceuticals and functional food ingredients. Antioxidant activity can be monitored by a variety of assays with different mechanisms, including hydrogen atom transfer (HAT), single electron transfer (ET), reducing power, and metal chelation, among others . (5)

2.2.Ascorbic Acid

Ascorbic acid, or vitamin C, is a water-soluble vitamin, meaning that your body doesn't store it. You have to get what you need from food, including citrus fruits, broccoli, and tomatoes. (6) (figure 4)

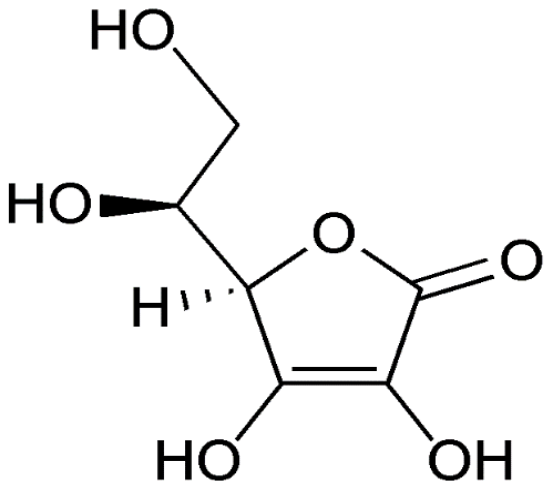


Figure 4

The vitamin C needs for the growth and repair of tissues in all parts of your body. It helps the body make collagen, an important protein used to make skin, cartilage, tendons, ligaments, and blood vessels. Vitamin C is needed for healing wounds, and for repairing and maintaining bones and teeth. It also helps the body absorb iron from nonheme sources.

Vitamin C is an antioxidant block some of the damage caused by free radicals, substances that damage DNA. The build up of free radicals over time may contribute to the aging process and the development of health conditions such as cancer, heart disease, and arthritis. (6)

2.3. Free Radical

Free radicals are chemical species containing one or more unpaired electrons, most of them being unstable and capable of abstracting electrons from other Molecules

The predominant reactive oxygen species generated by cell metabolism or by exogenous factors include hydrogen peroxide (H2O2), the hydroxyl radical (HO), the superoxide anion radical ($O2^{\bullet -}$). These free radicals have essential roles in cell signaling, apoptosis and gene expression. (6)

2.4.Targets of Free radical

The three main classes of biological macromolecules (lipids, nucleic acids, and proteins) are susceptible to free radical attack, and there is plentiful evidence that all suffer oxidative damage in vivo. (7)

2.5.Consequences of free radical damage

Free radicals can **damage DNA,** resulting in cell injury and mutagenesis, and **protein**, resulting in denaturation and, decreased enzyme activity. The **amino acids** histidine, tryptophan, methionine and cysteine are particularly prone to attack.

. Damage to carbohydrate particularly as glycoproteins can result in alteration of receptors and depolymerization of substances such as hyaluronic acid. Free radical - induced lipid oxidation can cause damage to the membrane directly by causing alterations in the PUFA and indirectly by formation of secondary products such as reactive aldehydes. (8)

3.1.Triazoles

Nowadays research is concentrated towards the introduction of new and safe therapeutic agents of clinical importance. The heterocycles are enjoying their importance as being the centre of activity. The nitrogen containing heterocycles are found in abundance in most of the medicinal compounds.(9)

Triazole nucleus is present as a core structural component in an array of drug categories such as antimicrobial, anti-inflammatory, analgesic, antiepileptic, antiviral, antineoplastic, antihypertensive, antimalarial, local anaesthetic, antianxiety, antidepressant, antihistaminic, antioxidant, antitubercular, anti-Parkinson′s, antidiabetic, antiobesity and immunomodulatory agents, etc . (10)

The simplest form of the triazole family is triazole itself.

Triazole is a white to pale yellow crystalline solid with a weak, characteristic odour, it is soluble in water and alcohol, melts at 120°C and boils at 260°C. (10)

It occurs as a pair of isomeric chemical compounds 1,2,3-triazole, **1**, and 1,2,4-triazole, **2** with molecular formula $C_2H_3N_3$, and a molecular weight of 69.06 .The two isomers are: (9)

Each exists in two disimilar tautomeric forms. The different isomers are characterized by the position of the nascent hydrogen. Thus, 1, 2, 4-triazoles exist in two isomeric forms, i.e., 1H and 4H. (9)

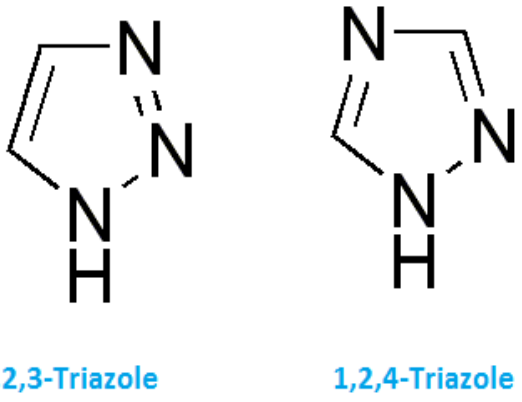


Figure 5 figure5

3.2.Pharmacological activitiey

The most relevant and recent studies have revealed that triazole derivatives have a broad spectrum of pharmacological activities which can be classified into the following categories: (10)

3.2.1.Antimicrobial activity

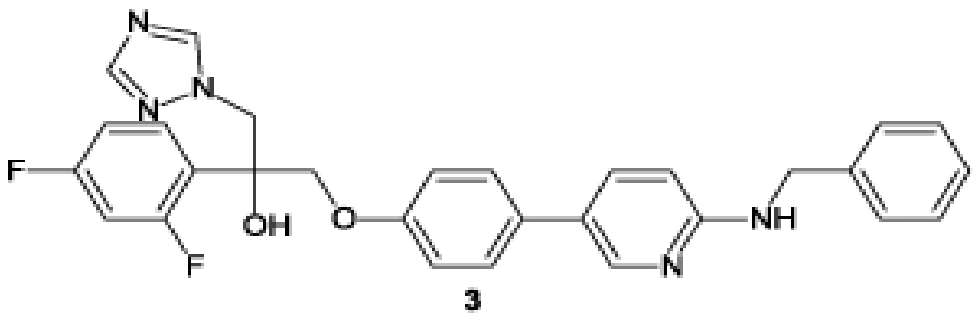
Systemic fungal infections are life-threatening and have become increasingly common in immuno-compromised hosts. Currently triazole drugs (fluconazole, itraconazole, voriconazole and posaconazole) are most frequently used antifungals in clinical therapy. They possess a broad spectrum of activity and reduced toxicity when compared with imidazole antifungals.(10)

However, resistance to azoles is emerging and may pose a serious health problem in future. In addition, triazole drugs are often associated with hepatotoxicity and have a limited antifungal spectrum

Consequently, it remains attractive to develop new triazole derivatives possessing broader antifungal spectra and higher therapeutic indexes.

The synthesis of a series of 1-(substituted biaryloxy)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl) propan-2-ol derivatives, , and their antifungal activity was evaluated against eight human pathogenic fungi *in vitro*.

Seventeen compounds showed activity between 4- and 64-fold higher than voriconazole against *Candida albicans*.(10) figure 6



3

Figure 6

3.2.2.Antineoplastic activity

Antineoplastic properties of triazole derivatives can most probably be attributed to their affinity to anticancer biotargets. It must be emphasised, that combination of the triazole template with other heterocycles is a well known approach for the build-up of drug-like molecules, which allows new pharmacological profiles to be achieved, either by strengthening their action or lowering of toxicity(10)

It has been found that the compound in the (figure 7) to be the most effective *in vitro* against human colon adenocarcinoma cell line (10)

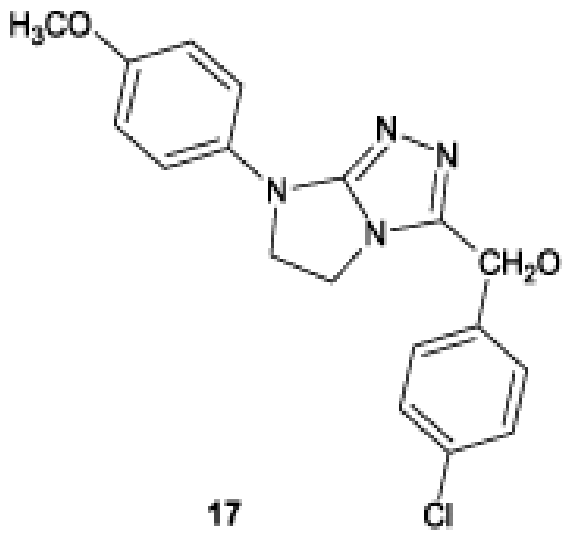


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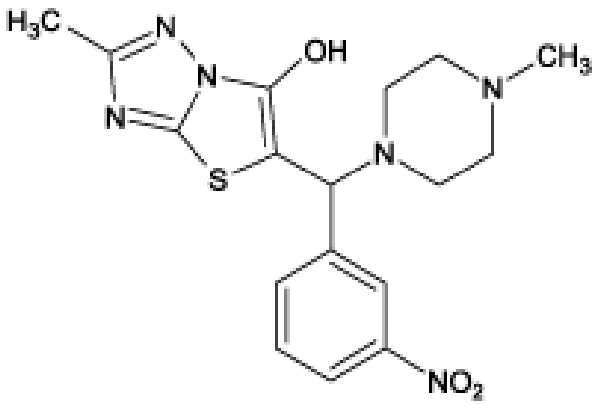
Figure 7

3.2.3.Antioxidant activity

1- it has been found that the compound in the (figure 8) has both antioxidant and anti-inflammatory activities with a lack of COX-1 enzyme inhibitory effect may improve the gastrointestinal safety profile of such compounds (10)



28. 2-methyl-5-[(4-methylpiperazin-1-yl)(3-nitrophenyl)methyl][1,3]thiazolo[3,2-*b*][1,2,4]triazol-6-ol

Figure 8

2- There were some searches for a new Antioxidant glucal-based triazoles compounds with anti-sickling activity, that could act on RBC to reduce the oxidative stress, being useful for the treatment of SCD. (11) (figure 9)

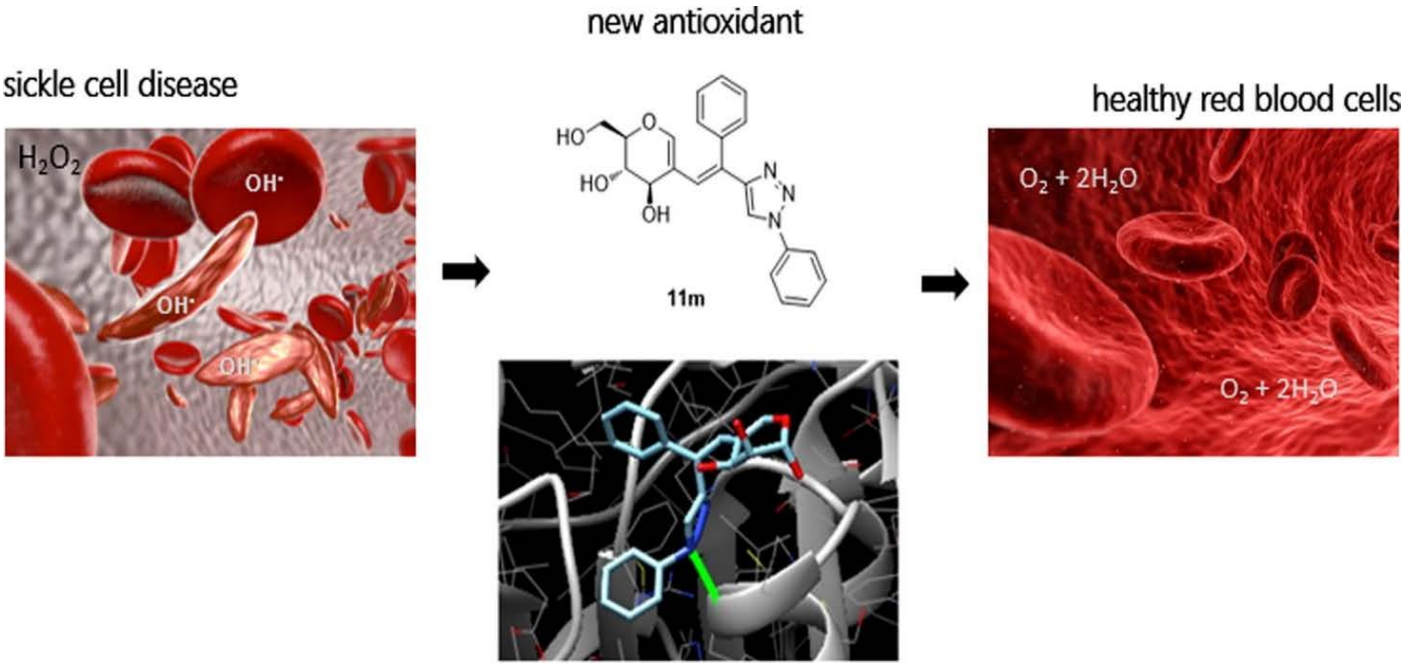
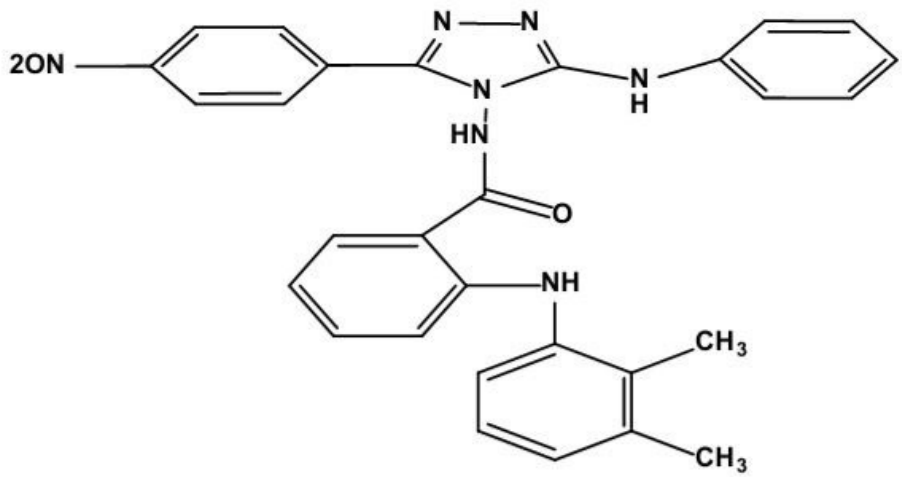


Figure 9

4.MATERIALS AND METHODS

4.1. The new compound

The new compound (figure10) that we studied its antioxidant activity in the laboratory contain a triazole functional group and mefenamic acid.



2-((2,3-dimethylphenyl)amino)- ***N*-(3-(4-nitrophenyl)-5-(phenylamino)-4-yl)benzamide** ***H*-1,2,4-triazol-4-**

Figure 10

4.2. Procedure

■Stock DPPH solution was prepared by dissolving 0.004 g of DPPH in 25mL of ethanol to give a concentration of $1* 10^{-3}$ mol L^{-1}.

Stock solutions(1000 μg mL–1) of test compounds and vitamin C were prepared and suitably diluted to get final concentrations of 250, 200, 150, 100, and 50 μg mL–1

Stock solutions of samples were prepared by dissolving 0.01g of test sample in 10 ml of ethanol

Stock solution of vitamin c were prepared by dissolving 0.01g in 10 ml ethanol.

2700 μL of each concentration was mixed with 300 μL of Stock DPPH solution.

Each concentration was carried out in triplicate.

The absorbance was taken immediately at 516 nm for a diluted solution (1*10-4 mol L–1) as a control reading.

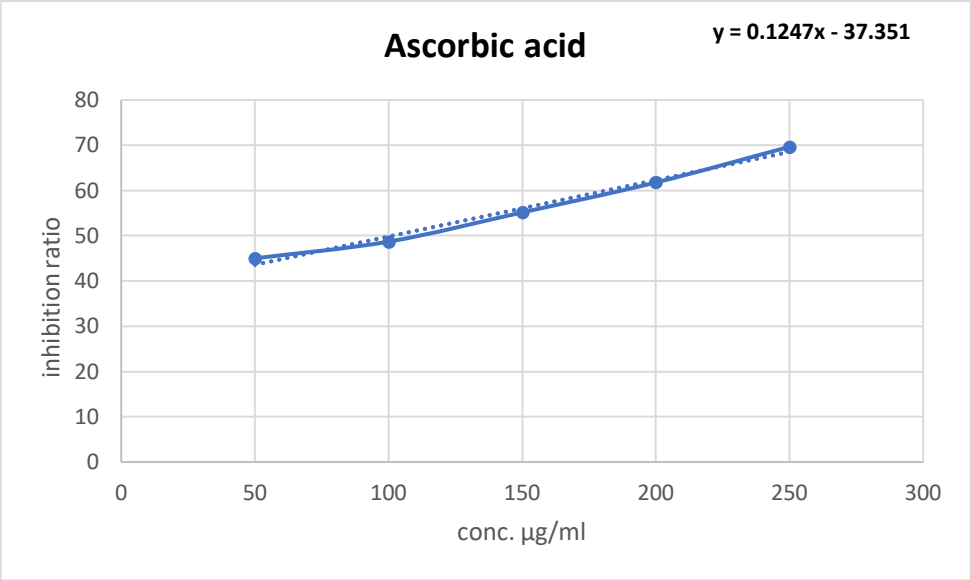
The solutions were incubated for 30 min and the absorbance of each one was measured at 516 nm.

The % antiradical activity and IC50 were calculated.

$$\%\text{Antioxidant} = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} \times 100\%$$

4.2.Results

Ascorbic Acid					
conc.	Abs. 1	Abs. 2	Abs. 3	Average	% inhibition
50µg/mg	0.526	0.529	0.527	0.527	45.01
100µg/mg	0.485	0.49	0.501	0.492	48.7
150µg/mg	0.425	0.435	0.43	0.43	55.16
200µg/mg	0.362	0.373	0.365	0.366	61.77
250µg/mg	0.29	0.288	0.295	0.291	69.66



Y=0.1247x + 37.351

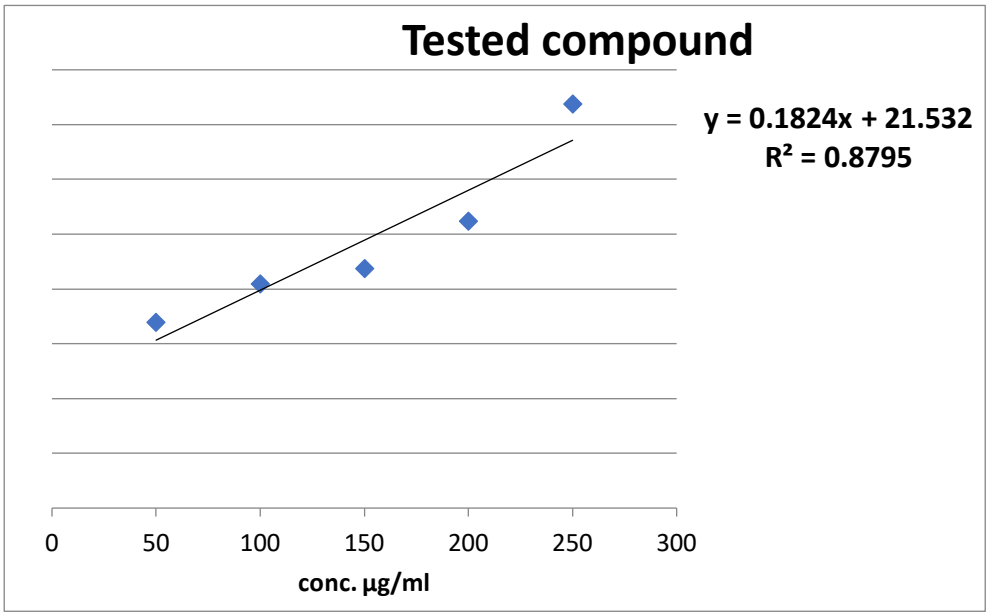
x + 37.3510.1247=50

x=50-37.3510.1247

X=101

IC50= 101 µg/ ml

TESTED SAMPLE					
conc.	abs. 1	abs. 2	abs. 3	average	% ratio
50µg/mg	0.043	0.695	0.61	0.534	33.82
100µg/ml	0.342	0.564	0.525	0.477	40.89
150µg/ml	0.456	0.454	0.454	0.454	43.74
200µg/ml	0.441	0.384	0.33	0.385	52.29
250µg/ml	0.111	0.223	0.302	0.212	73.72



Y= 0.1824 x + 21.532

50 = 0.1824 x + 21.532

$X = \frac{28.468}{0.1824} = 156.07$

IC50 = 156.07 µg/ml

It has been founded that this compound has a promising antioxidant activity according to this assay .

We need a further investigations to studying it’s stability , pharmacokinetics and pharmacodynamic.

Discussion

The present study was designed to investigate the anti-oxidant activity of new triazoles derivative

DPPH free radical scavenging activity DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 516 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 516 nm.

One parameter that has been introduced recently for the interpretation of the results from the DPPH method, is the “efficient concentration” or EC50 value (otherwise called the IC50 value).

This is defined as the concentration of substrate that causes 50% loss of the DPPH activity

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