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ANTIOXIDANTS AND ANTIFUNGAL ACTIVITIES OF SUBSTITUTED GUANIDINES AND THEIR COPPER COMPLEXES

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Abstract: A series of guanidines and their copper (II) complexes were investigated for their radical scavenging activity including peroxyl radicals (ROO·), superoxide anion ($O_2^{\cdot-}$), hydroxyl (·OH), and reactive hydrogen peroxide (H_2O_2) species. Among the Cu(II) complexes, Cu-MR-9-2 shows the highest, Cu-MR-9-3, Cu-MR-9-6 less and Cu-MR-9-1 least antioxidant potential. The Cu(II) complexes show better Fe^{2+}-chelating activity than that of ligands. Among the Cu(II) complexes Cu-MR-9-2 was found to have the highest, Cu-MR-9-6 moderate, MR-9-3 less and Cu-MR-9-1 least ferric reducing capacity. The IC_{50} values for ligands (MR-9-1, MR-9-2, MR-9-3, MR-9-6) were determined to be 197.53 ± 7.13, 189.07 ± 7.34, 207.98 ± 6.78 and 233.38 ± 6.37 µM, which showed lower antioxidant activity than their Cu(II) complexes. The IC_{50} values for ascorbic acid were found to be 51.60 ± 13.18 µM. The Cu(II) metal compounds (Cu-MR-9-1,Cu-MR-9-2, Cu-MR-9-3 and Cu-MR-9-6) were detected to be the most powerful scavengers of the hydroxyl radical with IC_{50} up to 108.03 ± 11.34 µM, 101.41 ± 12.10 µM, 90.59 ± 11.53 µM and 88.86 ± 13.16 µM, respectively.

Keywords: bioactivity of synthesized ligands and their complexes, antioxidants and antifungal, assays

Free radicals are reactive oxygen species (ROS), include peroxyl radicals ($ROO^{\bullet}$), superoxide anion ($O_2^{\bullet-}$), hydroxyl ($^{\bullet}OH$) and reactive hydrogen peroxide (H_2O_2) while reactive nitrogen species (RNS), include nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$) and peroxy nitrite anion ($ONOO^{\bullet}$) generated in the body either from the environment or during normal metabolic activities (1-3).

In living systems, stimulation of macrophages, leucocytes and aerobic respiration have endogenous sources, while the tobacco smoke, pollutants, ionizing radiations, organic solvents and pesticides are the major exogenous sources of free radicals production (4, 5). Excess production of these free radicals have a great impact on humans in the etiology of various diseases like cancer, cardiovascular diseases, liver injury (6), neurodegenerative, diabetes, rheumatism diseases (7), atherosclerosis (8), autoimmune disorders, aging (9), ischemia, asthma, anemia, arthritis, mongolism and Parkinson diseases (10-13). Although the body possesses defense mechanisms as antioxidant nutrients and enzymes, which arrest the damaging properties of free radicals (14, 15), continuous exposure to chemicals and contaminants may increase the amount of free radicals in the body beyond its ability to control and cause irreversible oxidative damages (16).

Therefore, antioxidants with free radical scavenging potential may be relevant in the therapeutic and preventions of diseases where free radicals are implicated (17). In addition to natural antioxidants such as vitamin C, vitamin E, carotenoids and flavonoids (18), a number of synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone have been prepared and their antioxidant capacity has been assessed for prevention of various diseases (19, 20). They were evaluated for having the possible antioxidant properties *in vitro*. *Alternaria brassicicola* is a well-known species of fungus which causes leaf blight to brassicas. The symptoms are the appearance of pin spot which cov-

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ered the photosynthetic area of the leaves leads to the reduction in productions of crops (21).

EXPERIMENTAL

Chemicals

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), ascorbic acid, ethylenediaminetetraacetic acid (EDTA), ferrous sulfate, Tris HCl buffer, ferric chloride ($FeCl_3$), o-phenanthroline, sulfuric acid, ammonium molybdate, potassium (mono- and di-) phosphate), hydrogen peroxide (H_2O_2), agar gel, ethanol were of analytical grade and were purchased from Sigma Aldrich, Pakistan.

Several selected methods were used to test the potency of the guanidine ligands and their copper(II) complexes because of the availability of the required materials and equipment in the lab so all determinatons were carried with full efficiency and observation led to excellent results in short time.

DPPH radical scavenging assay

The antioxidant activity of guanidines and their copper(II) complexes were assessed using the stable DPPH free radical according to Shanab et al. (22). Various concentrations (12.5, 25, 50, 100 and 200 µM) of guanidines and their copper(II) complexes were mixed with an ethanolic solution containing 510 µL of 85 µM DPPH radical. The mixture solutions were incubated for 30 min at room temperature and the decrease in absorbance was measured at 518 nm using an UV spectrophotometer. Ascorbic acid at the same concentrations as drugs was used as a positive control. The experiment was carried out in triplicate. Percentage inhibition of the drugs as well as ascorbic acid was calculated by using the following formula:

DPPH inhibition effect (%) = $(A_c - A_s / A_c) \times 100$

where A_c = absorbance reading of the control, A_s = absorbance reading of the sample.

Ferrous ion-chelating assay

The ferrous ion chelating activity of guanidines and their copper(II) complexes was evaluated by a standard method of Puntel et al. (23). Various concentrations (12.5, 25, 50, 100 and 200 µM) of guanidines and their copper(II) complexes were mixed with 0.2 mL of 3.6 mM ferrous sulfate, 0.3 mL of 100 mM Tris-HCl (pH = 7.4), 0.1 mL of 9 mM o-phenanthroline and diluted up to 3.0 mL with ultrapure distiled water. The reaction mixture was shaken vigorously, incubated for 10 min and the decrease in absorbance was determined at 510 nm. EDTA (ethylenediaminetetraacetic acid) at the same concentrations was utilized as a reference standard and sample without compounds complexes mixture served as control. The Fe^{2+} chelating capacity was calculated by using the following formula:

Chelating effect (%) = $(A_c - A_s / A_c) \times 100$

where A_c = absorbance reading of the control; A_s = absorbance reading of the sample.

Ferric reducing / antioxidant power assay

The ferric reducing power of the guanidines and their copper(II) complexes was determined as described by Kumar et al. (24). Different concentrations (12.5, 25, 50, 100 and 200 µM) of guanidines and their copper(II) complexes, 0.2 mL of 3.6 mM ferric chloride, 0.3 mL of 100 mM tris buffer (pH = 7.4), 0.1 mL of 9 mM o-phenanthroline and diluted up to 3.0 mL with ultra-pure distilled water was shaken vigorously and left to stand at room temperature for 10 min. The increase in absorbance of the sample solution was measured at 510 nm using the UV spectrophotometer. Ascorbic acid at the same concentrations was utilized as a reference standard and sample without compounds mixture served as control. The reducing power comparable with ascorbic acid was calculated by using the following formula:

Reducing power (%) = $(A_s - A_c / A_s) \times 100$

where A_c = absorbance reading of the control, $\mathbf{A_s}$ = absorbance reading of the sample.

Total antioxidant activity (phosphomolybdenum assay)

The total antioxidant capacity of guanidines and their copper(II) complexes were evaluated by phosphomolybdenum assay assessed by Sahaa et al. (25). Reagent solution containing various concentrations (12.5, 25, 50, 100 and 200 µM) of guanidines and their copper(II) complexes aliquot in ethanol, 0.7 mL of 0.6 M sulfuric acid, 1.0 mM ammonium molybdate, 1.0 mL of 28 mM potassium phosphate and ultra pure distilled water was incubated at 95°C for 90 min. After cooling to room temperature, the increase in absorbance of the mixture was measured at 695 nm using UV spectrophotometer. Ascorbic acid was utilized as reference standard and sample without compounds mixture served as control. The reducing power of drugs as well as ascorbic acid was calculated by using the following formula:

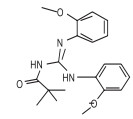
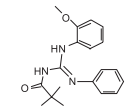
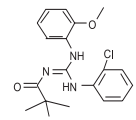
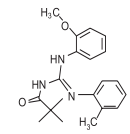
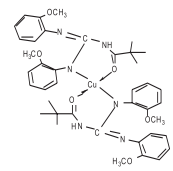
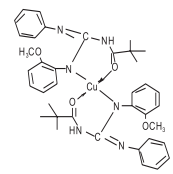
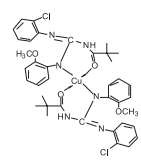
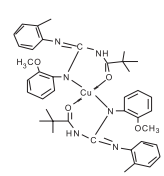
Reducing power (%) = $(A_s - A_c / A_s) \times 100$

where A_s = absorbance reading of the control, A_c = Absorbance reading of the sample.

Hydroxyl radical scavenging activity

The scavenging activity of hydroxyl radicals was measured with Fenton reaction described by Huo et al.

Table 1. Names and structures of the investigated ligands.

Comp. No.	Code	Name	Structures
1	MR-9-1	*N*-pivaloyl-*N′*,*N*''-bis-(2-methoxyphenyl)guanidine	
2	MR-9-2	*N*-pivaloyl-*N*'-(2-methoxyphenyl)-*N*''-phenylguanidine	
3	MR-9-3	*N*-pivaloyl-*N*'-(2-methoxyphenyl)-*N*''-(2-chlorophenyl)-guanidine	
4	MR-9-6	*N*-pivaloyl-*N*'-(2-methoxyphenyl)-*N*''-(2-tolyl)guanidine	
1a	Cu-MR-9-1	Bis(*N*-pivaloyl-*N*',*N*''-bis-(2-methoxyphenyl) guanidinato)-Cu(II)	
2a	Cu-MR-9-2	Bis(*N*-pivaloyl-*N*'-(2-methoxyphenyl)-*N*''-phenylguanidinato)-Cu(II)	
3a	Cu-MR-9-3	Bis(*N*-pivaloyl-*N*'-(2-methoxyphenyl)-] *N*''-(2-chlorophenyl)-guanidinato)-Cu(II)	
4a	Cu-MR-9-6	Bis(*N*-pivaloyl-*N*'-(2-methoxyphenyl)-*N*''-(2-toluyl)guanidinato)-Cu(II)	

(26). Reaction mixtures of various concentrations (12.5, 25, 50, 100 and 200 µM) of guanidines and their copper(II) complexes, 0.1 mL of 7.5 mM o-phenanthroline, 0.5 mL of 0.2 M phosphate buffer (pH 6.6), 0.1 mL of 7.5 mM ferrous sulfate and 0.1 mL of H_2O_2 (0.1%) were diluted up to 3 mL with distilled water. The reaction mixtures were incubated at room temperature for 30 min and the absorbance was measured at 510 nm using UV spectrophotometer. The reaction mixture without compounds complexes has been used as control and samples without compounds complexes and H_2O_2 served as a blank. The DPPH radical scavenging activity of compounds complexes and ascorbic acid were calculated using the following furmula:

Scavenging power (%) = $(A_s - A_c / A_b - A_s) \times 100$

where: A_s = absorbance reading of the sample, A_c = absorbance reading of the control, A_b = absorbance reading of the blank.

Antifungal activity

Method: agar well diffusion

Prepared was the stock solution of concentration 20000 ppm. The samples were incubated for 7 days. Sample Cu-MR- 9-1 showed slower growth (2.5 cm), Cu-MR- 9-6 showed 2.2 cm, Cu-MR- 9-3 showed 2.5 cm growth while sample MR-9-1 showed 4 cm, MR- 9-6 showed 2.07 cm, MR- 9- 3 showed 4 cm and MR-9-2 showed 2.12 cm growth. *A. brassicicola* showed 1.7 cm growth in standard (Mancozeb) plate and 4 cm growth in negative control plate. Standard showed 57.5% growth inhibition, sample Cu-MR-9-1 showed 37.5%, Cu-MR- 9-6 showed 45%, Cu-MR- 9-3 showed 37.5%, MR-9-1 showed 0%, MR- 9-6 showed 48.25%, MR-9-3 showed 0% and MR-9-2 showed 47% inhibition in growth against *A. brassicicola*. For the % inhibition the following formula was used:

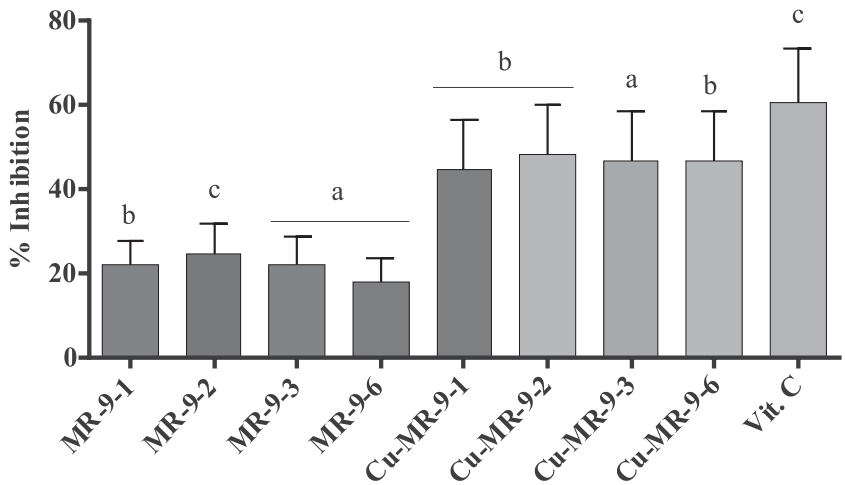


Figure 1. DPPH radical scavenging activity of guanidine ligands (**1** – **4**), their Cu(II) complexes (**1a** – **4a**) and Vit. C. Significance: $^{a}p < 0.0099$, $^{b}p < 0.0192$ and $^{c}p < 0.0226$

Table 2. DPPH radical scavenging activity of guanidine ligands (**1**-**4**), their Cu(II) complexes (**1a**-**4a**) and Vit. C.

Compound	% Inhibition (n = 3)					IC_{50} (µM) ± SEM
	12.5 µM	25 µM	50 µM	100 µM	200 µM	
1	5.270	12.776	21.860	30.763	39.136	245.91 ± 6.064
2	7.006	12.429	26.086	35.058	44.018	211.42 ± 6.877
3	6.732	13.299	20.108	29.705	42.098	232.71 ± 6.225
4	4.458	9.093	16.304	25.372	35.784	275.25 ± 5.639
1a	14.942	24.845	43.124	62.721	78.921	94.040 ± 11.79
2a	17.618	29.506	46.672	65.920	82.690	82.125 ± 11.81
3a	18.855	28.411	42.577	64.223	80.724	87.008 ± 11.38
4a	15.932	27.811	45.988	64.732	79.672	87.402 ± 11.65
Vit. C	24.951	40.611	62.899	77.641	96.997	47.188 ± 12.83

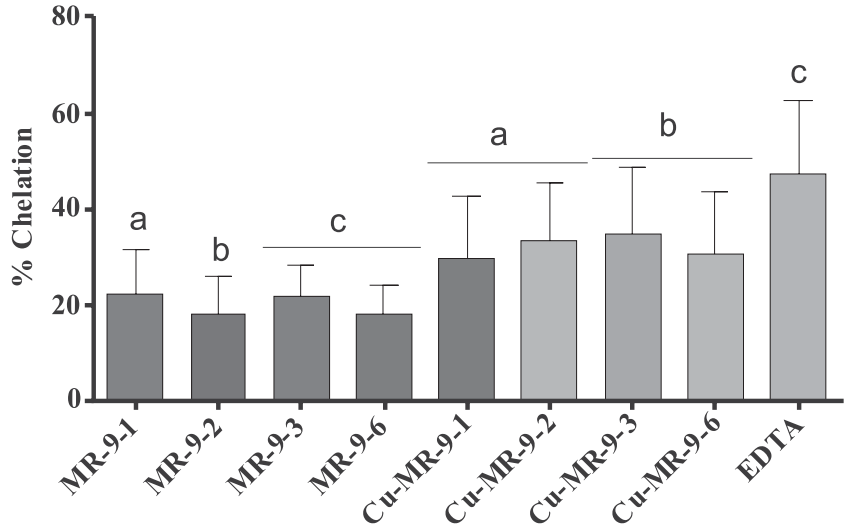


Figure 2. Ferrous ion chelation assay of guanidine ligands (MR-9-1, MR-9-2, MR-9-3, MR-9-6, their Cu(II) metal complexes (Cu-MR-9-1,Cu-MR-9-2, Cu-MR-9-3,Cu-MR-9-6) and EDTA. Significance: $^{a}p < 0.0001$, $^{b}p < 0.0010$ and $^{c}p < 0.0094$

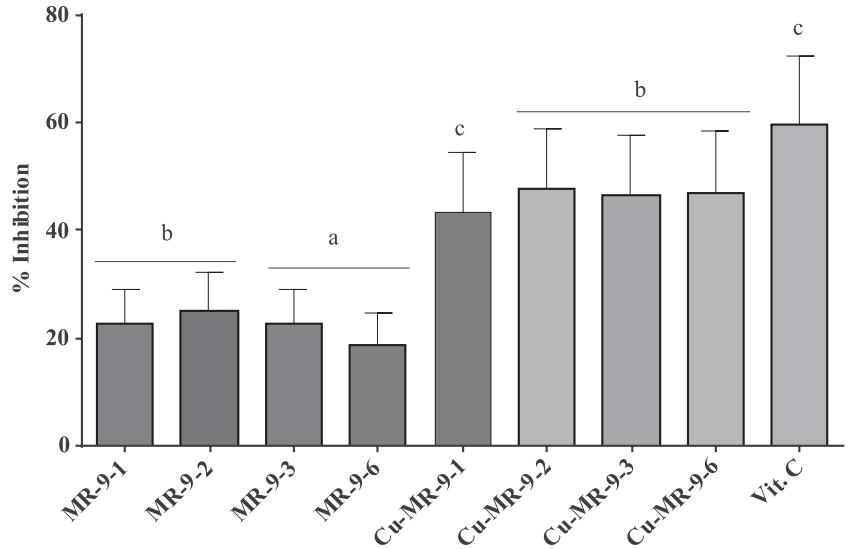


Figure 3. Ferric ion reductin assay of guanidine ligands (**1** – **4**), their Cu(II) complexes (**1a** – **4a**) and Vit. C. Significance: $^{a}p < 0.0068$, $^{b}p < 0.0199$ and $^{c}p < 0.0292$

$$\% \text{ Inhibition} = \frac{C - T}{C} \times 100$$

where: C = growth in control plate, T = growth in test plate.

Statistical analysis

Linear regression analysis was used to calculate IC_{50} ± SEM values from data and graphs by using Graph Pad prism 6 software. Significant differences among the means of data were tested by the one-way ANOVA followed by the Student´s t-test with significance level ($p < 0.05$). All the tests were conducted in triplicate.

RESULTS AND DISCUSSION

A series of guanidines and their Cu(II) complexes **1-8** were synthesized and their structures

were confirmed as described in the literature. All analytical and spectral data were in agreement with previously published data (27).

The antioxidant activities of ligands and complexes are dose dependent and the increase caused due to concentration for the complexes has shown more significant activities than those of ligands, The highest activities of the member among the same series of the complexes is due to the evolvement of protons donation or electrons which depends upon the nature of atoms in the structure.

Name and structures

Names and structures of the investigated ligands are shown in Table 1

DPPH radical scavenging assay

DPPH radical scavenging assay has been extensively used for screening antioxidant activity because it is sensitive enough to detect active ingredients at low concentration, the absorbance is reduced when encounter with a proton donating substance such as an antioxidant. Thus, the DPPH radi-

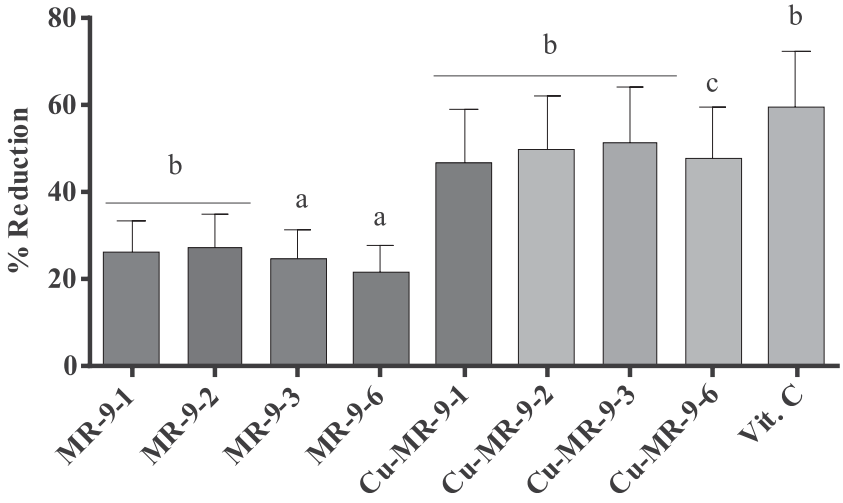


Figure 4. Molybdate ion reductin assay of guanidines (**1** – **4**), their copper(II) complexes (**1a** – **4a**) and Vit. C. Significance: [a] $p < 0.0058$, [b] $p < 0.0198$ and [c] $p < 0.0224$

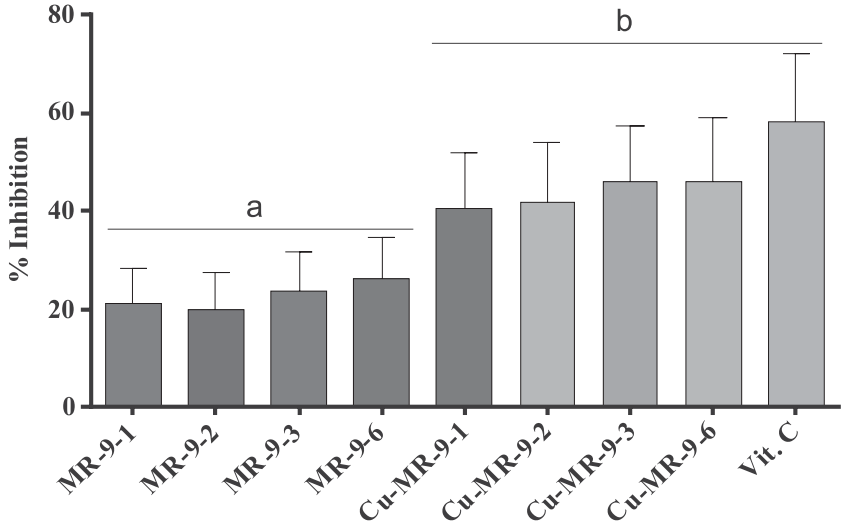


Figure 5. Hydroxyl ion scavenging assay of guanidine ligands (**1** – **4**), their Cu(II) complexes (**1a** – **4a**) and Vit. C. Significance: [a] $p < 0.0051$ and [b] $p < 0.0173$

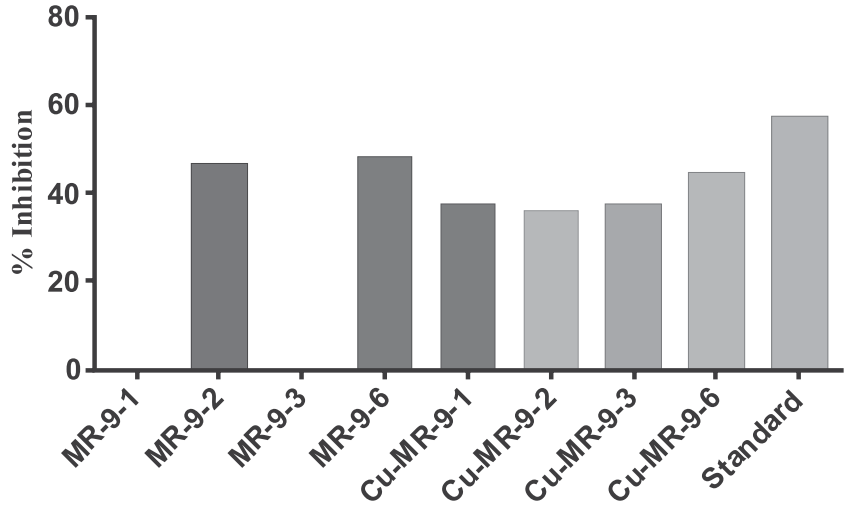


Figure 6. Antifungal activity of guanidine ligands (**1 – 4**), their Cu(II) complexes (**1a – 4a**) and Mancozeb (used as positive non-synthetic dithiocarbamate fungicide)

Table 4. Ferric ion reduction assay of guanidine ligands (**1**-**4**), their Cu(II) complexes (**1a**-**4a**) and Vit. C.

Compound	% Reduction (mean = 3)					IC_{50} (µM) ± SEM
	12.5 µM	25 µM	50 µM	100 µM	200 µM	
1	5.80	12.74	22.08	31.67	40.82	234.36 ± 6.30
2	7.09	11.85	26.27	34.99	45.14	205.97 ± 7.07
3	6.03	13.17	20.71	30.41	42.58	222.18 ± 6.43
4	5.39	9.20	17.47	25.65	36.42	272.06 ± 5.61
1a	14.90	24.43	43.69	62.42	71.98	99.72 ± 10.84
2a	16.71	29.47	46.04	65.16	80.08	85.40 ± 11.51
3a	18.19	28.09	42.02	64.83	78.66	89.11 ± 11.25
4a	15.55	28.78	46.08	64.75	79.16	87.37 ± 11.56
Vit. C	24.02	40.97	60.78	76.85	95.28	49.73 ± 12.62

cals have been widely used to investigate the scavenging activity of some synthetic compounds. In the present study, the IC_{50} values (illustrated in Table 2 and graphically represented in Fig. 1) for DPPH radicals with DCM solution of ligands (MR-9-1,MR-9-2, MR-9-3, MR-9-6) and their copper(II) (Cu-MR-9-1, Cu-MR-9-2, Cu-MR-9-3, Cu-MR-9-6) complexes were found to be 245.91 ± 6.064, 211.42 ± 6.877, 232.71 ± 6.225, 275.25 ± 5.639, 94.040 ± 11.79, 82.125 ± 11.81, 87.008 ± 11.38,87.402 ± 11.65, respectively. The free radicals were detected less efficient in decolorizing the pink color of the DPPH solution than its Cu(II) metal complexes. Among the Cu(II) complexes, Cu-MR-9-2 shows the highest, Cu-MR-9-3, Cu-MR-9-6 less and Cu-MR-9-1 least antioxidant potential. The complexation of ligand with Cu(II) metal confirmed a considerable increase in the percent scavenging activity. The IC_{50} for standard (ascorbic acid) was found to be 47.188 ±12.83. These activities were dose dependent and maximum DPPH scavenging activity was observed at higher concentration.

Ferrous ion-chelating assay

Table 3 shows the Fe^{2+}-chelating properties of DCM and ethanolic solution (1 : 1) of guanidines ligands and their copper(II) complexes and EDTA (ethylenediaminetetraacetic acid). Significances ($p < 0.05$) were determined by One-way ANOVA test for samples when compared to control. The guanidine ligands (illustrated in Table 3. and graphically represented by Figure 2) observed at lower Fe^{2+}-chelating

properties than metal complexes. The IC_{50} values for Fe^{2+}-chelating ability with DCM and ethanolic solution of ligands (MR-9-1, MR-9-2, MR-9-3, MR-9-6) and their Cu(II) metal complexes (Cu-MR-9-1, Cu-MR-9-2, Cu-MR-9-3, Cu-MR-9-6) were found to be 178.46 ± 9.43, 216.21 ± 7.84, 229.12 ± 6.59, 275.25 ± 5.63, 131.64 ± 12.80, 123.90 ± 12.45, 115.08 ± 13.58 and 83.45 ± 14.97 µM, respectively. The IC_{50} values Cu-MR-9-3 complex was determined to be the highest, Cu-MR-9-2 moderate, Cu-MR-9-6 less and Cu-MR-9-1 the least antioxidant activities. The Cu(II) complexes show better Fe^{2+}-chelating activity than that of ligands. The IC_{50} values for EDTA were 83.45 ± 14.97. These activities were dose dependent and maximum Fe^{2+}-chelating activity was observed at higher concentrations.

Table 5. Molybdate ion reduction assay of guanidine ligands (**1**-**4**), their Cu(II) complexes (**1a**-**4a**) and Vit. C.

Compound	% Reduction (n = 3)					IC_{50} (µM) ± SEM
	12.5 µM	25 µM	50 µM	100 µM	200 µM	
1	7.295	14.776	25.860	35.763	47.136	197.53 ± 7.13
2	8.056	15.429	27.086	39.058	48.018	189.07 ± 7.34
3	7.755	14.299	23.108	32.705	46.098	207.98 ± 6.78
4	6.668	11.093	18.304	29.372	41.784	233.38 ± 6.37
1a	14.956	25.845	45.124	65.721	81.921	85.50 ± 12.34
2a	16.678	31.506	47.672	68.920	84.690	77.80 ± 12.29
3a	18.838	32.411	48.577	69.223	88.724	73.06 ± 12.53
4a	14.948	29.811	46.988	66.732	80.672	84.17 ± 11.92
Vit. C	22.951	39.611	60.899	75.641	97.997	51.60 ± 13.18

Table 6. Hydroxyl ion scavenging assay of guanidine ligands (**1**-**4**), their Cu(II) complexes (**1a**-**4a**) and Vit. C.

Compound	% Reduction (n = 3)					IC_{50} (µM) ± SEM
	12.5 µM	25 µM	50 µM	100 µM	200 µM	
1	4.586	9.776	17.860	30.763	43.136	220.67 ± 7.03
2	3.046	7.429	14.086	29.058	45.018	212.99 ± 7.70
3	6.756	10.299	19.108	31.705	50.098	192.36 ± 7.90
4	7.458	13.093	22.304	35.372	52.784	178.56 ± 8.15
1a	10.945	21.845	39.124	55.721	73.921	108.03 ± 11.34
2a	12.678	19.506	40.672	58.920	77.690	101.41 ± 12.10
3a	14.838	29.411	43.577	60.223	80.724	90.59 ± 11.53
4a	11.945	23.811	44.988	63.732	84.672	88.86 ± 13.16
Vit. C	19.951	37.611	60.899	73.641	98.997	55.88 ± 13.78

Table 7. Antifungal activity of guanidine ligands (**1**-**4**) and their Cu(II) complexes (**1a**-**4a**).

Name of fungus	Diseases cause	Zone of inhibition (cm)								
		1	**1a**	**2**	**2a**	**3**	**3a**	**4**	**4a**	Mancozeb (standard)
Alternaria brassicacola	Leaf blight in Brassica plants	4.0	2.5	2.12	2.34	4.0	2.5	2.07	2.2	1.7
% Inhibition		0	47	0	48.25	37.5	35.9	37.5	45	57.5

Ferric reducing / antioxidant power assay

The significant ($p < 0.05$) values of ferric ion indicates the reductive capabilities of various evaluating guanidine – ligands and their Cu(II) metal complexes compounds. Table 4 illustrates the percentage reduction potential of different guanidine ligands and their Cu(II) complexes comparable with that of standard ascorbic acid. The reducing power increased with increasing concentration of the compounds which acts as dose dependent like the antioxidant activity. The DCM and ethanolic solution (1 : 1) of Cu(II) complexes showed higher reducing ability than all their ligand tested compounds. However, the activity was less than the standard, ascorbic acid (IC_{50} 49.73 ± 12.62 µM). The reducing capacity of guanidine ligands and Cu(II) complexes also was significant ($p < 0.05$). Among the Cu(II) complexes, Cu-MR-9-2 was found to have the highest, Cu-MR-9-6 moderate, MR-9-3 less and Cu-MR-9-1 the least ferric reducing capacity.

Total antioxidant activity (phosphomolybdenum assay)

Total antioxidant capacity of guanidine ligands and their Cu(II) complexes have been evaluated by using phosphomolybdate method with ascorbic acid as a standard. The Mo(VI) is reduced to Mo(V), in the presence of a drug which shows maximum absorbance at 695 nm. All the compounds tested by this method possessed significant ($p < 0.05$) antioxidant activity and the reducing power was dose-dependent which increased with increasing concentration of the compounds. The IC_{50} was calculated for each guanidine ligands and their Cu(II) metal complexes as well as ascorbic acid as standard and summarized in Table 5 and graphically represented in Figure 4. Among the drugs tested Cu-MR-9-3 was found to have better reduction potential (IC_{50} 73.06 ± 12.53 µM). Cu-MR-9-2 complex with IC_{50} 77.80 ± 12.29 µM showed lower antioxidant activity than Cu-MR-9-3 complex but higher than that of Cu-MR-9-6 and Cu-MR-9-1 compounds which show almost the same antioxidant activity with IC_{50} 84.17 ± 11.92 µM and 85.50 ± 12.34 µM, respectively. The IC_{50} values for ligands showed lower antioxidant activity than their Cu(II) complexes. The IC_{50} values for ascorbic acid were found to be 51.60 ± 13.18 µM.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the guanidine ligands and their Cu(II) complexes were investigated. All the compounds exhibited strong concentration-dependent scavenging significant ($p < 0.05$) properties for the hydroxyl radical demonstrated in Table 6 and graphically represented in Figure 5. The Cu(II) metal compounds (**1a** - **4a**) were detected to be the most powerful scavengers of the hydroxyl radical, with IC_{50} up to 108.03 ± 11.34, 101.41 ± 12.10, 90.59 ± 11.53 and 88.86 ± 13.16 µM. Among the complexes the weakest scavenger was found to be Cu-MR-9-1 complex. The IC_{50} value for ascorbic acid was found to be 55.88 ± 13.78 µM.

Antifungal activity

The antifungal activity of the guanidine ligands and their Cu(II) complexes were tested against fungus *Alternaria brassicicola*. The results (Table 7, Fig. 6) reveal that standard showed 57.5% growth inhibition, sample Cu-MR-9-1 showed 37.5%, Cu-MR-9-2 showed 5%, Cu-MR-9-6 showed 45%, Cu-MR-9-3 showed 37.5% while MR-9-1 showed 0%, MR-9-6 showed 48.25%, MR -9-3 showed 0% and MR-9-2 showed 47% inhibition in growth.

$$\% \text{ inhibition} = \frac{C - TX}{C} \times 100$$

C = growth in control plate, T = growth in test plate

CONCLUSIONS

In the present work, free radical scavenging, iron metal chelating and antioxidant activities of a series of guanidines and their Cu(II) complexes were examined by scavenging effect on the DPPH free radical, metal chelation effect, iron reducing and hydroxyl radical scavenging activities. The results shown that all the complexes were excellent scavengers as compared to the ligands. The antifungal activities were investigated against *Alternaria brassicicola* by agar well diffusion methods. The overall results strongly indicated that guanidines based Cu(II) complexes exhibit good activity.

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Conflict of interest

The authors declare no conflict of interest.

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