SYNTHESIS, CHARACTERIZATION AND EVALUATION OF BIOLOGICAL ACTIVITY OF SOME SUBSTITUTED(*E*)-2-BENZYLIDENEHYDRAZINECARBOTHIO AMIDES

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ABSTRACT. A series of substituted (*E*)-2-benzylidenehydrazinecarbothioamide compounds were synthesized by condensation of thiosemicarbazide with substituted benzaldehydes. The synthesized substituted (*E*)-2-benzylidenehydrazinecarbothioamide compounds were characterized by their physical constants, UV, IR and NMR spectra. The antimicrobial activities of these synthesized substituted (*E*)-2-benzylidenehydrazinecarbothioamide compounds have been screened by Bauer-Kirby method using human pathogenic bacteria and fungal species. The antimicrobial activities of all synthesized (*E*)-2-benzylidenehydrazinecarbothioamide compounds have shown significant activity.

1. INTRODUCTION

The synthesis, structure and biological activity of some new hydrazones prepared from aliphatic hydrazides has been the focus of research. Different methods have been employed to synthesize different types of hydrazones from different starting materials. Hydrazones are characterized by the presence of the triatomic grouping C=N-N-. They can be considered as Schiff bases derived from acid hydrazides. The most important property of hydrazones is their high physiological activity [1-6]. Extensive studies have revealed that the lone pair on trigonally hybridized nitrogen atom of the azomethine group is responsible [7-11] for the chemical and biological activity. It has been reported that metal complexes of hydrazones have diverse applications. They find use as plasticizers, polymerization inhibitors and antioxidants. They are used as fungicides and pesticides in biological and biochemical context.

Moreover, the hydrazone group plays an important role of the antimicrobial and possesses interesting antibacterial, antifungal [12-14] and anti-tubercular activities [15-20]. In addition, their varied coordinating behaviour makes them interesting candidates for metal-based drugs. Generally, the ligands act synergistically with metals towards their biological activity. These observations have guided the development of new hydrazones with varied biological activities [21]. The biological activity of complexes derived from hydrazones have been studied and contrasted with regard to their antibacterial, antitumoral, antiviral, antimalarial and antitubercular properties [22]. It has also been shown that the azomethine N, which has a lone pair of electrons in a sp² hybridised orbital, is biologically important [23].

Hydrazones constitute an important class of biologically active drug molecules [24] which has attracted attention of medicinal chemists due to their wide range of pharmacological properties. These compounds are being synthesized as drugs by many researchers in order to combat diseases with minimal toxicity and maximal effects. These predictions has provided therapeutic pathway to develop new effective biologically active hydrazones. A number of hydrazone derivatives have been reported to exert notably antimicrobial, antihypertensive, anticonvulsant, analgesic, anti-

inflammatory, antituberculosis, antitumoral, antiproliferative and antimalarial activities, biological activities of various hydrazones are well reported in literature. This review highlights diverse pharmacological activities shown by hydrazones.

Medicinal chemists have also carried out considerable research for novel antimicrobial and anticancer agents bearing hydrazone moiety. Some studies have confirmed that hydrazone derivatives exhibit antifungal and anticancer activities [25-29]. Some researchers have reported anticancer effects of some antifungal agents and carried out considerable research for deciphering the underlying mechanisms of antitumor activity [30-32]. In antifungal and anticancer drug design, the lack of selectivity of conventional chemotherapeutic agents and the acquisition of multiple-drug resistance are two major challenging problems. As a consequence of this situation, the search for new effective chemotherapeutic agents has attracted a great deal of interest [33-35].

Several hydrazone derivatives have been reported as insecticides, nematocides, herbicides, rodenticides and antituberculosis in addition to that some of the hydrazones were found to be active against leukemia, sarcoma and illnesses [36,37]. We now carry out another systematic study of their synthesis and biological activity. Herein, the synthesis of the substituted (*E*)-2-benzylidenehydrazinecarbothioamides are described and their antimicrobial properties are evaluated.

2. EXPERIMENTAL

2.1. General

All the chemicals used in the present investigation, have been procured from Sigma–Aldrich Chemical Company. Melting points of all substituted (*E*)-2-benzylidenehydrazinecarbothioamides have been determined in open glass capillaries on a Mettler FP51 melting point apparatus and are uncorrected. The UV spectra of all the substituted (*E*)-2-benzylidenehydrazinecarbothioamides have been recorded with ELICO-BL222 spectrophotometer (λ_{max} nm) in spectral grade methanol solvent. Infrared spectra (KBr, 4000–400 cm^{-1}) have been recorded on SHIMADZU Fourier transform spectrophotometer. Bruker AV400 NMR spectrometer operating at 400 MHz has been utilized for recording ^1H NMR spectra and 100 MHz for ^{13}C NMR spectra in DMSO solvent using TMS as internal standard.

2. 2. Synthesis of substituted (*E*)-2-benzylidenehydrazinecarbothio amides

A solution of equimolar quantities of thiosemicarbazide (0.01mol) with substituted benzaldehydes (0.01mol) acetic acid (two drops) and 10 ml of ethanol were shaken occasionally for 1 hour [38]. The completion of the reaction was monitored by TLC continuously. The resultant mixture was cooled at room temperature. Then the precipitate obtained, was filtered at the filter pump and washed several times with cold water then pale yellow solid was obtained as the final product. This crude product was recrystallized from ethanol and glittering colorless solid was obtained. The general scheme for preparation of substituted (*E*)-2-benzylidenehydrazinecarbothioamides has shown in **scheme-1**.

Scheme 1. Synthesis of substituted (*E*)-2-benzylidenehydrazinecarbothioamides

The physical constants of substituted (*E*)-2-benzylidenehydrazinecarbothioamide compounds presented in **Table 1**. The ultraviolet absorption maxima (λ_{max} , nm), infrared absorptions (ν , cm^{-1}) and NMR chemical shifts (δ , ppm) of substituted (*E*)-2-benzylidenehydrazinecarbothioamides are presented in Table 2.

Table 1.Physical constants of substituted (*E*)-2-benzylidenehydrazinecarbothioamides

Entry	X	M. F.	M. W.	Yield (%)	m.p. (C)
1	H	C ₈ H ₉ N ₃ S	179.24	89	157-158
2	3-Br	C ₈ H ₈ BrN ₃ S	258.13	91	209-210
3	4-Br	C ₈ H ₈ BrN ₃ S	258.13	88	197-198
4	3-Cl	C ₈ H ₈ ClN ₃ S	213.68	94	198-199
5	4-Cl	C ₈ H ₈ ClN ₃ S	213.68	92	210-211
6	4-F	C ₈ H ₈ FN ₃ S	197.23	87	195-196
7	4-OMe	C ₉ H ₁₁ N ₃ OS	209.26	84	182-183
8	4-Me	C ₉ H ₁₁ N ₃ S	193.26	90	169-170
9	3-NO ₂	C ₈ H ₉ N ₄ O ₂ S	224.23	95	215-216
10	4-NO ₂	C ₈ H ₈ N ₄ O ₂ S	224.23	91	223-224

(*E*)-2-benzylidenehydrazinecarbothioamide (1): Yield: 89%, m.p. 157-158°C. UV $\lambda_{\max}$ (nm): 309.50. IR (KBr, cm⁻¹): ν = 1643.35 (CH=N), 999.13 (N-N), 3387.00 (-NH), 3248.00(-NH₂). ¹H NMR (DMSO, ppm): δ =8.048 (S, 1H.CH=N), 7.387-7.801 (m, 5H Ar-H) 8.190 (S, 1H.-NH), 7.982 (S, 2H.-NH₂). ¹³C NMR (DMSO, ppm): δ (C₁) = 142.29(CH=N), 134.19 (C₂), 128.64(C₃), 127.30(C₄), 129.84(C₅), 128.66(C₆), 128.66 (C₇), 178.01(C=S), M.F = C₈H₉N₃S, M.W = (179.24).

(*E*)-2-(3-bromobenzylidene)hydrazinecarbothioamide (2): Yield: 91%, m.p. 209-210 °C. UV $\lambda_{\max}$ (nm): 318.00. IR (KBr, cm⁻¹): ν =1641.42 (CH=N), 997.20 (N-N), 3385.07 (-NH), 3250.05 (-NH₂). ¹H NMR (DMSO, ppm): δ =8.117 (S, 1H.CH=N), 7.323-7.667 (m, 4H, Ar-H), 8.171 (S, 1H.-NH), 8.567 (S, 2H.-NH₂). ¹³C NMR (DMSO, ppm): δ (C₁) = 140.75 (CH=N), 136.48 (C₂), 126.84 (C₃), 128.83 (C₄), 132.34 (C₅), 122.25 (C₆), 130.74 (C₇), 178.02 (C=S), M.F = C₈H₈BrN₃S. M/W = (258.13).

(*E*)-2-(4-bromobenzylidene)hydrazinecarbothioamide (3): Yield: 88%, m.p. 197-198°C. UV $\lambda_{\max}$ (nm): 317.50. IR (KBr, cm⁻¹): ν =1641.42(CH=N), 997.20 (N-N), 3385.00 (-NH), 3251.00(-NH₂). ¹H NMR (DMSO, ppm): δ =8.015 (S, 1H.CH=N), 7.283-7.864 (m, 4H Ar-H) , 8.239(S, 1H.-NH), 7.946 (S, 2H.-NH₂). ¹³C NMR (DMSO, ppm): δ (C₁) = 140.23 (CH=N), 132.23 (C₂), 128.50 (C₃, C₇)132.23 (C₄, C₅, C₆), 172.58 (C=S). M/F = C₈H₈BrN₃S. M/W = (258.13)

(*E*)-2-(3--chlorobenzylidene)hydrazinecarbothioamide (4): Yield: 94%, m.p. 198-199°C. UV $\lambda_{\max}$ (nm): 315.00. IR (KBr, cm⁻¹): ν =1685.79 (CH=N), 1078.21(N-N), 3388.93 (-NH), 3230.77 (-NH₂). ¹H NMR (DMSO, ppm): δ = 8.041 (S, 1H.CH=N), 7.403-7.654 (m, 4H Ar-H), 8.181 (S, 1H.-NH), 8.246 (S, 2H.-NH₂). ¹³C NMR (DMSO, ppm): δ (C₁) = 140.55(CH=N), 136.49(C₂), 129.41 (C₃), 126.62 (C₄, C₇), 130.46 (C₅), 133.78 (C₆), 178.21 (C=S). M/F = C₈H₈ClN₃S. M/W = (213.68).

(*E*)-2-(4--chlorobenzylidene)hydrazinecarbothioamide (5): Yield: 92%, m.p. 210-211°C. UV $\lambda_{\max}$ (nm):316.50. IR (KBr, cm⁻¹): ν =1689.64 (CH=N), 1078.21(N-N), 3388.93 (-NH), 3371 (-NH₂). ¹H NMR (DMSO, ppm): δ = 8.060 (S, 1H.CH=N), 7. 439-7.842 (m, 4H Ar-H) , 8.021(S, 1H.-NH), 8.222 (S,2H.-NH₂) . ¹³C NMR (DMSO, pm): δ (C₁) = 140.90 (CH=N),133.21 (C₂), 128.97 (C₃,C₇), 128.72 (C₄,C₆), 134.35 (C₅) ,178.10 (C=S). M/F = C₈H₈ClN₃S. M/W = (213.68).

(E)-2-(4--Fulorobenzylidene)hydrazinecarbothioamide (6): Yield: 87%, m.p. 195-196°C. UV $\lambda_{\max}$ (nm): 311.00. IR (KBr, cm^{-1}): ν = 1638.43 (CH=N), 1093.64 (N-N), 3388.33 (-NH), 3238.48 (-NH₂). ¹H NMR (DMSO, ppm): δ = 8.051 (S, 1H.CH=N), 7.367-7.819 (m, 4H Ar-H), 7.948 (S, 1H.-NH), 8.356 (S, 2H.-NH₂). ¹³C NMR (DMSO, ppm): δ (C₁) = 140.39(CH=N), 129.30(C₂), 133.80(C₃), 116.20(C₄), 163.33(C₅), 115.60(C₆), 133.70 (C₇) 178.43 (C=S). M/F = C₈H₈FN₃S. M/W = (197.23).

(E)-2-(4--Methoxybenzylidene)hydrazinecarbothioamide (7): Yield: 84%, m.p. 182-183°C. UV $\lambda_{\max}$ (nm): 320.50. IR (KBr, cm^{-1}): ν = 1625.99 (CH=N), 1087.85 (N-N), 3381.21 (-NH), 3221.12 (-NH₂). ¹H NMR (DMSO, ppm): δ = 8.101 (S, 1H.CH=N), 7.712-7.741 (m, 4H Ar-H) 3.786 (OCH₃), 7.906 (S, 1H.-NH), 7.990 (S, 2H.-NH₂). ¹³C NMR (DMSO, ppm): δ (C₁) = 142.36(CH=N), 126.78(C₂), 128.93(C₃, C₇), 114.18(C₄, C₆), 160.70(C₅), 55.30 (OCH₃), 177.62 (C=S). M/F = C₉H₁₁N₃OS. M/W = (209.26).

(E)-2-(4--Methylbenzylidene)hydrazinecarbothioamide (8): Yield: 90%, m.p. 169-170°C. UV $\lambda_{\max}$ (nm): 313.50 IR (KBr, cm^{-1}): ν = 1698 (CH=N), 1093.43 (N-N), 3368.43(-NH), 3258.84 (-NH₂). ¹H NMR (DMSO, ppm): δ = 8.150 (S, 1H.CH=N), 7.197-7.683 (m, 4H Ar-H) 2.491 (CH₃), 7.937 (S, 1H.-NH) 8.011(S 2H.-NH₂). ¹³C NMR (DMSO, ppm): δ (C₁) = 142.23 (CH=N), 131.47 (C₂), 127.30 (C₃, C₇), 129.31(C₄, C₆), 139.67 (C₅), 21.07 (CH₃), 177.83 (C=S), M/F = C₉H₁₁N₃S. M/W = (193.26).

(E)-2-(3--Nitrobenzylidene)hydrazinecarbothioamide (9): Yield: 95%, m.p. 215-216 °C . UV $\lambda_{\max}$ (nm): 311.50. IR (KBr, cm^{-1}): ν = 1643.35 (CH=N), 1099.43 (N-N), 3373.50(-NH), 3265.49 (-NH₂). ¹H NMR (DMSO, ppm): δ = 8.041 (S, 1H.CH=N), 7.203-7.874 (m, 4H, Ar-H), 8.032 (S, 1H.-NH), 8.185 (S, 2H.-NH₂). ¹³C NMR (DMSO, ppm): δ (C₁) = 140.03 (CH=N), 139.99 (C₂, C₃), 130.18 (C₄), 123.99 (C₅), 148.44 (C₆), 121.47 (C₇) 178.38 (C=S). M/F = C₈H₉N₄O₂S. M/W = (224.23).

(E)-2-(4--Nitrobenzylidene)hydrazinecarbothioamide (10): Yield: 91%, m.p. 223-224°C. UV $\lambda_{\max}$ (nm): 322.00. IR (KBr, cm^{-1}): ν = 1641.42 (CH=N), 1099.43 (N-N), 3387.50 (-NH), 3256.94 (-NH₂). ¹H NMR (DMSO, ppm): δ = 8.211 (S, 1H.CH=N), 7.183-7.971 (m, 4H Ar -H), 8.119 (S, 1H.-NH), 8.242 (S, 2H.-NH₂). ¹³C NMR (DMSO, ppm): δ (C₁) = 140.77 (CH=N), 139.63 (C₂), 123.84 (C₃, C₄, C₆, C₇), 151.76 (C₅), 178.42(C=S). M/F = C₈H₉N₄O₂S. M/W = (224.23).

Table 2. The ultraviolet absorption maxima ($\lambda_{\max}$, nm), infrared absorptions (ν , cm^{-1}) and NMR chemical shifts (δ , ppm) of substituted (E)-2-benzylidenehydrazinecarbothioamides

ENTRY	X	UV($\lambda_{\max}$)nm	IR v cm^{-1} (CH=N)	¹ H CH=N(ppm)	¹³ C C=N(ppm)
1	H	309.50	1643.35	8.048	142.29
2	3-Br	318.00	1641.42	8.117	140.75
3	4-Br	317.50	1641.42	8.015	140.23
4	3-Cl	315.00	1685.79	8.041	140.55
5	4-Cl	316.50	1689.64	8.060	140.90
6	4-F	311.00	1638.43	8.051	140.39
7	4-OMe	320.50	1625.99	8.101	142.26
8	4-Me	313.50	1698.00	8.150	142.23
9	3-NO ₂	311.50	1643.35	8.041	140.03
10	4-NO ₂	322.00	1641.42	8.211	140.77

3. RESULTS AND DISCUSSION

3.1. CHARACTERIZATION: UV SPECTRUM

In the UV–visible spectra of the compound (fig-1) below 350 nm, a single peak is observed for the of substituted (*E*)-2-benzylidenehydrazinecarbothioamides of (hydrazone) transition band due to π - π^* at 309.00 λ_{max} (nm). The observed peak is assigned to π - π^* transition. According to the valence Band theory, as the conjugation increases, the energy difference between the highest occupied and the lowest unoccupied π -orbitals decreases and hence the wave length of the absorption band increases.

Fig:1 The UV spectrum of the (*E*)-2-benzylidenehydrazinecarbothioamide

3.2. IR SPECTRUM

The important IR frequencies (fig-2) of substituted (*E*)-2-benzylidenehydrazinecarbothioamides formed due to the condensation of thiosemaicarbazide with benzaldehyde are present in the table-2. A strong band is observed bending vibration for (*E*)-2-benzylidenehydrazinecarbothioamides of (CH=N) at around 1620 -1700 cm^{-1} characteristic of the azomethine. The sharp peak at 3220 cm^{-1} corresponds to N–H stretching and the broad absorption band at 3385 cm^{-1} corresponds to the NH_2 stretching. An absorption band was observed around the N–N stretching has been observed at 999 cm^{-1} .

Fig: 2 The IR spectrum of (*E*)-2-benzylidenehydrazinecarbothioamide

3.3. ^1H NMR SPECTRUM

The complete assignment of the ^1H NMR spectra is given here (fig-3). The spectrum was recorded at 400 MHz. The assignment is done on the basis of chemical shifts, multiplicities and coupling constants. The ^1H NMR spectrum (*E*)-2-benzylidenehydrazinecarbothioamide in $\text{DMSO}-d_6$ shows the one singlet for $\delta=8.048$ (S, 1H.CH=N), (ppm), 8.190 (S, 1H. -NH) and 7.982 (S, 2H.-NH₂) respectively. For this compound multiplet are observed at 7.387 – 7.801ppm which is assigned to protons (5 protons) of the aromatic rings.

Fig. 3 The ^1H NMR spectrum of (*E*)-2-benzylidenehydrazinecarbothioamide

4. Antimicrobial activities

In an urge to develop new antimicrobial compound, a number of hydrazones were tested for their antimicrobial activities because of the evolution of drug-resistant microbial pathogens. The fast resistance of bacteria against antibiotics has become a widespread medical problem. Treatment options for these infections are often limited, especially in debilitated and immune compromised patients. The dramatically rising incidence of multi-drug resistant microbial infections in the past few decades has become a serious health care problem. The search for new antimicrobial agents will consequently always remain as an important and challenging task for medicinal chemists. The treatment of bacterial and fungal infectious diseases remains a challenging problem because of the increasing number of multi-drug microbial pathogens [39-41]. Nowadays, the design of new compounds able to deal with resistant bacteria, having new structures and new targets of action, has become one of the most important areas in the antibacterial research purpose [42].

4.1. Antibacterial sensitivity assay

Antibacterial sensitivity assay was performed using Kirby-Bauer [43] disc diffusion technique. In each Petri plate about 0.5 mL of the test bacterial sample was spread uniformly over the solidified Mueller Hinton agar using sterile glass spreader. Then the discs with 5mm diameter made up of Whatmann No.1 filter paper, impregnated with the solution of the compound was placed on the medium using sterile forceps. The plates were incubated for 24 hours at 37 °C by keeping the plates upside down to prevent the collection of water droplets over the medium. After 24 hours, the plates were visually examined and the diameter values of the zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure. The antibacterial screening effect of the synthesized substituted (*E*)-2-benzylidenehydrazinecarbothioamide compounds is shown in **Fig. 4** Plates (1-10).

The antibacterial activities of all the synthesized substituted (*E*)-2-benzylidenehydrazinecarbothioamide compounds have been studied against three gram positive pathogenic strains *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus* and two gram negative strains *Escherichia coli* and *Pseudomonas aeruginosa* species. The disc diffusion

technique was followed using the Kirby–Bauer [43] method, at a concentration of 250 $\mu\text{g/mL}$ with Ciprofloxacin used as the standard drug. The zone of inhibition is compared using **Table 3** and the corresponding Clustered column Chart is shown in **Fig. 5**. The substituents parent (H), 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH₃, 3-NO₂ and 4-NO₂ showed good activities against *Bacillus subtilis* species. All the substituents of (*E*)-2-benzylidenehydrazinecarbothioamides compounds showed good antibacterial activity against *S. aureus*. The only one 4-Cl substituent has shown very good antibacterial activity against *Streptococcus*. The parent (H), 3-Br, 4-Br, 3-Cl, 4-F, 4-CH₃, 3-NO₂ and 4-NO₂ substituents have shown good antibacterial activity. All the substituents of (*E*)-2-benzylidenehydrazinecarbothioamides compounds showed good antibacterial activity against *E.coli* species. The parent (H), 3-Br, 4-Br, 3-Cl, 4-Cl, 4-F, 4-CH₃, 3-NO₂ and 4-NO₂ substituents have shown good antibacterial activity against *P.aeruginosa* species. The only one 4-OCH₃ substituted compound has shown moderate antibacterial activity against *P.aeruginosa* species.

Plate-1

Plate-2

Plate-3

Plate-4

Plate-5

Plate-6

Plate-7

Plate-8

Fig. 4 Antibacterial activity of substituted (*E*)-2-benzylidenehydrazinecarbothioamide compounds

Table3. Antibacterial activity of zone of inhibition values of substituted (*E*)-2-benzylidenehydrazinecarbothioamides

S.No.	Substituents	Zone of inhibition(mm)				
		Gram positive Bacteria			Gram negative Bacteria	
		<i>B.subtilis</i>	<i>S. aureus</i>	<i>Streptococcus</i>	<i>E.coli</i>	<i>P.aeruginosa</i>
1	H	10	10	12	10	12
2	3-Br	8	9	10	12	10
3	4-Br	12	13	13	13	14
4	3-Cl	13	12	15	14	13
5	4-Cl	15	14	18	16	15
6	4-F	13	10	10	12	13
7	4-OCH ₃	8	9	8	15	6
8	4-CH ₃	14	12	12	12	10
9	3-NO ₂	12	11	11	11	11
10	4-NO ₂	15	13	14	15	13
Standard	Ciprofloxacin	25	19	22	24	23
Control	DMSO	0	0	0	0	0

Fig. 5 Antibacterial activity of substituted (*E*)-2-benzylidenehydrazinecarbothioamide compounds - Clustered column chart

4.2. Antifungal sensitivity assay

Antifungal sensitivity assay was performed using Kirby-Bauer [43] disc diffusion technique. PDA medium was prepared and sterilized as above. It was poured (ear bearing heating condition) in the Petri-plate which was already filled with 1 ml of the fungal species. The plate was rotated clockwise and counter clock-wise for uniform spreading of the species. The discs were impregnated with the test solution.

The test solution was prepared by dissolving 15 mg of the hydrazone in 1ml of DMSO solvent. The medium was allowed to solidify and kept for 24 hours. Then the plates were visually examined and the diameter values of zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

Table4. Antifungal activity of Zone of inhibition values of substituted (*E*)-2-benzylidenehydrazinecarbothioamides

S. No.	Substituents	Zone of inhibition(mm)		
		<i>A. flavus</i>	<i>A. niger</i>	<i>T. viride</i>
1	H	8	11	9
2	3-Br	10	10	12
3	4-Br	12	8	10
4	3-Cl	8	9	9
5	4-Cl	8	15	12
6	4-F	10	8	12
7	4-OCH ₃	11	10	9
8	4-CH ₃	10	8	12
9	3-NO ₂	8	8	9
10	4-NO ₂	9	10	4
Standard	Ciprofloxacin	16	16	17
Control	DMSO	-	-	-

Fig. 6 Antifungal activity of substituted (*E*)-2-benzylidenehydrazinecarbothioamide compounds

Antifungal assay has been performed using Kirby–Bauer [43] disc diffusion technique. The antifungal activities of the entire substituted (*E*)-2-benzylidenehydrazinecarbothioamide have been studied and are shown in **Fig. 6** for Plates (1–4). The zone of inhibition values of the antifungal activities is given in **Table 5**. The clustered column chart was shown in **Fig. 7** and it reveals that the 4-Br and 4-OCH₃ compounds have shown good activity against *A. flavus*. The 3-Br and 4-Cl substituted compounds have shown excellent activity against *A. niger*. Also, 3-Br, 4-Br, 4-Cl, 4-F and 4-CH₃ compounds have shown good activity against *T. viride*. The remaining compounds have shown moderate activity against all the fungi.

Fig. 7 Antifungal activity of substituted (*E*)-2-benzylidenehydrazinecarbothioamide compounds (Clustered column chart)

5. CONCLUSIONS

The authors have been synthesised some substituted (*E*)-2-benzylidenehydrazinecarbothioamide compounds using condensation method. The antimicrobial activities of all the synthesized hydrazone compounds have been evaluated using Bauer-Kirby disc diffusion technique. Most of the synthesized compounds found to shown moderate antibacterial as well as antifungal activity. The 4-Cl substituted compound has shown good activity against *Spectrococcus* and *A. niger* species.

REFERENCE

- [1] French F.A., Blanz E.J., *Journal of medicinal Chemistry*. 9 (1966) 585-589
- [2] Idem, *Cancer Research*., 26 (1966) 1638.
- [3] Idem, *Ibid*, 28 (1968) 2419.
- [4] Agrawal K.C., Booth A.B., Sartorelli AC., *Journal of medicinal Chemistry*. 11 (1968) 700.
- [5] M.Mohan, N.S.Gupta, M.P.Gupta, A Kurnar, M.Kurnar, N.K.Jha, *Inorganica.Chimica Acta.*, 152 (1988) 25-36
- [6] Mohan M., Kurnar A., Kurnar M., Jha N.K., *Ibid*, 136 (1987) 65.
- [7] Ma T.S., Tien T.M., *Antibiotics and Chemotherapy*. 3 (1953) 491.
- [8] Abbert Q., *Nature*. 9 (1961) 370.
- [9] Price IM., *Federation Proceedings*., 20 (1961) 223.
- [10] Sacconi L., *Journal of the American Chemical Society* ., 74 (1952) 4503
- [11] Korolkovas A., Burckhalter IH., *Essentials of Medicinal Chemistry*, Interscience Publication. N.Y(1976).
- [12] Loncle C., Brunel J.M., Vidal N., Dherbomez M., Letourneux Y., *European journal of Medicinal Chemistry* 39 (2004) 1067-1071.
- [13] Papakonstantinou-Garoufalias S., Pouli N., Marakos P., Chytyroglou-Ladas A., *Farmaco*. 57 (2002) 973-977.
- [14] B. K. Kaymakcioglu and S. Rollas, *Farmaco*, 2002, 57, 595-599.
- [15] Maccari R., Ottana R., Vigorita M.G., *Bioorganic Medicinal Chemistry Letters*. 15 (2005) 2509-2513.
- [16] Cocco M.T., Congiu C., Onnis V., Pusceddu M.C., Schivo M.L., De Logu A., *European Journal of Medicinal Chemistry*. 34 (1999) 1071-1076.
- [17] Rando D.G., Sato D.N., Siqueira L., Malvezzi A., Leite C. Q. F., de Amaral A. T., Ferreira E. I., Tavares L.C., *Bioorgani and Medicinal Chemistry* 10 (2002)557-560.
- [18] Patole J., Shingnapurkar D., Padhye S., Ratledge C., *Bioorganic Medicinal Chemistry Letters* 6 (2006) 1514-1517.
- [19] Maiti A., Ghosh S., *Journal of Inorganic Biochemistry* 36 (1989) 131-139.
- [20] Badiger D.S., Hunoor R.S., Patil B.R., Vadavi R.S., Mangannavar C.V., Muchchandi I.S., Patil Y.P., Nethaji M., Gudasi K.B., *Inorganica Chimica Acta*. 384 (2012) 197-203.
- [21] Rollas S., Kucukguzel S. G., *Molecules*, 12 (2007) 1910-1939.
- [22] Sreeja P. B., Kurup M. R. P., Kishore A., Jasmin C., *Polyhedron*. 23 (2004) 575-581.
- [23] Patai S., *Interscience*, NewYork, (1970) 149-180.
- [24] Seleem HS., El-Inany GA., El-Shetary BA., Mousa MA., *Chemistry Central Journal*. 5 (2011) 1186

-
- [25] R. Narang, B. Narasimhan, S. Sharma, *Current Medicinal Chemistry* 19 (2012) 569-612.
- [26] Terzioğlu N., Gürsoy A., *European Journal of Medicinal Chemistry*. 38 (2003) 781-786.
- [27] Vicini P., Incerti M., Doytchinova I., La Colla P., Busonera B., Loddo R., *European Journal of Medicinal Chemistry* 41 (2006) 624-632.
- [28] Gürsoy A., Terzioğlu N., Otuk G., *European Journal of Medicinal Chemistry* 32 (1997) 753-757.
- [29] Ulusoy N., Gürsoy A., Otuk G., *Il Farmaco* 56 (2001) 947-952.
- [30] Rochlitz C.F., Damon L.E., Russi M.B., Geddes A., Cadman E.C., *Cancer Chemother. Pharmacol.* 21 (1988) 319-322.
- [31] Procopio G., Guadalupi V., Giganti M.O., Mariani L., Salvioni R., Nicolai N., Capone F., Valdagni R., Bajetta E., *BJU International*. 108 (2010) 223-228.
- [32] Shi W., Nacev B.A., Aftab B.T., Head S., Rudin C.M., Liu J.O., *Journal of Medicinal Chemistry* 54 (2011) 7363-7374.
- [33] Bhaskar V.H., Mohite P.B., Optoelectron J., *Biomedical Materials*. 2 (2010) 249-259.
- [34] Galmarini C.M., Mackey J.R., Dumontet C., *Leukemia*. 15 (2001) 875-890.
- [35] Ghannoum M.A., Louis B.R., Clin., *Microbiological Reviews*. 12 (1999) 501-517.
- [36] Kitaev Y.P., Buzykin B.I., Troepol'skaya T.V., Russ., *Chemical Reviews*. 39 (1970) 441-456.
- [37] Wu A.M., Senter P.D., *Nature Biotechnology* 23 (2005) 1137-1146.
- [38] Nataliya P., Belskaya., Wim Dehaen., Vasiliy Bakuleva A., *Arkivoc* 1 (2010) 275-332
- [39] Diana Camelia Nuța., Carmen Balotescu Chifiriuc., Alexandru Vasile Missir., Ileana Cornelia Chirița., Carmellina Daniela Badiceanu., *Farmacia*. 58(1) (2010) 38-45.
- [40] Omar K., Geronikaki A., Zoumpoulakis P., Camoutsis C., Sokovic M., Ciric A., Glamoclija., J., *Bioorganic Medicinal Chemistry* 18 (2010) 426-432.
- [41] Ioana Moș., Otilia Micle., Mihaela Zdranca., Mariana Mureșan., Laura Vicaș., *Farmacia*, 58(5) (2010) 637-645
- [42] Zani, F., Vicini, P., Incerti M., *European Journal of Medicinal Chemistry*., 39 (2004) 135-140.
- [43] Bauer A. W., Kirby W. M. M., Sherris J. C., Truck M., *American Journal of Clinical Pathology* 45 (1966) 493-496.