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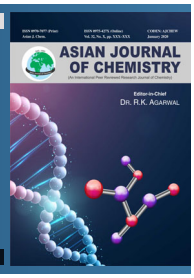

High Diffusivity Coefficient of Cyclic Voltammetric Behaviour of Semicarbazones and Thiosemicarbazones and their Antimicrobial Studies

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In this work, a series of *N*-hydroxy-2,6-diaryl-3-alkylpiperidin-4-one semicarbazones and thiosemicarbazones have been synthesized and also subjected towards cyclic voltammetric studies. The structure of reduced products was confirmed by FTIR, ^{1}H & ^{13}C NMR spectral analysis. Further products were also screened for antimicrobial activities and it shows high significant effect towards *Aspergillus niger* among the eight tested fungal species. The propency towards order of reduced products is observed sharply and it was established that irreversible reduction of two electron transfers has taken for all four synthesized compounds and highly pH dependent. Among the synthesized compound, thiosemicarbzones showed versatile features with high diffusion coefficient value in minimum power consumption of current function.

Keywords: *N*-Hydroxypiperdone, Semicarbazones, Thiosemicarbazones, Cyclic voltammetry, Diffusivity coefficient.

INTRODUCTION

Piperidine compounds comprises the biggest class of active heterocyclic groups, provoke its excellent functions in both 3- or 5- position of alkyl substitutions and 2- or 6-positions aryl substitutions, respectively [1-3]. Piperidin-4-one is simple, rapid and precursor among the most dominant natural bioactive elements with high potent of biological properties. It is reported that both semicarbazones and thiosemicarbazones are generously associated in electrochemical behaviour concepts and posses a wider scope in pharmacological applications with large complexion properties in high end analytical response [2-5]. Several analytical methods were also employed to select an active components from its mixtures through clinical and analytical analyzers like spectrophotometric, gravimetric, calorimetric methods, adaptive standard titrations, polaraographic and mass spectral analysis [4-6]. It is also revealed that the piperidin-4-ones showed an immense interest in anti-therapeutically activity to act as bioactive element. In addition various studies were examined scrupulously and extended their research contribution in formulation of drug ingredients. Thus, the drug have been synthesized and developed periodically to bring out the quality products under specified production rate of limited intermediate products [5-7].

Some reports [8-10] also established the essential presence of nitrogen compound in the aryl moeity for the phosphorous detector measurements as accuracy and product confirmation separator. In owing to natural and synthetic pharmaceutical applications, where piperidine derivatives were one of the active materials to act drug resistance and well documented in cure of wide variety infectious diseases [11,12]. Further piperidine scaffolds and its derivatives exhibits numerous biological applications and attracted towards anti-therapeutic, anti-arrhythmic, antiviral, anti-tumour, anti-inflammatory and antiseptics [13-16].

In few reports [16-19], it was stated that blocking of its C-4 positions also shown antimicrobial activity with quite interesting and provoking conformational features. The present

work envisage the comparative study of cyclic voltammetric effects on 2,6-diarylpiperidin-4-one semicarbazones and thiosemicarbazones. It is confirmed that no such existence of comparative analysis of the studied compound are reported so far and its characterization by FTIR, ^{1}H & ^{13}C NMR. Thus, in present work, a series of *N*-hydroxypiperidine semicarbazones as well as thiosemicarbazones have been synthesized and extended to subject in voltammetric studies at graphite electrode surface were carried out in the potential range of 100, 150 and 200 mV s^{-1} of mixed solvent as reported in earlier literature [16]. The work also extended to examine the redox conditions and its effect of diffusivity measurement and varied kinetic mechanism like charge transfer coefficient, diffusion coefficient and rate constant. The eight different test species were utilized for the screening of antimicrobial activities of synthesized compounds and their reduced products of cyclic voltammetric were also analyzed.

EXPERIMENTAL

Reagents and solvents were purchased from S.D. Fine, Qualigens & Merck with AR grade of purity more than 95%. These were used as such for the work without any further purification [10]. The atmospheric pressure and temperature under 40 ºC were maintained for the synthesis of the title compound. The semicarbazones and thiosemicarbazones were obtained at a refluxing temperature of 60 ºC [20,21]. The melting points were measured with open capillaries and are uncorrected. The yields of the product were calculated after purification by column chromatography.

Synthesis of *N*-hydroxy-3-alkyl-2,6-diarylpiperidin-4-one semicarbazones (2a-b) and *N*-hydroxy-3-alkyl-2,6-diarylpiperidin-4-one thiosemicarbazones (2c-d): The respective title compound of both the semicarbazone and thiosemicarbazone were synthesized in a similar method as reported in earlier [16]. The corresponding solids were filtered by Whatman filter no. 1 and washed thoroughly by absolute ethonol and dried *in vacco*. The physico-chemical analysis of the synthesized compounds are given in Table-1.

Electrochemical study: The cyclic voltammetric studies were carried out by the similar step up as reported earlier [6-9] with three different combination electrode. The working electrode potential was fixed slightly higher than that required for microelectrolysis. Through the passing of nitrogen gas for 5 min and then corresponding voltammogram data was recorded [21-24].

Media preparation: By using the *in vitro* disc diffusion technique proposed by Sabouraud, dextrose agar was employed to culture fungi maintained using periodic subcultures. The medium was created using inocula by adding 1 mL of the dilute test organism culture. The corresponding hydrochloride of thiosemicarbazones and semicarbazones (10 mg/mL stock solution) having various concentrations (100, 200 and 300 ppm) were dissolved in water. Ketoconazole, a standard antifungal drug, showed a 18 ± 5 mm inhibition zone against all the analyzed fungi for 250 µg/mL concentration.

RESULTS AND DISCUSSION

Chemistry: In the presence of sodium acetate, semicarbazide and *N*-hydroxy-3-ethyl-2,6-diarylpiperidin-4-one were refluxed for 2 h to obtain piperidin-4-one semicarbazones (**2a** and **2b**). Similarly, *N*-hydroxy-2,6-diarylpiperidin-4-one thiosemicarbazones were obtained (**2c** and **2d**). Subsequently, thiosemicarbazones (**2c** and **2d**) and semicarbazones (**2a** and **2b**) were subjected to CV analyses.

Cyclic voltammetry: The more negative values were observed because of geometry distortion demarcation on redox properties, where peaks depended on the scan rate; however the numbers of peaks were the same [21]. These results indicated irreversible two-electron transfer for the electroreduction of thiosemicarbazone and semicarbazone moieties [22-24]. Several oxidation kinetics, comprehensive investigations of azo groups, and synthesis have been reported to promote applications in various field [25-29]. With the irreversible reduction, higher shifts in the negative potential have occurred in *N*-hydroxy-3-alkyl-2,6-diphenylpiperidin-4-one thiosemicarbazones and semicarbazones [27]. The peak currents for thiosemicarbazone and semicarbazone compounds were higher because the reduction potential shifted to a more negative value. The presence of active thionyl and carbonyl groups in the synthesized thiosemicarbazone and semicarbazone compounds resulted in a shift of the potential peak towards a highly negative value. Hence, in semicarbazones, compared with aromatic substitution, heterocyclic substitution leads to a decrease in the anodic peak current [25].

NMR analysis: The structure of the compounds **3a-d** was confirmed by comparing the spectral characterization data of the compounds **2a-d** [23-26]. In ^{1}H NMR spectra of compounds **3a-d**, two new signals appeared corresponding to the shift in carbonyl and thionyl effects as a signal of methine proton. The ^{13}C NMR showed the disappearance of signal due to carbonyl and thionyl group and a new signal due to formation of C-OH and C-SH carbon owing to the reduction of carbonyl and thionyl group [29,30].

***N*-Hydroxy-3-ethyl-2,6-*bis*(*p*-methylphenyl)piperidin-4-one semicarbazone (2a):** ^{1}H NMR (DMSO-d_6) (δ ppm): 2.19-2.33 (m, 3H, 5H), 8.91 (s, NH_2), 4.11 (s, N-OH), 3.63-3.68

TABLE-1
ELEMENTAL ANALYSIS OF THE TITLE COMPOUND **3a-d**

Compound	m.w. (Kg/Kmol)	Yield (%)	Elemental analysis (%): Found (calcd.)		
			C	H	N
3a	196	74	73.09 (77.11)	6.24 (6.99)	7.11 (7.84)
3b	215	69	71.56 (77.00)	8.52 (8.17)	8.65 (9.01)
3c	202	77	67.07 (69.06)	6.44 (6.79)	7.37 (7.97)
3d	241	75	71.56 (73.00)	8.52 (8.17)	8.65 (9.01)

(d, 2H, 6H), 7.22-7.55 (s,ph), 1.12 (d, 3×CH_3), 1.56-1.69 (d, 3×CH_2), 2.15 (s, NH); ^{13}C NMR (δ ppm): 72.63 (2C, 6C), 161.85 (s, CO), 123.42-127.48 (Ph), 45.52 (3C, 5C), 15.5 (3-CH_3), 18.6 (3×CH_2).

Amino-(2-(*N*-hydroxy-3-ethyl-2,6-*bis*(*p*-methyl)-piperidin-4-one ylidene)hydrazinyl)methanol (3a): The structure of the reduced product **3a** was established by comparing the ^{1}H & ^{13}C NMR data with compound **2a**. ^{1}H NMR (DMSO-d_6) (δ ppm): 7.58-7.87 (s, Ph), 4.21-4.45 (d, 2H, 6H), 8.42 (s, NH_2), 4.25 (s, N-OH), 2.65-2.89 (m, 3H, 5H), 1.42 (d, 3×CH_3), 1.89-1.95 (d, 3×CH_2), 1.33 (s, NH); ^{13}C NMR (δ ppm): 62.56 (2C, 6C), 91.57 (s, -COH), 124.75-125.27 (Ph), 52.67 (3C, 5C), 11.67 (3×CH_3), 21.63 (3-CH_2).

It was observed that the multiplet peak around δ 2.69 ppm and a new singlet as around δ 3.43 ppm for the compound **3a**, a signal at δ 91.57 ppm was appeared, which assigned to C-OH, while the carbonyl group disappeared.

***N*-Hydroxy-2,6-*bis*(*p*-methylphenyl)-3-isopropylpiperidin-4-one semicarbazone (2b):** ^{1}H NMR (DMSO-d_6) (δ ppm): 3.84 (s, N-OH), 2.26-2.34 (m, 3H, 5H), 7.23-7.54 (s, Ph), 3.23-3.56 (d, 2H, 6H), 8.12 (s, NH_2), 1.67 (d, 3×CH_3), 1.56-1.68 (d, 3×CH_2), 1.81 (s, NH); ^{13}C NMR (δ ppm): 76.92 (2C, 6C), 161.39 (s, -CO), 122.66-128.20 (Ph), 42.02 (3C, 5C), 9.87 (3×CH_3), 18.4 (3×CH_2).

Amino(2-(*N*-hydroxy-3-isopropyl-2,6-*bis*(*p*-methylphenyl)piperidin-4-one ylidene)hydrazinyl)methanol (3b): The structure of the reduced product **3b** was established by comparing the ^{1}H & ^{13}C NMR data of compound **2b**. ^{1}H NMR (DMSO-d_6) (δ ppm): 4.31 (s, N-OH), 4.35-4.57 (d, 2H, 6H), 2.61-2.91 (m, 3H, 5H), 7.34-7.49 (s, Ph), 7.65 (s, NH_2), 1.23 (d, 3×CH_3), 1.38-1.43 (d, 3-CH_2), 1.32 (s, NH); ^{13}C NMR (δ ppm): 63.87 (2C, 6C), 90.13 (s, -CSH), 126.65-128.62 (Ph), 50.60 (3C, 5C), 11.22 (3×(CH_3)$_2$), 21.56 (3×CH_2).

By the appearance of a new singlet around δ 3.71 ppm and the multiplet at δ 2.41 ppm was assigned to the methine proton, a new signal at δ 90.13 ppm appeared is assigned to C-OH, while the carbonyl group disappeared.

***N*-Hydroxy-3-ethyl-2,6-*bis*(*p*-methylphenyl)piperidin-4-one thiosemicarbazone (2c):** ^{1}H NMR (DMSO-d_6) (δ ppm): 3.68-3.79 (d, 2H, 6H), 4.16 (s, N-OH), 2.78-2.89 (m, 3H, 5H), 7.23-7.31 (s, Ph), 8.69 (s, NH_2), 1.52 (d, 3×CH_3), 1.89-1.98 (d, 3×CH_2), 2.09 (s, NH); ^{13}C NMR (δ ppm): 76.89 (2C, 6C), 162.76 (s, -CS), 126.86-128.90 (Ph), 41.17 (3C, 5C), 9.76 (3×CH_3), 17.67 (3×CH_2).

Amino-(2-(*N*-hydroxy-3-ethyl-2,6-*bis*(*p*-methylphenyl)-piperidin-4-one ylidene)hydrazinyl)thiol (3c): The structure of the reduced product **3c** was established by comparing the ^{1}H & ^{13}C NMR data of compound **2c**. ^{1}H NMR (DMSO-d_6) (δ ppm): 2.89-3.52 (m, 3H, 5H), 4.25 (s, N-OH), 7.32-7.37(s, Ph), 7.82(s,NH_2), 1.26(d, 3×CH_3), 3.72-3.79 (d, 2H, 6H), 1.37-1.43 (d, 3×CH_2), 2.12 (s, NH), 2.33(*m*-CH); ^{13}C NMR (δ ppm): 62.45 (2C, 6C), 89.61 (s, -CSH), 126.8-129.1 (Ph), 52.39 (3C, 5C), 11.57 (3×CH_3), 22.63 (3×CH_2).

The formation of singlet and multiplet peak around δ 3.58 and 2.23 ppm assigned to the methine proton of compound **3c**, whereas a signal at δ 89.61 ppm was assigned to C-SH and the thionyl group got disappeared.

***N*-Hydroxy-2,6-*bis*(*p*-methylphenyl)-3-isopropyl-piperidin-4-one thiosemicarbazone (2d):** ^{1}H NMR (DMSO-d_6) (δ ppm): 2.87-2.89 (m, 3H, 5H), 7.37-7.41 (s, Ph), 8.54 (s, NH_2), 4.14 (s, N-OH), 1.39 (d, 3×C_3H_5), 3.74-3.79 (d, 2H, 6H), 2.12 (s, NH); ^{13}C NMR (δ ppm): 72.70 (2C, 6C), 161.29 (s, -CS), 123.58-126.78 (Ph), 15.67-21.71 (3,5-C_3H_7), 42.22 (3C, 5C).

Amino-(2-(*N*-hydroxy-3-isopropyl-2,6-*bis*(*p*-methyl-phenyl)piperidin-4-one ylidene)hydrazinyl)thiol (3d): The structure of the reduced product **3d** was established by comparing the ^{1}H & ^{13}C NMR data of compound **2d**. ^{1}H NMR (DMSO-d_6) (δ ppm): 3.79 (s, N-OH), 3.27-4.09 (d, 2H, 6H), 2.66-2.75 (m, 3H, 5H), 7.37-7.46 (s, Ph), 8.31(s, NH_2), 2.55 (m,C-H), 2.23 (d, 3×CH_3, 5×CH_3), 3.79 (s, C-SH), 2.19 (d, NH); ^{13}C NMR (δ ppm): 61.30 (2C, 6C), 80.67 (s, -C-SH), 123.09-127.60 (Ph), 15.91-16.51 (3×C_3H_7), 51.6 (3C, 5C).

A new singlet as around δ 3.69 ppm and the multiplet as around δ 2.65 ppm, there appeared a signal at δ 80.67 ppm assigned to C-SH and the thionyl group got disappeared.

Electrochemical behaviour: Compound **2a** having a potential range of -2200 to 2200 mV exhibited three cathodic and two anodic peaks in the forward and reverse scan, respectively. Thus, an irreversible reduction mechanism was observed during the shifts of potential values (Table-2). When the reaction occurred under stronger acidic conditions, the shift was higher. Although the number of crossing levels increased, the number of peaks did not vary. Hence, at the site of active carbonyl group of the selected compound **3a**, a highly irreversible redox path was observed. Compound **3b** showed a behaviour similar to compound **3a** but at single turn over, only one cathodic peak shifted to produce a novel compound under the same scan rate redox reaction. The degree significance of influence was **3a > 3b**.

Fig. 1d presents the voltammogram of compounds **3c** and **3d**. Compounds **3c** and **3d** exhibited one negative at the stop-crossing level. With an increase in the scan rate, the peak potential shifted towards a more negative value. The shift was higher when the reaction was performed under stronger acidic conditions.

TABLE-2
CYCLIC VOLTAMMOGRAM DATA OF 0.01 M *n*-HYDROXY-3-ETHYL-2,6-DIPHENYLPIPERIDIN-4-ONESEMICARBAZONE (**2a-b**) AND THIOSEMICARBAZONE (**2c-d**) DISSOLVED WITH 0.1 M OF ETHANOL AND DISTILLED WATER AT 200 mV s^{-1}

Compd.	100 mV s^{-1}					150 mV s^{-1}					200 mV s^{-1}				
	Epc_1 (V)	Epc_2 (V)	Epc_3 (V)	EpA_1 (V)	EpA_2 (V)	Epc_1 (V)	Epc_2 (V)	Epc_3 (V)	EpA_1 (V)	EpA_2 (V)	Epc_1 (V)	Epc_2 (V)	Epc_3 (V)	EpA_1 (V)	EpA_2 (V)
3a	0.2	-0.6	-1.6	-1.2	0.1	0.4	0.61	-1.81	-1.56	0.12	0.4	0.61	-1.82	-1.47	0.3
3b	-0.591	–	–	–	–	-0.752	–	–	–	–	-0.812	–	–	–	–
3c	-0.621	–	–	–	–	-0.742	–	–	–	–	-0.928	–	–	–	–
3d	-0.549	–	–	–	–	-0.692	–	–	–	–	-0.736	–	–	–	–

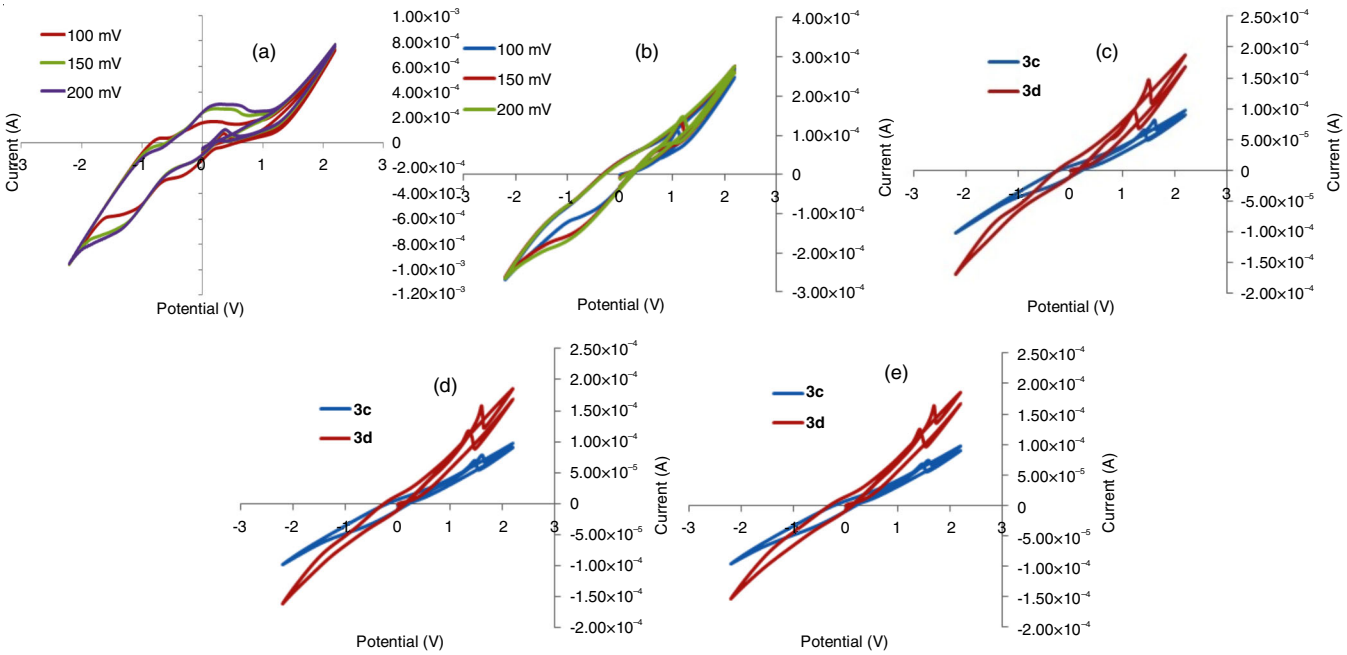

Fig. 1. Cyclic voltammogram of reduced product of 0.01 M *n*-hydroxy-3-ethyl-2,6-diphenylpiperidin-4-one semicarbazone (**2a-b**) and thiosemicarbazone (**2c-d**) dissolved with 0.1 M of ethanol and distilled water at different scan rate

The number of crossing levels increased; the number of peaks did not vary. The influence of both mechanisms of compounds **3c** and **3d** on the reduction that occurred was as follows: the order of the ease of product formation: **3c** > **3d**.

Compounds underwent an irreversible redox mechanism with larger potential shifts only when the reaction was conducted under mild acidic conditions in an electrolytic bath of the mixed solution of 0.1 M ethanol, title compound and distilled water. The potential shift and current peak increased linearly. The current function ($I_{pc}/v^{1/2}$) remained sustained during the electrode process under controlled diffusion. Compounds **2a-d** were observed and did not vary [28]. All the compounds were subjected to electrochemical reduction and proved that the mechanism of irreversible reduction occurs at both the active thionyl and carbonyl groups in the dual electron-transfer mode. For voltammetric response, the typical reaction and its corresponding cathodic potential increased with an increase in the scan rate. The mass transfer rate was considerably low for electron transfer on the reaction surface. Compared with an increase in the scan rate, the calculated diffusion coefficients exhibited distinct values (Table-3). For all compounds, the kinetic parameters, charge transfer coefficient ($D_o^{1/2}$) and diffusion coefficient were evaluated using the following reduction equations:

$$\left|E_p - E_{P/2}\right| = \frac{1.85RT}{F\alpha_n} = \left(\frac{47.7}{\alpha_n}\right)mV \quad (1)$$

$$I_p = 3.01 \times 10^5 \times n(\alpha_n)^{1/2} A C D_0^{1/2} v^{1/2} \quad (2)$$

The results indicated that the degree of irreversibility enhanced in the following order: **3c** > **3d** > **3a** > **3b**. The cyclic voltammetric data showed that the scan rate was dependent on ΔE_p values, which confirmed that hydrogen transfer occurs at electron deficient thionyl sites.

Antifungal activity: Thiosemicarbazones and semicarbazones (**3a-d**) were screened for their antifungal activity. Fungal strains, namely *Microsporum gypseum*, *Aspergillus niger*, *Candida albicans*, *Mucor*, *Aspergillus flavus* and *Rhizopus*, were studied using disc diffusion method. Fig. 2 illustrates the

TABLE-3
CV VALUES OF THE COMPOUND **3a-d** WITH VARIABLE SCAN RATE

Compound	Scan rate (mV/s)	E_{pc} (mV)	I_{pc} (μA)	$E_{p/2}$ (mV)	$I_{pc}/v^{1/2}$	α_n	$D_o^{1/2} \times 10^{-5}$ cm^2 s^{-1}
3a	100	-225	0.33	-120	0.023	0.396	1.4
	150	-208	0.38	-109	0.031	0.437	1.1
	200	-211	0.76	-101	0.050	0.472	2.6
3b	100	-221	3.89	-101	0.289	0.472	11.7
	150	-170	4.55	-80	0.360	0.596	11.9
	200	-136	6.18	-63	0.475	0.757	12.9
3c	100	-247	0.33	-120	0.028	0.398	1.7
	150	-229	0.47	-113	0.033	0.422	1.9
	200	-218	0.51	-107	0.039	0.445	1.6
3d	100	-241	0.38	-123	0.041	0.472	1.5
	150	-219	0.41	-102	0.023	0.433	1.43
	200	-211	0.87	-117	0.038	0.610	1.28

antifungal activity of the synthesized products. All values are the average obtained from three determinations. Compounds **3a**, **3b** and **3d** were slightly to moderately active against all fungi; however, compound **3a** is more active against *Aspergillus niger* than the other tested fungi (Fig. 2).

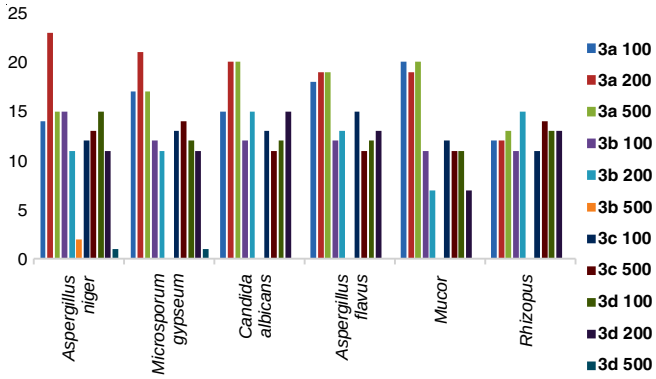

Fig. 2. *in vitro* Zone of inhibition profile of the *N*-hydroxy piperidone semicarbazone (**3a-b**) and thiosemicarbazone (**3c-d**) against tested fungi

Conclusion

The synthesized products undergo an irreversible reduction mechanism. The diffusion coefficient values and voltammetric data obtained in electrochemical studies for all compounds are acceptable. Studies were conducted under mild acidic conditions with a minimal working current of μA. For different alkyl-substituted compounds, the degree of irreversibility exhibited the following order: **3a > 3b > 3d > 3c**. Kinematic values such as diffusion coefficient ($D_o^{1/2}$) and charge transfer coefficient (α_n) of *N*-hydroxy-2,6-diaryl-3-alkylpiperidin-4-one semicarbazones and thiosemicarbazones were discussed. Compound **3a**, amino-(2-(*N*-hydroxy-3-ethyl-2,6-*bis*(*p*-methylphenyl)-piperidin-4-one-ylidene)hydrazinyl)methanol exhibited the highest inhibition against *A. niger* among all the compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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