UNLOCKING THE POTENTIAL: SYNTHESIS AND BIOACTIVITY ASSESSMENT OF NOVEL ISOXAZOLE DERIVATIVES

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Article History: **Received:12/09/22** **Revised:29/10/22** **Accepted: 15/11/22**

ABSTRACT

The several newly synthesized isoxazole compounds were produced using 3-methylacetophenone and substituted benzaldehyde derivatives. FTIR, 1H NMR, and mass spectroscopy were used to determine the products' spectra after the compounds were purified using the appropriate solvent and given a different color. The new isoxazole compounds (GS1-GS5) were examined for their antibacterial (in-vitro) activity against gram (+) bacteria in comparison to the widely used antibiotic ampicillin. Compounds 3-(4-methylphenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS5) and 3-(4-Bromophenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS2) have different levels of antibacterial activity when it comes to Escherichia coli. The most efficient antioxidant is 3-(4-chlorophenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS5), with an IC50 value of 76.29 g/ml. The least effective antioxidant for the hydrogen peroxide scavenging method is 4-(3-methylphenyl)-1,2-isoxazole (GS1), with an IC50 value of 373.544 g/ml.

Keywords: Isoxazole, Antioxidant, Scavenging methods, Antibacterial activity.

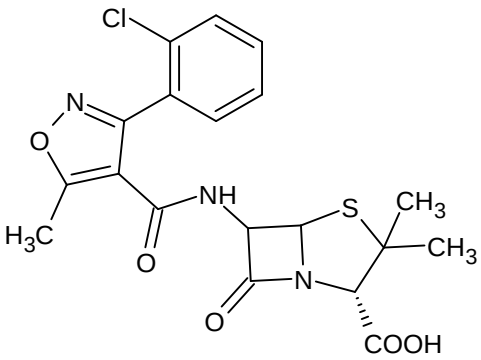
DOI: 10.48047/ecb/2022.11.12.80

INTRODUCTION

1.1. ISOXAZOLE

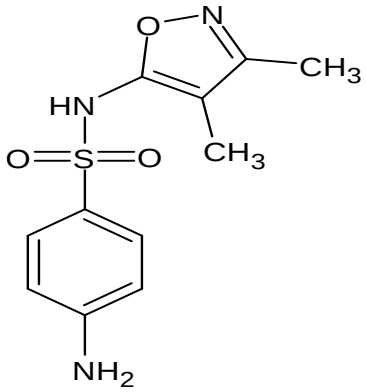
An extensive class of molecules with biological and therapeutic significance is composed of isoxazole ring structures, which primarily contain nitrogen and oxygen atoms**[1]**. A large variety of molecular scaffolds are represented by heterocyclic systems, the majority of which have five- and six-members **[2]**. Many of these heterocyclic scaffolds have been effectively incorporated into new drug leads and medicinal medicines **[3]**. A demonstration of the biological relevance and medicinal use of several heterocyclic derivative include substituted metronidazole. A thiabendazole derivative used as anthelmintic. A nitroimidazole derivative used as anti-amoebic **[4]**. The chemistry and biological significance of heterocyclic molecules

has long been an intriguing field of study. The biological significance of numerous structural derivatives of heterocyclic compounds has recently been studied in literature. Numerous biological applications, including antibacterial, antifungal, cancer-fighting, analgesic, and anti-inflammatory, have been associated with the condensed product of the aromatic imine and aromatic aldehydes **[5].** Due to the variety of their biological applications, heterocyclic compound containing nitrogen with an oxygen atom are regarded as important family of molecules in medicinal chemistry. Azole containing an oxygen atom adjacent to a nitrogen atom is called an isoxazole. Isotonic acid is one example of a natural substance that contains isoxazole rings. These are also serving as the foundation for several medications, including the nitric oxide donor furoxan and the cox-2 inhibitor. The univalent radical produced from isoxazole is called is oxazolyl. Many beta-lactamase-resistant antibiotics, including cloxacillin, dicloxacillin, and flucloxacillin, include an is oxazolyl group **[6]**. They produced 3,4,5-trimethyl isoxazole by heating nitroethane with aqueous alkalies to isolate a liquid base. Between 1930 and 1946, Qualco's research on synthesis of ring systems from nitrile oxides and unsaturated chemicals made a fundamental contribution to the development of isoxazole chemistry **[7]**. The derivatives of isoxazole demonstrate hypoglycemic, analgesic **,** anti-inflammatory, anti-fungal **[8,9]**, antibacterial, HIV-inhibitory, and antioxidant capacities **[10]**and an immunosuppressive disease-modifying antirheumatic drug [DMARD]**[11]**.



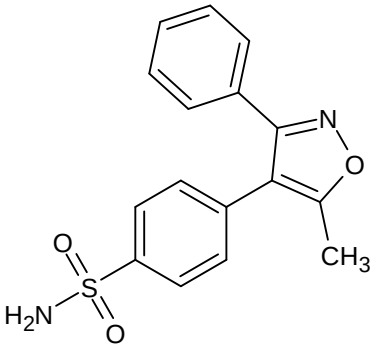
Cloxacillin

Antibacterial



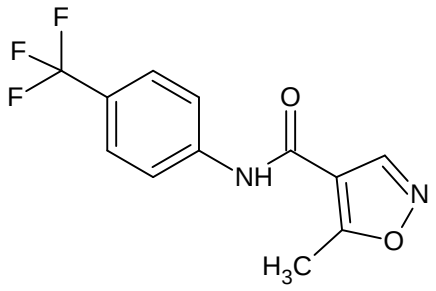
Sulfi-soxazole

Antibacterial



Valdecoxib

Selective COX-II inhibitor



Leflunomide

Immunosuppressive agent

Fig.1. Drugs containing heterocyclic isoxazole moiety

CHEMISTRY OF HETEROCYCLIC

While the oxygen atoms are more pronounced for its donating property of electron impact, the nitrogen hetero atom are more pronounced for its electron-withdrawing effect of electron-withdrawing. Isoxazoles, which are neutral compounds, are subject to electrophilic substitution at position 4 somewhat more easily than benzene. Their behavior may change as a result of substituent effects. In contrast to substituents at position 3, substituents at position 5 appear to have a stronger activating and deactivating effect. Isoxazoles are utilized as latent synthons in the synthesis of natural products, including masked aldol and related moieties, masked aromatic rings, masked fused rings, and novel heterocyclic rings **[12]**. Isoxazole can react in a variety of ways, including **[13].**

- Protonation
- Quaternization
- Complexation
- Oxidation
- Reduction
- Carbanionic Condensations
- Thermolysis
- Photolysis

1.2. CHALCONE

1.2.1. INTRODUCTION

Numerous in-depth scientific investigations have been conducted worldwide on the chemistry of chalcones. The synthesis of chalcones and their biodynamic properties have drawn a lot of attention. These compounds also known as benzylidene acetophenone and benzol acetophenone **[14,15]**. Chalcone has a very good synthon, making it possible to construct a wide range of new heterocycles with favorable medicinal properties. Chalcones are unsaturated ketones with the reactive keto ethylenic group. These substances are colored due to chromophore presence -CO-CH=CH-, and depends on the existence of another auxo chromes, is present **[16]**. There are various techniques available for making chalcones. The Claisen-Schmidt combination of equip-molar amounts of aryl-methyl-ketone and aryl-carbonyl in the presence of alcoholic alkali is the easiest approach **.**Chalcones are used to create a variety of derivatives, including pyrimidines with various heterocyclic ring systems, pyrazolines, and cyanopyridines **[17, 18]**.

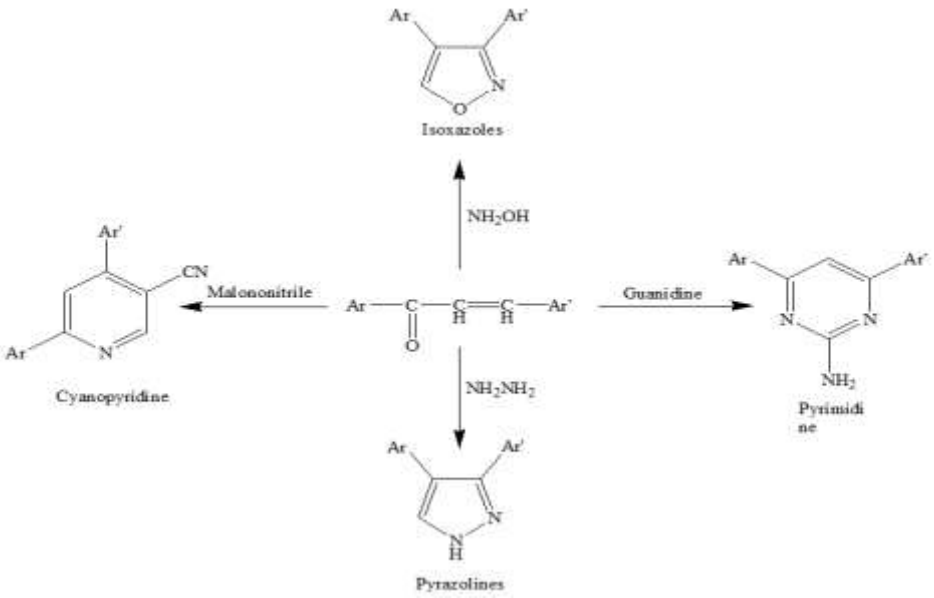


Fig.2. Formation of various rings from chalcone

1.2.2. SYNTHETIC METHODS OF PREPARING CHALCONES

1.2.2.1. REACTION OF CLAISEN-SCHMIDT

There are other ways to make chalcones, but the one that requires equimolar amounts of a substituted acetophenone and substituted aldehydes being Claisen-Schmidt condensed in aqueous alcoholic alkali solution is the most practical **[19]**. Typically, between 10% and 60% of alkali is employed in the Claisen-Schmidt process **[20]**. The reaction is conducted either at room temperature for a week or at 50°C for 12–15 hours. When these circumstances exist, the Cannizzaro reaction **[21]**and occurs, resulting in a reduction in the yield of the targeted product. It has been suggested that benzylidene-diacetate be used in place of aldehyde in the process above to prevent the disproportionation of aldehyde **[22]**.

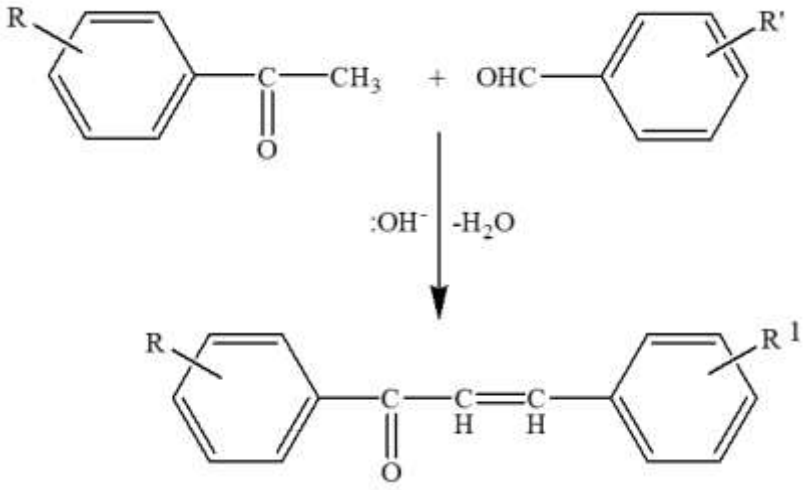


Fig.3.Formation of chalcone

1.2.3. IMPORTANCE OF CHALCONES

1. They are closely related to flavones, tetralones, aurones, and aziridines **[23,24]**.
2. Tetrahydroxy-4-propoxy-dihydrochalcone-4-'-neohesperdoside, is a synthetic sweetener that is many times sweeter compare to glucose, has been utilized **[25,26,27]**.
3. They have a keto-ethylenic group, making them reactive to a number of chemicals, containing 2-amino thiophenol and phenyl hydrazine etc. **[28,29]**.
4. Chalcones have been discovered to be helpful in revealing the structure of natural goods. like hemlock tannin, cyan maclurin phloretin, eriodyctiol andhomoeriodictyol, naringenin **[30, 31]** etc.

1.3. ANTIBACTERIAL ACTIVITY

The microbiological assay was built on a comparison between the inhibition of microorganism growth caused by measured concentrations of test substances and that induced by known amounts of a standard antibiotic **[32]**. Turbidimetric method and filter paper disc method were the two methods that were typically used. The turbidometric approach measures the amount of an antibiotic uniformly diluted in a fluid medium that inhibits the growth of microbiological cultures **[33]**. It was put up against chemicals that were synthesized. In this case, growth was measured to be present or absent. The cylinder plate method relies on diffusion of antibiotic through a hardened layer of agar on a petri dish or plate so that the development of additional microorganisms is completely blocked in a zone around the cylinder holding solution of the antibiotics **[34]**. To find the compound's interesting properties, antibacterial activity testing is required after the synthesis of desired derivatives. The method used here to determine antibacterial activity is a relative one rather than an absolute one. Comparing an organism's reaction to an unknown substance to its reaction to a standard preparation with a known composition and concentration. This determination reveals whether the organism is susceptible to the agent or resistant to it. Sensitive organisms are those that are inhibited by the antimicrobial agent at therapeutically achieved concentrations, resistant organisms are those whose growth is not stopped by the antimicrobial agent, and intermediate organisms are those that require specific handling in order to be employed effectively **[35]**.

1.3.1. CUP PLATE METHOD

In the cup-plate approach, an exact volume of the inoculums (microbial suspension) was added to the sterilized nutritional agar media (10°C to 40°C) then mixed well. 20 cc of this suspension were aseptically placed into the petri plates and allowed to sit there until solidifying **[36]**. Agar cups are created with the use of a borer after the agar has solidified, and solutions of the appropriate concentrations of the sample and standard transferred into cups, respectively, and incubated at 35°C to 36°C for one day **[37]**. Through the agar surrounding its cup, the antimicrobial agent diffuses and creates a zone of sample activity of bacterium sensitive inhibition. Zones of inhibition were calculated as a metric of synthetic compounds' antibacterial capabilities **[38]**. The cup-plate method relies on the antibiotic diffusing from a vertical cup through a layer of solidified agar in a petri dish or plate to the point where the development of further microorganisms is completely stopped in a zone surrounding the cap carrying the

antibiotic solution. The cup-plate approach is straightforward, and it was simple to measure the amount of microbial inhibition. Here, we applied this technique to check the test compounds for antibacterial resistance **[39]**.

1.3.2. IN-VITRO ANTIBACTERIAL SCREENING

The following common bacterial strains were used for the in vitro antibacterial screens of synthesized compounds: For microbiological screening, Gram-negative E. coli (MT-CC No. 521) and Gram-positive P. aeruginosa (MT-CC No. 1688) microorganisms were utilized **[40]**.

1.3.3. Growing and maintaining test organisms for antibacterial research

For antimicrobial test organisms, Escherichia coli (MTCC No. 521) and Pseudomonas aeruginosa (MTCC No. 1688) bacterial cultures were employed, which were obtained from the culture collection centre, department of applied botany and biotechnology, university of Mysore, India. Nutrient broth (NB) was used to keep the bacteria alive at 370°C.

1.3.4. Preparation of inoculum

The gm- microbes E. coli (MT-CC-521) and P. aeruginosa (MT-CC No. 1688) had been prepared in a nutrient broth over night with the help of a rotary shaker at room temperature. The pellet was put up in distilled water (double distillated), and the cell density was standardized by spectrophotometer (A610 nm). Using a sterile spatula, the conidia were scraped from the Petri dishes after being inundated with 8 to 10ml of water obtained from distillation. The density of each fungus' spore was adjusted with the help of spectrophotometer to produce a final concentration of roughly 105 spores/ml **[42]**.

1.4. ANTI OXIDANT ACTIVITY

Reactive species, including reactive nitrogen and oxygen species, have harmful effects, on humans' normal physiological function are considerably reduced by antioxidants, which are synthetic or natural substances or enzymes **[43]**. A scavenger of free radicals, antioxidant defense mechanism, and free radical reduction are all improved by antioxidants. It appears to be somewhat unrealistic and difficult to quantify the entire antioxidant capability of a substance using one approach. To assess total antioxidant capacity in vitro, a number of approaches have been published. In order to assess the antioxidant ability of isoxazoles for potential management benefits, hydrogen peroxide scavenging experiments are planned **[44]**.

1.4.1. OXIDATIVE STRESS

Oxidative stress and inflammation are closely associated, and numerous studies have shown that vascular inflammation can contribute to arterial disorders. When there is oxidative stress, the production of reactive species of oxygen and reactive species of nitrogen also plays a critical role for the signaling pathway activation that affect intracellular and extracellular pathways. Mast cells and leukocytes are formed at the site of inflammation, which causes a buildup of ROS. This leads to a "respiratory burst" due to an accelerated uptake of oxygen **[45]**.

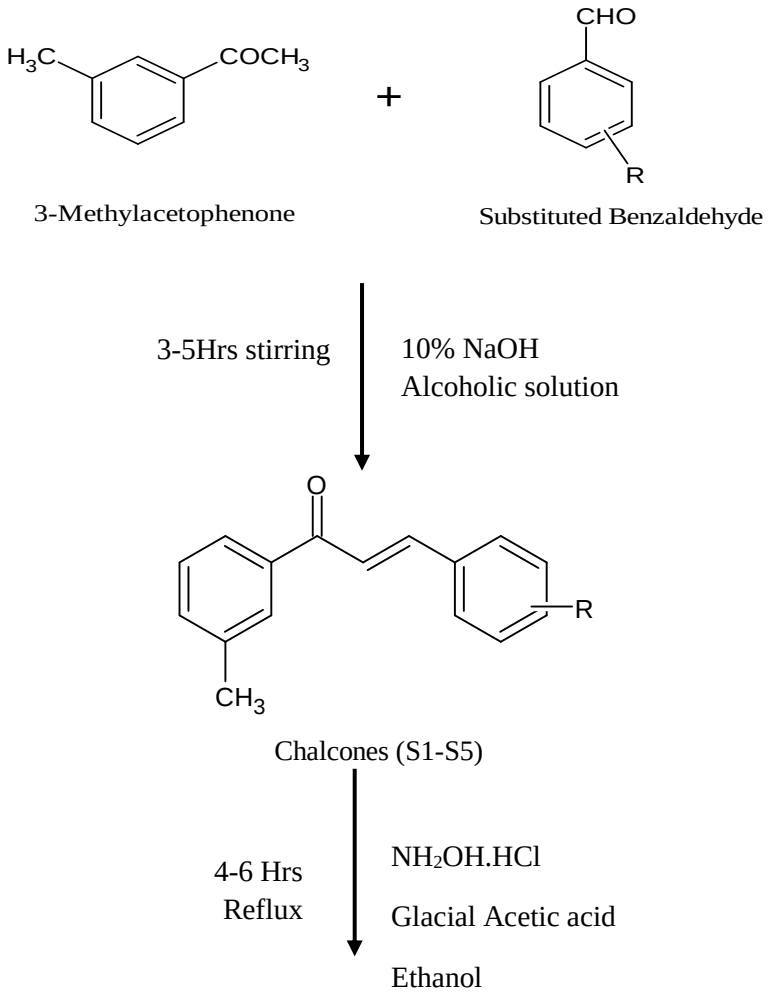
EXPREMENTAL WORK

3.1. SYNTHESIS AND CHARACTERIZATION OF COMPOUNDS (GS1-GS5)

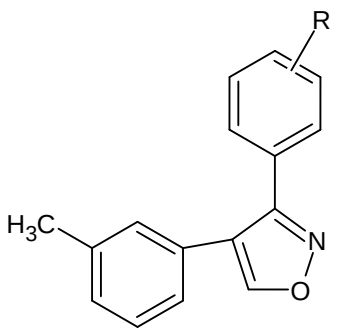
3.1.1. MATERIAL AND METHOD

Chemicals and solvents were purchased from Sigma-Aldrich (India), Spectro chem (India), Merck (India). These chemicals are used in this formulation 3- Methyl acetophenone, Benzaldehyde, Methanol, Sodium hydroxide, Ethanol, 4- chloro benzaldehyde, 4- bromo benzaldehyde, 4 Fluoro benzaldehyde, 4- hydroxyl benzaldehyde, 4- methyl benzaldehyde, Hydroxylamine hydrochloride, Glacial acetic acid, Silica gel Merck, Potassium bromide, DMSO (Dimethyl sulfoxide), Nutrient agar, Beef extract, Sodium chloride, Hydrogen peroxide, Ampicillin, Ascorbic acid

REACTION SCHEME: 1.



GS1	R	-Cl
GS2	R	-Br
GS3	R	-F



GS4	R	-OH
GS5	R	-CH_3

GS1-GS5

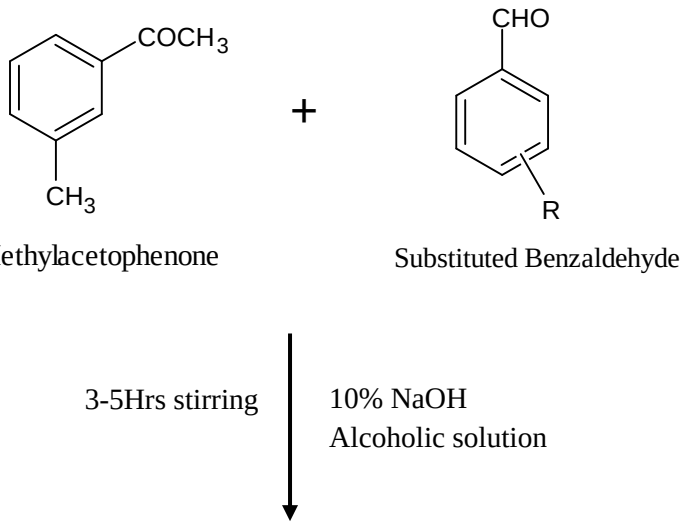
Scheme1. Synthesis of substituted Isoxazole compounds (GS1-GS5)

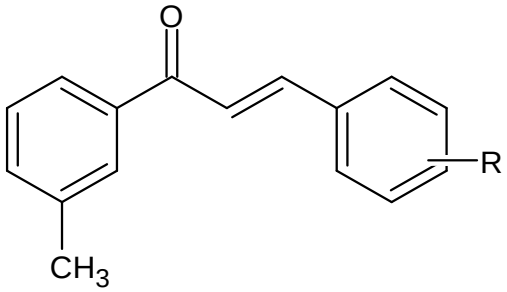
3.1.2. Procedure for the synthesis of compounds (GS1-GS5)

3.1.2.1. Synthesis of chalcones(S1-S5):

3-Methylacetophenone (0.02 mol) and substituted benzaldehyde (0.02 mol) were dissolved in CH_3-OH (40mL). 10% NaOH (Sodium hydroxide) alcoholic solution (40ml) was added slowly to previous solution and the mixture stirred for 3 to 4 hrs. The mixture was transferred into 415 mL of water with constant stirring and left overnight at 0℃. The obtained precipitate was filtered, cleaned, and recrystallized from ethanol. Substituted benzaldehydes which are used in the synthesis of chalcones are as: -

- ✓ 4-chlorobenzaldehyde
- ✓ 4-bromo benzaldehyde
- ✓ 4-fluoro benzaldehyde
- ✓ 4-hydroxy benzaldehyde
- ✓ 4-methyl benzaldehyde.

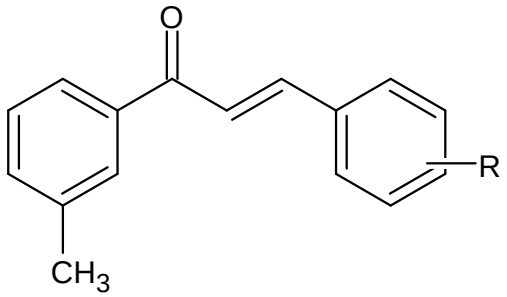




Chalcones (S1-S5)

3.1.2.2. Synthesis of isoxazole compounds [3-(Substituted phenyl)-4-(3-methylphenyl)-1,2-oxazole] (GS1-GS5) from3-(Substituted-phenyl)-1-(3-methylphenyl) prop-2-en-1-one (S1-S5): -

Chalcone (0.02 mol) and ($NH_2OH.HCl$) hydroxylamine hydrochloride (0.02 mol) was dissolved in 10mL of alcoholic Glacial Acetic acid solution and stirred for 4-6 hrs., then it was transferred into 415mL of water cold at 0°Cwithcontinuous stirring for 1 hr. then left overnight. The obtained precipitate was filtered, cleaned, and recrystallized from ethanol.



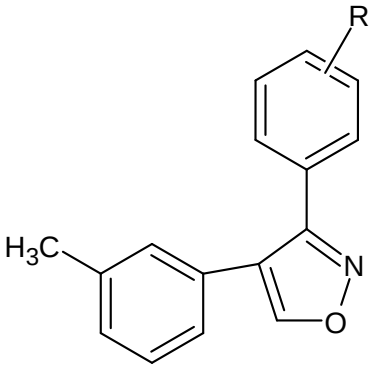
Chalcones (S1-S5)

4-6 Hrs
Reflux

$NH_2OH.HCl$

Glacial Acetic acid

Ethanol



GS1	R	-Cl
GS2	R	-Br
GS3	R	-F
GS4	R	-OH
GS5	R	$-CH_3$

Isoxazole compounds (GS1-GS5)

3.2. IDENTIFICATION OF COMPOUNDS (GS1-GS5)

The compounds were created, scaled for yield, and then purified through recrystallization using the proper solvent system. The following techniques are used to identify and characterize the purified chemicals:

1. Physical properties - Table no.1

2. Thin Layer Chromatography.

3. Melting point determination.

4. IR (Infra-Red Spectroscopy).

5. NMR (Nuclear Magnetic Resonance Spectroscopy).

6. Mass spectroscopy.

3.2.1. PHYSICAL PROPERTIES: -

Table no.1.Physical characteristics of all synthetic compounds

Compound	R	Molecular formula	Molecular weight	R_f value	Percentage yield (%)
GS1	-Cl	$C_{16}H_{12}ClNO$	269.72	0.63	54.35
GS2	-Br	$C_{16}H_{12}BrNO$	314.17	0.57	68.46
GS3	-F	$C_{16}H_{12}FNO$	253.27	0.62	70.52
GS4	-OH	$C_{16}H_{13}NO_2$	251.27	0.54	58.33
GS5	-CH3	$C_{17}H_{15}NO$	249.30	0.60	72.50

3.2.2.TLC (THIN LAYER CHROMATOGRAPHY)

In order to identify organic compounds with characteristic Rf values, thin layer chromatography (TLC) carried out on precoated silica gel plates (604 GF 254 Merck) with an appropriate solvent system, and the Rf values were recorded accordingly.

3.2.3. DETERMINATION OF MELTING POINT

The temperature was measured in capillaries using LABHOSP melting point instrument and documented in 0°C without rectification. It is a frequently used physical characteristic in the description of an organic compound.

COMPOUND-1. 3-(4-CHLOROPHENYL)-4-(3-METHYLPHENYL)-1,2-ISOXAZOLE (GS1)

Parameters- Color- Light Brownish, Melting point- 221-223°C, R_f value- 0.63, percentage yield- 54.35 %, Molecular formula- $C_{16}H_{12}ClNO$.

Characterization- FT-IR: 1644.40 cm-1 (Ar-H, stretching), 1477.65 cm^{-1}(C=C, ring stretching), 1599.93 cm-1 (C=N, ring stretching), 1266.98 cm-1 (CO, ring stretching), 1441.46 cm^{-1}(CH_3, stretching).

^{1}HNMR: (DMSOd6 - 500 MHz), δ 6.92-7.56 (m, 8H, Ar-H), 3.83 (s, 3H, -CH_3), 8.97(s,1H, -CH)

MS(m/z): MS(m/z): 269.06. M^+

COMPOUND-2. 3-(4-BROMOPHENYL)-4-(3-METHYLPHENYL)-1,2-ISOXAZOLE (GS2)

Parameters- Color- Greyish, Melting point- 256-258°C, R_f value- 0.57, percentage yield- 68.46 %, Molecular formula- $C_{16}H_{12}BrNO$.

Characterization-FT-IR: 1715.39 cm-1 (Ar-H, stretching), 1456.11 cm^{-1}(C=C, ring stretching), 1599.10 cm^{-1}(C=N, ring stretching), 1252.06 cm^{-1}(CO, ring stretching), 1408.37cm^{-1}(CH_3, stretching).

^{1}HNMR: (DMSO d6 - 500 MHz), δ 6.92-7.77 (m, 8H, Ar-H), 3.83 (s, 3H, -CH_3), 8.96(s,1H, -CH)

MS(m/z): 313.01. M^+

COMPOUND-3. 3-(4-FLUOROPHENYL)-4-(3-METHYLPHENYL)-1,2-ISOXAZOLE (GS3)

Parameters- Color- Light Reddish, Melting point- 211-213°C, R_f value- 0.62, percentage yield- 70.52 %, Molecular formula- $C_{16}H_{12}FNO$.

Characterization-FT-IR: 1701.79 cm-1 (Ar-H, stretching), 1509.02 cm^{-1}(C=C, ring stretching), 1604.82 cm^{-1}(C=N, ring stretching), 1259.57 cm^{-1}(CO, ring stretching), 1442.74 cm^{-1}(CH_3, stretching).

^{1}HNMR: (DMSO d6 - 500 MHz), δ 6.92-7.61 (m, 8H, Ar-H), 3.83 (s, 3H, -CH_3), 8.97(s,1H, -CH)

MS(m/z): 253.08. M^+

COMPOUND-4.3-(4-HYDROXYPHENYL)-4-(3-METHYLPHENYL)-1,2-ISOXAZOLE (GS4)

Parameters- Color- Creamish, Melting point- 189-191°C, R_f value- 0.54, percentage yield- 58.33 %, Molecular formula- $C_{16}H_{13}NO_2$.

Characterization-FT-IR: 1635.83 cm-1 (Ar-H, stretching), 1452.23 cm^{-1}(C=C, ring stretching), 1598.35 cm^{-1}(C=N, ring stretching), 1259.95 cm^{-1}(CO, ring stretching), 1452.23 cm^{-1}(CH_3, stretching), 3238.56 cm^{-1}(Ar-OH, stretching in ring)

^{1}HNMR: (DMSO d6 - 500 MHz), δ 6.73-7.42 (m, 8H, Ar-H), 3.83 (s, 3H, -CH_3), 8.97(s,1H, -CH)

MS(m/z): 251.09. M^+

Compound-5. 3-(4-methylphenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS5)

Parameters- Color- Blackish, Melting point- 199-201°C, R_f value- 0.60, percentage yield- 72.50 %, Molecular formula- $C_{17}H_{15}NO$.

Characterization-FT-IR: 1687.52cm^{-1}(Ar-H, stretching), 1500.27 cm^{-1}(C=C, ring stretching), 1618.44 cm^{-1}(C=N, ring), 1259.95 cm^{-1}(CO, ring), 1452.23 cm^{-1}(CH_3, stretching).

^{1}HNMR: (DMSO d6 - 500 MHz), δ 6.92-7.54 (m, 8H, Ar-H), 3.79-3.83 (d, 6H, -CH_3), 8.97(s,1H, -CH)

MS(m/z): 249.11. M^+

3.3. PHARMACOLOGICAL EVALUATION

3.3.1. ANTIBACTERIAL ACTIVITY

3.3.1.1. MEDIUM PREPARATION FOR CUP PLATE METHOD

All the ingredients were weighed out and dissolved in water obtained from distillation. Then the mixture was boiled on water bath until it became transparent. This nutrition medium was autoclaved at 121 °C and 15 pressure to sterilize it.

3.3.1.3. TECHNIQUE OF ANTIMICROBIAL ACTIVITY DETECTION

The Petri dishes was sterilized with the help of oven at 155°C for one hr. In an autoclave set to 121°C and 15 pressure, agar media, borer, and test solutions were sterilized. The sterilized nutritional agar media (cooled to42°C) was added to, and mixed well with, a predetermined volume of the microbial suspension (inoculums). The sterile petri dishes were filled aseptically with molten sterile agar [43].

The isoxazole synthesized compounds were dissolved in DMSO (Dimethyl sulfoxide) to obtain concentrations of 100 and 200 g/ml after the agar media was allowed to stand for cooling and solidify. Cups were then created with the aid of a borer and filled with the isoxazole derivatives.

The corresponding bores received 0.1 ml of each of the isoxazole derivative concentrations. As a standard reference, 0.1 ml of ampicillin at concentrations of 100 and 200 g/ml has also been used. For diffusion, the petri dishes were stored in a refrigerator (1hr). The petri dishes incubated at 35°C for antibacterial growth after diffusion, and the zone of inhibition was monitored and recorded.

3.3.2. ANTIOXIDANT ACTIVITY

Antioxidant activity of newly synthesized substituted isoxazole derivatives were evaluated by hydrogen peroxide scavenging method.

UV- spectrophotometer was used to observe absorbance of all synthesized compounds (GS1-GS5) and standard compound (Ascorbic acid).

3.3.2.1. ASSAYS FOR HYDROGEN PEROXIDE SCAVENGING

H2O2scavengingactivity of the synthesized compounds (GS1-GS5) were investigated according to the method of **[12]**. In potassium phosphate buffer with a pH of 7.3, a solution of H2O2 (40 mM) was made and 3.5 ml of different concentration of synthesized compound (GS1-GS5) in distillate water (200,300,400,500 and 600 µg/ml) were added toH2O2solution (0.7 ml). The absorbance value was measured at 230 nm after 10 minutes of incubation in comparison to a blank solution made up of phosphate buffer without H2O2. Ascorbic acid was used as a standard drug. The graph showing scavenging activity vs concentrations was used to get the IC50 value. These numbers represent the concentration of an extract with 50% scavenging.

3.3.2.2. PREPARATION OF 40 MM SOLUTION OF HYDROGEN PEROXIDE

Hydrogen peroxide Solution was prepared by the dilution of 1 ml 30% H_2O_2 with 244 ml distilled water.

The following formula was used to determine the capacity of compounds with hydrogen peroxide scavenging activity:

$$\%\ H2O2\ Scavanging\ Activity\ = \frac{Ab\ (Control) - Ab\ (Sample)}{Ab\ (Control)} \times 100$$

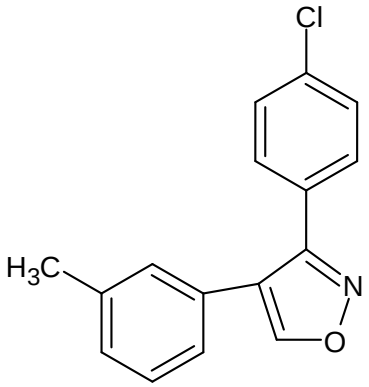
*Ab – Absorbance

Where Ab control is the absorbance of solution containing only potassium phosphate buffer and Hydrogen peroxide and Ab sample is the absorbance of the solution containing potassium phosphate buffer, hydrogen peroxide combined with a test substance that exhibited the anticipated hydrogen peroxide scavenging activity. Each experiment was performed three times, and the data were displayed on the graph as the mean± SD.

RESULT AND DISCUSSION

4.1. SYNTHETIC WORK

COMPOUND-1. 3-(4-CHLOROPHENYL)-4-(3-METHYLPHENYL)-1,2-ISOXAZOLE (GS1)



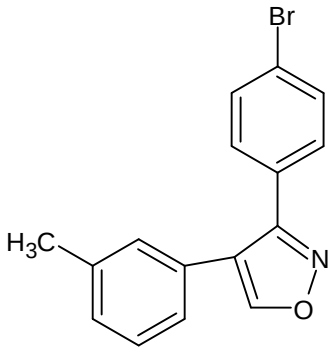
Parameters- Color- Light Brownish, Melting point- 221-223°C, R_f value- 0.63, percentage yield- 54.35 %, Molecular formula- $C_{16}H_{12}ClNO$.

Characterization- FT-IR: 1644.40 cm-1 (Ar-H, stretching), 1477.65 cm-^{1}(C=C, ring stretching), 1599.93 cm-1 (C=N, ring stretching), 1266.98 cm-1 (CO, ring stretching), 1441.46 cm-^{1}(CH_3, stretching).

^{1}HNMR: (DMSOd6 - 500 MHz), δ 6.92-7.56 (m, 8H, Ar-H), 3.83 (s, 3H, -CH_3), 8.97(s,1H, -CH)

MS(m/z): MS(m/z): 269.06. M^+

COMPOUND-2. 3-(4-BROMOPHENYL)-4-(3-METHYLPHENYL)-1,2-ISOXAZOLE (GS2)



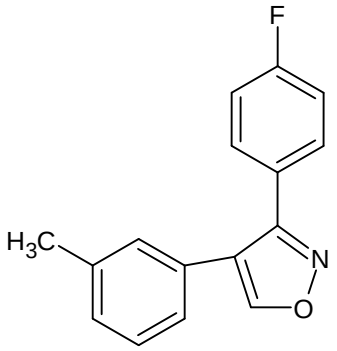
Parameters- Color- Greyish, Melting point- 256-258°C, R_f value- 0.57, percentage yield- 68.46 %, Molecular formula- $C_{16}H_{12}BrNO$.

Characterization-FT-IR: 1715.39 cm-1 (Ar-H, stretching), 1456.11 cm-^{1}(C=C, ring stretching), 1599.10 cm-^{1}(C=N, ring stretching), 1252.06 cm-^{1}(CO, ring stretching), 1408.37cm-^{1}(CH_3, stretching).

^{1}HNMR: (DMSO d6 - 500 MHz), δ 6.92-7.77 (m, 8H, Ar-H), 3.83 (s, 3H, -CH_3), 8.96(s,1H, -CH)

MS(m/z): 313.01. M^+

COMPOUND-3. 3-(4-FLUOROPHENYL)-4-(3-METHYLPHENYL)-1,2-ISOXAZOLE (GS3)



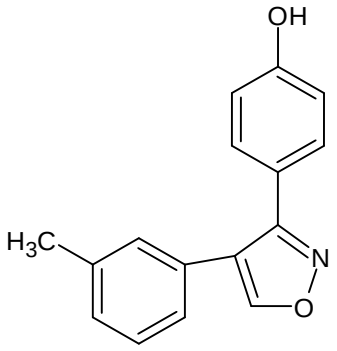
Parameters- Color- Light Reddish, Melting point- 211-213°C, R_f value- 0.62, percentage yield- 70.52 %, Molecular formula- $C_{16}H_{12}FNO$.

Characterization-FT-IR: 1701.79 cm-1 (Ar-H, stretching), 1509.02 cm-^{1}(C=C, ring stretching), 1604.82 cm-^{1}(C=N, ring stretching), 1259.57 cm-^{1}(CO, ring stretching), 1442.74 cm-^{1}(CH_3, stretching).

^{1}HNMR: (DMSO d6 - 500 MHz), δ 6.92-7.61 (m, 8H, Ar-H), 3.83 (s, 3H, -CH_3), 8.97(s,1H, -CH)

MS(m/z): 253.08. M^+

COMPOUND-4.3-(4-HYDROXYPHENYL)-4-(3-METHYLPHENYL)-1,2-ISOXAZOLE (GS4)



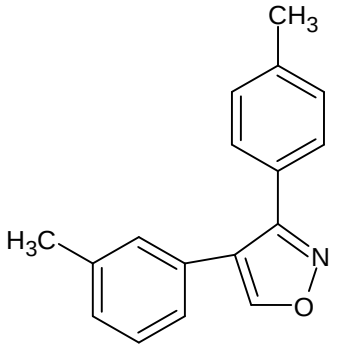
Parameters- Color- Creamish, Melting point- 189-191°C, R_f value- 0.54, percentage yield- 58.33 %, Molecular formula- $C_{16}H_{13}NO_2$.

Characterization-FT-IR: 1635.83 cm-1 (Ar-H, stretching), 1452.23 cm-^{1}(C=C, ring stretching), 1598.35 cm-^{1}(C=N, ring stretching), 1259.95 cm-^{1}(CO, ring stretching), 1452.23 cm-^{1}(CH_3, stretching), 3238.56 cm-^{1}(Ar-OH, stretching in ring)

^{1}HNMR: (DMSO d6 - 500 MHz), δ 6.73-7.42 (m, 8H, Ar-H), 3.83 (s, 3H, -CH_3), 8.97(s,1H, -CH)

MS(m/z): 251.09. M^+

COMPOUND-5. 3-(4-METHYLPHENYL)-4-(3-METHYLPHENYL)-1,2-ISOXAZOLE (GS5)



Parameters- Color- Blackish, Melting point- 199-201°C, R_f value- 0.60, percentage yield- 72.50 %, Molecular formula- $C_{17}H_{15}NO$.

Characterization-FT-IR: 1687.52cm-^{1}(Ar-H, stretching), 1500.27 cm-^{1}(C=C, ring stretching), 1618.44 cm-^{1}(C=N, ring), 1259.95 cm-^{1}(CO, ring), 1452.23 cm-^{1}(CH_3, stretching).

^{1}HNMR: (DMSO d6 - 500 MHz), δ 6.92-7.54 (m, 8H, Ar-H), 3.79-3.83 (d, 6H, -CH_3), 8.97(s,1H, -CH)

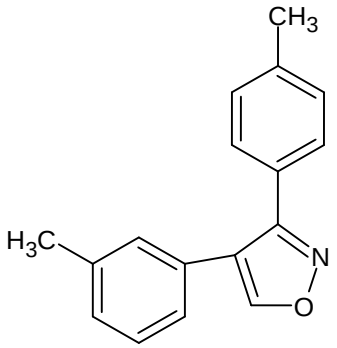
MS(m/z): 249.11. M^+

Characterization- FT-IR: 1635.83 cm-1 (Ar-H, stretching), 1452.23 cm-1 (C=C, stretching in ring), 1598.35 cm-1 (C=N, stretching in ring), 1259.95 cm-1 (CO, stretching in ring), 1452.23 cm-1 (CH_3, stretching), 3238.56 cm-1 (Ar-OH, stretching in ring);

^{1}HNMR: (DMSO d6 - 500 MHz), δ 6.73-7.42 (m, 8H, Ar-H), 3.83 (s, 3H, -CH_3), 8.97(s,1H, -CH)

MS(m/z): 251.09. M^+

COMPOUND-5. 3-(4-METHYLPHENYL)-4-(3-METHYLPHENYL)-1,2-ISOXAZOLE (GS5)



Parameters- Color- Blackish, Melting point- 198-200°C, R_f value- 0.60, percentage yield- 72.50 %, Molecular formula- $C_{17}H_{15}NO$.

Characterization- FT-IR: 1687.52 cm-1 (Ar-H, stretching), 1500.27 cm-1 (C=C, stretching in ring), 1618.44 cm-1 (C=N, stretching in ring), 1259.95 cm-1 (CO, stretching in ring), 1452.23 cm-1 (CH_3, stretching).

^{1}HNMR: (DMSO d6 - 500 MHz), δ 6.92-7.54 (m, 8H, Ar-H), 3.79-3.83 (d, 6H, -CH_3), 8.97(s,1H, -CH)

MS(m/z): 249.11. M^+

4.2. PHARMACOLOGICAL EVALUATION

4.2.1. Antibacterial activity

All the new synthesized isoxazole derivative compounds (GS1-GS5) were evaluated to antibacterial activity with the help of Cup Plate method.

Table no. 2. Antibacterial activity of compounds

COMPOUNDS CODE	Inhibited Zone (mm)		
	E. coli (MTCC-521) concentration in µg/ml		
	10	25	50
GS1	2	3	3
GS2	1	2	2
GS3	1	2	3
GS4	2	3	4
GS5	3	3	4
Ampicillin	2	4	5
Control	-	-	-

Fig.4. Antibacterial activity zone of inhibition

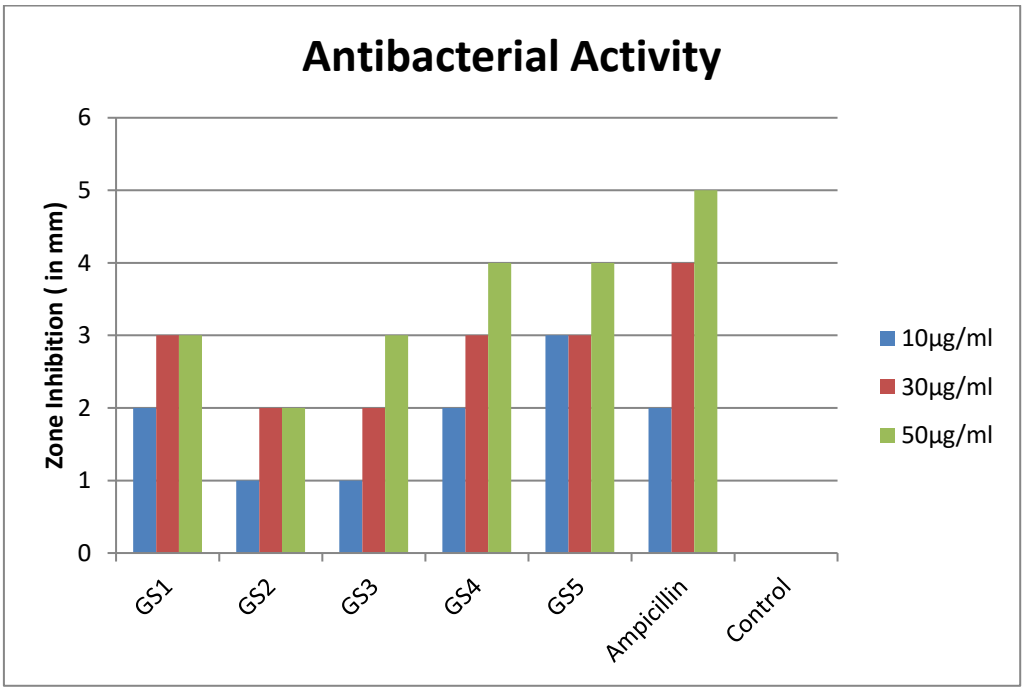


Fig.5. Antibacterial activity

4.2.2. ANTIOXIDANT ACTIVITY OF SYNTHESIZED COMPOUNDS (GS1-GS5)

All the synthesized isoxazole derivative compound (GS1-GS5) was evaluated for their antioxidant activity by hydrogen-proxied scavenging method.

4.2.2.1. HYDROGEN PEROXIDE SCAVENGING ASSAYS

Absorbance of synthesized compounds at various concentrations is observed through UV spectroscopy for Hydrogen peroxide scavenging assays.

Table: 3. Absorbance of compound at various concentrations in Hydrogen peroxide scavenging model

Sample No.	Concentration µg/mL	Absorbance at 230nm Mean ±SD					
		GS1	GS2	GS3	GS4	GS5	Ascorbic acid
1	25	0.275±0.002	0.288±0.009	0.298±0.019	0.264±0.004	0.247±0.003	0.263±0.002
2	50	0.253±0.012	0.266±0.014	0.244±0.017	0.168±0.008	0.136±0.005	0.151±0.011
3	100	0.221±0.006	0.226±0.011	0.209±0.013	0.135±0.006	0.108±0.004	0.125±0.017
4	250	0.204±0.004	0.208±0.010	0.195±0.006	0.112±0.012	0.086±0.010	0.092±0.002
Control	00	0.325±0.002	0.322±0.003	0.327±0.001	0.324±0.003	0.320±0.004	0.330±0.005

*Data expressed the means of three replicates Percentage inhibition of the synthesized compounds and ascorbic acid standard in hydrogen peroxide scavenging model and IC_{50} value.

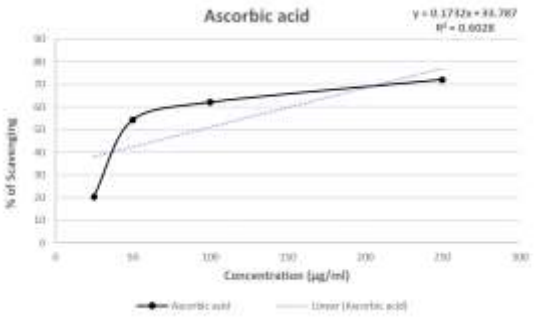


Figure 21. Scatter graph of Ascorbic acid

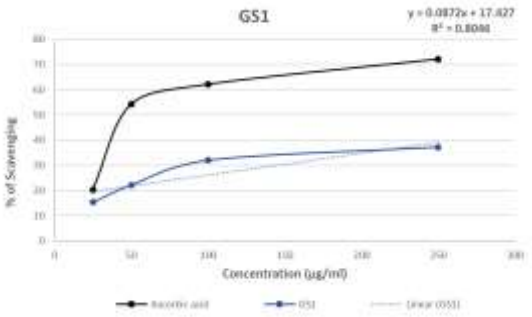


Figure 22. Scatter graph of GS1

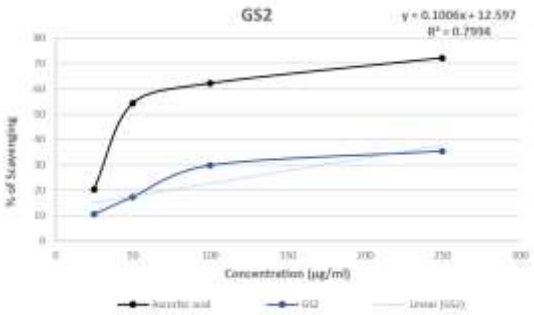


Figure 23. Scatter graph of GS2

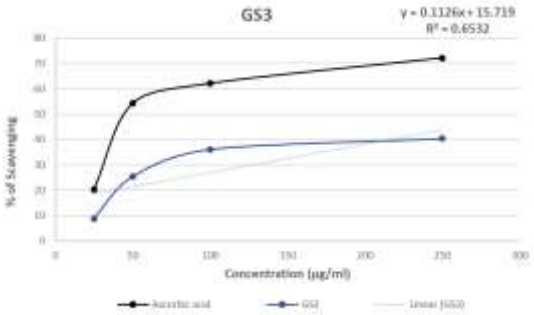


Figure 24. Scatter graph of GS3

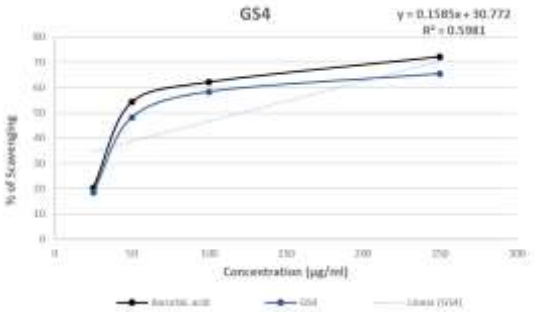


Figure 25. Scatter graph of GS4

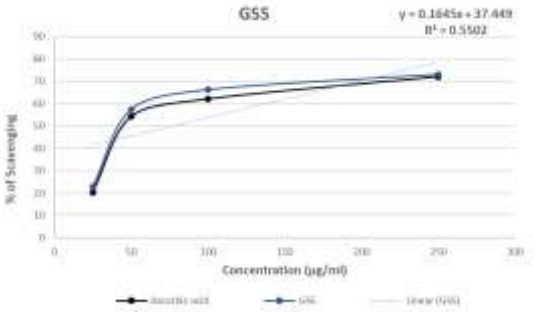


Figure 26. Scatter graph of GS5

To determine the IC50 value, the scatter graph of synthetic isoxazole derivative compounds (SG1-SG5) and ascorbic acid were utilized. The results are shown in table 4.

Table: 4. In-vitro antioxidant activity (IC_{50} value) of synthesized compounds (GS1-SG5) and standard in hydrogen-proxied scavenging method

S.no.	Compound	% SCV of different Concentration (µg/ml)					
		25	50	100	250	Blank	IC50
1	GS 1	15.38	22.15	32.00	37.23	00	373.544±1.20
2	GS 2	10.56	17.39	29.81	35.40	00	371.799±0.96
3	GS 3	8.87	25.38	36.09	40.37	00	304.449±0.77
4	GS 4	18.52	48.15	58.33	65.43	00	121.312±1.40
5	GS 5	22.81	57.50	66.25	73.13	00	76.298±0.75
6	Ascorbic acid	20.30	54.24	62.12	72.12	00	93.608±0.34

* Scavenging activity values were means of three replicates

Compounds GS1, GS2, GS3, GS4 and GS5 having IC50 values of 373.5, 371.8, 304.5, 121.3 and 76.3µg/mL respectively, compared to Ascorbic acid 93.6µg/mL.

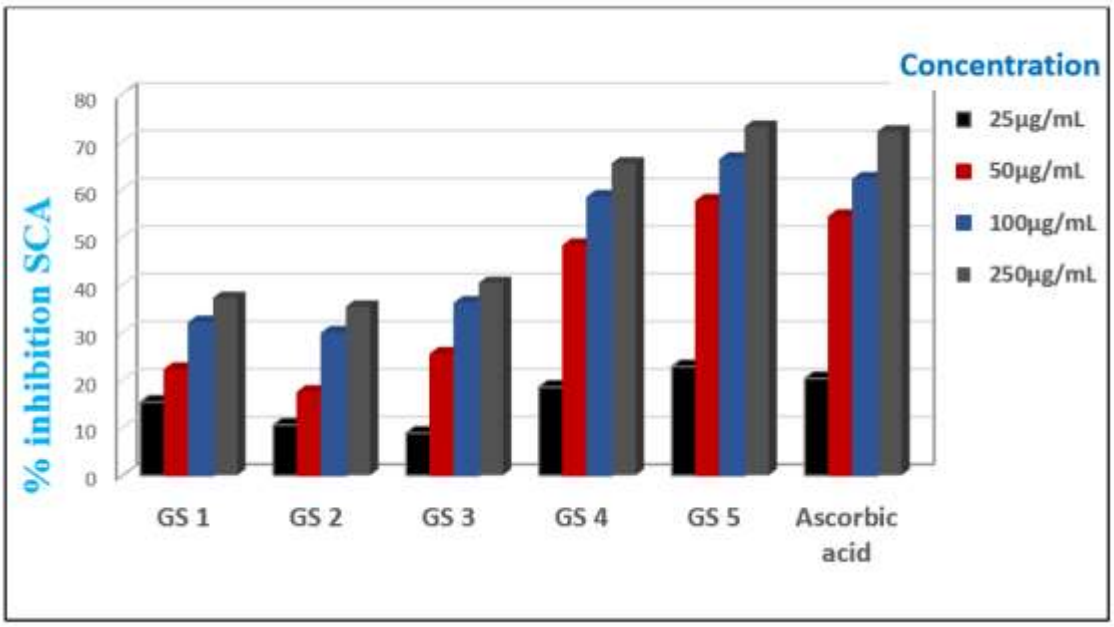


Figure. Antioxidant activity % comparison of synthesis isoxazole derivative compoundswith Ascorbic acid.

SUMMARY

A series of novel Isoxazole derivatives were synthesized by 3-Methylacetophenone with substituted benzaldehyde derivatives. All compound recrystallized with the help of ethanol and in-vitro antioxidant activity was performed by hydrogen peroxide scavenging activity assay. spectral data presented in tables and figures. Compound 3-(4-methylphenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS5) have highest antioxidant potency presented IC_{50} value 76.29µg/ml for hydrogen peroxide scavenging method. Compound 3-(4-methylphenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS5) have highest antibacterial activity against ***Escherichia coli.*** A number of novel isoxazole compounds were produced by mixing 3-methyl laceto phenome with substituted benzaldehyde derivatives. Tables and graphs were used to present the spectrum data. A hydrogen peroxide scavenging activity assay was used to determine the in-vitro antioxidant activity of each chemical after it was recrystallized using ethanol. Compound 3-(4-methylphenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS5), which has an IC50 value of 76.29 g/ml, has the highest antioxidant performance for the hydrogen peroxide scavenging method. Compound 3-(4-methylphenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS5) has the strongest antibacterial effects against Escherichia coli**.**

CONCLUSION

A series of novel Isoxazole derivatives were synthesized by 3-Methylacetophenone with substituted benzaldehyde derivatives. All compound recrystallized with the help of ethanol and in-vitro antioxidant activity was performed by hydrogen peroxide scavenging activity assay. Compound was characterized by FT-IR, 1H-NMR and MASS spectroscopy and spectral data presented in tables and figures. Compound 3-(4-methylphenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS5) have highest antibacterial activity and compound have lowest 3-(4-Bromophenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS2) against ***Escherichia coli***. Compound 3-(4-methylphenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS5) have highest antioxidant potency presented IC50 value 76.29µg/ml and compound3-(4-chlorophenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS1) have lowest antioxidant activity presented IC_{50} value 373.544µg/ml for hydrogen peroxide scavenging method. If the compounds further explored, they can be more potent for multi-target or disease. Every compound was recrystallized with the aid of ethanol, and hydrogen peroxide's antioxidant activity was tested. The compound was described using FT-IR, 1H-NMR, and MASS spectroscopy, and spectrum information was supplied in tables and figures. Compound 3-(4-methylphenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS5) has the maximum antibacterial activity against Escherichia coli, whereas compound 3-(4-Bromophenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS2) has the lowest antibacterial activity. The compound 3-(4-methylphenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS5) demonstrated the highest antioxidant potency, and the compound 3-(4-chlorophenyl)-4-(3-methylphenyl)-1,2-isoxazole (GS1) demonstrated the lowest antioxidant activity, with an IC50 value for hydrogen peroxide scavenging of 373.544 g/ml. If the chemicals are studied more, they might be more effective against a variety of targets or diseases.

CONFLICT OF INTEREST

The author has declared that no conflicts of interest
exist.

ACKNOWLEDGEMENTS

I would like to thanks to Dr. Vaishali (Guide), Rabiya (Co-guide), Dr. Arvind Kumar (Director) and my parents.

Funding

None.

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