

## EFFECTS OF ENAMINOTHIONES COMPOUNDS ON THE DERMATOPHYTES AND YEAST ISOLATES IN VITRO

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### الخلاصة

تم تحضير ثلاث مركبات تابعة لمجموعة اينا امينو ثيون وهي 5,5 ثنائي مثل-1-3N (ن،ن-ثنائي أثل) فينول امين -2- هكسان حلقي-1- ثيون ؛ 5,5 ثنائي مثل -3-ن(4-برومفينول) أمين-2- هكسان حلقي -1- ثيون و 5,5 ثنائي مثل (ن-فينول) امين -2- هكسان حلقي -1- ثيون ، كما تم قياس الامتصاصية لهذه المركبات باستخدام طيف الاشعة فوق البنفسجية وتحت الحمراء. اختبرت الفعالية التثبيطية لهذه المركبات تجاه عدد من الفطريات الجلدية الممرضة و الخميرة مختبريا ، أظهرت النتائج إن لهذه المركبات التأثير المثبط تجاه العزلات الفطرية المختبرة

### Summary :-

Three enaminothione compounds namely 5,5- Dimethyl -3 N (N,N-Diethyl) phenylamino -2- Cyclohexane -1- thione ; 5, 5- Dimethyl -3N (4-Bromophenyl) amino -2- Cyclohexane -1- thione ; 5,5- Dimethyl -3N-phenyl) amino -2- Cyclohexane -1- thione were prepared and their U.V. and I.R. spectra were measured. The inhibitory activity of these compounds were tested by disc diffusion method against pathogenic fungi ( dermatophytes and yeast infections ) in vitro . Results revealed that synthesized compounds have an inhibitory effect against the tested fungal isolates .

### Introduction :-

There has always a wide interest in certain type of schiff bases such as enaminothiones ( 1,2 ), and compounds containing thiol group because their inhibitory effects ( 3 ) . Superficial - Cutaneous mycotic infections constitute a group of microorganisms categorized as causing dermatophytosis and candidiasis (4). The development of new agents for the treatment of mycotic infections has been slow , and there are relatively few agents available , compared to the number that have been developed for treatment of bacterial infections (5). Recently , many synthetic drugs have been demonstrated to have in vitro and therapeutic activity against a variety of human fungal pathogens (6).

The aim of the present work is to study the in vitro sensitivity of the isolated fungal pathogens to the prepared enaminothiones as antifungal drugs using disc

diffusion method , in addition , to measured and discussed the I.R and U.V spectra of the prepared compounds .

### Materials and Methods :-

#### Preparation of studied compounds :-

The studied compounds were prepared according to method of Walter and Proll (7) from the reaction of enamines with Lawessons reagent as follows :

1- 5,5 - Dimethyl -3N(N,N-Diethyl) phenylamino-2-cyclohexane -1-thione :  
Reaction time 30 min, in benzene , dark-brown crystals from ethanol -water mixture , m.p. 162C. Anal Calcd . for C<sub>18</sub> H<sub>26</sub> N<sub>2</sub>S : C,71.52;H,8. N,9.27. Found : C,71.2;H,8.16;N,9.87.

2- 5,5-Dimethyl -3-N (4-Bromophenyl) amino-2- Cyclohexane -1 -thione:  
Reaction time 40 min, in benzene ,brown crystals from ethanol-water mixture , m.p. 188-189C .Anal Calcd . for C<sub>14</sub> H<sub>16</sub> NSBR : C,54.19; H,5.16; N,4.51. Found : C,54.89; H, 5.31; N, 4.32.

3- 5,5-Dimethyl (N-phenylamino)-2-cyclohexane -1- thione :  
Reaction time 30 min , in benzene , red crystals from ethanol -water mixture , m.p. 176C .Anal Calcd . for C<sub>14</sub> H<sub>17</sub> NS : C, 72.72; H, 6.06. Found : C, 73.23; H, 7.83; N, 5.57 .

#### Experimental: -

Elemental analysis were performed on aperkin-Elmer 240B Elemental analyzer . The I.R. spectra were recorded as KBr discs on a pye-Unicam SP3 -300 Spectrophotometer. The U.V. measurements have been obtained on Shimadzu recording Spectrophotometer UV-120 using matched quartz cells .

#### Fungal isolates :-

Thirty-five isolates of (3) dermatophyte species and (2) species of pathogenic yeasts ( Table 1), isolated from skin , hairs and nails were tested for sensitivity to prepared compounds , were grown on sabourauds Dextrose Agar ( SDA) ( Dextrose 40 g; peptone 10 g; Agar 20g; Distilled water 1000 ml, pH 5.4 ) .

#### Fungal inocula :-

Growth from a 12-14 day old culture of each isolates of dermatophytes grown on SDA , or 2-5 day old culture of each isolates of yeasts , was harvested in 5 ml or 0.85% sterile saline by gently scraping of the surface of the fungal colonies and two loopful of yeast with sterile nichrome wire loop . The suspensions were vigorously shaken and an adjusted suspension of 10( 5) conidia / ml obtained by counting a hemocytometer was used as the standard inoculum . From the control organism , *Candida albicans* ( strain No. 1066 ) or *Trichophyton mentagrophytes var. erinacei* ( strain No. 186 ) , a suspension

containing 10 ( 5) colony forming units (C.E.U) /ml was prepared by adjusting the suspension to a transmission of 90% at 530 nm in a Uvikin 1810 Spectrophotometer.

#### Test media and antifungal preparation :-

For the tests of fungi ( dermatophytes and yeasts ), Emniones sabourauds Dextrose Agar ( ESDA ; Dextrose 20 g ; peptone 10 g ; Agar 20 g ; Distilled water 1000 ml). Each antifungal agents ( prepared compounds ) was prepared as an initial concentration ( working stock solution ) of 10.000 ug / ml (8) as follows : 5 ml of 100% dimethyl-sulfoxide ( DMSO ) was poured into clean sterile screw - capped glass vials ; 50 ml of each antifungal was added to the 5 ml ( AMSO ) and shaken vigorously . This giving a final concentration of 10 . 000 ug / ml. The stock solution was allowed to sit at room temperature for 30 min prior to its use . This permits self-sterilization of the antifungal by (DMSO). The stock solution was frozen at ( - 20 C) until used .

#### Preparation of antifungal discs :-

These were prepared according to Gould and Bowie (9) with little modification as follows : In screw -capped bottles , dispense batches of 100 small discs of absorbant paper ( about 6.5 mm in diam ) and sterilized by dry heat at 180 C for 30 min . Form the stock solution (10.000 ug/ml) diluted solutions of antifungal were prepared so that one ml contain 100 times the amount of antifungals required in the discs ( e.g. 10 ug / disc ) . This is achieved by diluting the stocks with (DMOS) to a concenteration of 1000 ug/ml. One ml of the diluted solution was added to each bottle of 100 discs and as the whole of this volume will be absorbed , it was assumed that each disc would contain approximately 0.01 ml (10 ug / disc ) . These discs were stored in wet condition in screw - capped bottle tightly screwed at(- 20C) until used .

#### Disc diffusion method :-

To carry out the assays , the diffusion method as described by Casals (10) was used as follows : 0.2 ml of the fungal inocula suspensions (containing 10 conidia / ml) was pipetted on the surface of ( ESDA) which has been poured in sterile 10 X 10 cm petri dishes in a constant and equally thick volume . The inocula were evenly spreaded with sterile L-shaped glass rod . The inverted inoculated plates were left on the bench for 1hr before discs were placed on the surface with sterile forceps . One disc of each antifungal agent was placed on the medium . The petri dishes were placed in refrigerator for 1-3 hrs pre incubation . At the same time, growth controls (without antifungal discs ) for all strains on (ESDA) were carried out. Plates inoculated with moulds were incubated at 25-30 C while these with yeasts were incubated at 35-37 C . The assay was recorded after 2 , 5 or 10 days incubation . Zones of inhibition of growth were expressed as clear zones around the antifungal discs in mm . Duplicated plates were made .

### Results and discussion :

Some important infrared absorption bands of enaminothiones are listed in Table 2 . Bands found at (1100 cm<sup>-1</sup>) are easily assigned to (C=S) stretching vibration . The prepared enaminothiones in solid case ( KBr discs) are characterized by strong absorbance bands at region (13130-3150 cm<sup>-1</sup>) which agree with stretching vibrations of (N-H) linked with hydrogen bond as shown in Table 2 . All compounds under this study have similar spectra of configuration , and this mean they are containing the same chromophore that responsible for absorbance (Fig 1). The absorbance spectra of these compounds are characterized by double essential peak at about 400 and 260 nm , and according to absorbance indexes and solvents that effect on them may due to electron transitions . Table 3 shows the results of discs diffusion method performed for (35) isolates of (5) different fungal species . The results showed slightly wider zones of inhibition of growth .Generally , larger inhibition zones were obtained with antifungal discs containing 5,5-Dimethyl-3N(N,N-Diethyl)phenylamino-2-cyclohexane-1-thione while were smaller with discs containing 5,5-Dimethyl-3N(4-bromophenyl)amino-2-cyclohexane-1-thione . This may due to the presence of thiol (SH) group in all the prepared compounds and this support the medical importance to use as antimicrobial agents (11) . Susceptibility testing of fungi are variable and standardization is difficult and effected by many factors such as inoculum size , temperature duration of incubation and medium composition (12). Also the width of the zones of inhibition of growth depends upon variables that influence the diffusion of the drug such as pH, depth hydration and concentration of the agar medium (13) On the other hand Schaefer and Stuttgen (14) noticed that the concentrations of the drug in the skin in vivo by using radioactive labeled technique , range from 10000 ug \ ml of tissue in the upper horny layer down to 1 ug\ml or less in the deeper epidermis . This means that the agent will mainly be active in the horny layer and in the epidermis . In the present study , the results of disc diffusion were comparable with those shown by Hantschke (15) and Cabafies (16) were the inhibition zones were wider than 10 mm . Since the commercially prepared antifungal discs were not available due to the international blockade imposed on our country filter paper discs impregnated with a prepared compounds in the laboratory were used as a substituted antifungal agents .

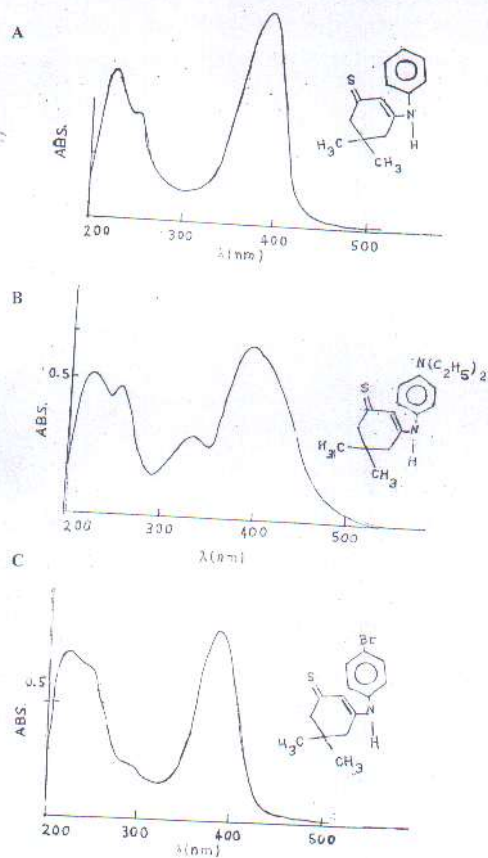


Fig. 1: Ultra - Violet spectra of prepared compounds in ethanol .  
(A= compound 1 ;B= compound 2;C= compound 3)

anol .

Table 1 : List of dermatophytes and yeast species and number of isolates with common diseases collected from different specimen sources .

Fungal species	Disease caused	Source of specimen	No. of isolates
<i>Epidermophyton floccosum</i> (Harz) Langeron & Milochev	Tinea pedis	Skin	8
<i>Microsporum canis</i> (Bodin) Bodin	Tinea capitis	Hair	6
<i>Trichophyton mentagrophytes</i> var. <i>mentagrophytes</i> (Bodin) Blanch	Tinea cruris	Skin	9
<i>Candida albicans</i> (Bodin) Bork	Cutaneous candidiasis	Nail	7
<i>Geotrichum candidum</i> (Bodin)	Dermatomycoses	Skin	5

Table 2: Data of infrared spectra of enaminothiones in KBR discs .

	Compound	Stretching vibration of (N-H.S) cm-1	Stre. vibration of (C-S. H) cm-1	Stre. vibration of C-C and aromatic ring (cm-1)
1	5,5-Diethyl-3N(N,NDiethyl) phenylamino-2-cyclohexane -1-thione	3100 wide medium	1030 sharp strong	1530-1430 wide
2	5, 5-Dlmethyl-3N (4-bromophenyl) amino-2-cyclohexane -1-thione	3125 wide medium	1040 sharp medium	1550-1460 wide
3	5,5-Dimethyl(N-phenyl) amino -2-cyclohexane-1-thione	3100 medium	1030 sharp strong	1570-1470 wide

Table 3 : zones of inhibition of growth of the tested isolates on ESDA .

Fungal species	Zones of inhibition of growth (mm)		
	Compound(1)	Compound(2)	Compound(3)
<i>E. floccosum</i>	*18	8	11
<i>M. canis</i>	10	5	9
<i>T. mentagrophytes</i> <i>var. mentagrophytes</i>	25	6	16
<i>C. albicans</i>	16	6	9
<b><i>G. candidum</i></b>	<b>11</b>	<b>7</b>	<b>10</b>

( \* ) represent the average of duplicates.

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