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ORIGINAL ARTICLE

Transition metal complexes with N, S donor ligands as synthetic antioxidants: Synthesis, characterization and antioxidant activity

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Abstract

Transition metal complexes containing bidentate N, S donor ligands i.e., carvone thiosemicarbazone [(RS)-5-isopropenyl-2-methylcyclohex-2-en-1-one thiosemicarbazone (IPMCHTSC)] and carvone N^1-phenylthiosemicarbazone [(RS)-5-isopropenyl-2-methylcyclohex-2-en-1-one phenylthiosemicarbazone (IPMCHPhTSC)] have been synthesized. All the metal complexes (**1**–**8**) have been characterized by elemental analysis, molar conductance measurement and various spectral studies [infrared (IR), electronic, fast-atom bombardment (FAB) mass and NMR (for ligands)] and thermogravimetric analysis. FAB mass spectroscopic studies of (**1**), (**3**), (**4**), (**5**), (**6**) (**7**), and (**8**) suggest their monomeric nature. Metal complexes are $[M(LH)Cl_2]$ and $[M(LH)_2Cl_2]$ type, where M = Fe(III), Co(II), and Cu(II) and LH = IPMCHTSC and IPMCHPhTSC. The proposed geometries of the complexes were octahedral for 1:2 complexes, square planar for 1:1 complexes and distorted octahedral for Cu(II) complexes (1:2). The free radical scavenging activity of ligands (IPMCHTSC and IPMCHPhTSC) and their metal complexes have been determined at the concentration range of 10–400 µg/mL by means of their interaction with the stable free radical 2,2′-diphenyl-1-picrylhydrazyl and 5–200 µg/mL by 2,2′-Azinobis-3-ethylbenzothiazoline-6-sulphonic acid. All the compounds have shown encouraging antioxidant activities.

Keywords: Molar conductance, FAB mass, radical scavenging activity, DPPH, ABTS

Introduction

During the recent years transition metal complexes with N, S donor ligands have attracted considerable interest because of their encouraging antibacterial and antifungal[1–4] activities than those of the parent ligands. Thiosemicarbazones are well established as an important class of sulfur donor ligands particularly for transition metal ions.[5–7] The activity of thiosemicarbazones and its substituted derivatives is usually increased by complexation therefore to understand the properties of both ligands and metal can lead to the synthesis of highly active compounds.[8–15] Previously, we have reported structural and spectral studies of transition metal complexes with semicarbazone and thiosemicarbazones of some terpenoids.[16–18] Our ongoing research work on transition metal complexes with thiosemicarbazones involving such systems led to describe the synthesis, characterization and antioxidant activity of some transition metal complexes with new thiosemicarbazones derived from carvone [(RS)-5-isopropenyl-2-methyl cyclohex-2-en-1-one]. Carvone is a monocyclic monoterpenone and important constituent of several essential oils, e.g. oil of caraway seeds (carum carvi).[19]

Antioxidants are the compounds that terminate the attack of reactive species like free radicals and prevent it from ageing and different disease associate with oxidative damage inside the body system.[20] Antioxidant activity of a synthetic compound can be measured using the scavenging potential of that compound for the trapping of free radicals. These free radicals can oxidize biomolecules viz. nucleic acids, proteins, lipids, DNA, tissue damage, and can initiate degenerative diseases, oxidative damage

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plays a significantly pathological role in human diseases such cancer, emphysema, cirrhosis, and arthritis etc.[21,22] Almost all organisms are protected upto some extent by free radical (peroxide, hydroperoxide, or lipid peroxyl) damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid (AA), tocopherols, phenolic acids, polyphenols, flavonoids, and glutathione.[23] Some compounds like rutin, quercetin having nitrogen, and glutathione having sulfur are also most effective antioxidants.[24–27]

However, antioxidant supplements or dietary antioxidants may be sources of protection that the body needs to protect against the damaging effects of free radicals.[28] Presently, synthetic antioxidants are widely used because they are effective and cheaper than natural antioxidants.

Material and methods

All the chemicals and reagents used were of AR grade. Solvents were dried by conventional methods and distilled prior to use. Metal contents were measured by complexometric titrations. Sulfur was estimated gravimetrically as $BaSO_4$ and chloride content was determined by Volhard's method.[29] Elemental analyses were carried out on thermoquest analyzer. The infrared (IR) spectra were recorded with KBr pellets in the 4000–225 cm^{-1} range on Nicolet Megna 550 FT-IR spectrometer. The ^1H and $^{13}C\{^1H\}$ NMR spectra of ligands were collected in $CDCl_3$ solvent using Tetramethylsilane (TMS) as internal standard on JEOL FX 300 FT NMR spectrometer at 300.4 and 75.45 MHz frequencies for ^1H and $^{13}C\{^1H\}$ NMR, respectively, electronic spectra were recorded on Agilent UV/visible spectrometer. Molar conductivity of 10^{-3} dimethylsulphoxide (DMSO) solutions were measured on a microprocessor based conductivity meter model 1601/E. Thermogravimetric analysis (TGA) was performed by PerkinElmer Thermal analyzer with the heating rate 35-900/20°C under nitrogen atmosphere. Mass spectra were recorded on Schimadzu mass spectrophotometer. Antioxidant activity was measured in spectro UV-Vis double beam PC scanning spectrophotometer (Labomed Inc., Culver City, CA) vortex (Spinix).

Synthesis of ligands

Carvone thiosemicarbazone (IPMCHTSC) and carvone N^1-phenylthiosemicarbazone (IPMCHPhTSC) were prepared according to known method.[15]

IPMCHTSC

Yield: 91% (2.01 gm); m.pt. 109°C; IR (cm^{-1}) 3441 s, 3240 s, br $\upsilon(NH_2)$; 3155 s, υ(NH); 1592, υ(C=N); 840 m, υ(C=S); ^1H NMR ($CDCl_3$, δ ppm): 9.05 (s, 1H, NH); 7.32, 7.05 (2 s, 2H, NH_2); 6.20–6.77 (m, 1H, =CH–CH_2); 4.83, 4.79 (2 s, 2H, =CH_2); 2.79 (dd, 1H, J = 3.0Hz, –CH_2–CH–CH_2–); 2.22–2.69 (m, 2H, CH_2); 2.09–2.18 (m, 2H, CH_2); 1.86 (s, 3H, CH_3); 1.75 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, δppm): 178.7 (C=S); 150.2 (C=N); 146.8 (C-7); 135.3 (C-2); 132.0 (C-3); 110.7 (C-8); 40.6 (C-5); 30.1 (C-4); 29.2 (C-6); 20.6 (C-9); 17.7 (C-10). Anal. Found for $C_{11}H_{17}N_3S$ (223.34): C, 59.40; H, 7.91; N, 18.01; S, 14.40%. Calcd. C, 59.15; H, 7.67; N, 18.81; S, 14.36%.

IPMCHPhTSC

Yield: 71% (2.19 gm); m.pt. 99°C; IR (cm^{-1}) 3280 s, 3150 s, br υ(NH); 3030w, υ(Ar-H); 1585 s, υ(C=N); 832 m, υ(C=S); ^1H NMR ($CDCl_3$, δ ppm): 9.36 (br, s, 1H, NHCS); 8.77 (br, s, 1H, NHPh); 7.20–7.69 (m, 5H, NHPh); 1.78, 1.92 (2 s, 6H, CH_3); 6.26 (t, 1H, =CH–CH_2); 4.85, 4.79 (2 s, 2H, =CH_2); 2.72–2.79 (dd, 1H, J=3.9Hz, –CH_2–CH–CH_2); 2.32-2.51 (m, 2H, CH_2); 2.10–2.20 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$, δppm): 175.8 (C=S); 149.0 (C=N); 146.7 (C-7); 137.9 (C-2); 135.3 (C-3); 131.8–123.8 (–C_6H_5); 110.8 (C-8); 40.5 (C-5); 30.4 (C-4); 29.1 (C-6); 20.5 (C-9); 17.6 (C-10). Anal. Found for $C_{17}H_{21}N_3S$ (299.44): C, 68.13; H, 7.10; N, 14.10; S, 10.78%. Calcd. C, 68.19; H, 7.06; N, 14.03; S, 10.70%.

Synthesis of complexes

Synthesis of [MCl_2(IPMCHTSC)$_n$]

To an ethanolic solution (~20 mL) of $CuCl_2.2H_2O$ (0.4310 g, 2.5 mmol), a hot ethanolic solution (~25 mL) of ligand (IPMCHTSC) (1.1167 g, 5 mmol) was added dropwise with constant stirring. After complete addition the reaction mixture was refluxed for 6 h and cooled to room temperature. The resulting greenish-yellow precipitate was filtered and washed with ethanol and diethyl ether and dried in vaccum to give greenish-yellow colored solid.

Similar route have been employed for the preparation of other IPMCHTSC complexes.

Synthesis of [MCl_2(IPMCHPhTSC)$_2$]

To an ethanolic solution (~20 mL) of $CuCl_2.2H_2O$ (0.5966 g, 2.5 mmol), a hot ethanolic solution (~25 mL) of ligand (IPMCHPhTSC) (1.3514 g, 5 mmol) was added dropwise with constant stirring for 6 h. After complete addition the reaction mixture was filtered and washed with ethanol and diethyl ether and dried in vaccum to give grayish-black colored solid.

Similar route have been employed for the preparation of other IPMCHPhTSC complexes.

Antioxidant activity

Antioxidant activity of the compounds was estimated by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging effect. The method for estimating free radical scavenging activity of the methanolic solutions of bioactive compounds were undertaken as suggested by Hatano et al. 1989.[30] The DPPH reagent evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidant.[31] To different concentration (10–400 μg/mL) of methanolic solution of test compound, 5 mL methanolic solution of DPPH (0.01 mm) was added and mixed thoroughly. The absorbance of the mixture was measured after 40 min at 517 nm against methanol as blank. AA and catachin (CAT) were used as reference standard. The radical scavenging activities (%) of

tested samples were evaluated by comparing with a control (5.0 mL DPPH and 0.5 mL of methanol). Each sample was then measured in duplicate and averaged.

The method of Re et al.[32,33] was adopted for ABTS radical scavenging assay. A solution containing a mixture of 7.0 mM ABTS and 2.4 mM potassium persulphate in equal measures was allowed to react for 12 h at room temperature in the dark. This working solution was diluted by mixing 1.0 mL of ABTS radical solution and 60.0 mL of methanol. AA and butylated hydroxytoluene (BHT) were used as standard antioxidants and the testing samples were compared with them. For this purpose solution of different concentrations range (5–200.0 μg/mL) were prepared for AA, BHT, and testing samples. A quantity of 1.0 mL of each sample was allowed to react with 1.0 mL of ABTS radical solution for 7.0 min and the absorbance was recorded at 734 nm. Percentage inhibition was calculated as ABTS radical scavenging activity.

The % scavenging activity was calculated by using the formula:

$$\%\ \text{Scavenging activity} = [(A_c - A_s/A_c)] \times 100$$

Where, A_c = Absorbance of control (DPPH or ABTS radical + methanol)
A_s = Absorbance of sample (DPPH or ABTS radical + sample/standard)

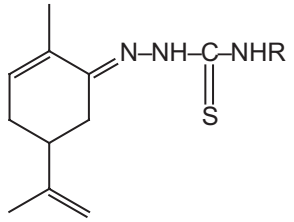
The scavenging activity was expressed as IC_{50}, which is defined as the concentration (μg/mL) of compound required for the 50% inhibition of the DPPH and ABTS radical. IC_{50} values were determined by linear regression analysis using at least five different concentrations in duplicate.[34]

Results and discussion

A systematic study of the reaction of metal chlorides with ligand IPMCHTSC in 1:1 and 1:2 and IPMCHPhTSC in 1:2 molar ratio in EtOH have been carried out. The reaction can be represented by following equation:

$$MCl_X.mH_2O + yLH \xrightarrow[\text{Reflux}]{\text{EtOH}} \left[M(LH)_y Cl_2\right]^{+n}$$

[Where M = Fe(III), Co(II), Cu(II); X = 2 for Co(II), Cu(II) and 3 for Fe(III); m = 0 for Fe(III), 2 for Cu(II) and 6 for Co(II); y = 1 or 2 for LH = IPMCHTSC, y = 2 for LH = IPMCHPhTSC; n = 0 for Co(II), Cu(II) and 1 for Fe(III)]



R=H (IPMCHTSC), Ph (IPMCHPhTSC)

Several analytical techniques were used to characterize the complexes including microanalysis (CHN), spectral studies (IR, fast-atom bombardment (FAB) mass, and UV), TGA and conductometric measurements. Analytical data for the newly synthesized complexes are given in Table 1. All the metal complexes are non-hygroscopic in nature, stable at room temperature, insoluble in water partially soluble in ethanol and methanol but completely soluble in DMSO.

The molar conductance values of the complexes are well presented in Table 1. The molar conductivity shows that all the complexes are non-electrolyte with λ = 17.4–31.8 Ω^{-1} cm^2·mol^{-1} in DMSO (10^{-3} M) solution at room temperature.

Electronic spectra

The electronic spectra of Fe(III) complexes show 810 nm and 570 nm may be assigned to $^6A_{1g} \rightarrow {}^4T_{1g}$ and $^6A_{1g} \rightarrow {}^4T_{2g}$ transitions, respectively, which suggest the octahedral geometry around Fe(III).[35] The electronic spectra of the Co(II) complexes exhibit four bands at 890, 685, 602, and 256 nm, which are assigned to $^4T_{1g} \rightarrow {}^4T_{2g}$ (F), $^4T_{1g} \rightarrow {}^4T_{1g}$ (P), $^4T_{1g} \rightarrow {}^4A_{2g}$ and charge transfer transitions of the d^7 system. Therefore, octahedral geometry was proposed for Co(II) complex.[35–37]

The Cu(II) complexes show bands at 930, 620, and 405 nm which are assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ (υ_1), $^2B_{1g} \rightarrow {}^2B_{2g}$ (υ_2), and ^{2}Eg (υ_3) transitions. The positions of these bands and their assignments suggest distorted octahedral geometry.[38] The absorption bands appearing in the UV domain are considered to the characteristics of ligand. The assignments of n-π* and π-π* transitions as being due to the (C=S) bond.

The electronic spectrum of [Co(IPMCHTSC)Cl_2] exhibits three bands at 1090, 985, and 650 nm. The first two bands are assigned to $^2B_{2g} \rightarrow {}^2E_g$ and $^2B_{2g} \rightarrow {}^2A_{1g}$ transitions, respectively, in a square-planner environment of Co(II).[39] The spectrum of [Cu(IPMCHTSC)Cl_2] shows a band 485 nm indicates square-planar geometry for the Cu(II) complex.[39,40]

IR spectra

The main IR spectral bands of complexes and their assignments are presented in Table 2. The ligands IPMCHTSC and IPMCHPhTSC exhibit bands at 840 and 832 cm^{-1}, which shifted to the downward region in complexes suggested the coordination of metal of through the C=S group. The spectra of both ligands exhibit a band in the 1580–1595 cm^{-1} region due to C=N mode of azomethine linkage. In the metal complexes this band shifted to lower frequency suggesting that the unsaturated nitrogen of azomethine linkage is coordination to metal. In IPMCHTSC the highest frequency bands observed in 3441 and 3240 cm^{-1} are assigned to asymmetric and symmetric stretching of terminal NH_2 group vibration. The second highest bands observed at 3280, 3150, and 3155 cm^{-1} due to NH group stretching vibrations in IPMCHPhTSC and IPMCHTSC, respectively. In IPMCHPhTSC a band observed at 3030 cm^{-1} due to Ar-H stretching vibration. In

Table 1. Analytical and physical data for complexes.

Compound	Color	Yield (%)	M. P.	Found (Calcd.) C	H	N	S	M	Cl	Molar conductance (Ω^{-1}·mol^{-1} cm^2)
$[Fe(IPMCHTSC)_2Cl_2]Cl$	Brown	82	95	43.01	5.11	13.10	11.01	9.02	17.41	
$[Fe(C_{11}H_{17}N_3S)_2Cl_2]Cl$ (**1**)				(43.39)	(5.62)	(13.80)	(10.53)	(9.17)	(17.46)	19.4
$[Fe(IPMCHPhTSC)_2Cl_2]$ $Cl[Fe(C_{17}H_{21}N_3S)_2Cl_2]Cl$ (**2**)	Dark brown	79	83	53.60	5.15	11.10	8.49	7.19	13.19	30.8
				(53.65)	(5.56)	(11.04)	(8.42)	(7.33)	(13.97)	
$[Co(IPMCHTSC)Cl_2]$ $[Co(C_{11}H_{17}N_3S)Cl_2]$ (**3**)	Blue	90	158	37.53	4.99	10.78	9.23	16.02	19.98	31.8
				(37.40)	(4.85)	(11.89)	(9.07)	(16.68)	(20.07)	
$[Co(IPMCHTSC)_2Cl_2]$ $[Co(C_{11}H_{17}N_3S)_2Cl_2]$ (**4**)	Greenish blue	87	148	45.18	5.19	14.41	11.10	10.09	12.20	22.9
				(45.83)	(5.94)	(14.57)	(11.12)	(10.22)	(12.29)	
$[Co(IPMCHPhTSC)_2Cl_2]$ $[Co(C_{17}H_{21}N_3S)_2Cl_2]$ (**5**)	Pale yellow	74	119	56.91	5.18	11.94	8.69	8.01	9.79	23.4
				(56.04)	(5.80)	(11.53)	(8.80)	(8.08)	(9.73)	
$[Cu(IPMCHTSC)Cl_2]$ $[Cu(C_{11}H_{17}N_3S)Cl_2]$ (**6**)	Dark green	85	163	37.68	4.72	12.0	8.28	17.10	18.91	19.2
				(36.92)	(4.78)	(11.74)	(8.96)	(17.76)	(19.81)	
$[Cu(IPMCHTSC)_2Cl_2]$ $[Cu(C_{11}H_{17}N_3S)_2Cl_2]$ (**7**)	Greenish yellow	88	138	45.11	5.18	14.54	11.10	10.19	12.29	17.4
				(45.47)	(5.89)	(14.46)	(11.03)	(10.93)	(12.20)	
$[Cu(IPMCHPhTSC)_2Cl_2]$ $[Cu(C_{17}H_{21}N_3S)_2Cl_2]$ (**8**)	Greyish black	90	85	55.18	5.71	11.38	8.71	8.50	9.59	22.1
				(55.68)	(5.77)	(11.45)	(8.74)	(8.66)	(9.66)	

Table 2. Main IR spectral bands for complexes.

Compound	$\nu(NH_2)$	ν(NH)	ν(Ar-H)	ν(C=N)	ν(C=S))	ν(M–N)	ν(M–S)	ν(M–Cl)
(**1**)	3441 as 3240 s	3152	—	1575	825	456	350	330
(**2**)	—	3280 3148	3032	1572	810	460	332	325
(**3**)	3439 as 3241 s	3155	—	1545	825	472	395	332
(**4**)	3440 as 3245 s	3151	—	1550	827	438	326	317
(**5**)	—	3281 3150	3035	1572	812	442	355	335
(**6**)	3440 as 3239 s	3150	—	1579	817	420	345	339
(**7**)	3445 as 3242 s	3156	—	1570	826	435	375	328
(**8**)	—	3280 3152	3030	1572	819	465	395	370

the complexes the above bands are not affected indicating non-participation of NH_2, NH and Ar-H in coordination. The non-ligand bands occuring the 420–472, 325–395, and 310–370 cm^{-1} regions are tentatively assigned to ν(M–N), ν(M–S) and ν(M–Cl) modes, respectively.

Thermal studies

The thermogram for complex (**3**) and (**6**) revealed three step decomposition behavior [Figure 1a and 1b]. These TG steps are connected with exothermic events caused due to the pyrolysis of organic by products. The thermogram,

also exhibits completion of the decomposition at 900°C. The residual for complex (**3**) was 29.85% corresponding to the formation of Co_2S_3 (Calcd. 30.29%). The residual for complex (**6**) was 21.60%, corresponding to the formation of Cu_2S (Calcd. 22.23%).

Mass spectra

The FAB mass spectra studies of seven of the representative compounds $[Fe(C_{11}H_{17}N_3S)_2Cl_2]$ (**1**), $[Co(C_{11}H_{17}N_3S)Cl_2]$ (**3**), $[Co(C_{11}H_{17}N_3S)_2Cl_2]$ (**4**), $[Co(C_{17}H_{21}N_3S)_2Cl_2]$ (**5**), $[Cu(C_{11}H_{17}N_3S)Cl_2]$ (**6**), $[Cu(C_{11}H_{17}N_3S)_2Cl_2]$ (**7**), and $[Cu(C_{17}H_{21}N_3S)_2Cl_2]$ (**8**) indicate their monomeric nature. The splitting patterns of mass spectra of compounds are shown in Table 3. The molecular ion peak of (**2**), (**5**), and (**6**) appears at m/z 608.0, 576.0, and 581.0, respectively, thus confirming the formation a metal ion complex in 1:2 ratio. Appearance of some molecular ion peaks at higher m/z than molecular ion in the FAB mass spectra may be due to re-association of fragments.

On the basis of above analysis, the following structural formula (Figure 2) may be suggested for the complexes.

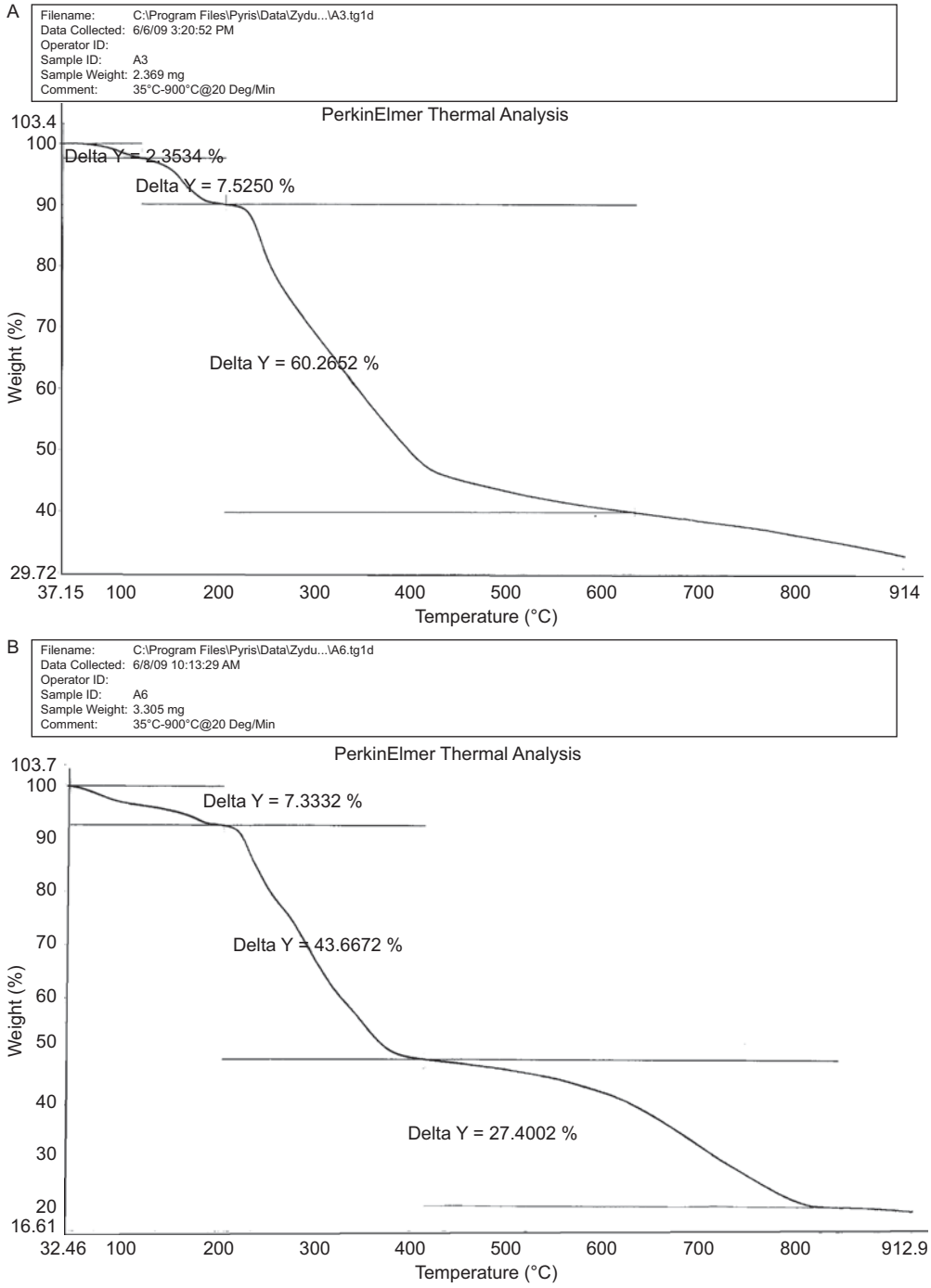


Figure 1. TGA curve of {weight (%) vs temperature (°C)} (A) $[Co(C_{11}H_{17}N_3S)Cl_2]$ and (B) $[Cu(C_{11}H_{17}N_3S)Cl_2]$. (See colour version of this figure online at www.informahealthcare.com/enz)

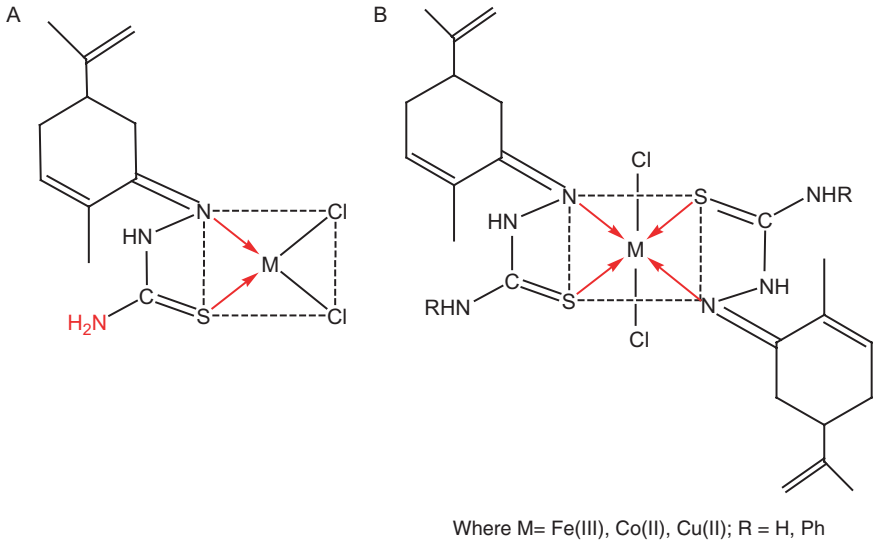
Table 3. Fragmented molecular ions vs m/z values of following metal complexes.

Complexes	Fragmented ions	m/z values
$[Fe(C_{11}H_{17}N_3S)_2Cl_2]Cl$ (**1**)	$[Fe(C_{11}H_{17}N_3S)_2Cl_2]Cl$	608
	$[Fe(C_{11}H_{17}N_3S)_2Cl_2]^+$	573
	$[Fe(C_{11}H_{17}N_3S)(C_{11}H_{17}N_3S)Cl]^+$	537
	$[Fe(C_{11}H_{17}N_3S)(C_{11}H_{16}N_3S)Cl]^+$	536
	$[Fe(C_{11}H_{17}N_3S)(C_{11}H_{16}N_3S)]^+$	501
	$[Fe(C_{11}H_{16}N_3)(C_{10}H_{14}N_2)]^+$	408
	$[Fe(C_{10}H_{15}N)(C_{10}H_{14}N_2)]^+$	367
	$[Fe(C_{10}H_{14}N)(C_{10}H_{14}N)]^+$	352
	$[Fe(C_7H_{14}N)(C_{10}H_{14}N)]^+$	316
	$[(C_7H_{14}N)(C_7H_{14}N)]^+$	224
	$[(C_7H_{13}N)(C_6H_{14}N)]^+$	211
	$[(C_7H_9N)(C_6H_{14})]^+$	193
	$[(C_{10}H_{14}N)]^+$	148
	$[(C_{10}H_{14})]^+$	134
	$[(C_7H_7)]^+$	91
$[Co(C_{11}H_{17}N_3S)Cl_2]$ (**3**)	$[Co(C_{11}H_{17}N_3S)Cl_2]$	353.17
	$[Co(C_{10}H_{17}N_3S)Cl]^+$	305.70
	$[Co(C_9H_{17}N_3S)Cl]^+$	293.68
	$[Co(C_5H_{17}N_3S)Cl]^+$	245.63
	$[Co(C_3H_{17}N_3S)Cl]^+$	221.60
	$[Co(C_2H_{15}N_3S)Cl]^+$	207.57
	$[Co(CH_{13}NS)Cl]^+$	165.53
	$[Co(CH_{11}S)Cl]^+$	149.50
	$[Co(CH_{10}S)Cl]^+$	148.49
	$[Co(CH_4S)]^+$	107.03
	$[Co(CS)]^+$	103.0
$[Co(C_{11}H_{17}N_3S)_2Cl_2]$ (**4**)	$[Co(C_{11}H_{17}N_3S)_2Cl_2]$	576
	$[Co(C_{11}H_{17}N_3S)(C_{11}H_{17}N_3S)Cl]^+$	541
	$[Co(C_{11}H_{17}N_3S)(C_{11}H_{16}N_3S)Cl]^+$	540
	$[Co(C_{11}H_{17}N_3S)(C_{11}H_{16}N_3S)]^+$	504
	$[Co(C_{11}H_{16}N_3)(C_{10}H_{14}N_2)]^+$	411
	$[Co(C_{10}H_{14}N)(C_{10}H_{14}N)]^+$	355
	$[Co(C_{10}H_{17}N_2)]^+$	224
	$[Co(C_{10}H_{14}N_2)]^+$	221
	$[Co(C_{10}H_{14}N)]^+$	207
	$[(C_{10}H_{14}N)]^+$	148
	$[(C_{10}H_{14})]^+$	134
	$[(C_7H_7)]^+$	91
$[Co(C_{17}H_{21}N_3S)_2Cl_2]$ (**5**)	$[Co(C_{17}H_{21}N_3S)_2Cl_2]$	729
	$[Co(C_{17}H_{21}N_3S)(C_{17}H_{21}N_3)Cl]^+$	662
	$[Co(C_{10}H_{18}N)(C_{10}H_{17}N)]^+$	362
	$[Co(C_{10}H_{17}N)(C_7H_6]^+$	300
	$[Co(C_{10}H_{17}N)(C_7H_5]^+$	299
	$[Co(C_{10}H_{17}N)(C_4H_2]^+$	260
	$[Co(C_{10}H_{14}N)]^+$	207
	$[Co(C_8H_{12}N)]^+$	181
	$[Co(C_7H_8N)]^+$	165
	$[(C_7H_8N)]^+$	106
	$[(C_6H_7N)]^+$	93
	$[(C_6H_5N)]^+$	91
$[Cu(C_{11}H_{17}N_3S)Cl_2]$ (**6**)	$[Cu(C_{11}H_{17}N_3S)Cl_2]$	357.79
	$[Cu(C_8H_{17}N_3S)Cl_2]^+$	321.75
	$[Cu(C_7H_{17}N_3S)Cl_2]^+$	309.73
	$[Cu(C_6H_{17}N_3S)Cl_2]^+$	297.71
	$[Cu(C_5H_{17}N_3S)Cl_2]^+$	285.69

Table 3. continued on next page

Table 3. Continued.

Complexes	Fragmented ions	m/z values
	$[Cu\ (C_5H_{16}N_3S)Cl_2]^+$	284.68
	$[Cu\ (C_5H_{11}N_3S)Cl_2]^+$	279.64
	$[Cu\ (C_4H_{10}N_3S)Cl_2]^+$	266.62
	$[Cu\ (C_4H_7N_3S)Cl_2]^+$	263.59
	$[Cu\ (C_2H_7N_2S)Cl_2]^+$	225.56
	$[Cu\ (C_2H_6N_2S)Cl_2]^+$	224.56
	$[Cu\ (C_2H_3NS)Cl_2]^+$	207.53
	$[Cu\ (CH_3NS)Cl_2]^+$	195.51
	$[Cu\ (HNS)Cl_2]^+$	181.48
$[Cu\ (C_{11}H_{17}N_3S)_2Cl_2]$ (**7**)	$[Cu\ (C_{11}H_{17}N_3S)_2Cl_2]$	581
	$[Cu\ (C_{11}H_{17}N_3S)\ (C_{11}H_{17}N_3S)Cl]^+$	545
	$[Cu\ (C_{11}H_{17}N_3S)\ (C_{11}H_{16}N_3S)Cl]^+$	544
	$[Cu\ (C_{11}H_{17}N_3S)\ (C_{11}H_{16}N_3S)]^+$	509
	$[Cu\ (C_{11}H_{16}N_3)\ (C_{10}H_{14}N_2)]^+$	416
	$[Cu\ (C_{10}H_{14}N)\ (C_{10}H_{14}N)]^+$	360
	$[Cu\ (C_{10}H_{14}N_2)]^+$	226
	$[(C_{10}H_{14}N)]^+$	148
	$[(C_{10}H_{14})]^+$	134
	$[(C_7H_9)]^+$	93
$[Cu\ (C_{17}H_{21}N_3S)_2Cl_2]$ (**8**)	$[Cu\ (C_{17}H_{21}N_3S)_2Cl_2]$	733
	$[Cu\ (C_{17}H_{21}N_3S)\ (C_7H_{14})]^+$	461
	$[Cu\ (C_{17}H_{21}N_3S)\ (C_4H_6)]^+$	417
	$[Cu\ (C_{17}H_{21}N_3S)\ (C_2H_5)]^+$	392
	$[Cu\ (C_{17}H_{21}N_3S)\ (C_2H_2)]^+$	389
	$[(C_{19}H_{19}N_2S)]^+$	307
	$[(C_{17}H_{19}N_2S)]^+$	283
	$[(C_{17}H_{18}N_2S)]^+$	282
	$[(C_5H_{18}N_2)]^+$	106
	$[(C_5H_{16}N)]^+$	90
	$[(C_4H_{16}N)]^+$	78



Where M= Fe(III), Co(II), Cu(II); R = H, Ph

Figure 2. Structural formula for the complexes (A) $[M(LH)Cl_2]$ and (B) $[M(LH)_2Cl_2]$.

Free radical scavenging activity of methanolic solutions using DPPH and ABTS assay

A variety of thiosemicarbazone complexes show antioxidant activity.[41–43] Various researchers have used scavenging effect of a chemical on DPPH and ABTS radicals as a quick and reliable parameter to assess the *in vitro* antioxidant activity. The results of free radical scavenging activity of methanolic solutions of compounds at different concentrations are shown in Tables 4–6. It is evident from results that free radical scavenging activity of these compounds was concentration dependent. Among the examined compound the complex

Table 4. Antioxidant activity of metal complexes and ligands (DPPH assay).

	Concentration (μg/mL)							
Compound	0	10	25	50	100	200	300	400
AA	0	7.91	12.80	21.30	41.05	69.11	80.05	85.82
CAT	0	5.18	10.75	13.78	33.72	66.37	79.66	87.09
$C_{11}H_{17}N_3S$	0	3.71	11.53	17.20	33.33	58.06	74.09	82.99
$C_{17}H_{21}N_3S$	0	9.65	16.78	37.75	68.58	96.00	96.87	97.56
(**1**)	0	3.12	10.75	17.69	28.44	48.68	85.53	93.64
(**2**)	0	6.84	13.39	21.99	32.64	51.90	89.73	95.60
(**3**)	0	9.77	13.00	31.28	56.59	70.08	80.35	87.78
(**4**)	0	12.00	22.63	31.31	70.34	88.09	95.31	96.97
(**5**)	0	10.06	30.59	44.28	58.55	89.93	95.21	95.99
(**6**)	0	25.41	50.92	62.85	77.61	96.08	96.48	96.67
(**7**)	0	22.77	47.99	60.70	77.12	96.38	96.77	96.96
(**8**)	0	6.82	14.04	23.12	35.41	50.04	56.87	63.31

Table 5. Antioxidant activity of metal complexes and ligands (ABTS assay).

	Concentration (μg/mL)						
Compound	0	5	10	25	50	100	200
AA	0	29.21	47.19	68.25	82.02	98.03	99.71
BHT	0	22.19	41.01	65.16	78.08	95.78	99.15
$C_{11}H_{17}N_3S$	0	32.30	49.30	57.25	62.48	69.24	80.33
$C_{17}H_{21}N_3S$	0	33.62	38.79	54.31	77.01	97.12	97.70
(**1**)	0	32.02	47.23	52.16	57.40	64.88	74.43
(**2**)	0	33.70	49.36	56.12	62.64	68.36	76.40
(**3**)	0	24.71	40.14	53.32	63.08	75.00	82.30
(**4**)	0	37.93	45.40	51.72	79.31	91.66	96.83
(**5**)	0	28.08	46.06	58.42	66.57	76.12	93.82
(**6**)	0	38.48	55.33	70.78	77.80	87.35	97.47
(**7**)	0	36.79	53.93	68.82	76.68	86.51	98.03
(**8**)	0	39.65	44.54	50.28	58.33	67.81	72.12

Table 6. IC_{50} values of test compounds (μg/mL).

Compound	IC_{50} (μg/mL) DPPH assay	IC_{50} (μg/mL) ABTS assay
AA	131.9	12.0
CAT	149.8	—
BHT	—	15.58
$C_{11}H_{17}N_3S$	167.4	11.32
$C_{17}H_{21}N_3S$	69.87	20.83
(**1**)	203.50	18.43
(**2**)	190.01	11.42
(**3**)	87.10	21.22
(**4**)	73.94	20.92
(**5**)	70.20	14.78
(**6**)	24.80	8.42
(**7**)	28.90	8.85
(**8**)	199.72	24.27

$[Co(C_{11}H_{17}N_3S)_2Cl_2]$ (**4**) and $[Cu(C_{11}H_{17}N_3S)_2Cl_2]$ (**7**) showed a strong interactive ability with DPPH and ABTS, respectively, compound (**4**) shows maximum free radical scavenging activity (96.97%) for DPPH radicals and compound (**7**) shows maximum free radical scavenging activity (98.03%) for ABTS radicals, followed by (97.56%), (97.70%) in IPMCHPhTSC for DPPH and ABTS radicals, respectively, while least activity (82.99%) and (80.33%) were observed from IPMCHTSC for DPPH and ABTS radicals, respectively. The complex (**4**) and (**7**) expressed an IC_{50} value of 73.94 μg/mL and 28.90 μg/mL for DPPH radical, lower than that of the standards [i.e. AA (131.9 μg/mL) and CAT (149.8 μg/mL)]. The compound IPMCHTSC, (**2**), (**6**), and (**7**) expressed an IC_{50} values of 11.32, 11.42, 8.42, and 8.85 μg/mL, respectively, for ABTS radical which is lower than that of the standards [i.e. AA (12.0 μg/mL) and BHT (15.58 μg/mL)].

The comparative antioxidant activity of compounds against AA, CAT, and BHT as a standard is shown by graphs (Figure 3).

All DPPH and ABTS scavenging activities were carried out only to evaluate the relative antioxidant activities. In the experiment standard antioxidants and other synthetic antioxidants were mixed with DPPH and ABTS in same fraction ratio which was not 1:1 ratio but actually it was 1:6 ratio that's why 50% scavenging was observed with high concentration of antioxidants and large IC_{50} values were observed although it was only to compare synthetic antioxidants with standard antioxidants.

Conclusion

The metal complexes isolated during the present study demonstrated that the interaction of metal chloride with thiosemicarbazones of carvone leads to complexes

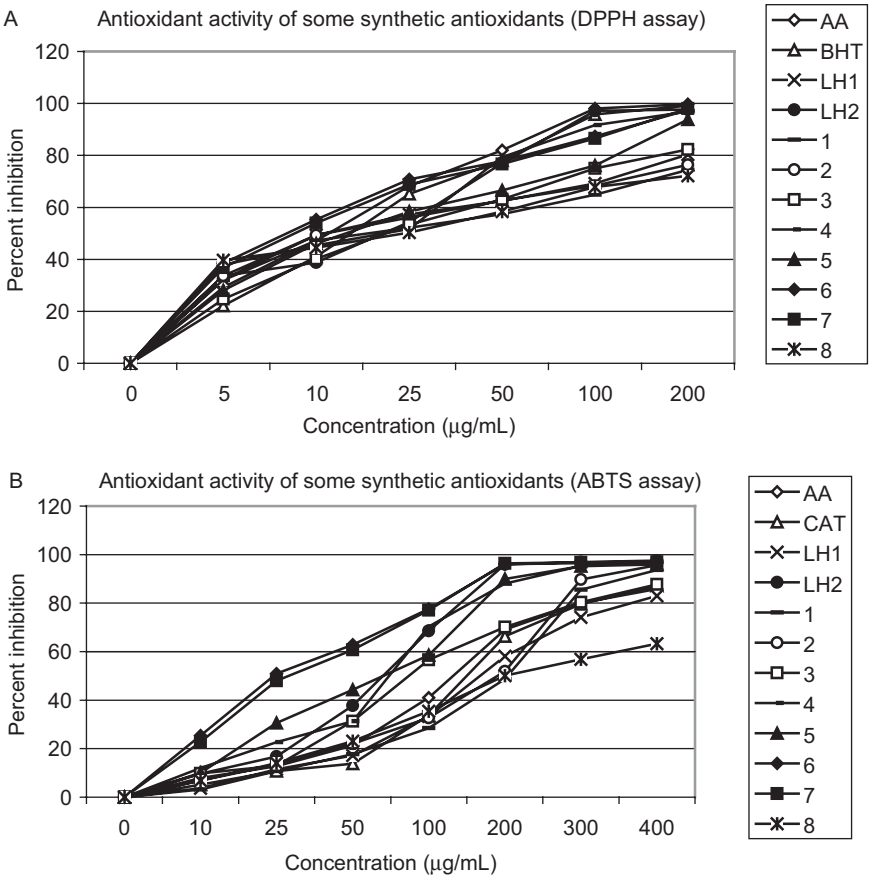


Figure 3. Free Radical Scavenging Activity of compounds (A) DPPH assay and (B) ABTS assay.

with 1:1 and 1:2 stoichiometry and are found to be mononuclear. The bidentate nature of both type of ligands have been suggested on the basis of spectral evidences. The antioxidants activity of considered compounds are very large which indicate that among the test compound ligand IPMCHTSC, IPMCHPhTSC and their complexes showed potent antioxidant activity. The free redical scavenging activity at different concentrations (10–400, 5–200 µg/mL) of metal complexes in DPPH and ABTS assay are clearly mentioned in Tables 4 and 5, respectively. The conditions for carrying out the antioxidant activities in the laboratory shows the results that indicate the good potentiality of the synthesized compounds as synthetic antioxidants and they can serve as dietary antioxidants accordingly but clinical trials are necessary to find out the Lethal Dose LD_{50} for the induction of any material in dietary supplements.

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Declaration of interest

The authors report no conflicts of interest.

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