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Sateydeo Singh
Research Scholor,
Department of Chemistry
S.K.M.U, University Dumka,
Jharkhand, India

Corresponding Author:
Sateydeo Singh
Research Scholor,
Department of Chemistry
S.K.M.U, University Dumka,
Jharkhand, India

Study of biological profile of metal and azo-metal complexes of embelin

Sateydeo Singh

Abstract
In this paper the emphasizes synthesis and bioprofiling of embelin, embelin-metal (EM) and embelin-azometal (EAM) complexes in detail. EM complexes were prepared using pure embelin and d-block transition elements, namely Mn, Fe, Co, Ni, Cu, and Zn. Similarly, EAM complexes were synthesized using phenyl azoembelin with the said transitionmetals. Embelin, EM, and EAM complexes were subjected to ultra violet visible spectroscopy, Fourier transform infrared spectroscopy, nuclear magnetic resonance, electrospray ionization mass spectrometry, thermogravimetric analysis, carbon hydrogen nitrogen sulfur analysis. The hemolytic activity studies suggested that both embelin and the metal complexes are non-hemolytic. The reason for the reduction in antioxidant and an increase in antimicrobial activities were discussed in detail.

Keywords: metal and azo-metal

Introduction
The biological activity of embelin is attributed to the formation of semiquinone radical. The presence of quinone carbonyl and enolic hydroxyl groups in the same molecule is believed to enhance its antioxidant activity. Embelin, displayed high affinity toward transition metal ions, which, results in the formation of embelinmetal (EM) complexes and the existing reports available on EM complexes dealt only the structural elucidation with physical characterization [1–6]. Though the biological properties of embelin had been widely studied, the unavailability of biological properties of metal complexes necessitates the present study. According to Chopra *et al.* [7], complexation with metals reduces the inherent biological activity of the drug molecule. In the case of tetracycline, on coordination with metals, the antimicrobial activity reduced considerably. According to Lecomte *et al.* [8] and Wallis *et al.* [9], cations such as magnesium and aluminum when complexed with fluoroquinolones, results with low oral bioavailability and causes therapeutic failures. However, Bakola-Christianopoulou *et al.* [10] reported that an increased antimicrobial activity by hydroxyquinone metal complexes and similarly Iqbal *et al.* [11] also reported an increased biological activity (antimicrobial activity) of cephalexin copper (II) complex.
Recent research on transition metal complexes suggests that the aryl-azo complexes of transition metal displayed interesting structural as well as functional properties. Moreover, beta-diketones and azo-compounds are reported as good ligands and studies on metal complexes with azo-derivatives may demonstrate important biological functions. Metal complexes of Lawsone, a napthoquinone demonstrated antibacterial activity [12] and Gokhale *et al.* [13] reported that the phenyl azo-metal complexes of Lawsone displayed antitumor properties against MCF-7. Till date no reports are available on EAM complexes; hence, an attempt on synthesis and evaluation of biological profile of both EAM and EM complexes has been made in the present study.
The present study includes the preparation, characterization and evaluation of major biological activities, namely antimicrobial, hemolytic, and antioxidant potential of embelin and its metal complexes, namely embelin–manganese, embelin–iron, embelin–cobalt, embelin–Nickel, embelin–copper and embelin–zinc. In addition, studies were further extended to EAM complexes for the above said metals for its biological activities.

Methods

Biological activity

Antioxidant activity: The free radical scavenging activity of embelin, EM, and EAM complexes was assessed by allowing the complex solution to react with a stable free radical, 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). The complexes were dissolved in dimethyl sulphoxide to the stock concentration of 1 gmL^{-1}. About 1.8mL of 0.3mM DPPH was added to different dilutions and left for incubation at 25^0C for 30–50 min. The scavenging activity was determined by measuring the absorbance at 517 nm, where DPPH alone serves as a negative control. The half-maximal inhibitory concentration (IC_{50}) of the antioxidant was calculated according to Amarowicz *et al.* [14].

Antimicrobial activity: Two Gram-positive and Gram-negative strains were used to examine the antimicrobial activity of embelin, EM, and EAM complexes according to the standard protocol suggested by Clinical and Laboratory Standards Institute (CLSI) guidelines using the broth dilution method. Bacterial strains of *Bacillus cereus* (ATCC 10876), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 4157), and *Shigella flexneri* (ATCC 9199) were employed for this study. Stock cultures of the bacterial strains were maintained on nutrient agar and checked for purity and stored at 4◦C. Sterile Mueller-Hinton broth media were prepared. The test compounds were dissolved in dimethyl sulfoxide (DMSO). Followed by inoculation, the required concentration of test compounds (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 1.75M) was added. Samples were incubated at 37◦C and the turbidity of the broth was measured at 600 nm after 24 h. The percentage reduction in absorbance was calculated accordingly. Samples without test compound served as control.

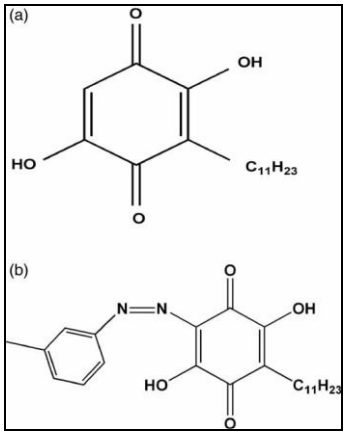

Fig 1: (a) Structure of embelin and (b) 2-(3-methyl phenyl azo) embelin

Hemolytic assay: The hemolytic activity of metal complexes was determined using the suspension of erythrocyte cells (RBC) according to WHO guidelines (1998). Blood samples from healthy volunteers with informed consent were collected as per Institutional Ethical committee guidelines and approval (vide. No. 466/160/1999/CPCSEA), later the samples were centrifuged at 10,000 rpm for 20 min at 4^0C, to remove the cell debris. Resultant pellet was washed (3–4 times) repeatedly with phosphate buffer saline (PBS; pH 7.4) to obtain RBC and was suspended in PBS containing test solutions at varying concentrations and incubated at room temperature for 10 min in the dark. At the end of incubation, tubes were centrifuged at 6000RPM for 20 min at 4^0C, in order to separate the intact cell and debris. The amount of releasing hemoglobin (Hb) in the supernatant was measured spectrophotometrically at 540 nm. The halfmaximal effective concentration (ED50) of hemolysis was then calculated.

Results and discussion

Biological activities

Antioxidant activity: In general, DPPH radical scavenging activity is a commonly employed assay in antioxidant studies of extracts across a short time scale and has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition unlike other hydroxyl radical and super oxide anion [15]. Furthermore, DPPH assay is known to give reliable information concerning the antioxidant ability of the tested compounds. To neutralize a free radical, an electron donating reducing agent can able to donate an electron to a free radical and the reduced species acquires proton from solution. In the present study, the antioxidant profile of embelin, EM, and EAM complexes were assessed using DPPH scavenging activity.

Table 1: Antioxidant profile of embelin, EM, EAM complexes in comparison with standard antioxidant ascorbic acid measured in terms of IC50 (μgmL^{-1}).

S. No	Test compounds	IC_{50} (μgmL^{-1})[a]
1	Embelin	27 ± 1
2	Emb-Mn	170 ± 0.5
3	Emb-Fe	44.56 ± 1
4	Emb-Co	57.46 ± 0.87
5	Emb-Ni	35.8 ± 0.9
6	Emb-Cu	87.7 ± 1
7	Emb-Zn	59.43 ± 0.4
8	Emb-Azo-Mn	200 ± 0.5
9	Emb-Azo-Fe	76.66 ± 0.5
10	Emb-Azo-Co	30 ± 0.4
11	Emb-Azo-Ni	76.9 ± 0.4
12	Emb-Azo-Cu	54.37 ± 0.6
13	Emb-Azo-Zn	127.81 ± 1.5
14	Ascorbic acid	2.9 ± 0.3

[a]Mean ± SD of triplicates.

Table 1 illustrates the antioxidant profile of embelin, EM, and EAM complexes. Embelin alone displayed IC_{50} of 27 ± 1μgmL^{-1} with reference to DPPH. The maximum antioxidant activity exhibited by embelin alone could be attributed to the presence of quinone and

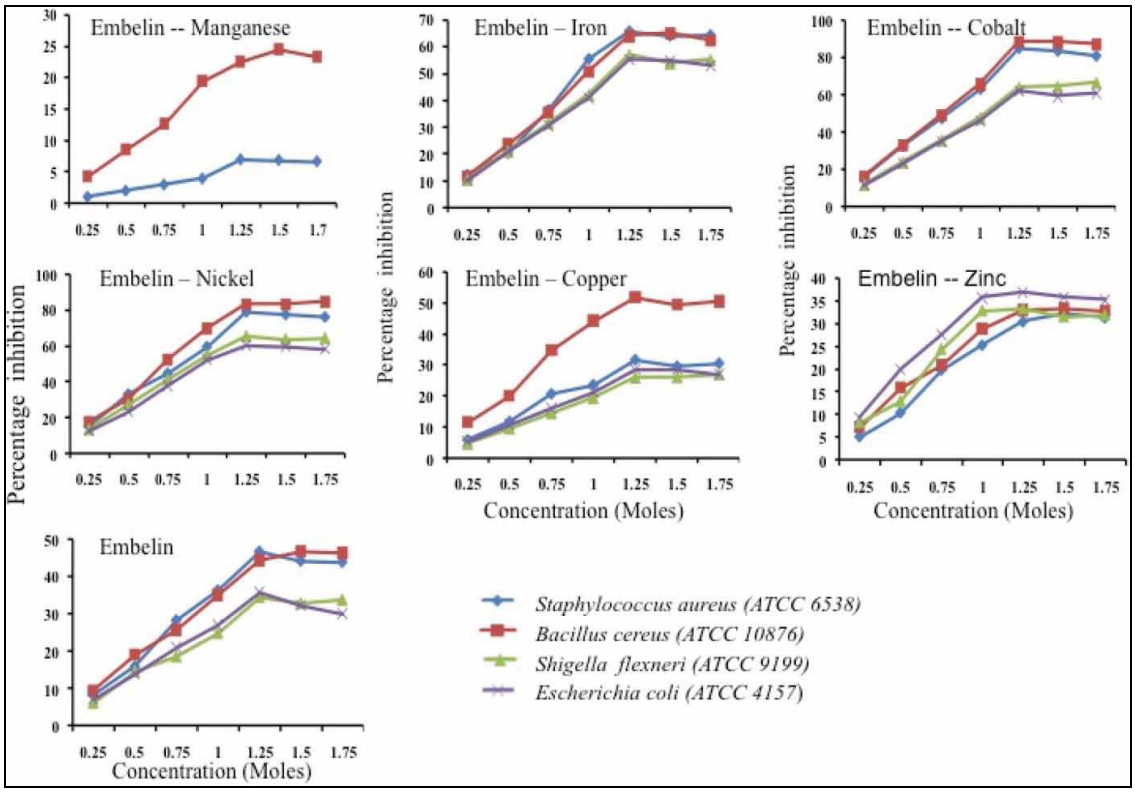

Fig 2: Antimicrobial activity profile of (a) EM and (b) EAM complexes

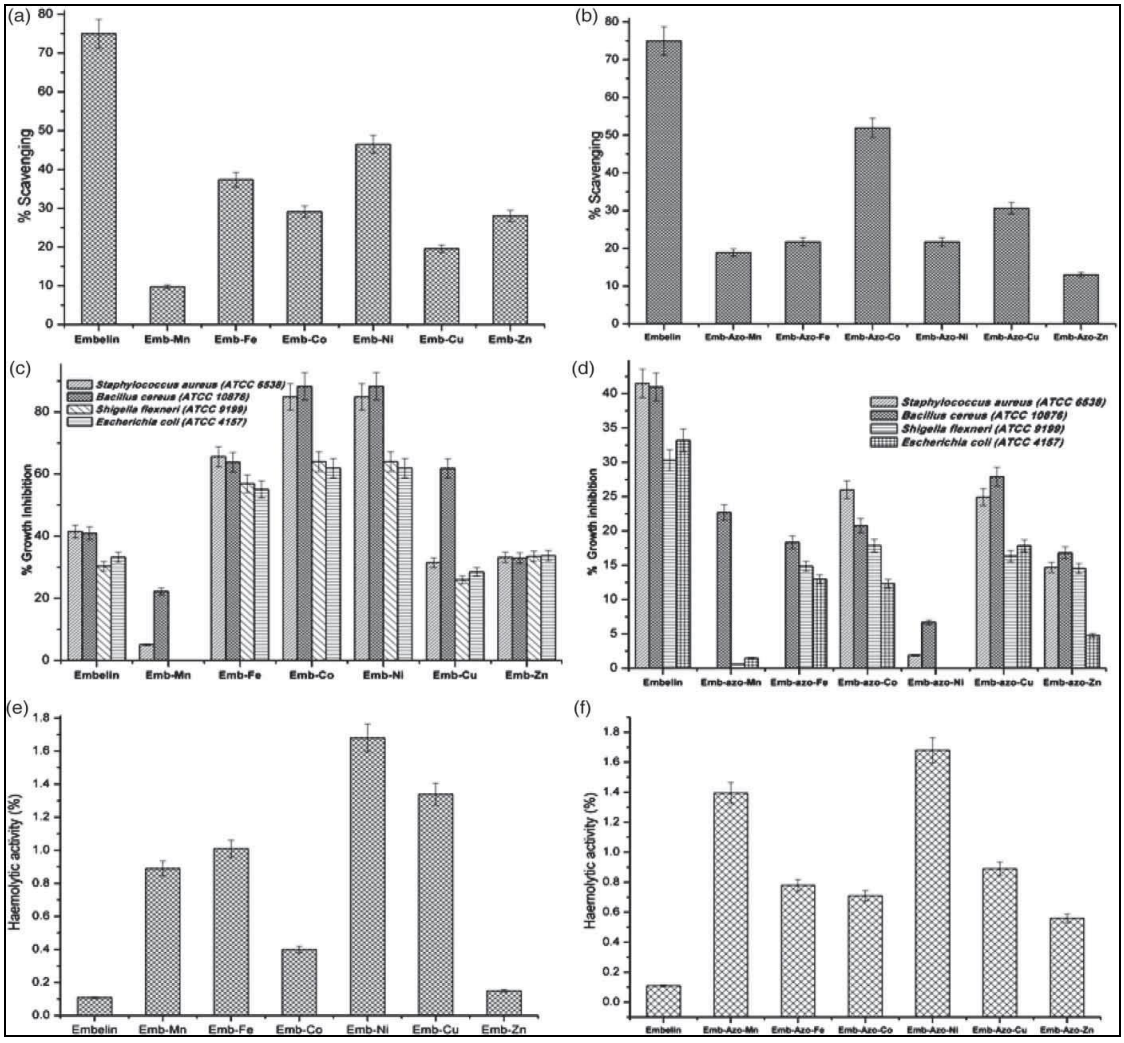

Fig 3: (a–f) A comparative analysis of biological profile (antioxidant, antimicrobial, hemolytic) of embelin, EM and EAM complexes. (a) Antioxidant profile of embelin and EM (b) antioxidant profile of embelin and EAM; (c) antibacterial profile of embelin and EM; (d) antibacterial of embelin and EAM; (e) heamolytic profile of embelin and EM; (f) heamolytic profile of embelin and EAM

hydroxyl moiety. Upon complexation with transition metals such as Mn, Fe, Co, Ni, Cu, and Zn, a decrease in scavenging behavior was observed. But, within the metal complexes, the maximum scavenging activity was observed in the order of Ni > Fe > Co > Zn > Cu > Mn (Figure 3(a)). This could be reasoned to the change in oxidation states in most of the transition metal ions, leading to instability in the d_{10} configuration, which makes the metal to scavenge the free radicals. Furthermore, the reduction in scavenging activity with embelin-transition metal complexes could be attributed to the absence of hydroxyl and quinone moieties because of chelation with metals.

Compared with EM complexes, a significant increase in antioxidant activity was observed with EAM complexes especially for the metals Mn, Co, and Cu and could be reasoned to the involvement of azo-group in chelation (Figure 4(b)),

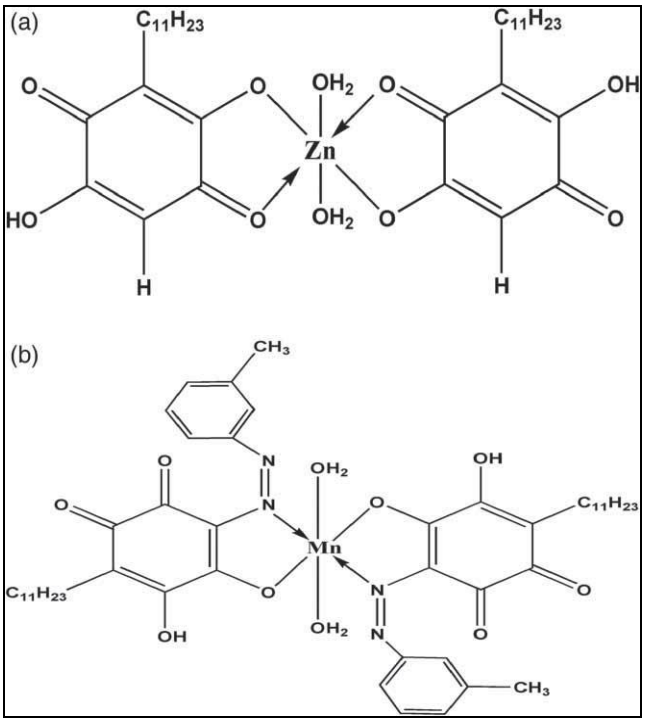

Fig 3: Schematic representation on synthesis of (a) embelin–zinc complex and (b) embelin-azo-manganese complex.

by which the benzene ring comes close to one of the ketone group of the embelin and forms an enol, thus facilitating the scavenging activity. The scavenging activity of EAM follows the order of Co > Cu > Ni > Fe > Mn > Zn as shown in Figure 3(b).

Antimicrobial activity: Figure 2(a) and 2(b) demonstrates antimicrobial profile of the chosen compounds embelin, EM, and EAM complexes. Embelin alone displayed 45% inhibition over Gram-positive bacterium and 34% growth inhibition over the Gram-negative bacterium. Similar to antioxidant profile, the antimicrobial activity displayed by embelin could also be reasoned to the presence of quinone moiety. Chitra *et al.* [16] reported antibacterial activity of embelin alone and suggested embelin showed bacteriostatic activity toward Gram-positive organisms and bactericidal toward Gramnegative organisms. Similar observations made by Radhakrishnan *et al.* [17]. Stern *et al.* [18] reported, quinone moieties complexes irreversibly with nucleophilic amino acids and inactivate the proteins. The authors also suggested that the probable targets in the microbial cell are adhesions, cell wall polypeptides and membrane bound enzymes.

Conclusions

In the present study, of biological profile of the metal complexes of embelin, a natural benzoquionone. Only six transition metals were chosen for the study and the corresponding metal complexes. In addition, the synthesis of embelin-azo complex was made. All the said complexes using instrumental techniques and confirmed the complexation and the presence of metals. Furthermore, these complexes were subjected to antioxidant, antimicrobial, and hemolytic activities for biological profiling. The antioxidant activity of embelin reduced significantly when it complexes with other metals or azo-metals. However, few metal complexes displayed a significant increase in antimicrobial activity compared with embelin alone. Hemolytic activity of embelin and its complexes were observed as meager. The present study concludes that upon complexation the antimicrobial activity of embelin increased significantly.

References

1. Dhar ML, Singh O. Inorg Chim Acta 1986;117:187.
2. Abdul Rashid KK, Chacko J, Nambisan PNK. Inorg Chim Acta 1988;151:1.
3. Abraham R, Yusuff KKM. J Mol Catal A Chem 2003;198:175.
4. Cherutoi JK, Cheruiyot LL. B Chem Soc Ethiopia 2005;19:295.
5. Rani VU, Jyothi G, Rao GN, Sailaja BBV. Acta Chem Slov 2010;57:916.
6. Rani VU, Rao MSP, Rao DV, Sailaja BBV. Pharm Chem 2010;2:89.
7. Chopra I, Hawkey PM, Hinton M, Antimicrob J. Chemother 1992;29:245.
8. Lecomte S, Baron MH, Chenon MT, Coupry C, Moreau NJ. Antimicrob Agents Chemother 1994;38:2810.
9. Wallis SC, Charles BG, Gahan LR, Filippich LJ, Bredhauer MG, Duckworth PA. J Pharm Sci 1996;85:803.
10. Bakola-Christianopoulou MN, Ecateriniadou LB, Sarris KJ. Eur J Med Chem 1986;21:385.
11. Iqbal MS, Ahmad AR, Sabir M, Asad SM. J Pharm Pharmacol 1999;51:371.
12. Amanda NP, Barbosa CC, Greco SJ, Vargas MD, Visentin LC, Pinheiro CB, Mangrich AS, Barbosae JP, da Costae GL. J Braz Chem Soc 2009;20:712.
13. Gokhale N, Padhye S, Newton C, Pritchard R. Met Based Drugs 2000;7:121.
14. Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Food Chem 2004;84:551.
15. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Hawkins Byrne D. J Food Compos Anal 2006;19:669.
16. Chitra M, Devi S, Sukumar E. Fitoterapia 2003;74:401.
17. Stern JL, Hagerman AE, Steinberg PD, Mason PK. J Chem Ecol 1996;22:1877.
18. Radhakrishnan N, Gnanamani A, Mandal AB. Biol Med 2011;3:1.