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Studies on synthesis, characterization and biological properties of Copper (II)-embelin complex

N. Radhakrishnan, V. Kavitha and A. Gnanamani*

Microbiology Division, Central Leather Research Institute (CSIR, New Delhi), Adyar, Chennai, Tamil Nadu, India

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ABSTRACT

The present study describes, preparation, characterization and biological properties (Antioxidant & and hemolytic activity) of Copper (II)-embelin complex. Copper Nitrate from commercial source and embelin (extracted & purified from E.ribes) at 1:2 ratio were used for the preparation. About 56% yield of the complex was obtained upon purification. The characterization studies (FT-IR) revealed the shift in wavelength of C=O stretching frequency. With regard to biological properties, assessment of antioxidant profile using DPPH radical revealed, the parent compound embelin displayed 50% inhibition of DPPH radical at 26 µg/ml, whereas the complex showed only 39% inhibition at 150 µg/ml. Results on hemolytic activity emphasizes, only 40% RBC lyses was observed even at 150 µg/ml concentration for the complex, whereas it was 109 µg/ml for the parent compound embelin. The results of the study infers though Copper and embelin alone demonstrated potential biological activities, however, when they are in the form of complex the biological activities were found inferior with respect to DPPH radical scavenging activity.

Keywords: Embelin, Copper (II)-embelin complex, Diphenyl picrylhydrazyl radical - Scavenging activity, Thermal analysis, hemolytic activity.

INTRODUCTION

Embelin (2, 5 dihydroxy-3-undecyl-1, 4 benzoquinone) is synthesis by plants of the family Myrsinaceae. Embelin is well recognized for its biological activities like, antitumor, anti-inflammatory and analgesic activity [1] antibacterial [2], anticancer [3], chemo –preventive [4] and antioxidant activity [5]. Recently, we have also reported the hemolytic activity of embelin [6]. Metal complexes play a vital role in agricultural, pharmaceutical and industrial sectors [7]. The metal complexes have gained much attention in recent years. These complexes play a key role not only in the development of coordination chemistry, but also in catalysis, enzymatic reactions, magnetism & molecular architectures [8-10] and moreover they also exhibit fascinating various biological activities [11-13]. A lot of metal ions especially copper (II) plays vital roles in biological process of human beings [14]. This prompted us to investigate semi-synthetic derivative of embelin [namely copper (II)-embelin complex] whether superior or inferior in its biological activities (antioxidant and blood compatibility) when compare to that of its parent. In the present study, we describe the preparation, physical characterization, antioxidant (Diphenyl picrylhydrazyl radical - Scavenging activity) and hemolytic activity of copper (II)-embelin complex.

EXPERIMENTAL SECTION

All the chemicals and solvents used in the present study were of Analytical grade.

Plant material
E. ribes berries was obtained from M/s Abirami Botanical Corporation, (Tuticorin, India, in April 2010) and authenticated by Dr. T. Anandan, Research Officer, Anna Hospital, Chennai, India.

Extraction and characterization of embelin
Extraction and characterization of embelin was carried out according to our previous report [2].

Preparation of the Copper (II)-embelin complex
Preparation of the copper (II)-embelin complex was carried out as per procedure of Cherutoi and co-workers [15].

Instrumental analyses
Instrumental analyses such as UV, FT-IR, DSC and TGA were carried as per standard procedures. UV-Visible spectrum was recorded using UV-2450 from Shimadzu (Japan) in the wavelength range between 200-600 nm. FT-IR spectral measurement was made using Spectrum One (Perkin-Elmer Co., USA). Thermal analysis was recorded using differential scanning calorimeter (DSC Q200 (V23.10 Build 79)) and thermal gravimetric analysis (TGA Q50 (V20.6 Build 31)). Copper element or metal content was determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-Elmer 5300 DV model)

Preparation of sample and analysis of copper (Cu) element
A known weight (about 135 mg) of the sample was digested with 5.0 ml of aquaregia in CEM microwave digester using MARSX press (self regulating microwave vessel) under the following conditions

Stage	Maximum power	% power	Ramp (min)	Temperature °C	Hold (min)
1	800 W	75	30	200	15

The digested solution was made up to 50 ml using de-ionized water and it was thoroughly filtered using Whatmann 40 filter paper and the clear solution was analyzed by ICP-OES and the following results were obtained. A blank solution was also prepared in a similar manner and the intensity values were subtracted. The results were weight percentage calculated using the following equation

$$\text{Weight percentage} = \frac{\text{ppm (mg/L)} * \text{Volume in mL} * \text{dilution factor} * 10^{-4}}{\text{Weight of sample in grams}}$$

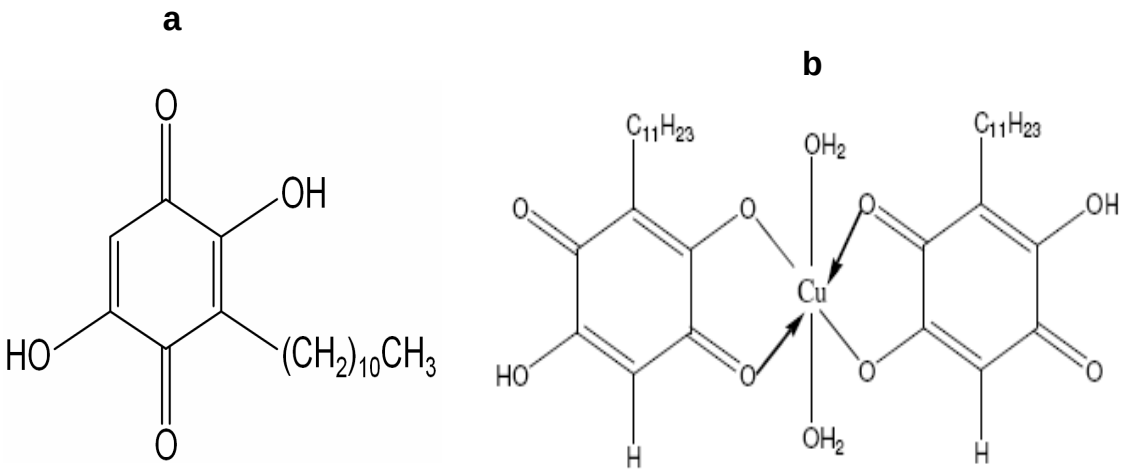
Diphenyl picrylhydrazyl (DPPH) radical - Scavenging activity
Diphenyl picrylhydrazyl (DPPH) radical - Scavenging activity was carried out as described by Yang and co-workers [16].

Hemolytic activity
The hemolytic activity of plant materials or its preparation was determined using suspension of erythrocytes cells (RBC), mixed with equal volumes of a serial dilution of the plant material according to WHO guideline [17].

RESULTS AND DISCUSSION

Figure 1a and 1b illustrated the structure of parent compound embelin and Copper (II) embelin complex.

Figure 1 Represents the a) Structure of embelin & b) Structure of Copper (II) embelin complex



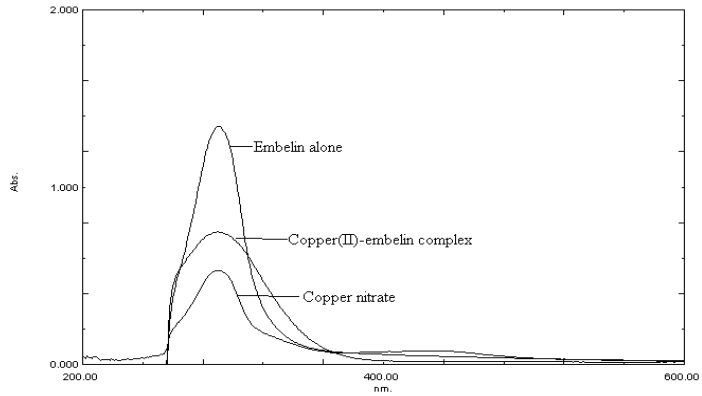
The yield of the metal complex was calculated as 56%. Table 1 describes the physical and chemical properties of Copper (II) embelin complex.

Table 1 Depicts physico-chemical analyses of Copper (II) embelin complex

Physico-chemical analyses	Characteristic features
Color and appearance	Green color solid in nature
Solubility	Insoluble in water, but precipitates with alcoholic solution. Sparingly soluble with organic solvents such as DMSO, ether and dioxane
UV spectrum	Exhibits λ_{max} at 290 nm
Melting point	280 ºC (as determined from DSC analysis)

The percentage of Copper in the complex obtained was calculated as 14.402% (ICP-OES analysis). Further, instrumental characterization studies revealed, the green colored product when subjected to UV-Visible spectral analysis displayed a shift in wavelength towards UV region (Figure 2) compared to the parent compound.

Figure 2 Represents the UV-Visible spectrum of embelin, Copper (II)-embelin complex and copper nitrate
(Spectrum was recorded in the wavelength region of 200-600 nm, using Shimadzu (Japan), UV-2450 model. Irrespective of sample, maximum absorbance was observed at ultra violet region).



FT-IR spectrum of copper (II)-embelin complex showed a considerable shift of the carbonyl absorption from 1610 cm^{-1} to 1460 cm^{-1} and of the sharp hydroxyl peak at 3300 cm^{-1} to a broad absorption in the region 3600-3050 cm^{-1} with a maximum at 3400 cm^{-1} as shown in Table 2.

Table 2 Represents the selected infrared bands of the embelin and Copper (II)-embelin complex

Sample	Infrared bands (cm^{-1})			
	ν (O-H)	ν (C-H str.)	ν (C=O str.)	ν(C-H out of plane)
Embelin	3300	2900,2850	1610	860, 825, 770
Copper (II)-embelin complex	3400	2900,2850	1460	860, 825, 770

(Infrared spectrum was recorded using Perkin-Elmer Co., (USA) Spectrum One model and resultant infrared bands were compared and tabulated).

This broad peak may also indicate the presence of coordinated water; similar observations were made by Cherutoi & Co-workers[15].

Thermal analysis of Copper (II) embelin complex (Figure 3) suggests melting point of embelin increases upon complexing with Copper.

Figure 3 Represents the differential scanning calorimetric analysis (DSC) of embelin, Copper (II)-embelin complex and Copper nitrate
(Differential scanning calorimetric analysis was analyzed up to 200 ° C using TA Instrument ((DSC Q200 (V23.10 Build 79) model).

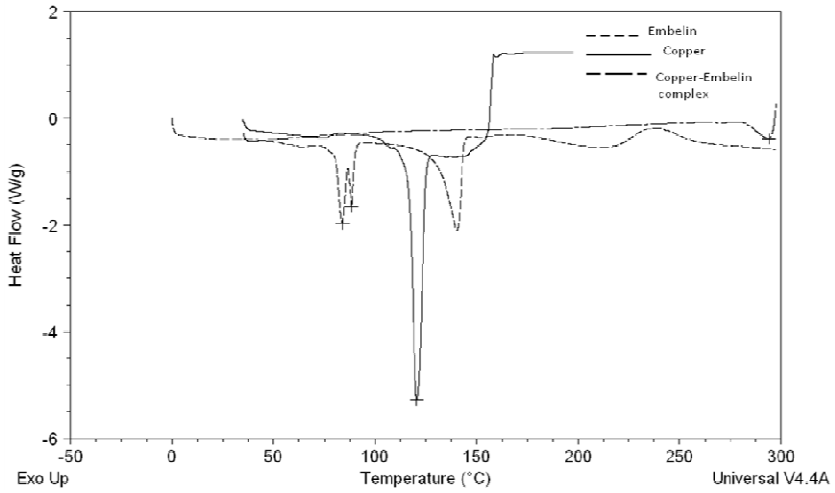
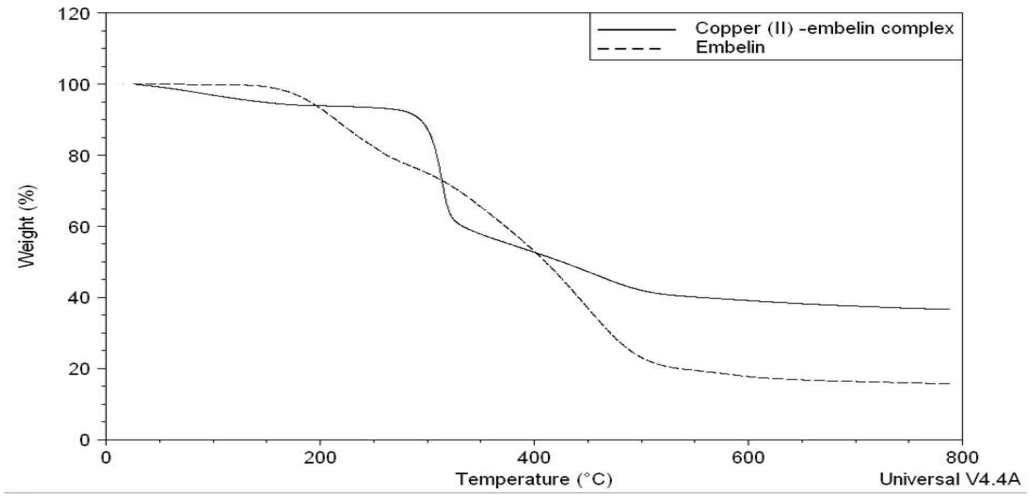


Figure 4 Represents the thermal gravimetric analysis (TGA) of embelin and Copper(II)-embelin complex
(Thermal gravimetric analysis was analyzed up to 800 ° C using TA Instrument (TGA Q50 (V20.6 Build 31) model).



Both Cu and embelin displayed sharp endothermic peak at 140ºC, whereas for the complex it was 280ºC. Similarly, thermo gravimetric analysis (TGA) suggests, increase in thermal stability of embelin after compelexing with Cu (Figure 4).

Embelin displayed stability up to 180ºC, whereas it increases to 360ºC for the complex prepared with Cu. Since, this report is the first kind with reference to the thermal analysis of the metal complex of embelin, no comparisons were made. The thermal stability of Copper (II)-embelin complex could be reasoned to the co-ordination of Copper with hydroxyl groups of embelin.

With reference to antioxidant activity, diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity for embelin and its Copper (II)-embelin complex using Vitamin-C as reference compound implies, the radical scavenging activity of embelin was better than its complex (as shown in Table.3) and it could be reasoned to the structural changes upon complexation and the reduced level of interaction of freely available –OH groups in the complex with DPPH.

Table 3 Represents the diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the embelin, copper (II)-embelin and Vitamin-C.

Sample	IC_{50} (in µg/ml)
Embelin	26 ± 1
Copper(II)-embelin complex	Only 39± 0.4 % even at 150 µg/ml
Vitamin-C	2.24 ± 0.2

(Free radical scavenging activity was determined by UV-Visible spectrophotometic method, using diphenyl-1-picrylhydrazyl (DPPH) radical & results were analyzed and tabulated).

Similar to the present findings, Sumino and co-workers [17] reported embelin exhibited DPPH free radical scavenging activity with 50% inhibitory concentration (IC_{50}) of 23.3 ± 0.5 µM. However, for Copper complex, even at the concentration of 150 ± 4 µg/ml only 39% inhibition was encountered and suggests the radical scavenging activity of the embelin reduced upon complexations with Copper.

Hemolytic activities for embelin as well as its complex namely Copper (II)-embelin results were illustrated Table 4.

Table 4. Represents the hemolytic activity of the embelin and Copper (II)-embelin Complex, the results were expressed as ED_{50} (in µg/ml).

Sample	ED_{50} (in µg/ml)
Embelin	109 ± 1
Copper(II)-embelin complex	Only 40± 0.5 % even at 150 µg/ml

(Hemolytic activity was determined by UV-Visible spectrophotometric method, using human RBC as substrate after getting volunteers consents for the experiments).

Embelin at high concentrations, ie., >100 µg found hemolytic [6], whereas the complexes display no heme lyses even at high concentration, > 100 µg.

CONCLUSION

The present findings suggest the biological profile of embelin was better than the Copper (II) embelin complex with respect to DPPH radical scavenging activity.

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