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SYNTHESIS AND EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF THIOSEMICARBAZONE DERIVATIVES

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ABSTRACT

Background: The threat of antimicrobial resistance has never been more acute, and the need for answers has never been greater. This work aimed to synthesize and evaluate the antimicrobial activity and antioxidant activity of novel thiosemicarbazones. **Method:** Seven novel thiosemicarbazone derivatives were synthesized using 4-phenylthiosemicarbazide as the main reactant and different aromatic aldehydes by a base-catalyzed nucleophilic addition reaction followed by a condensation reaction. Reactions were monitored using the thin-layer chromatography technique and the newly synthesized derivatives were characterized by UV-Visible, FTIR, and NMR spectroscopy. Prepared derivatives were screened for anti-microbial and anti-oxidant activity. **Results:** The proposed route for synthesis gave high to moderate yields of Thiosemicarbazone derivatives and showed promising antifungal, antibacterial, and antioxidant activity. **Conclusion:** These findings suggested that prepared compounds were showed promising antifungal and antibacterial activities and can be used as scaffolds to design new antimicrobial drugs.

KEYWORDS: Anti-fungal, anti-bacterial, anti-oxidant, thiosemicarbazones.

1. INTRODUCTION

1.1 Need for new antimicrobials

Antibiotic efficacy is being threatened by the fast rise of resistant microorganisms around the world. Antibiotics that are given incorrectly contribute to the occurrence of resistant microorganisms.[1] Subinhibitory and subtherapeutic antibiotic concentrations can promote the development of antibiotic resistance by supporting genetic alterations, such as changes in gene expression, HGT (Horizontal gene transfer), and mutagenesis. Changes in antibiotic-induced gene expression can increase virulence, while increased mutagenesis and HGT promote antibiotic resistance and spread.[2] Low levels of antibiotics have been shown to contribute to strain diversification in organisms such as *Pseudomonas aeruginosa*. Subinhibitory concentrations of piperacillin and/or tazobactam have also been shown to induce broad proteomic alterations in *Bacteroides fragilis.*[3]

Antibacterial products sold for hygienic or cleaning purposes may also contribute to this problem, since they may limit the development of immunities to environmental antigens in both children and adults. Consequently, immune-system versatility may be compromised, possibly increasing morbidity and mortality due to infections that wouldn't normally be virulent.[4]

So there have been recent efforts of the production of novel and hybrid antimicrobial compounds with the potential of being incorporated into the drug discovery process as drug candidates. As a part of ongoing efforts towards finding novel antimicrobial agents, it was thought worthwhile to synthesize derivatives of Thiosemicarbazone.

1.2 Thiosemicarbazone

Thiosemicarbazone is a potent intermediate for the synthesis of pharmaceutical and bioactive materials and thus, they are used extensively in the field of medicinal chemistry.[5] The complexes of nitrogen and sulfur atoms with metal ions may be considered a potential biological agent.[6] Numerous thiosemicarbazone derivatives are active for the control of some human pathogens and have received great attention because of their chemical and biological activities, such as antibacterial, anti-fungal, antiviral, antiamoebic, anti-inflammatory, antioxidant, and antitumor, antitubercular activities in human pharmacology.[7] Further, this class of compounds has been studied for its activity against tuberculosis, leprosy, psoriasis, rheumatism, trypanosomiasis, and coccidiosis however there is a waste scope of studies concerning their applications.[8]

2. MATERIALS AND METHODS

2.1 Material

The chemicals used during the synthesis and evaluation were purchased from Sigma Aldrich, Qualikem, Himedia Laboratories, Rankem, and Central Drug House Pvt. Ltd. and were used without further purification. Amoxicillin and Fluconazole were procured as a gift sample from Ramson Remedies, Amritsar. *Bacillus Subtilis, E.Coli, Candida Albicans, Aspergillus Nigerwere purchased from MTCC, Chandigarh.*

2.2.1 Method

2.2.1 Analytical methods

The melting point of prepared novel compounds was determined on Popular India Digital melting point apparatus.[9] UV spectra of synthesized compounds were measured by Ultra violet-visible (UV-vis) spectrophotometer of brand Labtronics, Model LT-2700.[10] All the synthesized compounds were characterized by the Furier Transform Infra Red spectroscopy (FTIR) of brand Thermo scientific, Model Nicolet iS5.[11] Plastic-backed pre-coated Thin layer chromatography (TLC) plates of silica gel 60 F_{254} (Merk) with 200 µm layer thickness were used for the TLC study. The mobile phase was selected according to the polarity of compounds.[12] ^{1}H Nuclear magnetic resonance spectra were obtained on Nuclear Meganetic Resonance spectroscopy of brand Brookfield using $CDCl_3$ and DMSO. The chemical shifts are reported in parts per million downfield from standard internal reference tetramethylsilane (TMS).[13]

2.2.2 Anti-microbial activity by Agar well diffusion method

The Agar well plate diffusion method was used to find the sensitivity of different bacteria (*E. coli, S. aureus*) and fungi (*Candida Albicans*, *Aspergillus Niger*). Nutrient agar media plates and Sabouraud Dextrose Agar plates were prepared, sterilized, and wells were dug in the plate with the help of a sterile cork borer after the solidification of media.[14] The microorganism was streaked on the plate from the periphery towards the center in a zig-zag manner. The synthesized compounds of 200µg/ml and 400 µg/ml concentration each (dissolved in toluene), Amoxycillin (dissolved in DMSO), and Fluconazole (dissolved in methanol) was filled in wells of the plate. Petri-plates were then incubated at 37^0 C for 24 hrs and zone inhibition was measured.[15]

2.2.3 Antioxidant screening by DPPH-Method

Ascorbic acid was used as a standard free radical scavenger. Five different concentrations (25µg/ml, 50µg/ml, 100µg/ml, 200µg/ml, and 400µg/ml) of compounds and standards were prepared in methanol.[16] In cleaned and labeled test tubes, 2 ml of DPPH solution (0.002%) in methanol was mixed with 2ml of different concentrations of compounds and standards separately.[17] The tubes were incubated at room temperature in dark for 30 min, and the optical density is measured at 517 nm using a UV-Vis spectrophotometer. The absorbance of the DPPH control was also noted.[18-21] The Radical scavenging activity (RSA) was calculated using the formula:

%RSA = Abs control - Abs sample/Abs control×100

1. RESULTS

3.1 Synthesis of Methyl phenyl carbamodithioate

To a solution of aniline (1.9gm, 0.02 mol) in DMSO (20ml), carbon disulfide (1.5gm,0.02mol), and 20M aqueous NaOH solution (1.2ml) were added dropwise over 30 min with continuous stirring at $5\text{-}10^0$C for 5 hrs. Completion of the reaction was monitored by TLC. Then dimethyl sulphate (2.5gm, 0.02mol) was added drop wise with continuous stirring for 3 hrs. below 10^0C. After 3hrs, reaction mixture was poured into ice-water. The product was filtered, dried in vacuum desiccators and recrystallized from acetone to give the compound; methyl phenyl carbamoditioate.

IR (cm^{-1}): 810cm^{-1} Aromatic, 3149cm^{-1} NH (stretching), 1290cm^{-1} C-N(stretching), 1247cm^{-1} C=S (stretching), 685cm^{-1} C-S(stretching), 1375cm^{-1} CH_3 (bending) ; $\lambda_{max:}$ 270nm ; Weight: 6.2gm; %Yield: 40.7% and m.p: 90-92^{0}C.

3.2 Synthesis of 4-Phenyl Thiosemicarbazide

Methyl phenyl carbamodithioate (1.5gm, 0.01 mol) was dissolved in absolute ethanol (50ml) and hydrazine hydrate (0.5gm, 0.01mol) was added drop-wise with stirring at 50^0C. Stirring was further continued for 5hr and then the reaction mixture was poured into ice –water. The solid obtained was filtered, dried and recrystallized using ethanol to give the compound; 4-phenyl thiosemicarbazide.

IR (cm^{-1}): 896cm^{-1} Aromatic, 3167cm^{-1} N-H (stretching), 1286cm^{-1} C=S (stretching), 1143cm^{-1} C-N (stretching), 3304cm^{-1} NH_2(bending); λ_{max}: 267nm ;Weight: 2.5gm; %Yield: 74.8% and m.p: 139-141^{0}C.

3.3 Synthesis of Thiosemicarbazone derivatives

Selected aldehyde solutions were added to a magnetically stirred solution of 4-phenylthiosemicarbazide (1 mmol) in ethanol (30ml) at room temperature. The mixture was refluxed for 6-9hrs. After completion of the reaction, the precipitated product was filtered and washed with ethanol (20ml) and dried at room temperature to give Thiosemicarbazone derivatives. Reaction completion of all the synthesized compounds was checked by performing TLC (Thin layer chromatography). Rf values of prepared compounds were calculated with selected mobile phase based on polarity are mentioned below in Table 3.

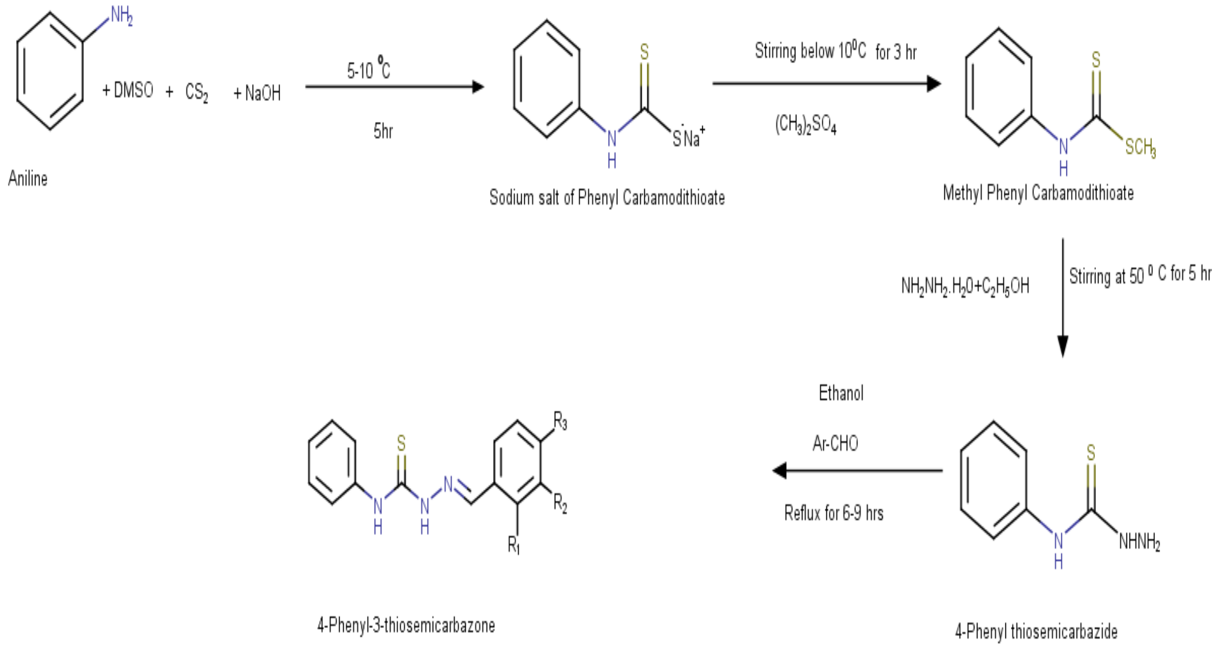


Figure 1: Scheme for the synthesis of Thiosemicarbazone.

A novel series of seven Thiosemicarbazone derivatives **(cp1-cp7)** were synthesized. The first step of the reaction was a base-catalyzed nucleophilic addition reaction in which DMSO is used as a solvent, Carbon disulfide was used as electrophile and Sodium hydroxide was used to neutralize the reaction. Dimethyl sulfate was used as an alkylating agent and Hydrazine hydrate was used as a reducing agent. Furthermore, the second step of the reaction was the condensation reaction in which ethanol was used for filtering and washing of synthesized derivatives The purity of the compounds synthesized was checked by performing TLC and determining the melting points. All the final synthesized compounds were purified by recrystallization using ethanol and were elucidated based on IR, ^{1}HNMR and UV-Vis spectral data.

The synthesized compounds were screened for anti-microbial and anti-oxidant activity. The anti-microbial activity was performed by using Agar well diffusion method and was evaluated by determining the zone of inhibition (mm). The antioxidant activity was performed using the DPPH method. All derivatives showed anti-bacterial activity against *B.subtilis* and *E.coli* using Amoxicillin as a standard drug showed antifungal activity against *Candida albicans* and *A.niger* using Fluconazole as standard drug.

Anti-oxidant activity at the different concentrations that is 25µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, and 400 µg/ml using ascorbic acid as a standard drug was evaluated. All compounds exhibited antioxidant activity with an appreciable percentage of radical scavenging. Results of different properties and activities of prepared novel Thiosemicarbazone are tabulated as follows.

Table 1: List of synthesized thiosemicarbazone derivatives.

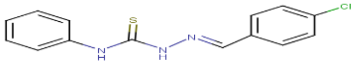
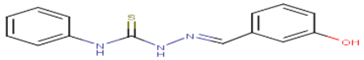
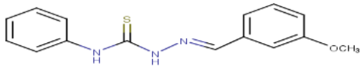
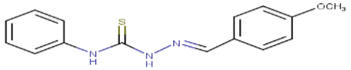
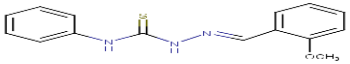
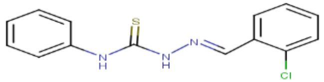
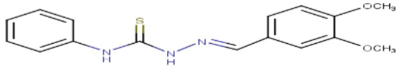
Sr.no.		Intermediate	Reactant	Product	IR (cm^{-1})	Structure
1	Cp 1	4-phenylthiosemicarbazide	4-chloro benzaldehyde	4-chloro benzaldehyde-4-phenyl-3-thiosemicarbazone	774cm^{-1} Cl-C (stretching), 1605cm^{-1} Aromatic, 1033cm^{-1} NH (stretching), 1206cm^{-1} C=S (stretching), 2260 cm^{-1} C=N (stretching), 1290cm^{-1} C-N (stretching), 1550cm^{-1} N-H (bending); UV λ_{max}: 200nm	 R1= -H R2= -H R3= -Cl
2	Cp 2	4-phenylthiosemicarbazide	3-hydroxy benzaldehyde	3-hydroxy benzaldehyde-4-phenyl-3-thiosemicarbazone	3302cm^{-1} OH(stretching), 1610cm^{-1} Aromatic, 1033cm^{-1} NH (stretching), 1206cm^{-1} C=S (stretching), 2260 cm^{-1} C=N (stretching), 1290cm^{-1} C-N (stretching), 1550cm^{-1} N-H (bending); UV λ_{max}: 200nm ;	 R1= -H R2= OH R3= -H
3	Cp 3	4-phenylthiosemicarbazide	3-methoxy benzaldehyde	3-methoxy benzaldehyde-4-phenyl-3-thiosemicarbazone	2822cm^{-1} OCH$_3$, 1590cm^{-1} Aromatic, 1033cm^{-1} NH (stretching), 1206cm^{-1} C=S (stretching), 2260 cm^{-1} C=N (stretching), 1290cm^{-1} C-N (stretching), 1550cm^{-1} N-H (bending); UV λ_{max}: 216nm	 R1= -H R2=OCH3 R3= -H
4	Cp4	4-phenylthiosemicarbazide	4-methoxy benzaldehyde	4-methoxy benzaldehyde-4-phenyl-3-thiosemicarbazone	2822cm^{-1} OCH^3, 1590cm^{-1} Aromatic, 1033cm^{-1} NH (stretching), 1206cm^{-1} C=S (stretching), 2260 cm^{-1} C=N (stretching), 1290cm^{-1} C-N (stretching), 1550cm^{-1} N-H (bending); UV λ_{max}: 216nm	 R1= -H R2= -H R3= OCH3
5	Cp 5	4-phenylthiosemicarbazide	2-methoxy benzaldehyde	2-methoxy benzaldehyde-4-phenyl-3-thiosemicarbazone	2822cm^{-1} OCH$_3$, 1590cm^{-1} Aromatic, 1033cm^{-1} NH (stretching), 1206cm^{-1} C=S (stretching), 2260 cm^{-1} C=N (stretching), 1290cm^{-1} C-N (stretching), 1550cm^{-1} N-H (bending); UV λ_{max}: 216nm	 R1= OCH3 R2= -H R3= -H
6	Cp 6	4-phenylthiosemicarbazide	2-chloro benzaldehyde	2-chloro benzaldehyde-4-phenyl-3-thiosemicarbazone	774cm^{-1} Cl-C (Stretching), 1560cm^{-1} Aromatic, 1033cm^{-1} NH (stretching), 1206cm^{-1} C=S (stretching), 2260 cm^{-1} C=N (stretching), 1290cm^{-1} C-N (stretching), 1550cm^{-1} N-H (bending); UV λ_{max}: 200nm	 R1= OCH3 R2= -H R3= -H
7	Cp 7	4-phenylthiosemicarbazide	3,4-dimethoxy benzaldehyde	3, 4-dimethoxy benzaldehyde-4-phenyl-3-thiosemicarbazone	2822cm^{-1} OCH$_3$, 1590cm^{-1} Aromatic, 1033cm^{-1} NH (stretching), 1206cm^{-1} C=S (stretching), 2260 cm^{-1} C=N (stretching), 1290cm^{-1} C-N (stretching), 1550cm^{-1} N-H (bending); UV λ_{max}: 216nm	 R1= -H R2= OCH3 R3=OCH3

Table 2: Physicochemical properties of synthesized thiosemicarbazone derivatives.

Synthesized compounds	**Molecular Formula**	**Molecular weight**	**Yield**	**Melting point**
Methyl phenyl carbamodithioate	$C_8H_9NS_2$	183.02g/mol	40.7%	90-92^{0}C
4-phenyl thiosemicarbazide	$C_7H_9N_3S$	167.05g/mol	74.8%	139-141^{0}C
Cp1	$C_{14}H_{12}ClN_3S$	289.78g/mol	78.5%	141-143^{0}C
Cp2	$C_{14}H_{13}N_3OS$	271.34g/mol	98.3%	140-142^{0}C
Cp3	$C_{14}H_{13}N_3OS$	271.34g/mol	66.1%	138-140^{0}C
Cp4	$C_{15}H_{15}N_3S$	269.37g/mol	51.4%	139-141^{0}C
Cp5	$C_{15}H_{15}N_3S$	269.37g/mol	80.8%	137-139^{0}C
Cp6	$C_{14}H_{12}ClN_3S$	289.78g/mol	92.8%	137-139^{0}C
Cp7	$C_{16}H_{17}N_3S$	283.39g/mol	93.7%	136-138^{0}C

Table 3: Ratio of mobile phase and RF value of synthesized compounds.

Compound	**Mobile phase**	**R_f value**
Methyl phenyl carbamodithiote	Hexane:Ethyl acetate (6:4v/v)	0.57
4-phenyl thiosemicarbazide	Toluene:Ethylacetate:Formicacid:Methanol (3:3:0.8:0.2v/v)	0.83
Cp1	Hexane:Ethyl acetate(6:4v/v)	0.62
Cp2	Toluene:Ethylacetate:Formicacid:methanol (4:2:0.6:1v/v)	0.78
Cp3	Hexane:Ethyl acetate (6:4v/v)	0.70
Cp4	Hexane:Ethyl acetate (6:4v/v)	0.66
Cp5	Hexane:Ethyl acetate (6:4v/v)	0.75
Cp6	Hexane:Ethyl acetate (6:4v/v)	0.70
Cp7	Hexane:Ethyl acetate (6:4v/v)	0.66

Table 4: Antibacterial activity of the synthesized compounds.

Synthesized Compounds	**Concentration (µg/ml)**	**Bacterial species**	
		B.subtilis	***E.Coli.***
cp1	200	22.6±0.75	26.02±0.85
	400	24.8±1.50	21.2±0.92
cp2	200	25.4±0.80	22.6±0.80
	400	27.6±0.45	23.5±0.76
cp3	200	21.9±0.79	26.6±1.50
	400	22.5±0.45	23.2±0.89
cp4	200	23.1±0.56	21.5±0.65
	400	20.2±0.55	21.4±0.67
cp5	200	21.9±0.79	26.6±1.50
	400	22.5±0.45	23.2±0.89
cp6	200	23.1±0.56	21.5±0.65
	400	20.2±0.55	21.4±0.67
cp7	200	23.4±0.56	20.6±0.76
	400	25.5±0.87	26.2±0.45
Amoxicillin	200	30.4±0.78	32.8±0.66
	400	34.9±0.32	37.9±.78

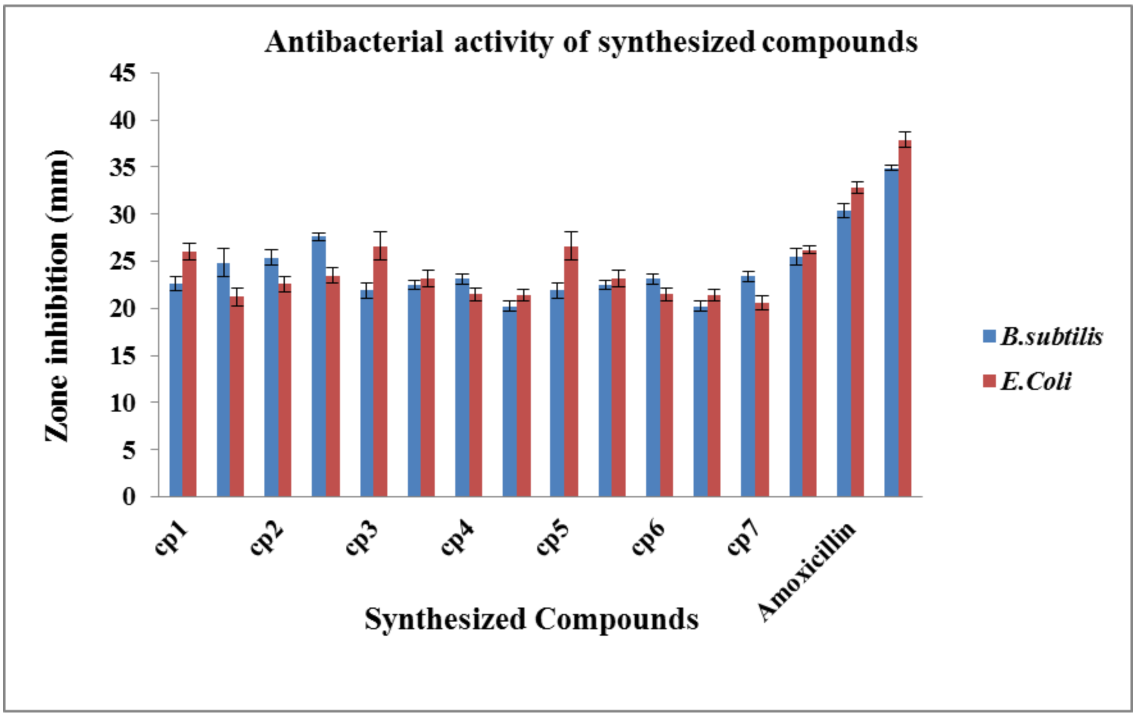


Figure 21: Graph predicted the antibacterial activity of synthesized compounds.

Table 5: Antifungal activity of the synthesized compounds.

Synthesized Compounds	**Concentration (µg/ml)**	**Fungal species**	
		C.albicans	***A.niger***
cp1	200	22.9±1.30	24.8±0.94
	400	23.7±1.12	26.6±1.11
cp2	200	31.7±1.15	36.5±1.20
	400	26.4±0.95	28.8±1.36
cp3	200	23.9±0.42	26.4±1.11
	400	32.1±1.10	38.9±1.12
cp4	200	18.4±0.61	27.6±1.05
	400	27.8±1.50	29.2±1.05
cp5	200	29.4±0.09	34.6±0.85
	400	21.4±0.85	24.6±1.15
cp6	200	18.3±0.80	22.1±1.43
	400	25.4±0.83	33.6±0.95
cp7	200	22.6±0.75	26.2±0.85
	400	24.8±1.50	28.2±0.85
Fluconazole	200	43.5±1.15	48.7±1.15
	400	44.9±1.23	49.9±1.14

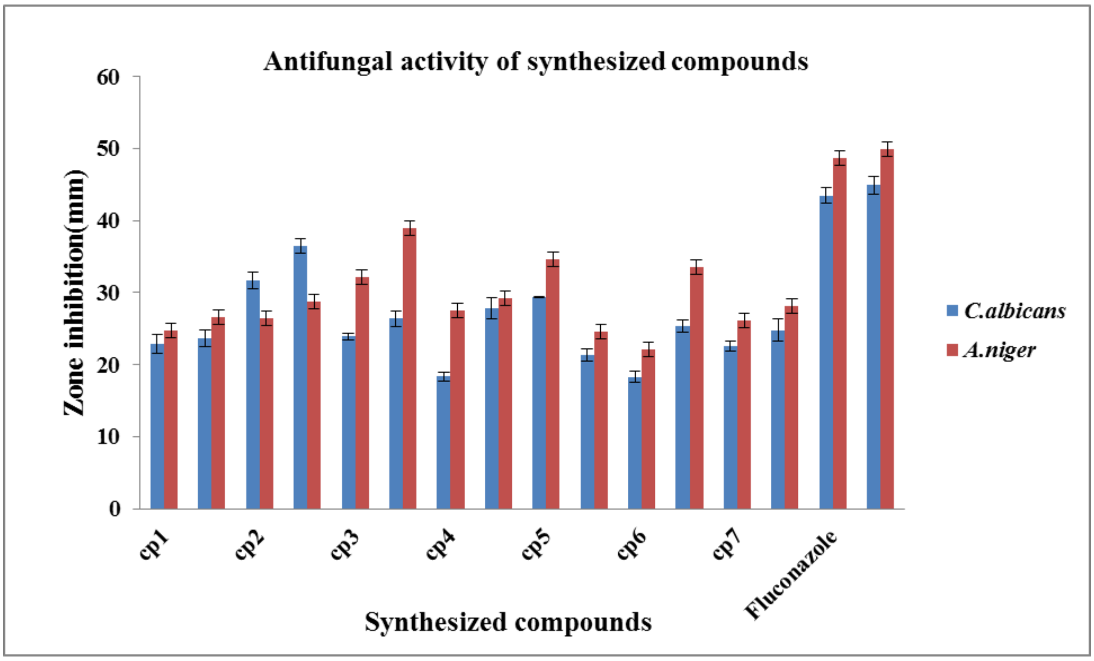


Figure 3: Graph predicted the antifungal activity of synthesized compounds.

Table 6: Antioxidant activity of synthesized compounds.

Synthesized Compounds	25µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml
cp1	25.03	29.18	34.72	39.25	44.70
cp2	30.75	34.66	39.74	44.64	48.70
cp3	33.65	37.90	41.18	45.87	51.39
cp4	38.90	42.74	46.88	50.56	57.63
cp5	43.76	47.95	51.72	54.94	58.70
cp6	51.82	56.21	62.58	69.56	73.25
cp7	56.65	61.74	66.65	71.18	77.63
Ascorbic acid	68.69	73.53	78.87	83.24	88.24

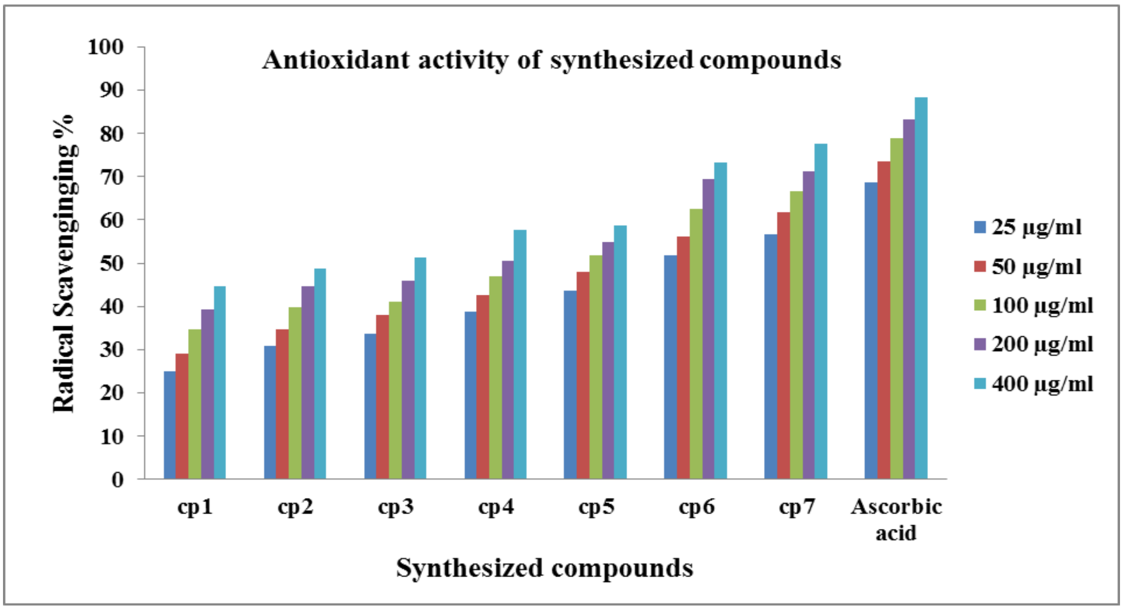


Figure 4: Graph predicted the antioxidant activity of synthesized compounds.

2. DISCUSSION

New series of seven thiosemicarbazone derivatives were synthesized and elucidated on the basis of IR, ^{1}HNMRand UV-Vis spectral data. Prepared derivatives were evaluated for their inhibitory effects on pathogenic bacteria including *Bacillus subtilis and E.coli and on the* antifungal effects on Candida albicans & Aspergillus niger using Agar well diffusion method. Prepared compounds were screened for Anti-oxidant activity using DPPH method.

All derivatives showed anti-bacterial activity against *B.subtilis* and *E.coli*using Amoxicillin as standard drug. Anti-bacterial activity was evaluated by determining zone of inhibition (mm). It was found that compound **CP1** and **CP3** showed maximum anti-bacterial activity while compound **CP2, CP5, CP6 and CP7** showed moderate anti-bacterial activity and compound **CP4** showed least anti-bacterial activity.

All derivatives showed anti-fungal activity against *Candida albicans* and *A.niger*using Fluconazoleas standard drug. Anti-fungal activity was evaluated by determining zone of inhibition (mm). It was found that compound **CP2** and **CP3** showed maximum anti-fungal activity while compound **(CP4-CP6)** showed moderate anti-fungal activity and compound **CP1 and CP7** showed least anti-fungal activity.

Synthesized compounds **(CP1-CP7)** were evaluated for anti-oxidant activity at the different concentrations that is 25µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, and 400 µg/ml using ascorbic acid as standard drug. All compounds exhibited appreciable antioxidant activity. Anti-oxidant activity was evaluated by determining percentage Radical scavenging (%). It was found that compound **CP6 and CP7** showed maximum anti-oxidant activity and compound **(CP2-CP5)** showed moderate anti-oxidant activity and compound **CP1** showed least anti-oxidant activity.

5. CONCLUSION

The objective of this study was to synthesise and investigate the antibacterial, antifungal and antioxidant activity of some new thiosemicarbazone derivatives**.** The obtained results clearly revealed that most of these derivatives showed promising broad spectrum antibacterial, antifungal and antioxidant activities compared to amoxicillin, fluconazole and ascorbic acid respectively.

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