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Antifungal activity of some synthesized metal complexes of embelin

V.Usha Rani[1], T.Nautha Mary Thambi[2], T.Suresh[1], G.Girija Shankar[2], B.B.V.Sailaja[1]*

[1]Department of Inorganic and Analytical Chemistry, Andhra University, Visakhapatnam-530 003, (INDIA)

[2]Department of Pharmaceutical Biotechnology, University college of Pharmaceutical sciences, Andhra University, Visakhapatnam-530 003, (INDIA)

E-mail : sailaja_bbv@yahoo.co.in

ABSTRACT

The antifungal activity of embelin (2,5 dihydroxy 3-undecyl- p-benzoquinone) and its complexes with Co (II), Ni (II), Cu (II) and Zn (II) have been evaluated against *Candida albicans* and *Aspergillus niger* by using agar diffusion method. These compounds showed significant enhancement in activity at higher concentrations of the complexes but moderate activity as compared to fluconazole and clotrimazole.

KEYWORDS

Embelin;
Metal complexes;
Antifungal activity;
Agar diffusion method.

INTRODUCTION

Embelia ribes belongs to the family *Myrsinaceae* is a benzoquinone derivative used as herbal medicine. It possesses great medicinal importance. Embelin is used as antihelmithic and to cure skin diseases in Ayurveda, siddha as well as in Unani medicine[1]. Dried berries of this 4 plant have traditional reputation for showing anti-fertility activity[2]. Embelin also shows anti-inflammatory[3], antitumor[4] and free radical scavenging activities[5]. It was reported that the extracts of *E.ribes* were useful in the treatment of abdominal tumors and cystic tumors[6] The derivatives of embelin also play an important role in the anticancer activity[7]. It was reported that the phospholipid complex of embelin acts as an effective drug delivery tool[8] and embelin is used as a cathode in zinc based secondary battery using $ZnCl_2$-NH_4Cl as an electrolyte[9]. Copper (II) complexes of embelin have been found to display fairly good catalytic activity for the reduction of molecular oxygen[10]. The authors have reported the speciation of binary complexes of embelin with some biologically important metal ions[11]. Based on the stability constants of binary complexes of embelin with some biologically important metal ions obtained by speciation studies and its relevance in biological systems the authors have synthesized, characterized and reported the Ni (II)[12], Cu (II)[13] and Zn (II)[14] complexes of embelin in 1:2 molar proportion. The authors have also reported the antibacterial activity of these metal complexes[15]. Different solvent extracts of *Embelia ribes* were screened for antifungal activity[16]. In view of the various bioactivities exhibited by embelin, its complexes and derivatives we attempted to investigate the antifungal activity of embelin and its binary complexes with Co (II), Ni (II), Cu (II) and Zn (II).

MATERIALS AND METHODS

Isolation of embelin

Embelin was isolated from *Embelia ribes.* The

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seeds of *Embelia ribes* (500 g) were purchased from the local market and it was cleaned, dried, powdered and subjected to extraction by n-hexane in a soxhlet assembly[17]. 500 g of powdered *Embelia berries* were soaked in n-hexane for 7 days. The level of n-hexane was maintained 1 inch above the powdered berries. The contents were then refluxed for 2 hours. It is filtered by applying vacuum sucssion while it is hot. Upon cooling embelin precipitates out, which was filtered and filtrate was again used to soak the material for second extraction for another 7 days and the process is repeated. The crude embelin obtained from the extraction was recrystallized from benzene. The recrystallization process was repeated until golden yellow crystals of embelin (9g) were obtained. The purity of the compound was determined by thin layer chromatography on oxalic acid coated silica gel plates, using hexane-ethylacetate (9:1 and 9:2) solvent systems showed a single spot and it is checked with authenticated sample. The melting point was observed to be 142^0C.

Synthesis of complexes

Preparation of the Dichlorobis (embelinate) metal (II): Hydrated metal chloride (5 mmol) and embelin (10 mmol) were dissolved in ethanol (20 cm^3) separately and mixed. Acetone (10 cm^3) was added to the solution and stirred for 30 minutes. The precipitated complex was filtered, washed thoroughly with ethanol and the collected precipitate was dried under vacuum at room temperature.

Tested compounds

Embelin was isolated from the berries by a method described previously. Metal complexes were synthesized by the above method. Solutions of all these compounds were prepared at the concentrations of 5 mg/ml, 10 mg/ml and 20 mg/ml using dimethyl sulfoxide.

Antifungal activity

This activity was determined by employing the agar diffusion method[18]. All the metal complexes were evaluated for *in vitro* antifungal activity against *Candida albicans* and *Aspergillus niger.* Sabouraud's dextrose agar was used as a medium for cultivating fungi in the laboratory. The inoculated medium was transferred in to sterile petridishes, evenly distributed and allowed to solidify. Thereafter the cups (8 mm) were made with a sterile stainless steel borer. Into each of these cups 50 µL of the test compound was added. Each 50 µL of the standard and control were used. Dimethyl sulphoxide used as a control.

Fluconazole and clotrimazole were used as standards for *A. niger* and *C. albicans* respectively. Inhibition zone diameters were recorded after 48 hours. All the zone diameters were average of three readings.

The activity of each compound was evaluated at 250, 500 and 1000 µg mL^{-1} concentrations. Fluconazole and clotrimazole as standards were also tested under similar conditions for the comparison of the results. Fungi used in this procedure were obtained from the Institute of Microbial Technology and National Collection of Industrial Microorganisms.

a) *Aspergillus niger* (NCIM no. 548)
b) *Candida albicans* (MTCC no. 227)

RESULTS AND DISCUSSION

Inhibition zone diameters were measured and reported in TABLE 1 and shown in Figure 1. In the case of *Aspergillus niger* it is observed that by increasing the concentration the zone of inhibition is also increased in Co, Ni and Zn complexes of embelin. Co and Ni complexes of embelin showed feeble activity. It is observed that the complexes of embelin with Zn exhibited activity against *Candida albicans* and *Aspergillus niger* at 1000 µg mL^{-1}. Embelin and its Cu-complex did not exhibit any activity against *Candida albicans* and *Aspergillus niger* even at higher concentrations. Complexes of embelin with Zn also exhibited moderate activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 200 µg mL^{-1}(15). Zinc (II) complexes with organic ligands play important role in clinical medicine. Complex of zinc (ll) acetate with erythromycin is used for acne therapy[19]. According to the general statements made about the differences in sensitivity of different groups of microbes, it was reported that fungi are more resistant than bacteria[20-22]. In our observation, it is observed that fungi, *Candida albicans* and *Aspergillus niger* required very high concentration of the complex when compared to bacteria. This may be due to the nature of fungal cell wall which is made up of chitin, the hard cover of the exoskeletons of the arthropods are also made up of chitin, which is relatively resistant, including microbial decomposition.

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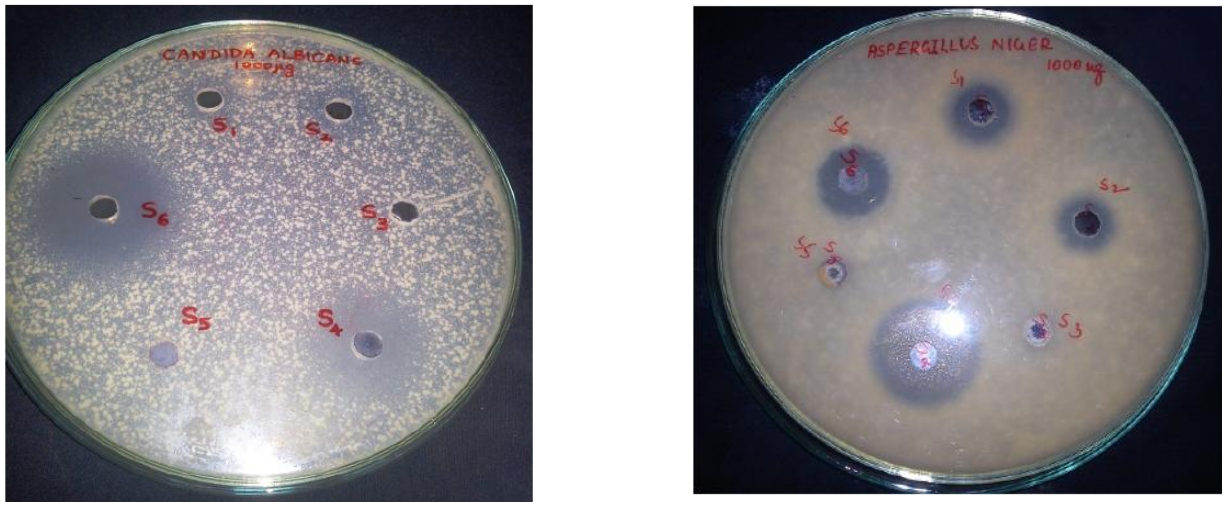
TABLE 1 : Effect of embelin and its complexes on fungi

Compound	Zone of inhibition (mm) (±SD)					
	Candida albicans			*Aspergillus niger*		
	250 µg mL^{-1}	500 µg mL^{-1}	1000 µg mL^{-1}	250 µg mL^{-1}	500 µg mL^{-1}	1000 µg mL^{-1}
S_1- Co $(C_{17}H_{25}O_4)_2Cl_2$	00	00	11 (±0.58)	00	14 (±0.58)	16 (±0.58)
S_2- Ni $(C_{17}H_{25}O_4)_2Cl_2$	00	00	15 (±1.00)	12 (±0.58)	13 (±1.00)	15 (±0.58)
S_3- Cu $(C_{17}H_{25}O_4)_2Cl_2$	00	00	00	00	00	00
S_4- Zn $(C_{17}H_{25}O_4)_2Cl_2$	00	00	23 (±0.58)	00	19 (±0.00)	22 (±0.58)
S_5- $C_{17}H_{26}O_4$ (Embelin)	00	00	00	00	00	00
Standard (Fluconazole)	-	-	-	16 (±1.00)	16 (±0.58)	17 (±1.00)
Standard (Clotrimazole)	35 (±0.58)	34 (±0.58)	35 (±1.00)	-	-	-
Control (DMSO)	-	-	-	-	-	-

S_1: Co-Complex, S_2: Ni - Complex, S_3: Cu-Complex, S_4: Zn Complex, S_5: Embelin, S_6: Fluconazole or Clotrimazole and SD: Standard deviation.



S_1: Co-Complex, S_2: Ni - Complex, S_3: Cu- Complex, S_4: Zn Complex, S_5: Embelin, S_6: Clotrimazole (*C. albicans*) or Fluconazole (*A. niger*).

Figure 1 : Inhibition zone diameters of embelin and its complexes at 1000 µg mL^{-1}.

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