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Antifungal Activity of Dibutyltindisalicylates and Clotrimazole Against Selected Pathogenic Fungi

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Abstract

In-vitro comparative study of antifungal activities of Dibutyltindisalicylates (DBTDS) and Clotrimazole (CLOT)-dermatophytic drugs of choice against clinical isolates of *Trichophyton rubrum, Trichophyton violaceum, Trichophyton verricosum, Microsporum canis, Cladosporium werneckii Penicillium citrinum, Aspergillus niger* and *Aspergillus flavus* has been carried out. In-vitro estimation of minimum inhibitory concentration (M.I.C) and minimum fungicidal concentration (M.F.C.) was by agar dilution method. The fungistatic activities of DBTDS against the five tested dermatophytes was 200 to 500ug /ml while that of CLOT ranges from 400 to 500ug/ml against the same test organisms. The minimum fungicidal concentration of DBTDS and CLOT against the test fungi followed the same pattern. Comparative antifungal activity of DBTDS and CLOT against the three phytopathogenic fungi also showed that the MIC of DBTDS ranges from 200.0ug/ml to 300.0ug/ml while that of CLOT was 500.0ug/ml. Using a concentration of 1.2mM, the two test compounds posses rapid fungicidal activity against the test fungal spores. For example 1.2mM of CLOT [407.0ug/ml] and DBTDS [600.00ug/ml] effected about 2.5 and 2.0 log cycle reduction of 10^8 cFu/ml of *T. violaceum* spores within five minutes contact time. Furthermore, rate of kill of test fungi spores at different fungicidal concentrations of these two test compounds appear to suggest that the fungitoxic activity of DBTDS compared favourably with CLOT commonly used in the management of the superficial fungal infection in man.

Keywords: Antifungal, Dibutyltindisalicylate, Activity, Clotrimazole, Dermatomycoses

Introduction

Combating fungal infections with chemical antifungal compounds has been practised since ancient times. In several countries, fungi have been reported to evolve resistance strategy to evade antifungal drugs which has become the most important obstacle to the control of fungal infections. This development probably resulted from the selection of increasingly less vulnerable fungi in individual human hosts, followed by their spread by active transmission.

Some causative pathogenic fungi of the vagina, and mouth infections have been reported to be satisfactorily treated with topical antifungal agent with tin compounds. (1,2, and 3) Tri-organotin compounds enjoy substantial applications as agricultural fungicides in various formulations such as pastes and powders, but very little has been reported about the antidermato-mycotic activity of Dibutyltindisalicylates (DBTDS).

We report in this paper our findings on the antifungal activity of synthesized DBTDS and purchase Clotrimazole (CLOT) a widely reported drug on clinical superficial fungal isolates namely *Trichophyton rubrum, Trichophyton verrocosum, Microsporum canis, Cladosporium werneckii, Penicillium citrinum, Aspergillus niger* and *Aspergillus flavus.*

Materials and Methods

A. Preparation of di-n-butyltindisalicylate

Salicylic acid (2.76g, 0.02M) and silver oxide (2.32g, 0.01M) were mixed in a 250ml conical flask wrapped with aluminium foil. Dry benzene (150ml) was added and the mixture stirred magnetically for 24 hours. The mixture was then evaporated to dryness on a rotary evaporator.
The dry powder was washed with dry benzene in order to remove untreated acid and dried. To the

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dry powder in a 250ml conical flask was added dibutyltin dichloride (4.56g, 0.015M), followed by dry dichloromethane (150ml). The mixture was stirred magnetically for 24 hours under aluminium foil and then filtered. The filtrate was evaporated to dryness; the residue (2.28g) dried *in vacuo*, and recrystallized from hexane.

m.p. 73 - 75°C.

Elemental anal.: Found % C, 52.06; H,5.52: Sn, 23.01

Calc.% C, 52.10; H,52,10; H,5.57; Sn, 23.40

IR, (KBr disc.): Fourier Transform 1710;

1580 cm^{-1}(M) COO asym

1420 cm^{-1}(M) COO asym

995 cm^{-1}(W) SnOC

640 cm^{-1}(W) Sn-Bu

B. Determination of Antifungal properties of Dibutyltindisalicylate and Clotrimazole

The determination of Minimum inhibitory concentration (MIC) was by graded concentration of each test-compound ranging from 100.00 to 600.00ug/ml were mixed with molten Sabouraud Dextrose agar at 45°C in sterile petri dishes and allowed to set.

Sterile paper discs were placed aseptically on the dried agar plates and inoculated with spectrophotometrically standardized spores of test fungi to 10^6cfu/ml in triplicates[5]. These were incubated at 30^{etch} for 3 days. Control was also set up i.e. Sabouraud Dextrose Agar plate without drug but inoculated with the test-fungi spores. The lowest concentration of each drug that inhibited the growth of the test fungi spores under this experimental conditions were recorded as M.I.C.

The minimum fungicidal concentration (MFC) of each test compound was determined by inoculating the discs showing no visible growth in MIC determination aseptically into 10ml recovery broth (sabouraud liquid medicine supplemented with 30% tween 80 and 0.5% egg lecithin). These were then incubated at 30^{etch} for 5 days (Olurinola, *et al*, 1991). The minimum concentration of each test compound as examined which prevent visible growth in the recovery broth was regarded as the M.F.C.

The effect of graded fungicidal concentrations of DBTDS and CLOT on the viability of *T. vop;aceim* and *P.citrnum* was studied. The desired concentrations of the test compounds were prepared in 19ml volume of 25% acetone in sterile normal saline medium containing 0.05% Tween 80 in Amber coloured bottle. These concentrations were inoculated with 1ml of 10^8cful/ml spores of test fungi at 27 + 2^{etch} room temperature.

At specified time interval, ten-fold dilution of 1.0ml of the reaction mixture was carried out using sterile inactivating diluent. These dilutions were plated out in triplicated and incubated at 30^{etch} for 5 days. The viability of the fungal spores was determined from the incubated plates.

Result and Discussion

The results of the antifungal activities of DBTDS in this report showed that this test compound have some degree of activity on wide range of fungi which are widely reported to be pathogenic to animals and plants (Olurinola *et al* 1991, Ehinmidu 1992). Furthermore this activity on the test fungi spores as shown in *table I* appeared to be greater than that elicited by the currently used antifungal agent i.e clotrimzole against dermatoplytes. For example, the fungistatic concentration of DBTDS and CLOT against *T violaceum* one of the most resistant test fungi spores was observed to be the same (500.00ug/ml).

The rate of kill of *T.violaceum* by clotrimazole and DBTDS at different concentration are as illustrated in figure 1.0 and 2.0. In both cases; the rate of kill and extent of kill appear to be concentration dependent. Generally, there was a very rapid on set of kill followed by a slow gradual rate of kill of test fungal spores. For example 1.2mM of CLOT and DBTDS effected about 2.5 and 2.0 log cycles reduction of *T. violaceum* spores within five minutes contact time. Within 45minutes contact time, the same concentrating of CLOT and DBTDS effected approximately 6 and 3 log cycles of kill (Fig. 3). The rapidity and intensity of onset of action of the two test chemical compounds at different- concentration showed clearly that these antifungal agents are strongly fungicidal.

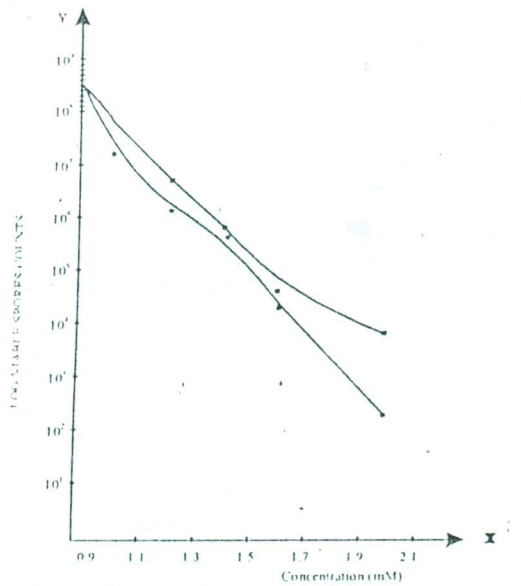


Fig 1: Influence of various concentration of DBTDS (·-·) and clotrimazole (- -) on viability of *T.violaceum* spores at 25°C

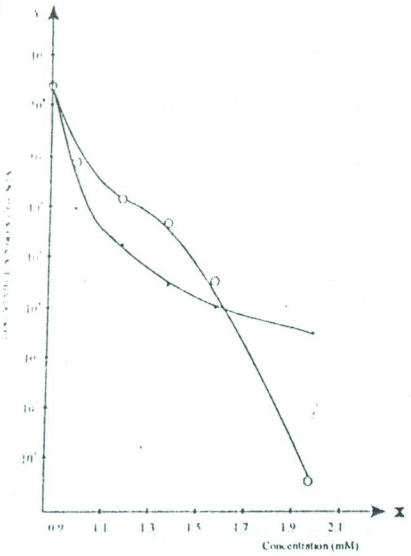


Fig 2: Influence of various concentration of DBTDS (* – *) and clotrimazole (o – o) on viability of *P.citrinum* spores at

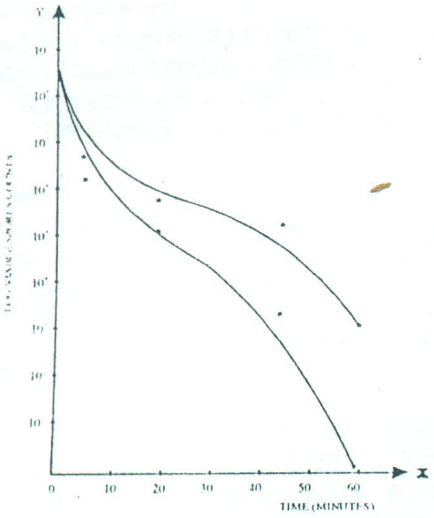
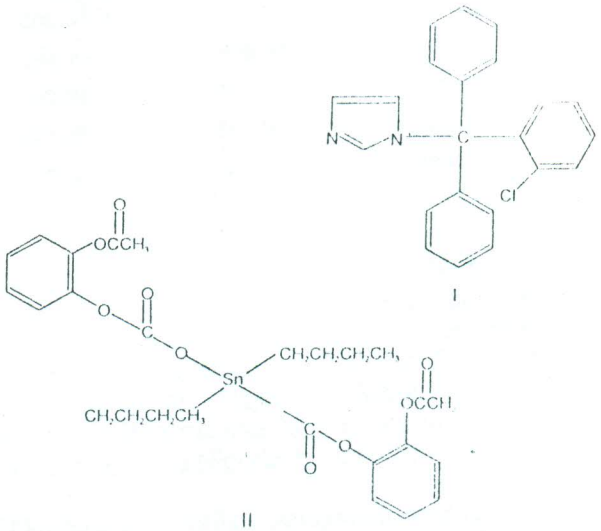


Fig 3: The rate kill of *T.violaceum* spore suspension treated with 1.2mM of DBTDS (* - *) and clotrimazole (• – •).

The shape of the death curve produced by DVTDS and CLOT are similar. This probably indicates similar mechanism of action although different chemical structures. It has been reported that imidazole derivative disrupt cell membrane of fungi with resultant leakage of vital cell constituent (Odds *et al* 1985, Surarit and Shepherd 1987).

Similarly, Cooney and Wuertz (1989) in their review of antimicrobial activity of organotin compounds reported that the mechanism of actions of these compounds is thought to be due to:

a) Intercalation of diorganotin with mitochondrial membranes which cause swelling and subsequent physical disruption of mitochondrial membrane;
b) Secondary effects due to chiorganotin disruption of mitochondrial lipid bilayer resulting to derangement of mitochondrial function through mediation of Chloride or Hydroxyl ions exchange across the lipid membrane;
c) Diorganotins ability to inhibit the fundamental energy Conservating processes involved in the biosynthesis of ATP and ADP by reacting with histidine and sulphur containing amino acids enzymes involved in these processes.



I

II

Clotrimazole (I), an imidazole and an organic compound, most probably has its imidazole functional group as the active site of the molecule.

'Dibutyltin disalicylate[11] on the other hand, an organometallic compound, has the Sn centre as the most active centre which forms loose bonds with electron donor sites in enzymes and other proteins. The role played by the salicylate nucleophile is still very much unexplored and therefore unclear, but the whole molecule would be expected to have the antifungal behaviour of the dibutyltin plus the salicylate moieties.

The differing natures and structures of the test compounds therefore might make their sites of action on the test organism differ, although the overall observable effects on the test organisms have appeared similar. All these are suggestions, as the actual biochemical reactions responsible for the observations are yet to be fully understood.

In-vitro fungicidal activity of DBTDS when compared with clinically, used chemotherapeutic drug such as CLOT appear to suggest that it a probable potential alternative antifungal agent to the highly expensive clotrimazole. Furthermore, the effectiveness of DBTDS against environmental mycelia bearing fungi such as *A niger, A. Flavus* and *P. citerinum* implicated as causative agents of yam rot (4), point to it's usefulness as another agricultural fungicide in crop protection.

TABLE 1: Susceptibilities of 10^6cFu/ml Test Fungi Spores to the Chemical Antifungal Agents Under Study.

Test fungi	Dibutyltin Disalicylate (DBTDS)		Clotrimazote (CLOT)	
	MIC	MFC	MIC	MFC
Trichophyton Rubrum	500.00	600.00	400.00	500.00
Trichophyton violaceum	500.00	600.00	500.00	600.00
Trichophyton verricosum	200.00	600.00	400.00	500.00
Microsporum canis	500.00	600.00	400.00	500.00
Cladosporum wereckii	200.00	600.00	400.00	500.00
Penicillium citrinum	200.00	500.00	500.00	600.00
Aspergillus niger	300.00	500.00	500.00	600.00
Aspergillus flavus	200.00	600.00	500.00	600.00

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