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Synthesis and Antimicrobial Evaluation of Some Simple Phenylhydrazones

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Abstract

The frequency of relapse and severity of some microbial infections call for continuous screening of natural and synthetic compounds for super inhibitory activity. In this research work, phenylhydrazine was reacted stoichiometrically with appropriate mole of acetone to give 1.19 g of compound A_1 (acetone phenylhydrazone, a yellow oily product) with percentage yield of 87%. Compounds A_2 (acetophenone phenylhydrazone), A_3 (acetylacetone phenylhydrazone) and A_4 (cyclohexanone phenylhydrazone) were similarly obtained by reacting the phenylhydrazine with acetophenone, acetylacetone and cyclohexanone respectively. The product yield for compounds A_2, A_3 and A_4 were 1.112, 4.198 and 1.637 g with percentage yields of 89, 81 and 94% respectively Infrared (FT-IR) spectra of the compounds showed peaks at 1612, 1608, 1597 and 1603 cm^{-1} which were absorptions due C=N, for compounds A_1, A_2, A_3 and A_4 respectively. Purity of the compounds was ascertained using thin layer chromatography (TLC). The retention factors (R_f values) of the compounds were found to be 0.82, 0.82, 0.77 and 0.87 for compounds A_1, A_2, A_3 and A_4 respectively. The four synthesized phenylhydrazones were individually screened against five bacteria and two fungi strains using the Disc Diffusion Method. Minimum Inhibitory Concentrations (MICs) of the compounds against the growth of the microbes were determined and zones of inhibition for susceptible strains recorded. The synthesized compounds generally exhibited weak antibacterial activity compared to the standard drug (10 µg/ml Ciprofloxacin) used. However, compound A_1 inhibited the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi* at MIC of 125 µg/ml.

Keywords: Phenylhydrazone, Synthesis, Phenylhydrazones, antibacterial, antifungal, drug-discovery

Introduction

The development of new drugs with less toxicity and desirable potency using the most efficient and cost-effective means is central in the practice of pharmaceutical chemistry. The innate capacity of microorganisms to undergo structural and physiological modifications and adapt to changing physiological environment in their host is the crux of most therapeutic failures. The complications and the attendant increase in cost of antibiotic therapy compounded by multi-drug resistant pathogens in recent times have increased the need for efficient, broad-spectrum drugs against Gram positive and Gram negative bacteria and pathogenic fungi [1]. Some of the pathogens implicated in a number of ailments are *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa.* Some fungi such as *Candida albicans* and *Aspergillus niger* have also been reported in fungal infections. One of the compounds that has extensively been used in organic synthesis is phenyl derivative of hydrazine known as phenylhydrazine which forms hydrazones when it reacts with aldehydes and ketones. Much research interests and efforts have been geared toward the study of phenylhydrazones and their derivatives due to their fascinating therapeutic properties

that have been reported. For instance, hydrazones have been found to be a potent source of antiplatelet aggregation, antifungal, antimalaria, anti-hypertension, anti-inflammatory and anti-oxidant compounds [2]. The side effects of some drugs after prolonged usage and the need for a cheap alternative, are some of the reasons for renewed interest in the search for antimicrobial agents.

Series of hydrazone derivatives have been prepared and screened for their antibacterial properties; ρ-trifluoromethyl-benzoyl phenylhydrazone, a derivative of hydrazone was found to exhibit appreciable antibacterial activity [3].

Although much has been known about the reactions of phenylhydrazine with aldehydes and ketones, and their reactions with aldose and ketose to prepare their corresponding osazones, not much, however, has been reported on the antimicrobial properties of simple acyclic and cyclic phenylhydrazones. Though some complex ones have been reported to possess appreciable antimicrobial activity [10]**.** Hence, it is worthwhile to explore synthesis and screening of simple phenylhydrazones of acetone, acetophenone, acetylacetone and cyclohexanone for their antimicrobial properties.

Materials and Methods

Reagents

Ethyl acetate, ethanol, acetone, glacial acetic acid, acetylacetone, cyclohexanone, phenylhydrazine and acetophenone used in this research were analytical grade obtained from Lobachemie Products with percentage purity ranging from 98-99.5%. Dimethylsulphoxide (DMSO) was obtained from Sigma-Aldrich with percentage purity of 99%.

Instruments

Melting points of crystals were determined in open capillary tubes using a Cole-Parmer Analog Melting Point Apparatus (WZ-03013-0). The values obtained were uncorrected. Infrared Spectra of the synthesized products were obtained using Fourier Transform Infrared (FT-IR, 600) Spectroscopy, Biotec Engineering.

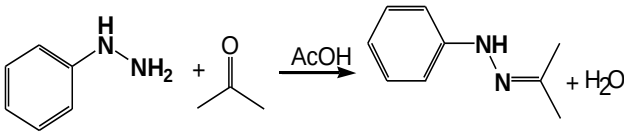
One-dimension analytical thin layer chromatography (TLC) was carried out on silica gel Merck F_{254} precoated plates. Mobile phase used was ethyl acetate:n-hexane (2:3). Detection was made under ultraviolet light at wavelength 254nm; and retention factors (R_f values) calculated.

Experimental

The syntheses were carried out using established procedures with some modifications [5].

Synthesis of compound A_1 (Acetone phenylhydrazone)

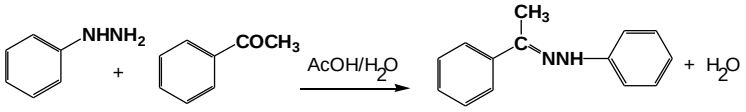
The reaction was carried out by transferring phenyl hydrazine (1.0 g; 0.00924 mol; 0.912 mL) into a boiling tube containing 0.92 mL of glacial acetic acid and diluted with 1.0 mL of water. To this solution, 0.678 mL (0.536 g; 0.00924 mol) of acetone was added and swirled for 5 minutes until the reaction began (Scheme 1 & 5). A yellow oily product which separated out at the end of the reaction was extracted using 10 mL of diethyl ether. Diethyl ether was removed under vacuum and the product was further dried over an anhydrous potassium carbonate in a desiccator to give 1.19 g (percentage yield 87%) of an oily product.



Scheme 1: Reaction of phenylhydrazine with acetone

Synthesis of compound A_2 (Acetophenone phenylhydrazone)

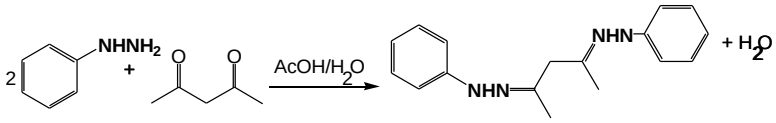
Phenylhydrazine (1.0 g; 0.00925 mol) was transferred into a boiling tube containing 2 mL acetic acid and the solution was further diluted with 2 mL of distilled water. To this mixture, acetophenone (1.112 g; 0.00925 mol) was added. The mixture was then allowed to react for 10 minutes at room temperature and cooled in an ice bath (Scheme 2 & 5). A gray crystalline product was obtained after filtration. The product was recrystallized from 6 mL of ethanol. Product yield was 1.73 g.



Scheme 2: Reaction of phenylhydrazine with acetophenone

Synthesis of compound A_3 (Acetylacetone phenylhydrazone)

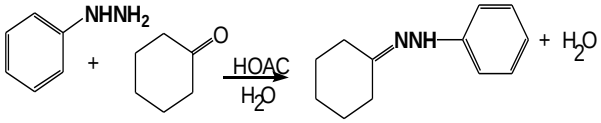
Acetylacetone (0.9261 g; 0.00925 mol) was transferred into a boiling tube containing phenylhydrazine (2.0 g; 0.0185 mol) dissolved in a 2.0 mL of acetic acid (Scheme 3 & 5). The reaction mixture was then allowed to react for 5 minutes with evolution of heat. To the reaction mixture, 2.0 mL of distilled water was added to separate out a light-yellow oily product. Product of the reaction was then extracted using about 10 mL of diethyl ether. The extract was then made solvent-free by removing diethyl ether under pressure (using a rotary evaporator) to give 4.198 g of a light-yellow oily compound.



Scheme 3: Reaction of phenylhydrazine with acetylacetone

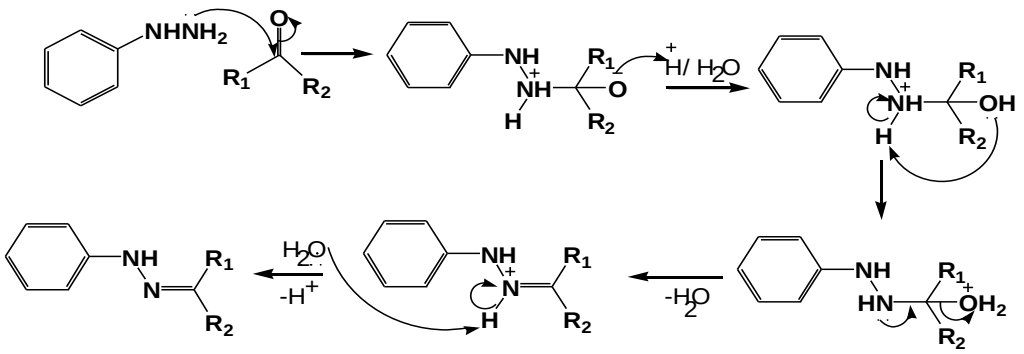
Synthesis of compound A_4 (cyclohexanone phenylhydrazone)

Cyclohexanone (0.91 g; 0.00927 mol) was transferred to a boiling tube containing phenylhydrazine (1.0 g; 0.00925 mol) in a 2.0 mL of acetic acid and was swirled for 8 minutes (Scheme 4 & 5). The reaction mixture was cooled in an ice bath. Addition of about 7 mL of water brought about the separation of colourless crystals. The crystals were then recrystallized in 6 mL absolute ethanol, filtered and dried (in a desiccator) over anhydrous sodium sulphate to yield 1.637 g of the product.



Scheme 4: Reaction of phenylhydrazine with cyclohexanone

Purity of the compounds was ascertained using thin layer chromatography (TLC). The mobile phase was ethyl acetate - n-hexane in the ratio of 2:3. The retention factors (R_f values) were also calculated in each case.



Scheme 5: Proposed mechanism for the reaction of phenylhydrazine with ketones

Antimicrobial Activities

The antimicrobial assays were carried out using standard procedures [6-7].

Antibacterial assay

Müeller-Hinton agar of about 28 g was dissolve in 1 liter of distilled water and autoclaved at 121 °C for 15 minutes. The solution was then cooled to 45 °C. About 30 mL of the solution was then dispensed into each of the autoclaved Petri dishes. The dishes were then dried and inoculated with freshly sub-cultured strains of *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis*. To the inoculated plates, Whatman No 1 filter paper discs (6 mm in diameter) impregnated with different concentrations (500, 250, 125 and 62.5 µg/mL) of the synthesized compounds were placed on 4 of the 6 portions to which the plates were demarcated. While the other 2 portions had discs impregnated with the solvent (DMSO) and a standard drug (ciprofloxacin 10 µg/mL) placed on each of them. The plates were then incubated at 35 °C in inverted position and observed for growth inhibition after 24 hours [6].

Antifungal assay

Standard procedure [7] was used in the screening of the phenylhydrazones for their antifungal properties. Sabouraud dextrose agar (32.5 g) and chloramphenicol (0.025 g) were dissolved in 500 mL of distilled water contained in 1 liter capacity conical flask and heated to ensure uniform dissolution; and afterwards autoclaved at 121 °C for 15 minutes. About 30 mL of the solution cooled to 45 °C was then poured into each of the autoclaved Petri dishes and allowed to solidify. Inoculation of freshly grown strains of *Candida albicans* and *Aspergillus niger* on the plates and the subsequent introduction of discs impregnated with the synthesized compounds was carried out as described in the antibacterial procedure.

Results and Discussion

Antibacterial potency of synthesized phenylhydrazones

Results of the antibacterial susceptibility screening against the tested bacteria strains are presented in Tables 1-5.

Table 1: Antibacterial Activity of the Synthesized Phenylhydrazones Against *Escherichia coli*

Conc. (µg/mL)	Zone of Inhibition (mm)					
	A_1	A_2	A_3	A_4	CPFX	DMSO
500.0	9±0.37	8±0.57	7±0.19	10±0.87		
250.0	9±0.50	-	-	7±0.34		
125.0	8±0.71	-	-	-		
62.5	-	-	-	-		
10.0					12±0.37	-

A_1=acetonephenylhydrazone; A_2=acetophenone phenylhydrazone; A_3 = acetylacetone phenylhydrazone; A_4 = cyclohexanone phenylhydrazone; CPFX = Ciprofloxacin(10 µg/mL); (-) = No inhibition

Table 1 shows that the Minimum Inhibitory Concentrations (MICs) are 125, 500, 500 and 250 µg/mL for compounds A_1, A_2, A_3 and A_4 respectively. This indicates that compounds A_2 and A_3 had the lowest

antibacterial activity against *E. coli*, while compound A_1 with MIC of 125 µg/mL had a moderate antibacterial activity.

Table 2: Antibacterial Activity of the Synthesized Phenylhydrazones Against *Staphylococcus aureus*

Conc.	Zone of Inhibition (mm)					
(µg/mL)	A_1	A_2	A_3	A_4	CPFX	DMSO
500.0	10.0±0.47	8±0.57	7.0±0.19	-		
250.0	9.0±0.70	-	-	-		
125.0	8.0±0.11	-	-	-		
62.5	-	-	-	-		
10.0					10±0.13	-

A_1=acetonephenylhydrazone; A_2=acetophenone phenylhydrazone; A_3 = acetylacetone phenylhydrazone; A_4 = cyclohexanone phenylhydrazone; CPFX = Ciprofloxacin (10 µg/mL)**;** (-) = No inhibition

In screening the compounds against *Staphylococcus aureus* (Table 2), it was observed that compounds A_1, A_2 and A_3 had MICs of 125, 500 and 500 µg/mL respectively indicating that *Staphylococcus aureus* is more susceptible to compound A_1 than compound A_2 and A_3. Compound A_4 did not inhibit the growth of the strain even at concentration of 500 µg/mL.

Table 3: Antibacterial Activity of the Synthesized Phenylhydrazones Against *Salmonella typhi*

Conc.	Zone of Inhibition (mm)					
(µg/mL)	A_1	A_2	A_3	A_4	CPFX	DMSO
500.0	-	-	7.0±0.11	-		
250.0	-	-	-	-		
125.0	-	-	-	-		
62.5	-	-	-	-		
10.0					10±0.54	-

A_1=acetonephenylhydrazone; A_2=acetophenone phenylhydrazone; A_3 = acetylacetone phenylhydrazone; A_4 = cyclohexanone phenylhydrazone; CPFX = Ciprofloxacin (10 µg/mL)**;** (-) = No inhibition

It may be observed from Table 3 that compounds A_1, A_2 and A_4 could not inhibit the growth of *S. typhi* even at high concentration of 500 µg/mL. The growth of the microbe was inhibited by compound A_3 only, and with high MIC of 500 µg/mL. Average zone of inhibition obtained for the standard drug (Ciprofloxacin 10 µg/mL) was found to be low (8.0 mm), when compared to other strains. The synthesized compounds were generally not active against *S. typhi*.

Results of antibacterial activities of the synthesized compounds against *P. aeruginosa* are presented in Table 4.

Table 4: Antibacterial Activity of the Synthesized Phenylhydrazones Against *Pseudomonas aeruginosa*

Conc.	Zone of Inhibition (mm)					
(µg/mL)	A_1	A_2	A_3	A_4	CPFX	DMSO
500.0	-	8±0.57	7±0.19	9±0.20		
250.0	-	8±0.81	-	8±0.31		
125.0	-	-	-	-		
62.5	-	-	-	-		
10.0					11±0.84	-

A_1=acetonephenylhydrazone; A_2=acetophenone phenylhydrazone; A_3 = acetylacetone phenylhydrazone; A_4 = cyclohexanone phenylhydrazone; CPFX = Ciprofloxacin (10 µg/mL)**;** (-) = No inhibition

As shown in Table 4, *Pseudomonas aeruginosa* showed resistance to compound A_1, but compounds A_2, A_3 and A_4 inhibited the growth of *P. aeruginosa* at MIC of 250, 500 and 250 µg/mL respectively. These concentrations are much higher than that of the standard drug, ciprofloxacin (10 µg/mL), indicating that the compounds are not active enough against the growth of *P. aeruginosa.*

Table 5: Antibacterial Activity of the Synthesized Phenylhydrazones Against *Bacillus subtilis*

Conc.	Zone of Inhibition (mm)					
(µg/mL)	A_1	A_2	A_3	A_4	CPFX	DMSO
500.0	-	8±0.90	7.0±0.52	-		
250.0	-	7±0.31	-	-		
125.0	-	-	-	-		
62.5	-	-	-	-		
10.0					11±0.71	-

A_1=acetonephenylhydrazone; A_2=acetophenone phenylhydrazone; A_3 = acetylacetone phenylhydrazone; A_4 = cyclohexanone phenylhydrazone; CPFX = Ciprofloxacin (10 µg/mL)**;** (-) = No inhibition

In Table 5, compound A_2 with an MIC of 250 µg/mL showed mild activity. However, compound A_3 also inhibited moderately at high concentration with MIC of 500 µg/mL to give a zone of inhibition of 7.0 ± 0.52 mm. These compounds may not be used as antibacterial agents against *Bacillus subtilis* because they showed very low to no inhibition to the growth of *B. subtilis*.

Antifungal potency of synthesized phenylhydrazones

Candida albicans and *Aspergillus niger* were subjected to susceptibility test to the synthesized compounds. The results of the test are as presented in Tables 6 and 7.

Table 6: Antifungal Activity of the Synthesized Phenylhydrazones Against *Candida albicans*

Conc. (µg/mL)	Zone of Inhibition (mm)					
	A_1	A_2	A_3	A_4	KTCZ	DMSO
500.0	8.0±0.61	-	-	-		
250.0	7.0±0.12	-	-	-		
125.0	-	-	-	-		
62.5	-	-	-	-		
10.0					8.0±0.21	
						-

A_1=acetonephenylhydrazone; A_2=acetophenone phenylhydrazone; A_3 = acetylacetone phenylhydrazone; A_4 = cyclohexanone phenylhydrazone; KTCZ = Ketoconazole (15 µg/mL)**;** (-) = No inhibition

Candida albicans, as shown in Table 6, was mildly susceptible to compound A_1 (zone of inhibition of 7.0 ± 0.12 with MIC of 250 µg/mL). The standard antifungal drug used was ineffective in inhibiting the growth of the fungus. For instance, 15µg/mL of the standard drug, ketoconazole, could only inhibit the growth of *Candida albicans* minimally with an average zone of inhibition of 8.0 ± 0.12 mm. *Candida albicans* could not be inhibited by compounds A_2, A_3 and A_4.

Table 7: Antifungal Activity of the Synthesized Phenylhydrazones Against *Aspergillus niger*

Conc. (µg/mL)	Zone of Inhibition (mm)					
	A_1	A_2	A_3	A_4	KTCZ	DMSO
500.0	-	-	-	-		
250.0	-	-	-	-		
125.0	-	-	-	-		
62.5	-	-	-	-		
10.0					-	
						-

A_1=acetonephenylhydrazone; A_2=acetophenone phenylhydrazone; A_3 = acetylacetone phenylhydrazone; A_4 = cyclohexanone phenylhydrazone; KTCZ = Ketoconazole (15 µg/mL)**;** (-) = No inhibition

In table 7, *Aspergillus niger* showed no susceptibility to the compounds and to the standard drug, this may be due to acquired resistance to the drug.

Properties of the synthesized phenylhydrazones

Compound A_1

Compound A_1 (1.19 g) was obtained as a yellow oily liquid product with a percentage yield of 87% having R_f value of 0.82. Synthesis of acetone phenylhydrazone as a light-yellow liquid has been previously reported [5]. The chromatogram spots were made visible with the aid of UV light. Compound A_1 showed FT-IR absorption spectrum peaks at 1614 and 2878 cm^{-1} which correspond to C=N and C-H stretches respectively of acetone phenylhydrazone (Table 8). This spectrum peaks are similar to the ones obtained for phenylhydrazine by the National Institute of Standards and Testing Maryland, USA [8]. The antibacterial susceptibility test of compound A_1 showed a generally moderate activity. *Bacillus subtilis* and *Pseudomonas aeruginosa* exhibited resistance to compound A_1.

Compound A_2

Compound A_2 (1.73 g) was obtained as a grey white crystal with melting point of 104-106 °C and percentage yield of 89%. Similar result was reported by the Royal Society of Chemistry [9]. The R_f value of the compound using ethyl acetate - n-hexane (2:3) as the eluting solvent was 0.82 when it was viewed under UV light. FT-IR (KBr, cm^{-1}) spectrum of the compound showed peaks at 3314 cm^{-1} for N-H bond and 1608 cm^{-1} due to C=N of an azomethine (Table 8). The 1608 cm^{-1} due to C=N peak is absent in the phenyl hydrazine confirming that compound A_2 was indeed synthesized. Absorption at these peaks were also observed in the IR analysis of series of phenyl hydrazones reported by Sileshi *et al.* [10]. The antimicrobial activity of compound A_2 against the pathogens screened was poor compared to the standard drug. This may be due to drug resistance by

the organisms or that the compound was not sufficiently functionalized.

Compound A_3

Compound $\mathbf{A}_3$ (4.198 g) was also obtained as a light-yellow oily product with percentage yield of 81% and R_f value of 0.77. The nature of the compound could not be corroborated with literature because there seemed not to be any literature on it presently. However, the IR spectrum of the compound exhibited peaks at 1597 cm^{-1} which is assignable to C=N, and at 2918 cm^{-1} due to $(C\text{-}H)_{sp3}$ absorption (Table 8). The IR absorption range for C=N is in the region of 1690-1590cm^{-1} [11]. The antimicrobial assay of the compound revealed that it has mild antimicrobial properties against the tested bacteria strains; this could be due to the fact that the pathogens screened, being clinical isolates, may have acquired resistance to the hydrazones. The presence of two azomethine in compound A_3 did not necessarily bring about better antimicrobial outcome.

Compound A_4

Compound A_4 (1.637 g) was successfully synthesized with about 94% yield as colourless crystals. Its melting point range was 77-79 °C. Royal Society of Chemistry [9] reported similar result for some phenylhydrazones of cyclohexanone. The retention factor (R_f) of the compound was 0.87 in ethyl acetate - n-hexane, 2:3. FT-IR spectrum of the compound exhibited peaks at 1603, 3329 and 3088 cm^{-1} which are assignable to C=N, N-H and C-H bonds respectively (Table 8). The absorption ranges of these functional groups are 1690-1590, 3500-3180 and 2970-2950 cm^{-1} respectively [11]. The observed peaks may be attributable to the presence of an azomethine (-NHN=CH-) functional group in the phenylhydrazone. Similar result was obtained by previous researchers [12]. The poor antimicrobial activities of the compound may be due to the compound not being adequately functionalized or that the strains had acquired resistance to the compound [13].

The general poor antifungal performance may be attributable to acquired resistance to the synthesized phenylhydrazones or that the strain could not be inhibited at the tested concentrations. *Aspergillus niger* showed resistance to all the synthesized compounds.

FT-IR spectrum of phenylhydrazine which was the starting material for the synthesis of compounds A_1 - A_4 gave a broad absorption peak at 3332 cm^{-1} (Table 8) which is assignable to N-H of phenylhydrazine ($PhNH\text{-}NH_2$). C=N bond is absent in the phenylhydrazine spectrum which corroborates the formation of an azomethine. The broad absorption peak at 3332 cm^{-1} due to the presence of $\text{-}NH\text{-}NH_2$ was expectedly reduced in the spectra of all the synthesized compounds (A_1 - A_4). The presence of only one secondary amine nitrogen-hydrogen bond ($\text{-}NH\text{-}N{=}CR_1R_2$) indicates the formation of an azomethine compound.

Table 8: Summary of FT-IR Spectra Data of the Synthesized Phenylhydrazone

Functional Group of Interest	Observed FT-IR Spectral Peaks (cm^{-1})					
	A_1	A_2	A_3	A_4	Phenylhydrazine	literature
C=N	1612	1608	1597	1603	-	1690-1590 [11]
N-H	3313	3314	3357	3329	3332	3500-3180 [14]
C-H sp^2	3092	3018	-	3088	3105	3010-3100 [15]
C-H sp^3	2878	2901	2918	-	-	2970-2950 [11]

A_1 = acetone phenylhydrazone; A_2 = acetophenone phenylhydrazone; A_3 = acetylacetone phenylhydrazone; A_4 = cyclohexanone phenylhydrazone; (-) = No absorption peak observed

Conclusion

Phenylhydrazones of acetone, acetophenone, acetylacetone and cyclohexanone were successfully synthesized and in good yields. FT-IR spectra of the compounds gave absorption peaks similar to those of reported azomethine group. Their antimicrobial assay was also carried out successfully. From the results of the antibacterial and antifungal assays, it may be concluded that phenylhydrazones of acetone, acetophenone, acetylacetone and cyclohexanone do not possess appreciable inhibitory activity against the tested pathogens at the concentrations evaluated.

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References

1. G. Verma, A. Marella, M. Shaquiquzzaman, M. Akhtarand and M. R. Ali (2014). A Review Exploring Biological Activities of Hydrazones. J*ournal of Pharmaceutical Bioallied Science*, 6:69-80.
2. R. Rajeshkumar and M. Sundararaman (2012). Emergence of *Candida* spp. and Exploration of Natural Bioactive Molecules for Anticandidal Therapy-Status Quo. *Mycoses*, 55:60-73.
3. M. Kratky, S. Bosze, Z. Baranyai, J. Stolarikova and J. Vinsova (2007). Synthesis and Biological Evolution of Hydrazones Derived from 4-(trifluoromethyl) benzohydrazide. *Bioorg. Medical Chemistry Letter*, 27:5185-5189.
4. L. M. Lima, F. S. Frattani, S. J. L. Dos, H. C. Castro and C. A. Fraga (2008). Synthesis and Antiplatelet Activity of Novel Arylsulfonate-acylhydrazone Derivatives designed as Antithrombotic Candidates. *European Journal of Medical Chemistry*, 43:348-356.
5. W. M. Cumming (I937). Systematic Organic Chemistry, 1st Edition, New Jersey, USA. 290.
6. Clinical and Laboratory Standards Institute CLSI (2002). Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints, and Quality Control Limits for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Third Informational Supplement Document M44-S3. Wayne. 18-23.
7. Clinical and Laboratory Standards Institute CLSI (2017). Performance Standards for Antifungal Susceptibility Testing of Yeasts M 60. Wayne, PA, USA. 10-17.
8. National Institute of Standard and Testing (2011). IR spectra of phenyl hydrazone https://www.webbook.nist.gov.cgi/cbook.cgi?.Gettn.ch1cl03026 retrieved on 24th October 2018.
9. Royal Society of Chemistry RSC (2005). Supplementary Material (ESI) for Chemical Communications. Retrieved on 25th October 2018 from www.rsc.org/suppdata/cc/c0/c0cc05440b/c0cc05440b.pdf
10. A. Sileshi, D. Tegene and E. Milkyas (2018). Synthesis and Antibacterial Activity of Phenylhydrazone Schiff Base Derivatives and their Copper (II) Complexes. *Chemistry and Materials Research*, 10 (6):2224-3224.
11. C. John (2000). *Interpretation of Infrared Spectra, a Practical Approac*h. In Meyers (Ed.) R. A. Encyclopedia of Analytical

Chemistry, John Wiley & Sons Ltd, Chichester, USA. 10815-10837.

12. N. P. Belskaya, W. Dehaen and V. A. Bakulev (2010). Synthesis and Properties of Hydrazones Bearing Amide, Thioamide and Amidine Functions. *Arkivoc*, 1:275-332.
13. S. M. Sondhi, M. Dinodia and A. Kumar (2006). Synthesis, Anti-inflammatory and Analgesic Activity Evaluation of Some Amidine and Hydrazone Derivatives. *Bioorg Medical Chemistry*, 14:4657-4663.
14. G. Neil (2005). IR Absorptions for Representative Functional Groups. Retrieved from http://www.chemistry.ccsu.edu/glagovich/teaching/472/ir/table.html on 20th August, 2018.
15. R. M. Silverstein, G. C. Bassler, and T. C. Morrill (1981). Spectrometric Identification of Organic Compounds, 4th edition, John Wiley and Sons; New York. 1272.