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SYNTHESIS AND CHARACTERIZATION OF TRIAZOLE COMPOUNDS DERIVED FROM OXADIAZOLE MOIETY FOR ITS ANTIMICROBIAL AND ANTI-OXIDANT EVALUATION

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ABSTRACT

The broad spectrum and excellent property of triazole compounds have led to the various synthetic researches in the derivation of these compounds. Also because they have an immense biological activity, therefore they promise to possess significant pharmacological activity. A new technique for the synthesis of new triazole derivatives from a heterocyclic nucleus oxadiazole has been developed by binding two types of amino acids in the oxadiazole molecule. The name of the final compounds as triazole compounds are 4-(1`indoline propionic acid)-3(3-pyridyl)-5-thione-1, 2, 4-triazole and4-(2`-methyl pentanoic acid)-3(3-pyridyl)-5-thione-1, 2, 4-triazole. The reaction and purity of the compounds is checked by TLC and melting point. The characterization of the compounds is performed by 1D-NMR and IR. The antibacterial activity is done on two strains of bacteria gram negative and gram positive and the antifungal activity has been performed by the strains of A.niger. The antioxidant activity has been done by *in-vitro* DPPH method.

Keywords: Triazole, oxadiazole, DPPH, antibacterial, NMR, IR .

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INTRODUCTION

Triazole and Oxadiazole

There has been a major focus in the chemical nature of five-member heterocyclic compounds and their substituted derivatives like triazole, tetrazole [1]. The ring is also known as azole ring and it readily interacts with various enzymes and receptors thus possessing various biological activity [2]. Triazole contains two carbon atoms and three nitrogen atoms. The triazole compounds are considered to have antibacterial [3], anti-fungal [4], antioxidant [5], anti-leishmanial and antimalarial activity [6].

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1, 3, 4 oxadiazole is a heterocyclic compound which is derived from furan by substituting two methylene group (=CH) with two pyridine type nitrogen (-N=). The two of its isomers 1, 3, 4-oxadiazole and 1, 2, 4-oxadiazole possess useful chemical and biological properties. Because of the tendency of 1, 3, 4-oxadiazole compounds to undergo various chemical reaction, it is very useful for planning of molecule due to its highly privileged structure to have enormous biological potential [7].

BIOLOGICAL ACTIVITY

Antibacterial Activity

The anti-bacterial agents are designed to inhibit or kill the infecting organism and to have minimal effect on the recipient. This type of treatment is called chemotherapy which is the treatment of systemic infections with the specific drugs that selectively suppress the infecting micro-organism with affecting the host. It

involves the action of drug on a component of the microbe or metabolic processes.

Antibiotics are the substances produced by micro-organism which selectively suppresses the growth or kill the other micro-organism at very low concentration [8]. Tubidometric method, agar streak dilution method, serial dilution, agar diffusion method are some of the evaluation techniques [9].

ANTI-OXIDANT

Anti-oxidant may be defined as the substance which, delays or inhibit oxidation of the substrate extensively when it is present in low concentration with the addition of oxdizable substrate. Anti-oxidant substances can be divided into two classes: primary or chain breaking anti-oxidant and secondary or preventative antioxidant. The aim of an anti-oxidant can be achieved in a number of ways that consists of removal of substrate or singlet oxygen that quenches primary antioxidant that can lead to either delay or inhibit the initiation step of a reaction with a lipid radical or by inhibition of the propagation step on reacting with the peroxyl or alkoxyl radicals, when present in trace amount [10].

MATERIAL AND METHOD

The synthesis was performed by the following procedures listed and the chemical reaction involved with mechanism was shown in scheme. The reaction involves four steps in which the three steps result in the formation of intermediates and the final steps results in the formation of the different compounds. The completion of the reaction was monitored by TLC using suitable solvents and melting point determination.

SYNTHESIS OF THE THREE INTERMEDIATE.

Synthesis of ethyl pyridine 3-caboxylate was performed using nicotinic acid as starting material a white solid compound is obtained which was then refluxed with hydrazine hydrate to produce pyridine-3-carbohydrazide, in third step 5-(3-pyridinyl)-1,3,4-oxadiazole-2(3H)-thione was prepared from above synthesized compound by the addition of carbon disulphide in presence of KOH. The completion for the synthesis of all the intermediate was determined by TLC and melting point. The chemical reaction shows the synthesis of the intermediates.

General procedure for the synthesis of triazole derivatives (A1 and A2)

A mixture of 5-(3-pyridinyl)-1,3,4-oxadiazole-2(3H)-thione(0.02 mol) and tryptophan (0.02 mol) is placed in an Erlenmeyer flask respectively fitted with a loose top cap and heated in a commercial microwave oven for 3 mins operating at 2450 MHz by setting the power range to medium high(70% of total power). The reaction mixture turned into compound after cooling the reaction mixture is extracted with ethanol (20 ml) recrystallized and solvent removed. The compound is checked by TLC in which solvent system is chloroform and methanol (7:3). The same reaction is being performed by substituting iso-leucine (0.02 mol) in place of tryptophan.

ANTIMICROBIAL ACTIVITY

The bacterial static properties of the compounds were evaluated by disc diffusion method. Compounds were taken at concentration of 0.1 mg/ml for testing antibacterial activity. Standard strains used for screening of antibacterial activities were E.Coli (gram negative) and S.aureus (gram positive). All the micro-organism was spot inoculated on the drug plates, containing different concentration of compounds. Then the plates were incubated at 37^0C for 24 hrs, after 24 hrs the presence or absence of bacterial growth in different plates was observed. The minimum inhibitory concentration (MIC) of the compounds was determined against the bacterial strains as per NCCLS guidelines (National Committee for Laboratory Standards). For screening of anti-fungal activity screening is done with taking the different concentration of different derivatives. Compounds of different concentration were prepared and mixed with different sterile Sabourand dextrose agar media and drug plates were prepared. For the preliminary screening, I species of gram positive fungi was taken. All the microorganisms were spot inoculated in drug plates containing different concentration of compounds. Then the plates were incubated at 22^oC for 24 hrs and incubated for further 7 days for fungi. After 24 hrs of incubation, the presence or absence of fungi growth in different plates were observed.

ANTI-OXIDANT ACTIVITY

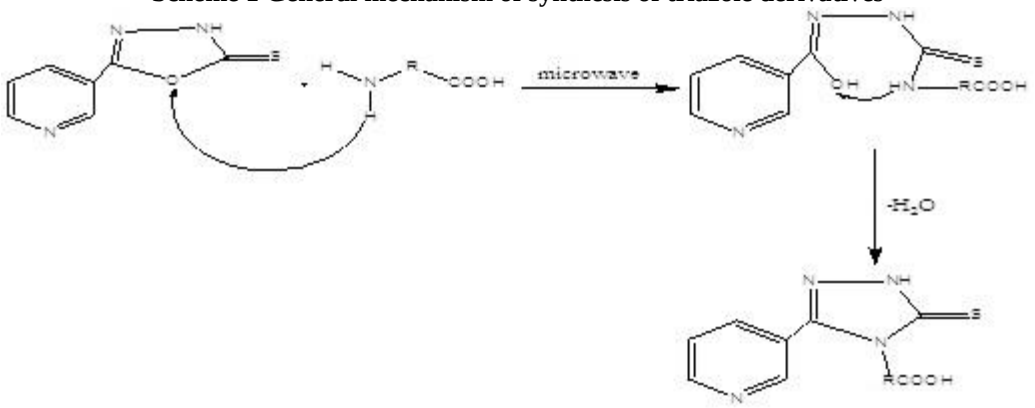
In- Vitro methods were employed in anti-oxidant studies. 4.8 mg of DPPH (1, 1-Diphenyl-2-picrylhydrazyl) was dissolved in 20 ml methanol, it was protected from light by covering the test tubes with aluminum foil. 0.1 ml of DPPH solution was added to 3 ml of methanol and absorbance was taken immediately at 517 nm for control reading. 0.1-0.5 ml of various concentration of unusual amino acids as well as of standard compound (ascorbic acid) was taken and volume was made uniformly using methanol. Each of the samples was further diluted with methanol up to 3 ml and to each 0.1 ml of DPPH was added. Absorbance was taken after 15 mins at 517 nm using methanol as blank on UV-visible spectrometer. IC50 for each compound as well as standard preparation were calculated (Ahmed D et al 2015). The DPPH free radical scavenging activity was calculated using formula:

% scavenging= [absorbance of control – absorbance of test sample/ absorbance of control] X 100.

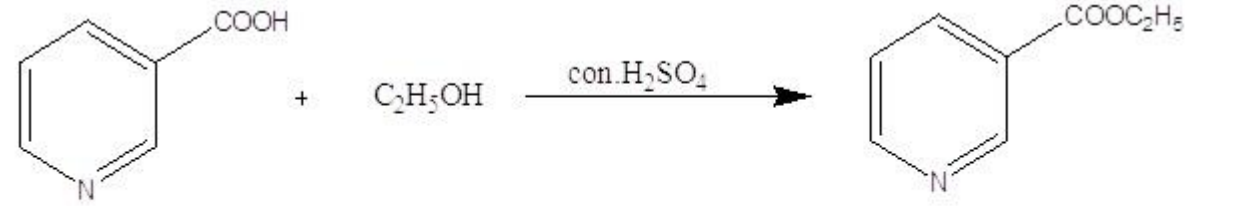
RESULT AND DISCUSSION

We developed new methodologies for the synthesis of new triazole derivatives from oxadiazole molecule. The starting material nicotinic acid was reacted with ethanol and undergoes esterification reaction which further undergoes hydrazinolysis and then reacted with carbon disulphide and lastly binds with the amino acid under microwave to yield the new derivatives. The confirmation of the intermediate reaction was checked by TLC and melting point also characterized by NMR.

Scheme 1 General mechanism of synthesis of triazole derivatives

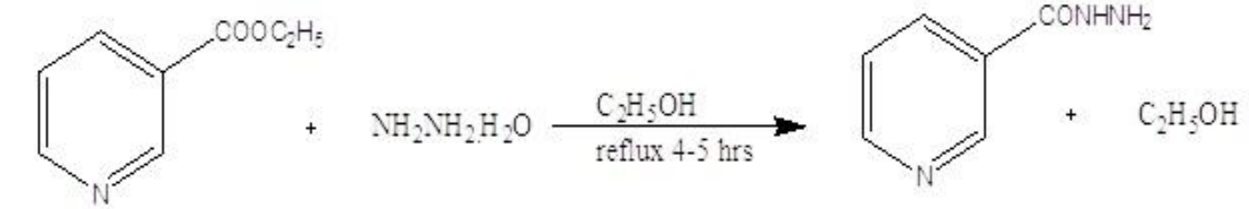


Scheme 2. synthesis of ethyl nicotinate (STEP I)



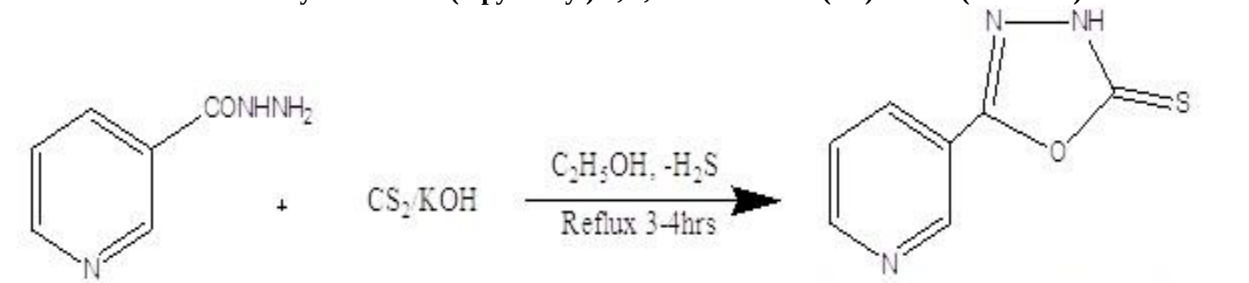
nicotinic acid (0.09 mol) | ethanol 25 ml | ethyl pyridine 3-carboxylate

Scheme 3. synthesis of pyridine-3-carbohydrazide (STEP II)



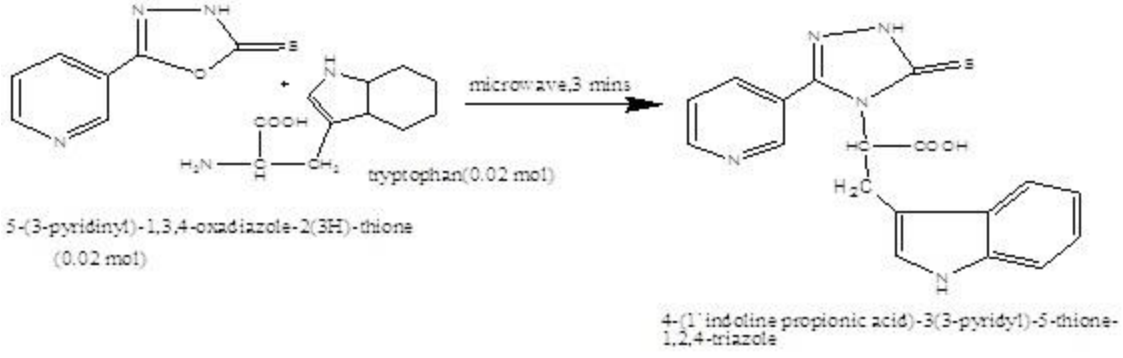
Ethyl pyridine3-caboxylate (0.03 mol) | hydrazine hydrate (0.06 mol) | pyridine-3-carbohydrazide

Scheme 4. synthesis of 5-(3-pyridinyl)-1, 3, 4-oxadiazole-2(3H)-thione (STEP III)



pyridine-3-carbohydrazide | carbon disulphide | 5-(3-pyridinyl)-1,3,4-oxadiazole-2(3H)-thione

Scheme 5. synthesis of compound A1



Scheme 6. synthesis of compound A2

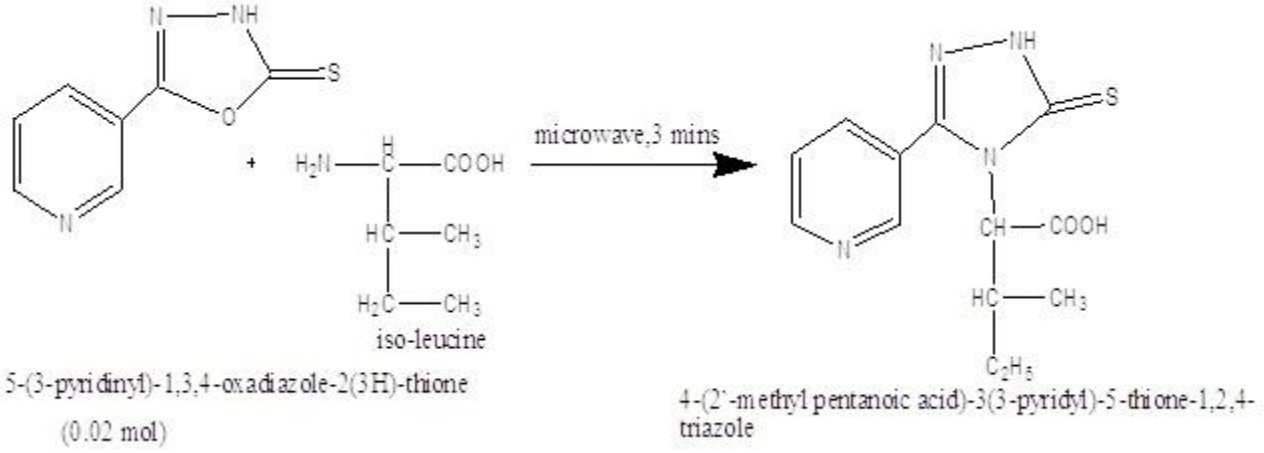
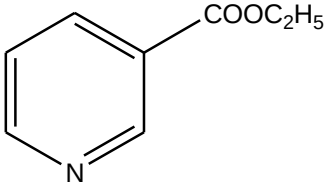


Table 1. Physical Properties of the Synthesized Compounds

COMPOUND	MELTING POINT (^{0}C)	APPEARNCE	YIELD (%)	MOLECULAR FORMULA
Ethyl pyridine-3-carboxylate**[I]**	9-11	White crystals	68.6%	$C_8H_9NO_2$
Pyridine-3-carbohydrazide**[II]**	110-115	Off white powder	95%	$C_6H_7N_3O$
5-(pyridyl)-1,3,4-oxadiazole-2(3H)-thione**[III]**	175-180.0	Light green powder	80%	$C_6H_6N_2O_2S$
4-(1`indoline propionic acid)-3(3-pyridyl)-5-thione-1,2,4-triazole**[A1]**	250-260	Brown powder	75%	$C_{18}H_{18}N_5O_2S$
4-(2`-methyl pentanoic acid)-3(3-pyridyl)-5-thione-1,2,4-triazole**[A2]**	210-215.0	Yellow powder	94.1%	$C_{13}H_{17}N_4O_2S$

Table 2. IR Data of the Synthesized Compounds

COMPOUND NO.	STRUCTURE	TYPE OF VIBRATION	REGION (cm^{-1})
I		CH(aromatic in pyr) C=C and C=N(ring in pyr) C-H(out of plane in pyr) C=O C-H in CH_3 C-H in CH_2	$3055cm^{-1}$ $1474cm^{-1}$ $742cm^{-1}$ $1591cm^{-1}$ $2874cm^{-1}$ $1420cm^{-1}$

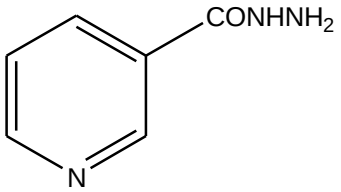
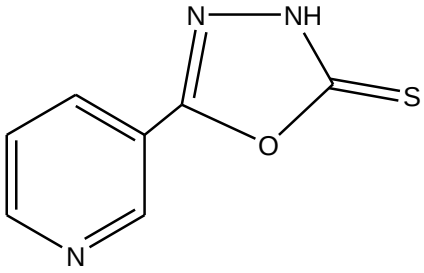
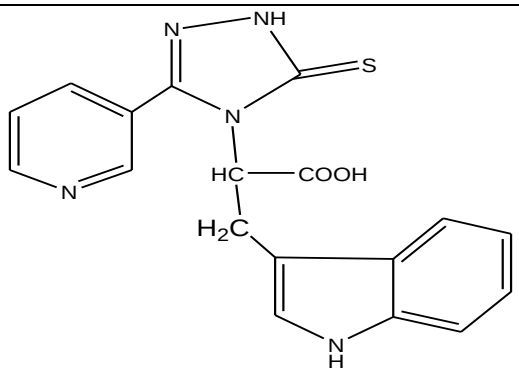
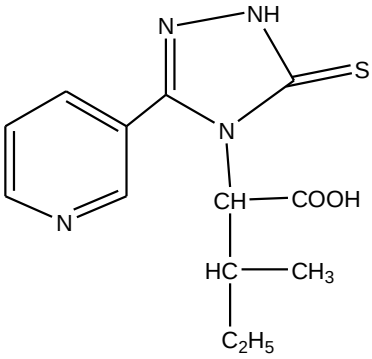
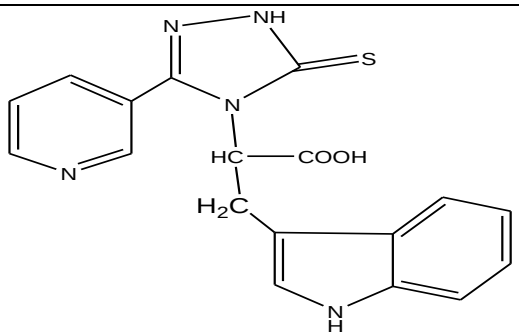
II		CH(aromatic in pyr) C=N(ring in pyr) CO NH	3354cm^{-1} 1480cm^{-1} 1636cm^{-1} 2937cm^{-1}
III		CH(aromatic in pyr) C=C and C=N(ring in pyr) C-H(out of plane in pyr) C-C N-H N-N C=N C-O C=S	3309 cm^{-1} 1551 cm^{-1} 706 cm^{-1} 1298 cm^{-1} 3383 cm^{-1} 3368 cm^{-1} 1648 cm^{-1} 1592 cm^{-1} 762 cm^{-1}
A1		CH(aromatic in pyr) C=C and C=N(ring in pyr) C-H(out of plane in pyr) O-H &C=O(COOH) N-H C-H C=S C-N C=N(indole) C-C (CH_2COOH) N-N	3048 cm^{-1} 1458cm^{-1} 743cm^{-1} 2527cm^{-1} 1559cm^{-1} 3402 cm^{-1} 2966cm^{-1} 765cm^{-1} 847cm^{-1} 1659cm^{-1} 1112cm^{-1} 1585cm^{-1}
A2		CH(aromatic in pyr) C=C and C=N(ring in pyr) C-H(out of plane in pyr) O-H &C=O(COOH) N-H C-H in CH_3 C-H in CH_2 C=S C-N C-C in ($CHCOOH$),(C_2H_5) N-N	2962 cm^{-1} 1562 cm^{-1} 706 cm^{-1} 2538 cm^{-1} 1503cm^{-1} 3372cm^{-1} 2877cm^{-1} 1417cm^{-1} 762cm^{-1} 1596cm^{-1} 1134cm^{-1} 1108cm^{-1} 1596cm^{-1}

Table 3. ^{1}H NMR data and interpretation of synthesized compounds

Compound No.	STRUCTURE	δ ppm	Group	Number of H
A1		7.03 11.13 2.51-3.92 6.93-7.93 7.61-9.02	-NH -COOH CH,CH_2 Indole Pyr	1 1 3 5 4

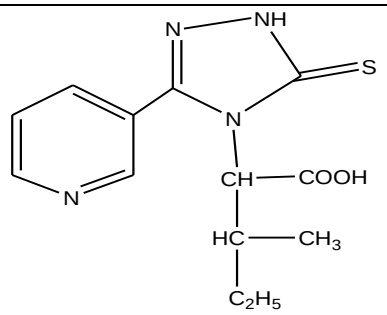
A2		2.54-3.99 0.82-1.94 7.30 7.85-9.00	-CHCOOH C_4H_9 -NH Pyr	2 9 1 4

Table 4. Zone of inhibition after 24 hrs in concentration µg/ml (mm)

Compound No.	Gram +ve Bacteria	Gram –ve Bacteria	Standard (ciprofloxacin)
	S.aureus	E.Coli	
A_1	18	14	
A_2	26	17	32

Table 5. Screening results of the newly synthesized Compounds as antifungal ActivityZone of inhibition after 24 hrs in concentration µg/ml (mm)

Compound No.	Fungi	Standard (ciprofloxacin)
	S.aureus	
A_1	24	
A_2	20	30

Table 6. Absorbance of drug and standard with DPPH

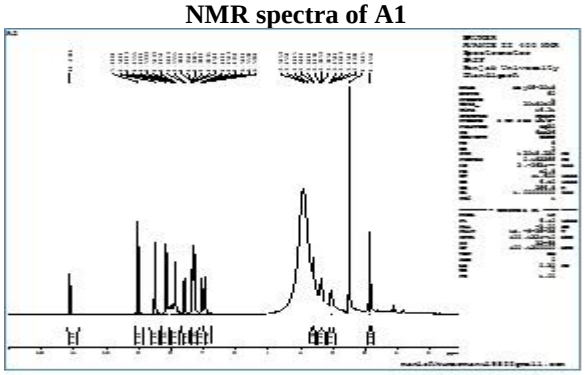
Concentration (mg/ml)	**A_1**	**A_2**	**Ascorbic acid**
0.02	0.493	0.315	0.302
0.04	0.456	0.273	0.134
0.06	0.281	0.199	0.070
0.08	0.123	0.142	0.033
0.10	0.110	0.093	0.011

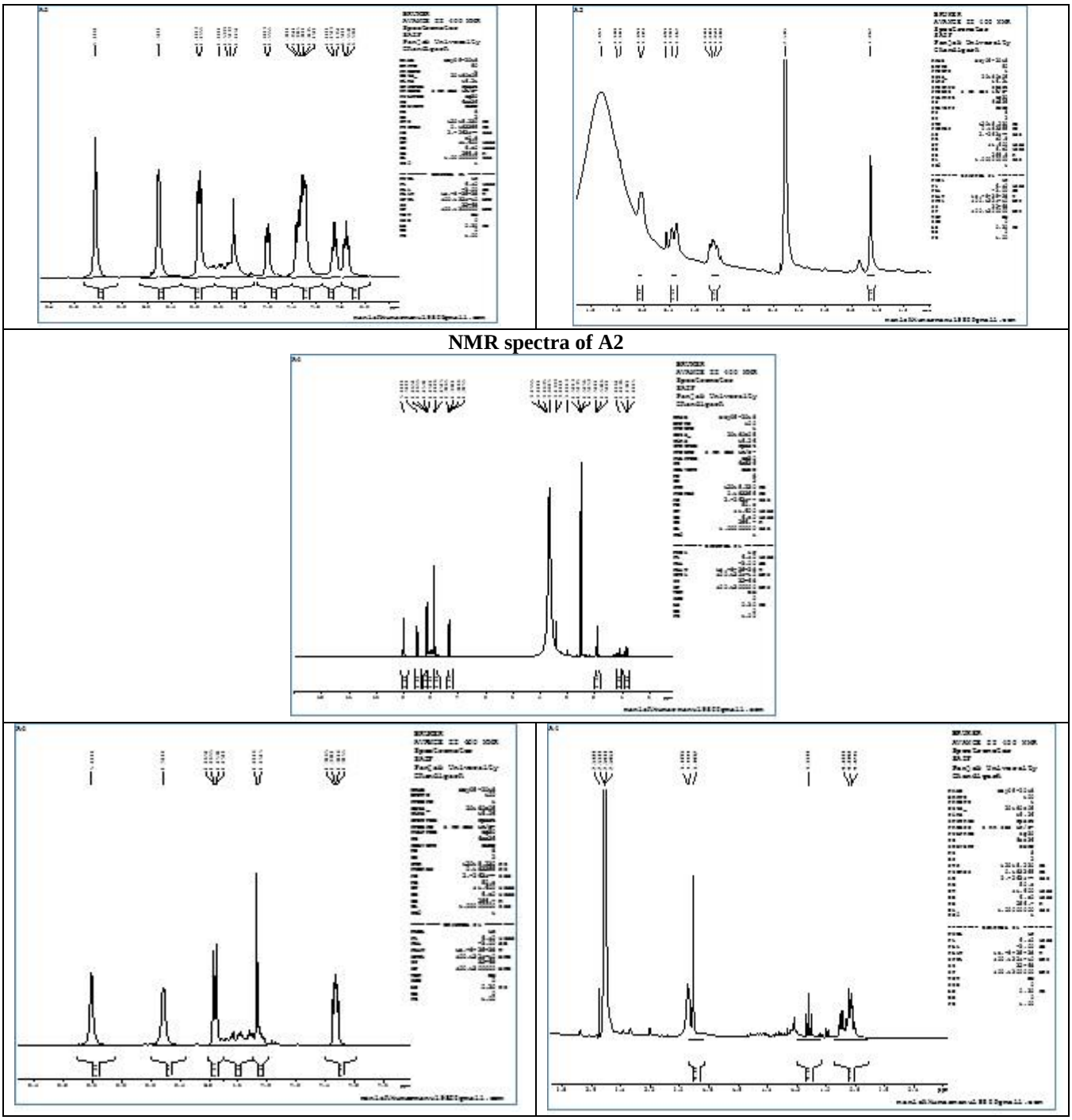
Absorbance of control = 0.521

Table 7. *In vitro* free DPPH free radical inhibition activity (% inhibition) of different derivatives

Concentration (mg/ml)	**A_1**	**A_2**	**Ascorbic acid**
0.02	5.4	39.6	42.1
0.04	12.4	47.7	74.8
0.06	46.1	61.9	86.6
0.08	76.4	72.7	93.6
0.10	78.9	84	97.9

NMR spectra of A1





NMR spectra of A2

Figure 1. Zone of inhibition in S.aureus

Figure 2. Zone of inhibition in gram E.coli.

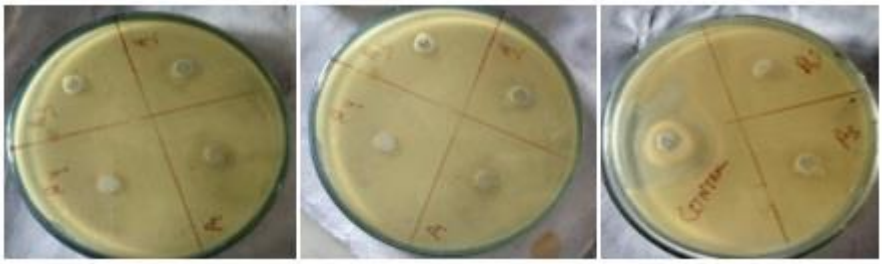


Figure 4. zone of inhibition in Aspergillusniger.

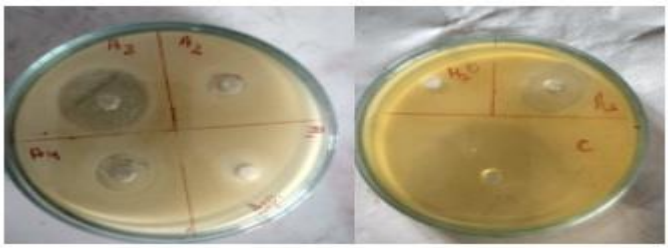


Figure 3. Bar diagram of zone of inhibition of compounds with standard

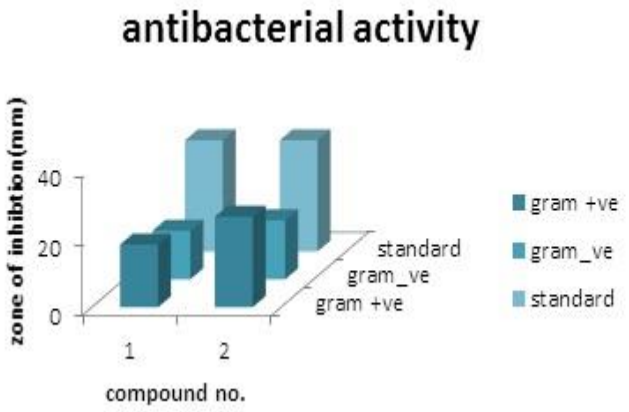


Figure 5. bar diagram of zone of inhibition of compounds with standard

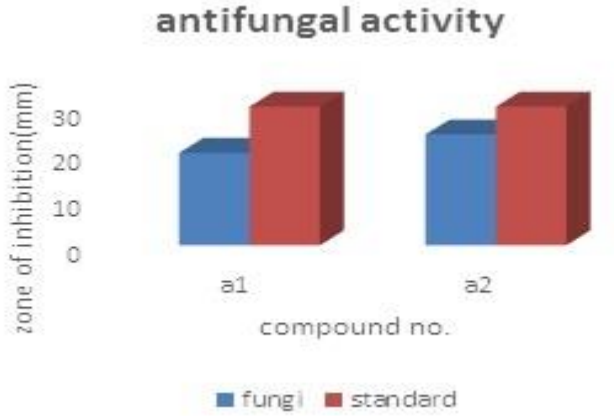


Figure 6. DPPH free radical % inhibition Vs concentration

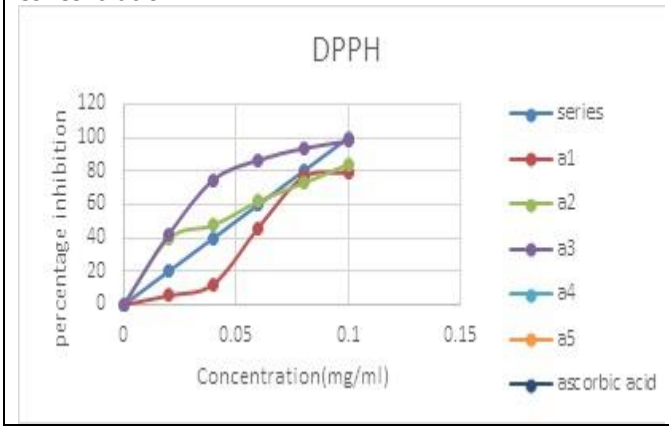
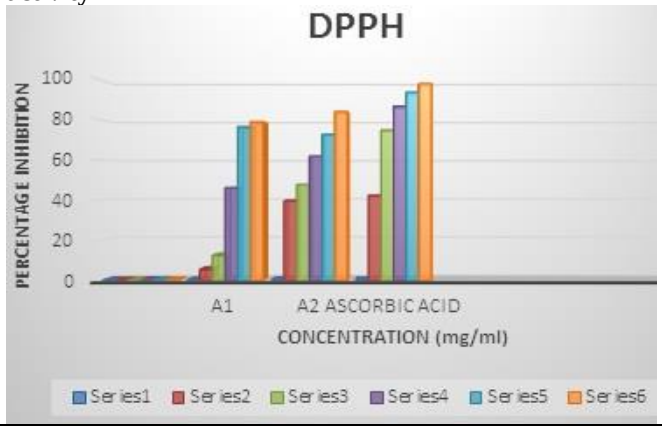


Figure 7. Bar diagram of DPPH radical scavenging activity



CONCLUSION

Both the compounds have been screened for both antibacterial and anti-fungal activities using agar disc diffusion method by measuring the zone of inhibition in mm. Ciprofloxacin (µg/ml) was used as standard drug for anti-bacterial activity and itraconazole as a standard drug

for antifungal activity. The compounds were screened for anti-bacterial acitivity against *E.coli* and *S.aureus* in nutrient agar media and for antifungal activity against A.niger in sabouarud`dextrose agar medium.

These sterilized agar media were spread with the help of sterilized loop. The disc of 5mm was used. Both the compounds were dipped in disc for diffusion for 1 hr. Water was used as a solvent for all compound and as control. These plates were incubated at 37^0C for 24 hr and 28^0C for 48 hr for antibacterial and antifungal activities respectively. The zones of inhibition were observed around the disc after the incubation and were measured. Compounds showed minimum inhibitory concentration against *E.coli*, *S.aureus* and *A.niger* when compared against standard. Compound A2 the maximum showed maximum MIC against *S.aureus*. These data support our research that a heterocyclic compound as triazole can have a biological activity by observing the result of synthesized derivatives. Both compounds have been screened for anti-bacterial, anti-fungal and anti-oxidant activities. The free scavenging activities of compounds were done by DPPH radical scavenging method. Figures show the DPPH radical scavenging activity with respect to ascorbic acid, used as a reference compound in the study. For DPPH radical scavenging activity, the compounds were prepared at the concentration ranges from 0.02-0.10 mg/ml. it was observed from the study that as the concentration of the compound increases the percent inhibition of the compounds also increases. The DPPH radical scavenging of the compounds was slightly lower than the ascorbic acid. The compound A2 showed maximum percentage inhibition against DPPH.

AKNOWLEDGEMENT

Nil

CONFLICT OF INTEREST

Nil

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