SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF SOME TRANSITION METAL COMPLEXES WITH TERPENOID DERIVATIVES

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ABSTRACT

A series of transition metal complexes of Fe(III), Co(II) and Cu(II) containing the bidentate N,O and N,S donor ligand, camphor semicarbazone (1,7,7-trimethylbicyclo [2,2,1]heptanesemicarbazone, TBHSC) and camphor thiosemicarbazone (1,7,7- trimethylbicyclo [2,2,1]heptanethiosemicarbazone, TBHTSC) have been synthesized and characterized by elemental analysis, molar conductance measurement and various spectral studies (IR, electronic and FAB Mass) and thermogravimetric analysis (TGA). All the metal complexes **(1-8)** are $[M(LH)Cl_2]$ and $[M(LH)_2Cl_2]$ type, where M = Fe(III), Co(II) and Cu(II); LH = TBHSC and TBHTSC. TBHSC and TBHTSC act as neutral bidentate ligands in all the complexes. FAB mass spectroscopic studies of the three representative complexes **(1), (2), (4), (5)** and **(8)** suggest their monomeric nature. The proposed geometries of the complexes were octahedral geometry 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 newly synthesized ligands (TBHSC, TBHTSC) and their metal complexes have been determined at the concentration range of 50-1000 µg/ml by means of their interaction with the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). All the compounds have shown encouraging antioxidant activities. The least IC_{50} value (111.0 µg/ml) for compound **(5)** showed the potent scavenging property compared to other test compounds.

Keywords: molar conductance; thermogravimetric analysis; radical scavenging activity; DPPH.

INTRODUCTION

Thiosemicarbazones are well established as an important class of sulfur donor ligands particularly for transition metal ions.[1-6] During the last few decades there has been a growing interest in the pharmacological properties of semicarbazones, thiosemicarbazones and their metal complexes due to their ability to function as antiviral, antibacterial, antifungal, anticancer and antioxidant agents.[7-13] The activity is usually increased by complexation therefore to understand the properties of both ligands and metal can lead to the synthesis of highly active compounds.[14-20] The influence of certain metals on the biological activity of these compounds and their intrinsic chemical interest as multidentate ligands has prompted a considerable increase in the study of their coordination behavior.[21-23] Previously, we have reported the structural and spectral studies of transition metal complexes of some acyclic monoterpenic constituents of essential oils.[24,25] Our ongoing research work on transition metal complexes with semicarbazones and thiosemicarbazones involving such systems led us to describe the synthesis, characterization and antioxidant activity of some transition metal complexes with semicarbazone and thiosemicarbazone derived from camphor (1,7,7-trimethylbicyclo [2,2,1] heptane-2-one). Camphor is a bicyclic monoterpenone and important constituent of several essential oils, e.g. sage oil.[26] The preparation and study of inorganic compounds containing biologically important ligands is made easier because metal ions used are active in many biological processes.[27-29] The fact that transition metals are essential metallic elements and exhibit great biological activity when associated with certain metal electronic transfer reactions or the storage of ion[30-32] has created attention in the study of system.

Drugs with antioxidant mechanisms are being widely proposed as starting point for the development of new therapeutic interventions in several pathological disorders associated with oxidative damage, caused by reactive oxygen species (ROS), including hydrogen peroxide, superoxide anion and hydroxyl radical, under conditions of 'oxidative stress'.[33,34] This term refers to an imbalance between ROS production and detoxification, in favour of the former, and it is characterized by excessive production of ROS and reduction in the responsible for their metabolism antioxidant defences.[35,36]

Antioxidants are the compounds, which terminate the attack of reactive species like free radicals and prevent it from ageing and different disease associated with oxidative damages inside the body system.[37] 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 plays a significantly pathological role in human diseases such as cancer, emphysema, cirrhosis, atherosclerosis and arthritis etc.[38,39] Almost all organisms are protected to some extent by free radical (peroxide, hydro-peroxide or lipid peroxyl) damage by enzymes such as super-oxide dismutase and catalase or compounds such as ascorbic acid, tocopherols, phenolic acids, polyphenols, flavonoids and glutathione.[40] However, antioxidant supplements or dietary antioxidants may be sources of protection that the body needs to protect against the damaging effects of free radicals.[41] Presently, synthetic antioxidants are widely used because they are effective and cheaper than natural antioxidants.

EXPERIMENTAL SECTION

Material and Methods

All the chemicals and reagents used were of AR grade. Solvents were dried by conventional methods and distilled prior to use. Ligands were prepared by method reported earlier.[24,42] Metal contents were measured by complexometric titration[48]. Sulfur was estimated gravimetrically as $BaSO_4$ and chloride content was determined by Volhard's Methods.[43]

Elemental analyses were carried out on Thermoquest analyzer. The 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 $CDCl_3$ solution using 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 and electronic spectra were recorded on a Agilent UV/Visible spectrometer. Molar conductivities of 10^{-3} M DMSO solutions were measured on a microprocessor based conductivity meter model 1601/E. Thermogravimetric analysis was performed by Perkin Elmer Thermal Analyzer with the heating rate 35-800/10ºC under nitrogen atmosphere. Mass spectra were recorded on Shimadzu Mass Spectrophotometer. Antioxidant activity was measured on Spectro UV-Vis double beam PC scanning spectrophotometer (LABOMED. INC) Vortex (SPINIX).

Synthesis of Ligands

Synthesis of 1,7,7-trimethylbicyclo [2,2,1]heptanethiosemicarbazone (TBHTSC)

The equimolar quantity of (±) camphor (3.04g, 20 mmol) and thiosemicarbazide (1.83g, 20 mmol) was dissolved in ethanol (50 mL) and few drops of conc. H_2SO_4 were added. The reaction mixture was refluxed for 6 h and then kept in ice for overnight. The resulting white solid was filtered, recrystallized from water and ethanol mixture and dried over P_2O_5. Yield: 75% (3.3 g); M. Pt. 137-139ºC; IR (cm^{-1}): 3425s, 3225s, br $\nu(NH_2)$; 3195s, n(NH); 1595s, n(C=N); 875m, n(C=S); 945, n(N-N); ^1H NMR ($CDCl_3$, d ppm): 0.74

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(s, 3H, H-8); 0.94 (s, 3H, H-10); 0.98 (s, 3H, H-9); 1.18-1.26 (m, 1H, H-5 endo); 1.33-1.42 (m, 1H, H-6 endo); 1.70-1.79 (m, 1H, H-6 exo); 1.80-1.85 (m, 1H, H-5 exo); 1.87-1.92 (m, 1H, H-4); 2.02-2.05 (m, 1H, H-3 endo); 2.36-2.44 (1H, H-3 exo); 7.27, 7.23 (2s, 2H, NH_2); 9.45 (s, 1H, NH-C=S); ^{13}C NMR ($CDCl_3$, d ppm): 11.0 (C-10); 18.5 (C-8 or C-9); 19.4 (C-8 or C-9); 27.1 (C-5); 32.4 (C-3); 33.8 (C-6); 43.9 (C-4); 47.9 (C-7); 52.3 (C-1); 167.2 (C=N); 177.4 (C=S). Anal. Found for $C_{11}H_{19}N_3S$ (225.35): C, 58.57; H, 8.43; N, 18.63; S, 14.31. Calcd. C, 58.62; H, 8.49; N, 18.64; S, 14.22 %.

Synthesis of 1,7,7-trimethylbicyclo [2,2,1]heptanesemicarbazone (TBHSC)

An aqueous solution (50 mL) of semicarbazide hydrochloride (3.34 g, 30 mmol) and crystalline sodium acetate (4.08 g, 30 mmol) was added dropwise with constant stirring to an ethanolic solution (50 mL) of camphor (4.56 g, 30 mmol) and refluxed. After 7 h refluxing TBHSC precipitated as white solid on cooling. It was filtered and recrystallized from water-ethanol mixture and dried in vacuum. Yield: : 90% (5.7 g); M. Pt. 238-239ºC; IR (cm^{-1}): 3455s, 3260s, br $\nu(NH_2)$; 3232s, n(NH); 1698, n(C=O); 1553s, n(C=N); 960, n(N-N); ^1H NMR ($CDCl_3$, d ppm): 0.74 (s, 3H, H-8); 0.94 (s, 3H, H-10); 0.98 (s, 3H, H-9); 1.18-1.26 (m, 1H, H-5 endo); 1.33-1.42 (m, 1H, H-6 endo); 1.70-1.79 (m, 1H, H-6 exo); 1.80-1.85 (m, 1H, H-5 exo); 1.86-1.94 (m, 1H, H-4); 2.02-2.05 (m, 1H, H-3 endo); 2.37-2.44 (m, 1H, H-3 exo); 7.28, 7.22 (2s, 2H, NH_2); 8.40 (s, 1H, NH–C=O); ^{13}C NMR ($CDCl_3$, d ppm): 11.0 (C-10); 18.6 (C-8 or C-9); 19.4 (C-8 or C-9); 27.2 (C-5); 32.5 and 33.5 (C-3 and C-6); 43.9 (C-4); 47.9 (C-7); 52.3 (C-1); 158.1 (C=N); 163.5 (C=O). Anal. Found for $C_{11}H_{19}N_3O$ (209.28): C, 63.06; H, 9.07; N, 20.06. Calcd. C, 63.12; H, 9.15; N, 20.07 %.

Preparation of metal complexes in (1 : 1) and (1 : 2) molar ratio with camphor thiosemicarbazone and (1 : 2) with camphor semicarbazone

To an ethanolic solution (~ 20 ml) of $CoCl_2.6H_2O$ (2.39 g, 10 mmol), a hot ethanolic solution (~ 25 ml) of ligand (TBHTSC) (2.28 g, 10 mmol) was added dropwise with constant stirring. After complete addition the reaction mixture was refluxed for 6h. and cooled to room temperature. The solvent was evaporated under vacuum and the residue dark blue precipitate was washed with anhydrous ethanol and diethyl ether and dried in vacuum to give dark blue coloured solid. Similar route have been employed for the preparation of Cu(II) complex.

To an ethanolic solution (~ 20 mL) of $CoCl_2.6H_2O$ (1.19 g, 5 mmol), a hot ethanolic solution (~25 mL) of ligand (TBHTSC) (2.25 g, 10 mmol) was added dropwise with constant stirring. After complete addition the reaction mixture was refluxed for *ca.* 4 h and cooled to room temperature. The resulting dark blue precipitate was filtered, washed several times with anhydrous ethanol and dried under reduced pressure.

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

ANTIOXIDANT ACTIVITY

Antioxidant activity of the compounds was estimated by DPPH radical scavenging effect. The method for estimating free radical scavenging activity of the methanolic solutions of bioactive compounds were under taken as suggested by Hatano *et al*. (1988).[44] The DPPH reagent evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants.[45]

The % scavenging activity was calculated (Table 6) by using the formula:

% Scavenging Activity = $[(A_c-A_t) / A_c] \times 100$

Where, A_c = Absorbance of the control sample

A_t = Absorbance of the test sample after 40 min.

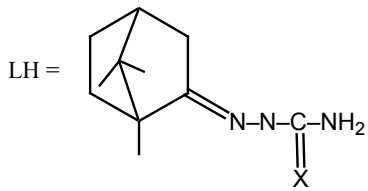
The scavenging activity was expressed as IC_{50} value (Table 7), which is defined as the concentration (µg/ml) of compound required for scavenging of DPPH radicals by 50%. IC_{50} values were determined by linear regression analysis using at least five different concentrations in duplicate.[46]

RESULTS AND DISCUSSION

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

$$MCl_x.mH_2O + y\ LH \xrightarrow[\text{Reflux}]{\text{EtOH}} [M(LH)_yCl_2]^{+n}$$

{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 = TBHTSC, y = 2 for LH = TBHSC; n = 0 for Co(II), Cu(II) and 1 for Fe(III)



Where X = O (TBHSC)
X = S (TBHTSC)

Several analytical techniques were used to characterize the complexes including microanalysis (CHN), spectral studies (IR, electronic and FAB mass), thermogravimetric analysis (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 but soluble in DMSO, THF and CH_2Cl_2.

The molar conductivity shows that all the complexes are non-electrolytes with l = 17.8-23.7 Ω^{-1} cm^2 mol^{-1} in DMSO (10^{-3} M) solution at room temperature.

Infrared spectra

The main IR spectral bands of complexes and their assignments are presented in Table 2. In the spectrum of TBHSC amide (C=O) band observed at 1698 cm^{-1} is shifted to lower frequency due to coordination of amido oxygen to metal ion[24]. The ligand TBHTSC shows band at 875 cm^{-1} which shifted to the downward region in the complexes suggests the coordination of metal ion through the C=S group[24]. The spectra of both ligands exhibit a band in the 1553-1580 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 coordinated to metal[24,42]. In ligands the highest frequency bands observed in 3425-3455 cm^{-1} and 3225-3260 cm^{-1} regions are assigned to asymmetric and symmetric stretching of terminal NH_2 group vibration. The second highest band observed at 3195-3232 cm^{-1} region due to the imino group stretching vibration. In the complexes the above bands are not affected indicating non-participation of amino and imino nitrogen atoms in coordination.

In the IR spectra of all the metal complexes, the bands observed in the regions 420-480 and 320-382 cm^{-1} can be assigned to M-N and M-Cl stretching respectively[4]. The bonding of oxygen to the metal ions in the corresponding semicarbazone complexes is proved by the occurrence of the ν(M-O) band at the 425-472 cm^{-1} region[24]. The bonding of sulfur to the metal ion in the corresponding thiosemicarbazone complexes is indicated by the occurrence of the ν(M-S) band at the 322-392 cm^{-1} region[4].

Electronic Spectra

The electronic spectrum of Fe(III) complexes shows 801 nm and 575 nm may be assigned to $^6A_{1g} \rightarrow {}^4T_{1g}$ and $^6A_{1g} \rightarrow {}^4T_{2g}$ transitions, respectively, in an octahedral geometry around Fe(III).[47] The electronic spectrum of the cobalt(II) complexes exhibit four bands at 907, 682, 608 and 260 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 cobalt(II) complex.[48-50]

The Cu(II) complexes shows bands at 948, 621 and 408 nm which are assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ (ν_1), $^2B_{1g} \rightarrow {}^2B_{2g}$ (ν_2) and ^2Eg (ν_3) transitions. The positions of these bands and their assignments suggest distorted octahedral geometry.[51] The absorption bands appearing in the UV domain are considered to the characteristics of the ligand. The assignment of n-p* and p-p* transition as being due to the (C=O) and (C=S) bond. The electronic spectrum of $[Co(TBHTSC)Cl_2]$ exhibits three bands at 1098, 980 and 675 nm. The first two bands are assigned to $^2B_{2g} \rightarrow {}^2E_g$ and $^2B_{2g} \rightarrow {}^2A_{1g}$ transitions, respectively, in a square planar environment of Co(II)[51]. The spectrum of $[Cu(TBHTSC)Cl_2]$ shows a band at 489 nm indicates square planar geometry for Cu(II) complex[52,53]. (Table 3).

Table 1. Physical and analytical data for complexe.

Compound (Empirical formula)	Colour	M. Pt.	Yield (%)	Molar conductance ($^{-1}$ mol^{-1} cm^2)	Found (Calcd.) %					
					C	H	N	S	M	Cl
[Co(TBHTSC)Cl_2] **(1)** [Co ($C_{11}H_{19}N_3S$)Cl_2]	Dark blue	172	85	17.9	37.50 (37.19)	5.10 (5.39)	11.40 (11.82)	9.10 (9.02)	15.96 (16.59)	19.78 (19.96)
[Cu(TBHTSC)Cl_2] **(2)** [Cu ($C_{11}H_{19}N_3S$)Cl_2]	Green	262	78	18.4	36.50 (36.72)	5.01 (5.32)	11.98 (11.67)	7.99 (8.91)	16.75 (17.66)	18.76 (19.70)
[Fe(TBHTSC)$_2Cl_2$]Cl **(3)** [Fe($C_{11}H_{19}N_3S$)$_2Cl_2$]Cl	Brown	81	89	19.2	43.18 (43.11)	7.03 (6.24)	13.60 (13.71)	10.04 (10.46)	8.93 (9.11)	17.63 (17.35)
[Co(TBHTSC)$_2Cl_2$] **(4)** [Co($C_{11}H_{19}N_3S$)$_2Cl_2$]	Dark blue	194	85	23.7	45.15 (45.51)	6.93 (6.59)	14.11 (14.47)	11.19 (11.04)	10.02 (10.15)	12.29 (12.21)
[Cu(TBHTSC)$_2Cl_2$] **(5)** [Cu ($C_{11}H_{19}N_3S$)$_2Cl_2$]	Yellow	118	88	19.9	45.11 (45.15)	6.49 (6.54)	14.42 (14.36)	10.45 (10.95)	10.11 (10.85)	13.12 (12.11)
[Fe(TBHSC)$_2Cl_2$]Cl **(6)** [Fe($C_{11}H_{19}N_3O$)$_2Cl_2$]Cl	Brown	199	85	17.8	45.11 (45.49)	5.95 (6.59)	14.49 (14.46)	-	9.10 (9.61)	17.99 (18.31)
[Co(TBHSC)$_2Cl_2$] **(7)** [Co($C_{11}H_{19}N_3O$)$_2Cl_2$]	Pink	231	74	22.9	48.53 (48.18)	6.43 (6.98)	15.15 (15.32)	-	9.83 (10.74)	14.07 (12.92)
[Cu(TBHSC)$_2Cl_2$] **(8)** [Cu ($C_{11}H_{19}N_3O$)$_2Cl_2$]	Parrot green	194	90	18.9	47.38 (47.78)	6.81 (6.92)	15.40 (15.19)	-	11.30 (11.49)	13.10 (12.82)

Table 2. Main IR spectral vibrations for complexes.

Compound	*ν*(NH_2)	*ν*(NH)	*ν*(C=N))	*ν*(C=O)	*ν*(N–N)	*ν*(C=S)	*ν*(M–O)	*ν*(M–N)	*ν*(M–S)	*ν*(M–Cl)
(1)	3420 3222	3190	1580	-	945	845	-	434	392	330
(2)	3432 3223	3192	1582	-	942	842	-	450	380	360
(3)	3423 as 3225 s	3182	1560	-	940	869	-	455	322	382
(4)	3420 as 3225 s	3175	1542	-	942	862	-	445	335	365
(5)	3425 as 3165 s	3190	1535	-	930	865	-	480	330	325
(6)	3455 as 3265 s	3221	1565	1640	950	-	472	465	-	320
(7)	3450 as 3254 s	3225	1562	1665	945	-	425	420	-	
(8)	3440 as 3270 s	3230	1556	1650	930	-	438	425	-	345

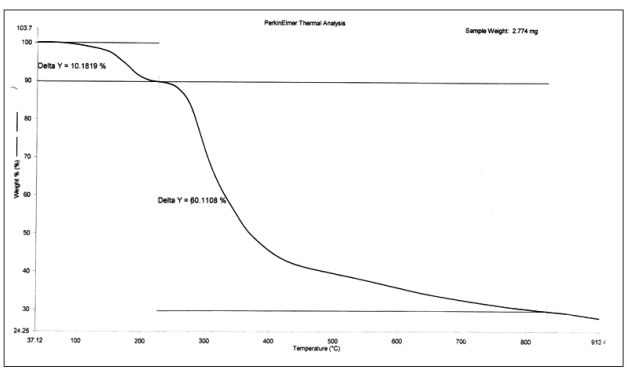
Table 3. Electronic absorption bands in complexes

Compound	Electronic spectral bands
(1)	1098; 980; 675
(2)	489
(3)	825; 575
(4)	881; 660; 620; 252
(5)	930; 625; 441
(6)	840; 562
(7)	892; 685; 608; 254
(8)	942; 612; 415

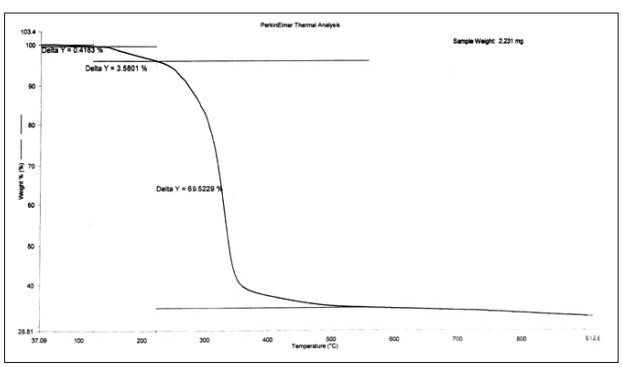
Thermal Studies

The thermogram for complexes $[Co(TBHTSC)Cl_2]$ (**1**) and $[Cu(TBHTSC)Cl_2]$ (**2**) revealed three step decomposition behavior (Fig. 2a,b). These TG steps are connected with exothermic events caused due to the pyrolysis of organic byproducts. The thermogram, also exhibits completion of the decomposition at 900°C. The residual for complex (**1**) was 29.70% (obs.), corresponding to the formation of Co_2S_3 (calcd. 30.13%). The residual for complex (**2**) was 26.48% (obs.), corresponding to the formation of CuS (calcd. 26.57%).

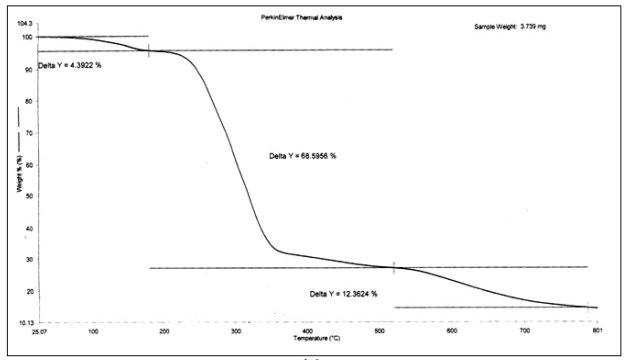
The thermogram for complex $[Cu(C_{11}H_{19}N_3S)_2Cl_2]$ **(5)** (Fig. 2c) revealed a three step decomposition behavior. These TG steps are connected with exothermic events caused due to the pyrolysis of organic byproducts. The thermogram, also exhibits completion of the decomposition at 800˚C. The residual was 14.65%, corresponding to the formation of Cu_2S being the final product (Calcd. 13.59 %,).



(a)



(b)



(c)

Fig. 2: TGA curve of {weight (%) vs temperature (°C)} (a) $[Co(TBHTSC)Cl_2]$, (b) $[Cu(TBHTSC)Cl_2]$ and (c) $[Cu(TBHTSC)_2Cl_2]$

Mass spectra

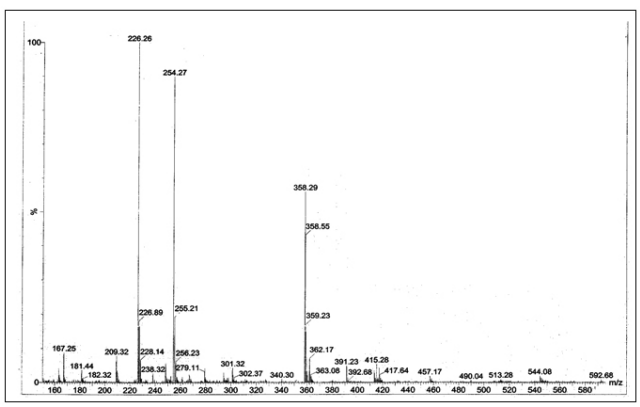
The FAB mass spectral studies of three of the representative compounds**,** $[Co(C_{11}H_{19}N_3S)Cl_2]$ **(1),** $[Cu(C_{11}H_{19}N_3S)Cl_2]$ **(2),** $[Co(C_{11}H_{19}N_3S)_2Cl_2]$ **(4),** $[Cu(C_{11}H_{19}N_3S)_2Cl_2]$ **(5)** and $[Cu(C_{11}H_{19}N_3O)_2Cl_2]$ **(8)** (Table 4) indicate their monomeric nature. The molecular ion peaks of **(2)** and **(5)** appears at m/z 359.58 and 585.0, thus confirming the formation of a metal complexes in 1:1 and 1:2 ratios. (Table 4 and Fig. 3a-e).

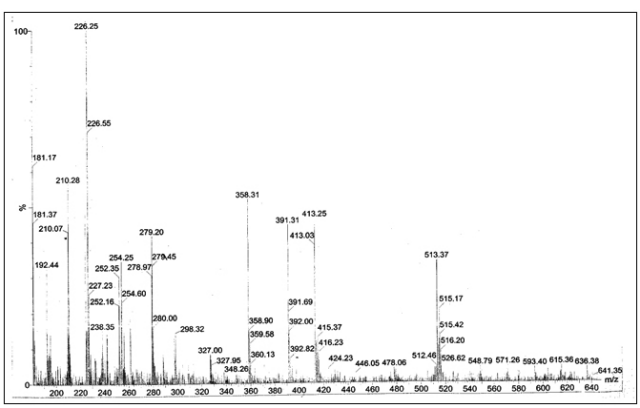
Table 4. Fragmented molecular ions *vs* m/z values of $[Co(C_{11}H_{19}N_3S)Cl_2]$ (1), $[Cu(C_{11}H_{19}N_3S)Cl_2]$ (2) $[Co(C_{11}H_{19}N_3S)_2Cl_2]$ (4), $[Cu(C_{11}H_{19}N_3S)_2Cl_2]$ (5) and $[Cu(C_{11}H_{19}N_3O)_2Cl_2]$ (8).

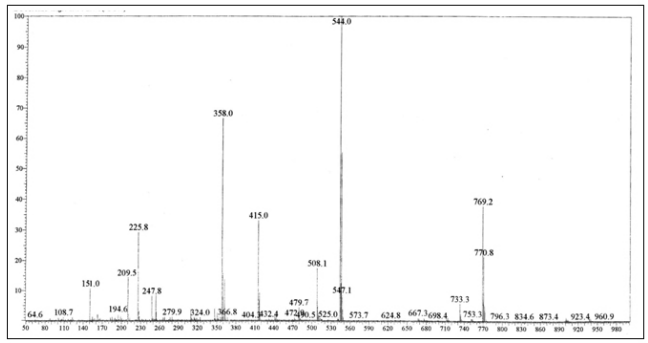
Compound	Fragmented ions	m/z value
$[Co(C_{11}H_{19}N_3S)Cl_2]$**(1)**	$[Co(C_{11}H_{19}N_3S)Cl_2]$	355.19
	$[Co(C_{10}H_{17}N_3S)Cl_2]^+$	341.16
	$[Co(C_8H_{17}N_2S)Cl_2]^+$	303.13
	$[Co(C_6H_{17}N_2S)Cl_2]^+$	279.10
	$[Co(C_4H_{17}N_2S)Cl_2]^+$	255.07
	$[Co(C_4H_{16}N_2S)Cl_2]^+$	254.06
	$[Co(C_3H_{14}NS)Cl_2]^+$	228.04
	$[Co(C_3H_{11}S)Cl_2]^+$	209.00
	$[Co(CH_9S)Cl_2]^+$	181.95
	$[Co(H_7S)Cl_2]^+$	167.93
$[Cu(C_{11}H_{19}N_3S)Cl_2]$ **(2)**	$[Cu(C_{11}H_{19}N_3S)Cl_2]$	359.80
	$[Cu(C_{11}H_{18}N_3S)Cl_2]^+$	358.72
	$[Cu(C_6H_{18}N_3S)Cl_2]^+$	298.67
	$[Cu(C_5H_{12}N_3S)Cl_2]^+$	280.62
	$[Cu(C_5H_{11}N_3S)Cl_2]^+$	279.61
	$[Cu(C_5H_{10}N_3S)Cl_2]^+$	278.61
	$[Cu(C_3H_{10}N_3S)Cl_2]^+$	254.59
	$[Cu(C_3H_8N_3S)Cl_2]^+$	252.58
	$[Cu(C_3H_8N_2S)Cl_2]^+$	238.58
	$[Cu(C_2H_8N_2S)Cl_2]^+$	226.58
	$[Cu(C_2H_6NS)Cl_2]^+$	210.56
	$[Cu(CH_2H_3S)Cl_2]^+$	193.54
	$[Cu(CH_3S)Cl_2]^+$	181.53
$[Co(C_{11}H_{19}N_3S)_2Cl_2]$ **(4)**	$[Co(C_{11}H_{19}N_3S)_2Cl_2]$	580
	$[Co(C_{11}H_{19}N_3S)(C_{11}H_{19}N_3S)Cl]^+$	545
	$[Co(C_{11}H_{19}N_3S)(C_{11}H_{18}N_3S)Cl]^+$	544
	$[Co(C_{11}H_{19}N_3S)(C_{11}H_{18}N_3S)]^+$	509
	$[Co(C_{11}H_{17}N_3)(C_{10}H_{17}N_2)]^+$	415
	$[Co(C_{10}H_{16}N)(C_{10}H_{16}N)]^+$	359

	$[Co(C_{10}H_{16}N)(N)]^+$	223
	$[Co(C_{10}H_{16}N)]^+$	209

	$[(C_{10}H_{16}N)]^+$	150
$[Cu(C_{11}H_{19}N_3S)_2Cl_2]$ **(5)**	$[Cu(C_{11}H_{19}N_3S)_2Cl_2]$	585
	$[Cu(C_{11}H_{19}N_3S)_2Cl]^+$	550
	$[Cu(C_{11}H_{19}N_3S)_2]^+$	514
	$[Cu(C_{11}H_{19}N_3S)(C_{11}H_{18}N_3S)]^+$	513
	$[Cu(C_{11}H_{16}N_3)(C_{10}H_{16}N_2)]^+$	418
	$[Cu(C_{11}H_{16}N_3)(C_{10}H_{16}N)]^+$	404
	$[Cu(C_{10}H_{16}N)(C_{10}H_{16}N)]^+$	364
	$[Cu(C_{10}H_{16}N)(N)]^+$	228
	$[Cu(C_{10}H_{16}N)]^+$	214
	$[(C_{10}H_{16}N)]^+$	150
$[Cu(C_{11}H_{19}N_3O)_2Cl_2]$ **(8)**	$[Cu(C_{11}H_{19}N_3O)_2Cl_2]$	553
	$[Cu(C_{11}H_{19}N_3O)(C_{10}H_{16}N)Cl_2]^+$	494
	$[Cu(C_{11}H_{17}N_2O)(C_{10}H_{16}N)Cl_2]^+$	478
	$[Cu(C_{11}H_{17}N_2O)(C_{10}H_{16}N)Cl]^+$	443
	$[Cu(C_{11}H_{16}N_2O)(C_{10}H_{16}N)Cl]^+$	442
	$[Cu(C_{10}H_{16}N_2)(C_{10}H_{16}N)Cl]^+$	414
	$[Cu(C_{10}H_{16}N)(C_{10}H_{16}N)]^+$	364
	$[Cu(C_{10}H_{16}N)(N)]^+$	228
	$[Cu(C_{10}H_{16}N)]^+$	214
	$[(C_{10}H_{16}N)]^+$	150







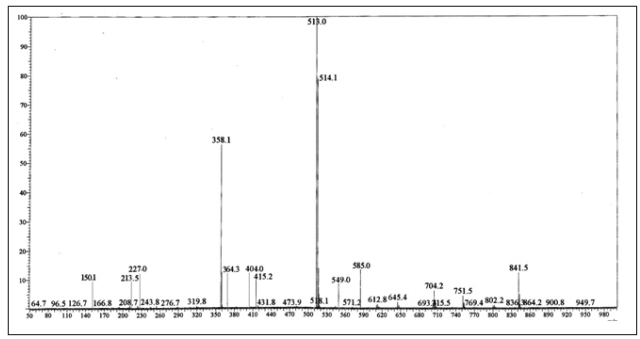


Fig.3a-e: Splitting patterns of FAB mass of {intensity vs m/z } (a) $[Co(TBHTSC)Cl_2]$, (b)$[Cu(TBHTSC)Cl_2]$,(c)$[Co(TBHTSC)_2Cl_2]$, (d) $[Cu(TBHTSC)_2Cl_2]$, and (e) $[Cu(TBHSC)_2Cl_2]$

On the basis of above analysis, the following structural formula (Fig. 1) may be suggested for the complexes.

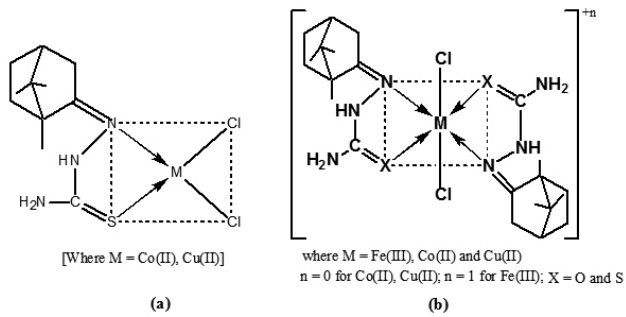


Fig. 1: Proposed structural formula for the complexes (a) $[M(LH)Cl_2]$ and (b) $[M(LH)_2Cl_2]$

Free radical scavenging activity of methanolic solutions using DPPH assay

Antioxidants can deactivate radicals by two major mechanisms, HAT and SET. Proton-coupled electron transfer and HAT reactions may occur in parallel, and the machanism dominating in a given system will be determined by antioxidant structure and properties, solubility and system solvent. Bond dissociation energy (BDE) and ionization potential(IP) are two major factors that determine the mechanism and the efficiency of antioxidants.[54]

HAT-based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation (AH= any H donor).

$$X^{\bullet} + AH \longrightarrow XH + A^{\bullet}$$

Relative reactivity in HAT methods is determined by the BDE of the H-donating group in the potential antioxidant, dominating for compounds with DDE of -10 kCal/mol and DIP of < - 36 kCal/mol.[54] HAT reaction are solvent and P^H independent and are usually quite rapid, typically completed in seconds to minutes.

1,1-diphenyl-2-picryl hydrazyl (DPPH) Assay:

The DPPH (Fig.4) radical is one of the few stable organic nitrogen radicals,

which bears a green color. It is commercially available and does not have to be generated before assay like $ABTS^{+}$. This assay is based on the measurement of the reducing ability antioxidants toward DPPH. The ability can be evaluated by electron spin resonance (EPR) or by measuring the decrease of its absorbance. Antioxidant assay are based on measurement of the loss of DPPH color at 517nm after reaction with test compound.

The percentage of the DPPH remaining is calculated as

$$\% \, DPPH^{\bullet}_{REM} = 100 \times [DPPH^{\bullet}]_{REM} / [DPPH^{\bullet}]_{T=0}$$

The percentage of remaining $DPPH^{\bullet}$ ($DPPH^{\bullet}_{REM}$) is proportional to the antioxidant concentration, the concentration that causes a decrease in the initial $DPPH^{\bullet}$ concentration by 50% is defined as IC_{50}.

Advantages of the DPPH assay: The test is simple and rapid and needs only a UV-vis spectrophotometer to perform, which probably explains its wide spread use in antioxidant screening. DPPH is a stable nitrogen radical that bears no similarity to the highly reactive and transient peroxyl radicals involved in lipid peroxidation. Many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH due to steric inaccessibility. DPPH also is decolorized by reducing agents as well as H transfer, which also contributes to inaccurate interpretations of antioxidant capacity.

Various researchers have used scavenging effect of a chemical on DPPH radical 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 Table 5 and 6. It is evident from results that free radical scavenging activity of these compounds was concentration dependent. Among the examined compound the complex $[Cu(C_{11}H_{19}N_3S)_2Cl_2]$ **(5)** showed a strong interactive ability with DPPH which was concentration dependent and this compound expressed an IC_{50} value (Table 7) of 111.0 µg/ml lower than that of ascorbic acid(136.0 µg/ml) and catachin (203.0 µg/ml) which were used as standard. Maximum free radical scavenging activity (96.09%) was found in compound **(5)** followed by (91.10%) in TBHTSC while least activity (8.60%) was observed from TBHSC. The comparative antioxidant activity of compounds against ascorbic acid and catachin as a standard is shown by graphs (Fig. 5).

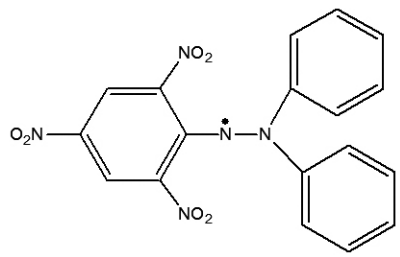


Fig. 4. Structure of 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$).

Table 5. Absorbance of compounds at different concentration at 517.0 nm

Compound	Concentration (µg/ml)							
	0	**50**	**100**	**150**	**200**	**250**	**500**	**1000**
Ascorbic acid (AA)	1.023	0.809	0.600	0.475	0.325	0.255	0.105	0.051
Catachin (CAT)	1.023	0.887	0.689	0.524	0.350	0.230	0.081	0.045
TBHTSC	1.023	0.847	0.682	0.522	0.429	0.317	0.121	0.091
TBHSC	1.023	0.992	0.974	0.968	0.958	0.945	0.939	0.935
$[Fe(TBHTSC)_2Cl_2]Cl$ **(3)**	1.023	0.840	0.659	0.530	0.488	0.441	0.345	0.268
$[Co(TBHTSC)_2Cl_2]$ **(4)**	1.023	0.934	0.831	0.760	0.9957	0.708	0.671	0.630
$[Cu(TBHTSC)_2Cl_2]$ **(5)**	1.023	0.752	0.547	0.384	0.281	0.200	0.075	0.040
$[Fe(TBHSC)_2Cl_2]Cl$ **(6)**	1.023	0.831	0.661	0.560	0.500	0.465	0.395	0.348
$[Co(TBHSC)_2Cl_2]$ **(7)**	1.023	0.989	0.970	0.954	0.935	0.938	0.930	0.924
$[Cu(TBHSC)_2Cl_2]$ **(8)**	1.023	0.985	0.934	0.908	0.905	0.887	0.866	0.836

Table 6. Antioxidant activity of ligands and metal complexes at different concentrations using DPPH assay.

Compound	Concentration (µg/ml)							
	0	**50**	**100**	**150**	**200**	**250**	**500**	**1000**
AA	0	20.92	41.35	53.57	68.23	75.07	89.74	95.01
CAT	0	13.29	32.65	48.78	65.79	77.52	92.08	95.60
TBHTSC	0	17.20	33.33	48.97	58.06	69.01	88.17	91.10
TBHSC	0	03.03	04.79	05.38	06.35	07.62	08.21	08.60
$[Fe(TBHTSC)_2Cl_2]Cl$ **(3)**	0	17.89	35.58	48.19	52.30	56.89	66.28	73.80
$[Co(TBHTSC)_2Cl_2]$ **(4)**	0	8.70	18.77	25.71	26.67	30.79	34.41	38.42
$[Cu(TBHTSC)_2Cl_2]$ **(5)**	0	26.49	46.53	62.46	72.53	80.45	92.67	96.09
$[Fe(TBHSC)_2Cl_2]Cl$ **(6)**	0	18.77	35.39	45.26	51.12	54.54	61.39	65.98
$[Co(TBHSC)_2Cl_2]$ **(7)**	0	03.32	05.18	06.74	08.60	08.31	09.09	09.68
$[Cu(TBHSC)_2Cl_2]$ **(8)**	0	03.71	08.70	11.24	11.53	13.29	15.35	18.28

Table 7. IC_{50} values of test compounds (µg/ml).

Compound	AA	CAT	TBHTSC	TBHSC	(3)	(4)	(5)	(6)	(7)	(8)
IC_{50} (µg/ml)	136.0	203.0	157.0	>1000	172.0	>1000	111.0	190.0	>1000	>1000

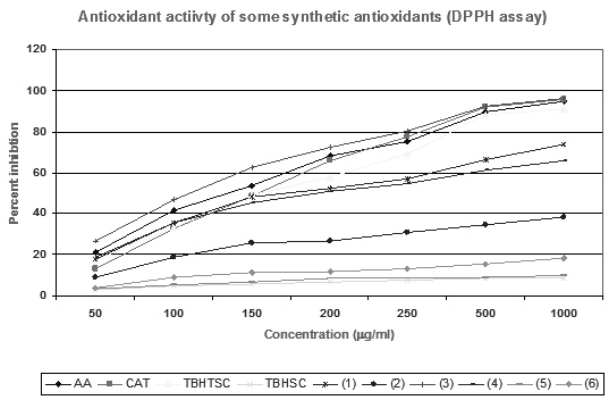


Fig. 5 : Graphical representation of % antioxidant of ligands and metal complexes (3-8) with respect to standards (Ascorbic acid and Catachin).

CONCLUSION

The metal complexes isolated during the present study demonstrated that the interaction of metal chloride with semicarbazone/thiosemicarbazone of camphor leads to complexes with 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. Results of the antioxidant activity experiments carried out in the laboratory clearly indicate that among the test compound ligand TBHTSC and complexes **(3)**, **(5)** and **(6)** showed potent antioxidant activity. All the compounds under study are synthetic in nature and thus these compounds can be referred as synthetic antioxidants.

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