Iraqi Journal of Biotechnology, 2016, Vol. 15, No. 3, 40-50

Evaluation of Maleamic Acid Derivatives Against Some Pathogenic Fungi

Batol Imran Dheeb[1],Ghassaq Tariq Sadiq[2], Taghreed Uloom Mohammed[3], Nasr Saadon Abid[4],Basim M. Kashman[5] , Sanaa Huseein Mohamad[6] , Mohammed A. Nouri[7]

[1] Biology Department, College of Education, Iraqia University
[2] College of Medicine, AL-Nahrain University
[3] Chemistry Department, College of Education, Iraqia University
[4] Depart of chemistry, College of Education for Science Ibn AL-Haitham, University of Baghdad
[5] College of Dentistry , University of Baghdad
[6] Biology Department, College of Education, University of Kirkuk
[7] Biotechnology Department, College of Science, University of Baghdad

Received: March 10, 2016 / **Accepted**: August 23, 2016

Abstract: Five maleamic acid derivatives were prepared: Bis –maleamic acid(compound A); Ethylene –Bis–maleamic acid(compound A1); 1,4 –phenylene (2,2- diamino –Bis (1,3,4- thiadiazol- 5-yl))malaemic acid(compound A2) ; 4-N(2,3 – dimethyl- 1- phenyl – pyrazolin-5- one- 4-yl) maleicacid (compound A3) and 5-methyl -3- sulphanilamide maleamic acid(compound B). These were examined to study their antifungal activity against six pathogenic fungi, some of them causing superficial infection like *T. rubrum*, *T. violaceum* and *T. soudanese*, while others are responsible for invasive infection(lower respiratory tract) like *A. fumigatus*, *A . flavus* and *P. marneffei.* Results showed that the first three derivatives A, A1 and A2, are active against both the dermatophytes and invasive fungi, remarkably A derivative is highly active (100% growth inhibition) against *T. soudanese* and in three different concentrations and have the same activity against *T.violaceum* and *Aspergillus* species but only at the highest concentration. Derivative A1 have the highest effect (100% growth inhibition) against *T. rubrum* and *T. violaceum* and *P. marneffei*at the highest concentration only, while the third derivative A2 showed the same activity against *T. violaceum* at the highest concentration and against *A. fumigatus* at the three highest concentrations. On the other hand the two derivatives A3 and B found to have a considered antifungal effect against dermatophytes (100% growth inhibition), that A3 derivative showed is active against *T. rubrum* in its tow highest concentrations and against *T. violaceum* only at the highest concentration, they were recorded also to have antifungal effect against other studied species but to less extent .Finally, derivative B is recorded to be active effect against *T.rubrum* (100% growth inhibition) at its highest concentration and also has a considered effect against *T. violacem* in its tow highest concentration. Maleamic acid derivatives appeared to be strong antifungals , and their activity increasing gradually with concentration and with a significant difference between the activities of the compounds ($p \leq 0.05$). It is concluded that those compounds may have uses as antifungals in the future.

Key words: Pathogenic fungi, Antifungal activity, Maleamic acid compounds

Corresponding author: should be addressed (Email: batoolomran@yahoo.com)

Introduction

Infectious diseases caused by fungi are called mycoses, and are often chronic in nature. Some mycotic infections are superficial that involve the epidermis only, cutaneous mycoses , such fungi are called dermatophytes, other fungi may penetrate the skin reaching subcutaneous tissues, causing subcutaneous infections, while other fungal agents may cause deep infections resulting in systemic mycoses, such fungi are called invasive fungi, such infection is the most difficult form to be treated, and is often life threatening (1). The development of laboratory diagnostic tests and classification systems and initial epidemiologic and ecologic investigations, led to the realization that fungal diseases are prevalent more than expected and are becoming more so with advances in medicine (2). Antifungal compounds are important weapons for fighting fungal infection and have greatly benefited the health related quality of human life since their introduction (3). Although, the number of agents available to treat invasive fungal infections has increased by 30% since the turn of the millennium, this still only brings the total number of approved systemic antifungal drugs to 14, with the possibility of one more product emerging later this year. These recent additions have provided clinicians with alternative therapeutic tools that were previously lacking in the management of these life-threatening infections (4).
Maleimides are widely known as active electrophilic reagents with the ability to react readily with a variety of dien S and 1,3- dipoles including azomethineylide, carbonylylide and nitorenes, leading to various heterocycles(3). The biological effects of maleamic acid derivatives are quite different and have been investigated in many laboratories (2).
The aim of this study is to evaluate the antifungal activity of several maleamic derivatives including Bis –maleamic acid; Ethylene –Bis –maleamic acid; 1,4 – phenylene (2,2- diamino –Bis (1,3,4)- thiadiazol- 5-yl) malaemic acid; 4-N (2,3 – dimethyl- 1- phenyl – pyrazolin- 5- one- 4-yl)maleamicacid and 5- methyl -3- sulphanilamide maleamic acid.

Materials and Methods

Fungal Isolates

All fungal isolates (*Trichophyton rubrum, Trichophyton violaceum, Trichophyton soudanese ,Aspergillus fumigates, Aspergillus flavus, Penicillium maeneffei)* were obtained from the laboratories of the Biotechnology Department, College of Science, Baghdad University. All the organisms were standardized within the laboratory, the isolates were identified according to (5,6), that a small piece of transparent-adhered tape was touched to the surface of the suspected colony, and then adhered to the surface of a microscope slide to which a drop of lactophenol cotton blue was added. Shape and arrangement of the spores were examined microscopically, as listed in (table-1).

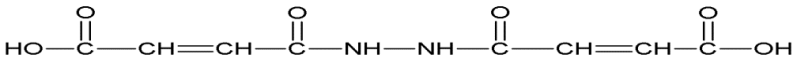
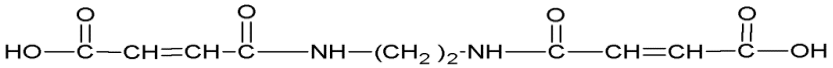
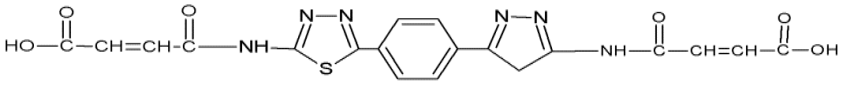
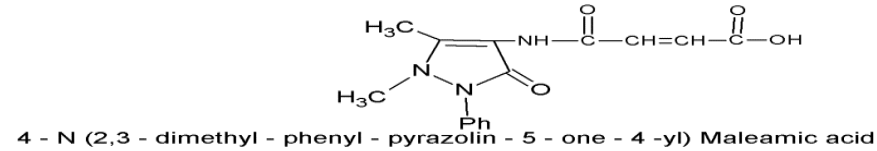
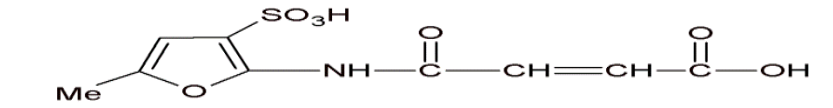
(Table-1): Fungal isolates and their sources

Fungus Species	Sources of Isolation
Trichophyto nrubrum	Dermal infection
Trichophyton violaceum	Dermal infection
Trichophyton soudanese	Dermal infection
Aspergillus fumigatus	Lower respiratory tract infection
Aspergillus flavus	Lower respiratory tract infection
Penicillium maeneffei	Lower respiratory tract infection

Maleamic Derivatives

The chemicals used in this work were from BDH and Fluka and were used without further purification, their chemical composition are illustrated in (table-2).

(Table-2) Structure of maleamic acid derivatives

Derivative A	 Bis - Maleamic acid
Derivative A1	 Ethylen - Bis Maleamic acid
Derivative A2	 1,4 Phenylene [2,2 - diamino - Bis (1,3,4 - thiodiazole - 5¯ yl] Maleamic acid
Derivative A3	 4 - N (2,3 - dimethyl - phenyl - pyrazolin - 5 - one - 4 -yl) Maleamic acid
Derivative B	 5 - Methyl -3 - sulfanilamido isooxazole Maleamic acid

Preparation of Maleamic Acid

The method used in preparation of maleamic acid (A and A1) is described in details by (5) with some modifications, that amine derivatives (0.05mole) were dissolved in dioxane and then added to a solution of maleamic acid (0.05mole) diamine and the mixture was dissolved in methanol, added drop by drop, with constant stirring. The mixture was then stirred at (0-5 °C) for 2 hrs. The precipitate was filtered and washed with diethyl ether (table-3).

The (A2 – B) maleamic acid derivatives were dissolved in acetic anhydride and 8% wt of an hydrous sodium acetate and the mixture was put in a water bath until the colour had changed. The solution was cooled by being poured in to an ice bath and stirred. The maleamic acid precipitate is then had been filtered, washed with sodium bicarbonate solution, dried and recrystallized with a suitable solvent (table-2).

All concentrations of the derivatives (0.03, 0.06, 0.3 and 0.6 M) were prepared by using dimethyl sulphoxide(DMSO) using the following equation.$C_1V_1 = C_2V_2$

DMSO was used as control in this study.

(Table-3) preparations of chemical compounds

Compounds	ῡ N— H Amide	ῡ C = O Amide	α N—H	ῡ C— N
Compound A	3060 CM^{-1}	1681CM^{-1}	1552CM^{-1}	1253CM^{-1}
Compound A_1	3461CM^{-1}	1716CM^{-1}	1558CM^{-1}	1272CM^{-1}
Compound A_2	3377CM^{-1}	1699CM^{-1}	1560CM^{-1}	1274CM^{-1}
CompoundA_3	3288CM^{-1}	1631CM^{-1}	1542CM^{-1}	1269CM^{-1}
Compound B	3417CM^{-1}	1670CM^{-1}	1556CM^{-1}	1269 CM^{-1}

The prepared derivatives were analysed using Infrared analysis (IR) through hydrolyzing each derivative with 5ml of 7NH Clean sealed glass vial and kept for 2hr. at 100℃ then each derivative was ground separately with KBr. The spectrum was recorded in Perkin–Elmer 377 spectrophotometer then evaluated against fungi using the agar dilution technique according to (6), different volumes of the derivatives were prepared and each of these volumes was mixed separately with 100 ml of SDA (Sabouraud Dextrose Agar) in order to prepare the required concentrations of these compounds for the test (0.03, 0.06, 0.3, 0.6 M). The SDA mixture were shaken well before being poured into petri dishes and left to solidify in sterile a condition, 8 mm piece from the mycelial growth of 15 days old mould culture was deposited in the centre of each plate. The inoculated plates were incubated at 28 ˚C for 7-10 days. The

diameters of the fungal colonies were measured, and then the antifungal activity of each concentration was calculated by measuring the growth inhibition percentage using the following equation.

Growth inhibition% = (Growth in control - Growth in treatment)/Growth in control) × 100 (6).

Statistical Analysis

The values of the investigated parameters were given interms of mean ± standard error and the differences between the means were assessed by analysis of variance (ANOVA) using the SAS computer program version 7.5. Differences in results were considered significant at probability value equal or less than 0.05 (SAS,2004) (7).

Results and Discussion

The first tested maleamic acid derivatives (Bis –maleamic acid) exhibited an absolute antifungal effect against *T.soudanense* with a 100% inhibitory effect at concentrations of 0.06M, 0.3M and 0.6M the same antifungal activity was detected against *A.flavus* but at concentration of 0.6M. Also, high inhibitory effect 99.6% and 99% was detected against *T.violaceum* and *A. fumigates*, respectively and 96% effect against *P.marneffei* at both 0.3M and 0.6M concentrations. In contrast, its highest effect against *T.rubrum* was only 89% and that was at the 0.6 M concentration.

The second derivative (Ethylen –Bis – Maleamic acid) demonstrated 100% efficacy against *T.rubrum, T.violaceum* and *P.marneffei* at 0.6M, and a high inhibition effect 97% against *T.violaceum* at 0.3M, also showed 95% effect at the 0.6M against *A.fumigatus*, while the same concentration showed 82% and 81% inhibitory effect against *T.soudanese* and *A.flavus*, respectively.

The third derivative (1,4-phenylene (2,2 –di amine –Bis(1,3,4)-thiadiazole -5- yl) had a 100% inhibitory effect at 0.6M against *T.violaceum* and also 100% effect against *A. flavus* at both 0.3M and 0.6M and 99.8% inhibitory effect to the same fungus at 0.03 M, an effect of 99.5%, 93.6% and 91% was detected against *T. Soudanese, A.flavus*, and *P.maneffei*, respectively at 0.6M concentration. A weaker effect of this derivative was detected against *A.rubrum* 89% and that was at its highest concentration 0.6M.

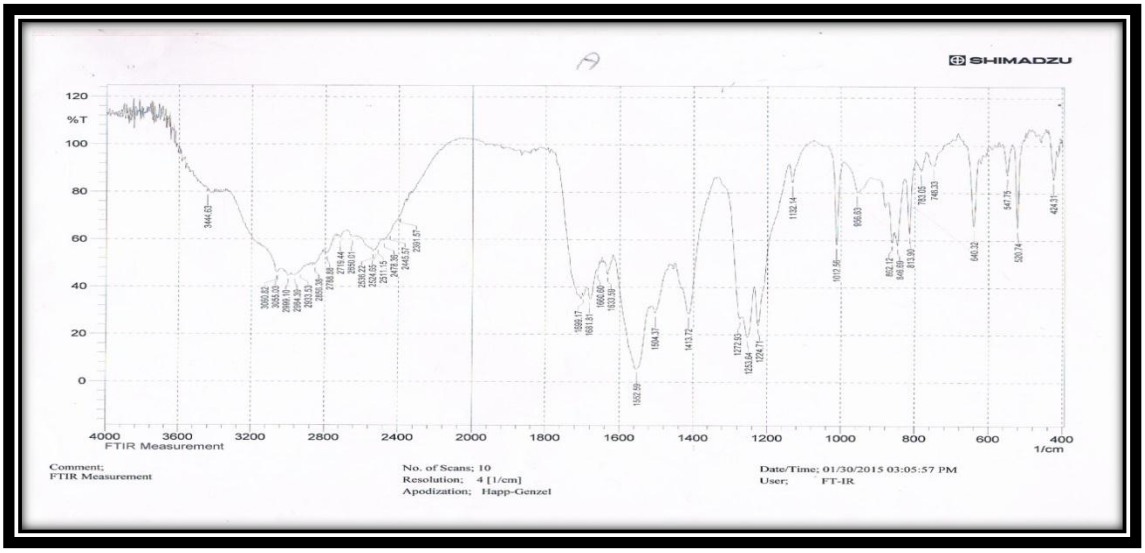
The fourth derivative,(4- N 2,3-dimethyl-1-phenyl-pyrazolin-5-one-4-yl) demonstrated 100% antifungal activity against *T.rubrum* at 0.6M and 99.9% activity at 0.3M and also a high activity 99.8% against *T.violaceum*at in the same concentration. The effect against *p.marneffei, A.flavus* and *T.soudanese* was 95%, 91% and 89% respectively at the 0.6M, while a weaker effect was detected against *A.fumigatus* which was only 50% also at the derivative highest concentration.

Finally, the fifth derivative (5- methyl-3- sulphanilamideisoxazole) exhibited 100% inhibition activity against *T.rubrum* at 0.6M also a good inhibitory effect 96% against *T.violaceum* at both 0.3M and 0.6M .(Table-4).

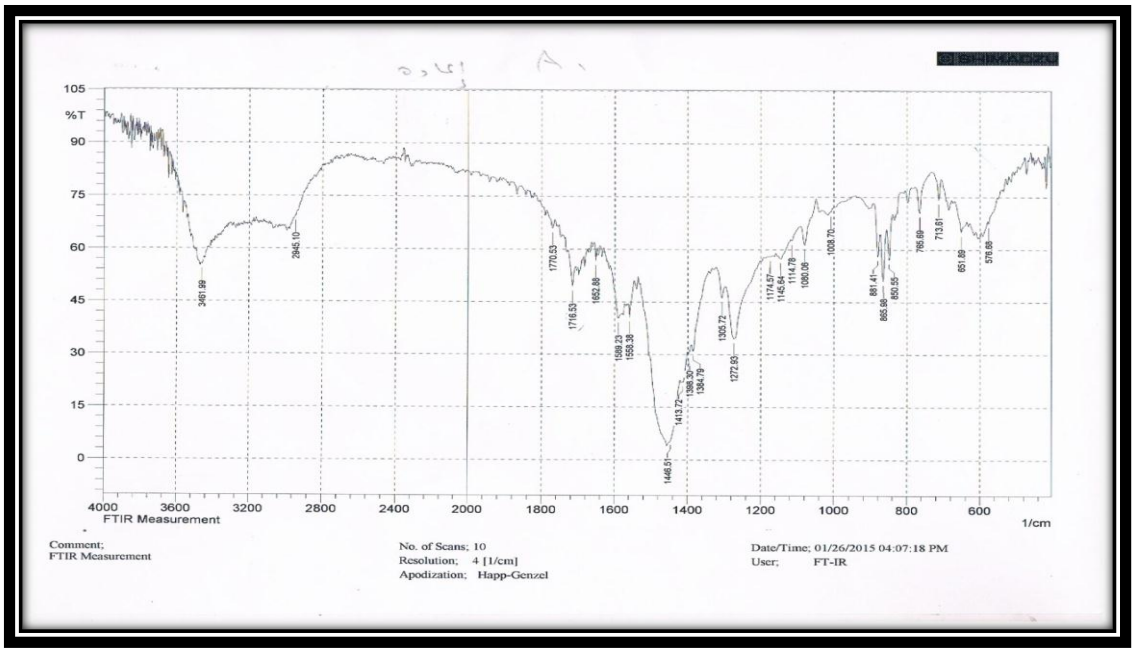
Infrared analysis IR is regarded as the most informative method for structural analysis of maleamic acid derivatives; therefore, this method was applied. The overlay of the IR spectra of synthetic derivatives exhibited wave numbers at3381,2927,1867,1531,1404,1073 and 651 cm-1.

The wave band can be ascribed to the

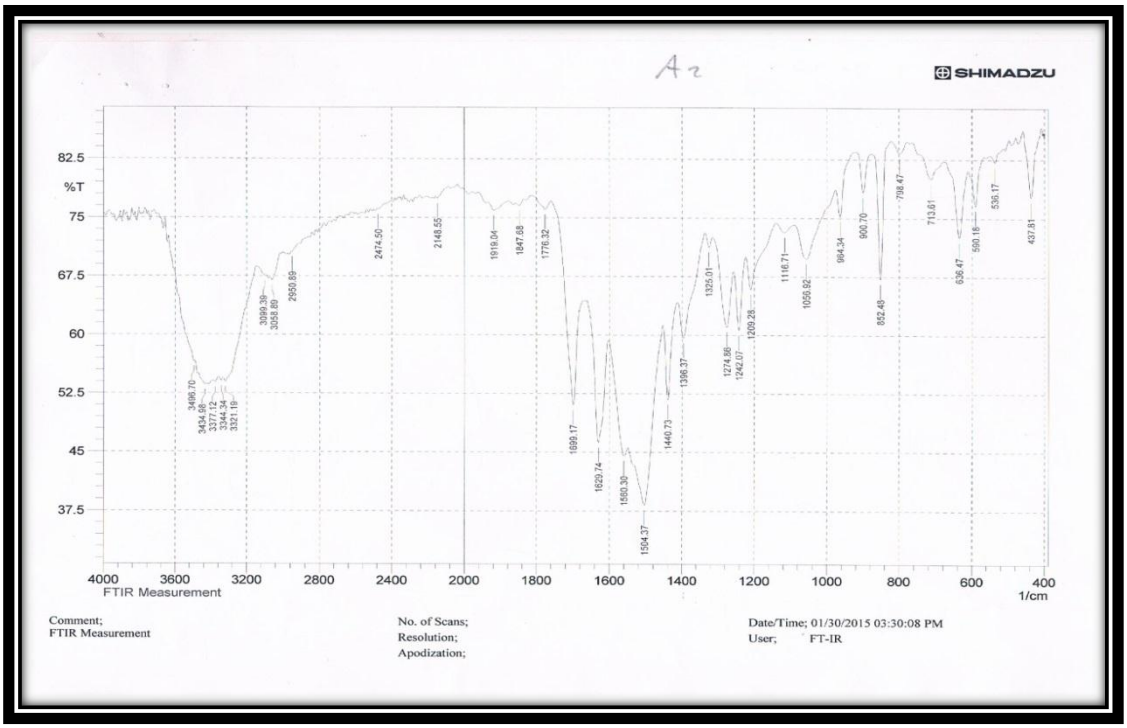
following chemical groups: 3381 cm-1 was attributed to OH bonds, 2927 cm-1 to H-C or H-C=0 bonds 1404 (C-CH3), and 651 cm-1 to (acyclic) CH2 bonds as shown in Figure (1,2,3,4,5) .



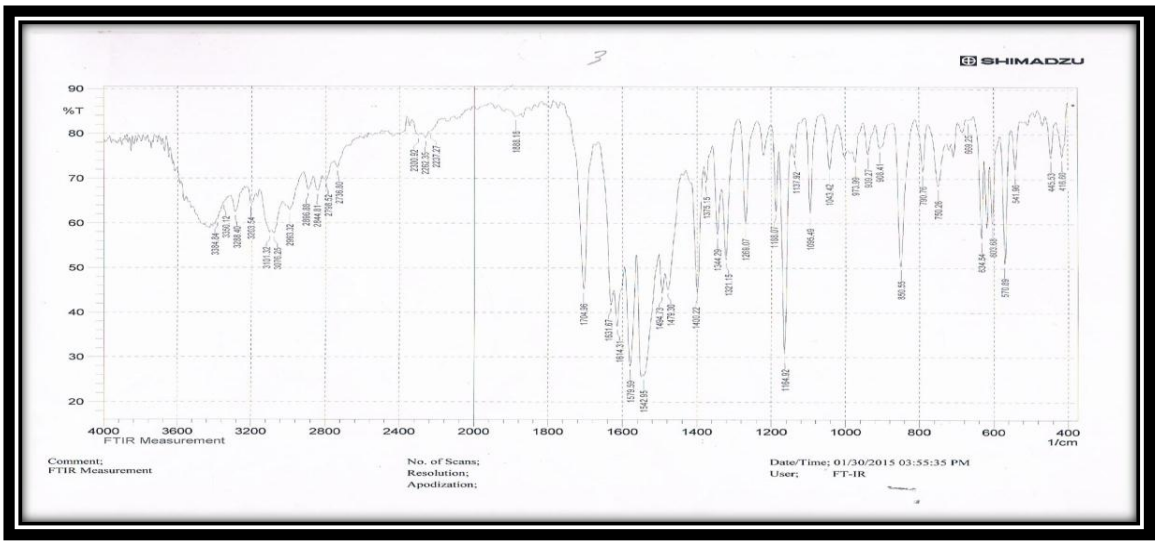
(Figure-1) Infrared analysis of Bis –malaemic acid



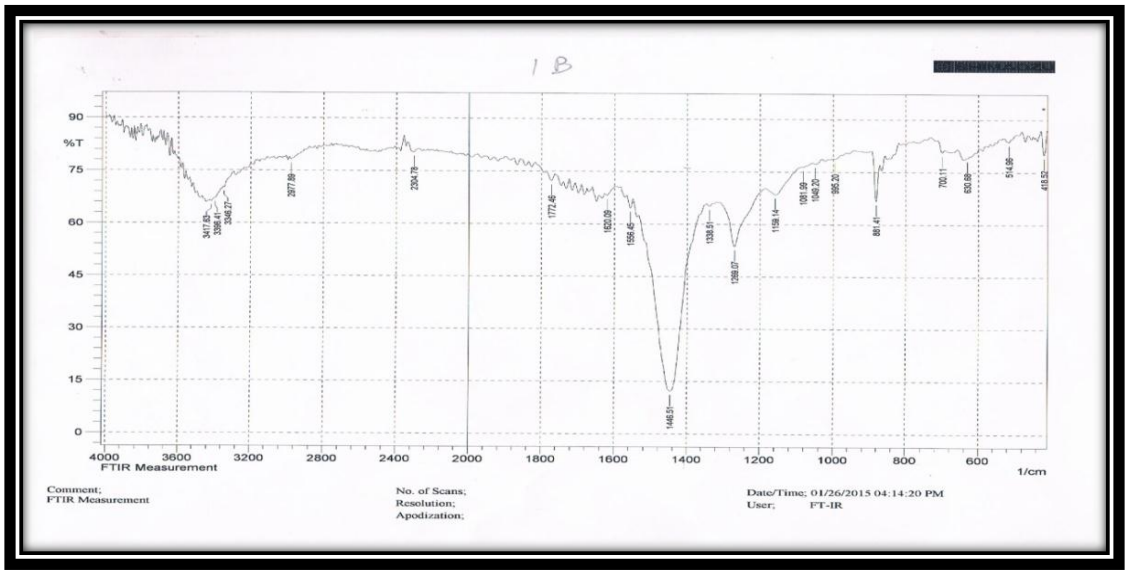
(Figure-2) Infrared analysis of Ethylene-Bismaleamic acid



(Figure-3) Infrared analysis of 1,4 phenyl(2,2-diamon-Bis(1,3,4-thiodiazole-5yl)



(Figure-4) Infrared analysis of 2,3 dimethyl-phenyl-pyrazolin-5- one-4-yl maleamic acid



(Figure-5) Infrared analysis of 5- methyl-3-sulphanilamido isooxazole malaemic acid

With the increase in drug resistance demonstrated among divers fungal species, the need for new agents is increasingly in need and it is often assumed that the clinical outcome of a treatment can be predicted from the result obtained of in vitro testing of patients isolates against a panel of suspected drug followed by demonstration of correlation with clinical outcome *in vivo* (8).

In the submitted research, five chemical derivatives of maleamic acid were tested against six pathogenic fungi, three are classified as dermatophytes and three others classified as invasive fungi.

Results showed that the first three derivatives A, A1 and A2, are active against both the dermatophytes and invasive fungi, remarkably A derivative is highly active (100% growth inhibition) against *T. soudanese* and in three different concentrations and have the same activity against *T.violaceum* and *Aspergillus* species but only at the highest concentration.

Derivative A1 have the highest effect (100% growth inhibition) against *T.rubrum, T. violaceum* and *P.marneffei* at the highest concentration only, while the third derivative A2 showed the same activity against *A. fumigates* only but at three different concentrations.

These results indicate that those derivatives have a wider range activity and may have a promising therapeutic role against both dermatophytes and invasive fungi.

On the other hand the other two compounds A3 and B found to have a considered antifungal effect against dermatophytes (100% growth inhibition), especially against *T. rubrum* and *T. violaceum*, they were recorded also to have antifungal effect against other studied species, but to less extent. That indicates the importance of those two derivatives in treating dermatomycosis more than systemic mycosis.

The importance of studying maleimides comes from the fact that the maleamic acid derivatives are found to be chemically stable and biologically active (9,10), the maleamic acid compounds stabilizer reasonable biological activity due to the presence of either –NH or C=O group in its structure (11).

However, the results obtained are in concordance with some researches documented that dimethyl maleimides have a perfect inhibitory activity for *Sclerotinia sclerotiorum* by using the mycelia growth rate method (12), on the other hand, the results of current study do not agree partially with results obtained in a Brazilian study in 2013 ,that reported the insignificant role of one of maleimic acid derivative ,N-alkyl maleamic acid as antifungal compound (13).Those differences in results are thought to due to the difference in the preparation steps or the chemical structure of the derivative itself, even if these structural differences are just minor, since each derivative has it own properties and each research examine a different structure.

It is assumed that the anti-fungal activity of maleamic acid is mediated through disruption of the cell membrane structure, causing leakage and cell death; blocking of membrane synthesis; inhibition of spore germination, fungal proliferation and cellular respiration(14) fungal cell membrane is composed of sterols lacking C-4 methyl group such as ergosterol, which is critical to the membrane integrity, regulating membrane fluidity and asymmetry (15) and is not present in mammalian cells and thus is an ideal target for antifungal activity. Indeed, most currently available antifungal drugs interact with or inhibit the synthesis of ergosterol, bind directly to membrane sterols (ergosterol) and form Ionic trans-membrane channels, that increase in membrane permeability that provokes leakage of the intracellular contents, which eventually leads to cell death (16) Further investigations are needed to precisely detect the target for each derivative, *in-vivo* studies are recommended to demonstrate the clinical outcome and possible side effects.

Given the need for more antifungal medications, these results suggest that pharmaceutical companies should conduct more research on these derivatives.

(Table-4)) : The Minimal Inhibitory Concentration (MIC) and Maximum Fungicidal Concentration (MFC) of Maleamic acid derivatives against some pathogenic fungi

Mean percentage of growth inhibition for fungal isolates (%) mean ± SE							
Maleamic acid derivatives	**Concentrations (M)**	***T. rubrum***	***T.violaceum***	***T. soudanese***	***A. fumigatus***	***A. flavus***	***P. marneffei***
Drivative A Bis-Maleamicacid	**0.03**	**16.4±0.33**	**13.5 ±0.80**	**44.6 ±0.77**	**12.6 ±0.12**	**24.3± 0.00**	**33.2± 0.45**
	0.06	**67.8±0.58**	**43±0.23**	**100±0.00**	**75±0.00**	**89±0.00**	**92.2±0.65**
	0.3	**78.5 ±0.12**	**62 ±0.88**	**100 ±0.38**	**92.6± 0.26**	**95± 0.00**	**96 ±0.12**
	0.6	**89.2 ±09**	**99.6 ±0.00**	**100 ±0.38**	**99 ±0.00**	**100± 0.09**	**96±0.76**
Derivative A1 Ethylene-Bis-Maleamic acid	**0.03**	**12.1 ± 0.31**	**43.1 ±0.23**	**10.3+ 0.56**	**33± 0.10**	**22.6 ± 0.23**	**33.2± 0.87**
	0.06	**81.1 ±0.48**	**93 ±0.26**	**50±0.78**	**85± 0.00**	**65.5± 0.34**	**80± 0.00**
	0.3	**87 0± 0.11**	**97 ±0.55**	**82±0.00**	**90 ±0.00**	**75.5± 0.99**	**92 ±0.98**
	0.6	**100±0.88**	**100 ±0.29**	**82±0.00**	**95.6± 0.34**	**81.2 ±0.00**	**100± 0.00**
Derivative A2 1,4-phenylene (2,2-diamino-Bis(1,3,4-thiadiazole -5-yl) Maleamic acid	**0.06**	**23.5 ±0.13**	**17.2 ±0.38**	**13.9 ±0.19**	**20 ±0.12**	**39.6± 0.00**	**18±0.00**
	0.03	**73.8 ±0.18**	**56 ±0.19**	**55 ±0.34**	**65.4 ±0.56**	**99.8± 0.09**	**67.5± 0.00**
	0.3	**78.5±0.34**	**72 ±0.45**	**74.6±0.68**	**75 ±0.44**	**100± 0.00**	**79.9± 0.87**
	0.6	**89 ±0.11**	**100 ±0.38**	**99.5±0.88**	**93.6± 0.89**	**100 ±0.00**	**91.5± 0.23**
Derivative A3 4-N (2,3-dimethyl- 1-phenyl –pyrazolin-5-one-4-yl) Maleamic	**0.03**	**34.6 ±0.23**	**23.3 ±0.00**	**14±0.00**	**19.1 ±0.87**	**24.4±0.98**	**33 ±0.35**
	0.06	**95 ±0.78**	**72.9 ±0.56**	**63 ±0.15**	**68 ±0.00**	**69±0.11**	**85 ±0.23**
	0.3	**99.9 ±0.83**	**89 ±0.11**	**78 ±0.99**	**50±0.03**	**79±0.65**	**90 ±0.22**
	0.6	**100 ±0.85**	**99.8 ±0.16**	**89 ±0.87**	**50±0.3**	**91± 0.07**	**95.6± 0.23**
Derivative B1 5-methyl-3-sulphanilamideMaleamic	**0.03**	**23.3 ±0.00**	**33.2 ±0.56**	**21.6± 0.67**	**50±0.6**	**17.1± 0.00**	**25.4±0.00**
	0.06	**81.4 ±0.18**	**92±0.00**	**70± 0.12**	**55±0.6**	**75±0.00**	**75±0.00**
	0.3	**92.1 ±0.12**	**96± 0.00**	**84.3± 0.44**	**87.2± 0.65**	**83.3±0.87**	**81.2±0.00**
	0.6	**100 ±0.00**	**96± 0.00**	**95.1± 0.23**	**93.1± 0.76**	**93.5± 0.23**	**87.5± 0.09**
DMSO		**0.00**					

References

1. Oskay, M. (2009) .Antifungal and antibacterial compounds from streptomycesstrains, *African Journal of Biotechnology*,8 (13):3007–3017.
2. Mercurio, M. G. and Elewski, B. E. (1993).Therapy of sporotrichosis. *Semin Dermatol*., 12:285.
3. Nayon, R.; Bhalodia, P. and Nariya, B. (2011).Antibacterial and antifungal activity from flower extracts of cassia fistula. *International Journal of Pharm Tech Research*, 3 (1): 160-168.
4. Elizabeth, S.; Dodds, A. and Russell, L. (2006). Pharmacology of systemic antifungal agents. *The infectious Diseases of America*, 43: 28-39.
5. Larone, D. H. (1993).Medically important fungi - a guide to identification. 2nd edition, American Society for Microbiology, Washington, D. C.
6. Kirk, P. M.; Cannon, P. F.; David, J. C. and Stalpers, J. A.(2008) . Ainsworth an Biby's dictionary of the fungi. 9th edition . CABI Bioscience , Oxon , UK . P.452.
7. Ahmed, S. and Fatima, S. (2014). Synthesis of some new maleimide

derivatives. *Journal of Applicable Chemistry*,3(1): 56- 63.

8. Al Zoraky, N. and Nakahara, K. (2003).Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *International Journal of Food Microbiology*, 80:223-230.
9. SAS users.(2004). Guide personal computer (ver.7) inst. Inc. Cary.Nc. USA.
10. kauffman, C.; Pappas, P.; Sobel, J. and Dismukes, W. (2011).Essentials of clinical mycology.Second edition.Springer Science +Business media, USA.
11. Wang, S.; Wu, C.; Chu, F.; Chien, S.; Kuo, Y.; Shyur, L. and Chang, S. (2005). Chemical composition and antifungal activity of essential oil isolated from *Chamaecyparis formosensis* Matsum. Wood. *Holzforschung*, 59: 295–299
12. Harris, R. (2002).Progress with superficial mycoses using essential oils. *International Journal of Aromatherapy*, 12:83-91.
13. Inouye, S. (2003). Laboratory evaluation of gaseous essential oils. (part 1). *International Journal of Aromatherapy*, 13:95-107.
14. Zhenzhon, S.; Yong, F.; Fuge, L.; Xiaolong, C. and Yinchu, S.(2012). Synthesis of N-substituted dimethylmaleides and their antifungal activities against *Sclerotinia sclerotiorum.Journal of Pest Science.*86 (2): 353-360.
15. Belinelo, V.; Tacchicapus, M.; Autunes, R.; Assenco, R.; Vieira Filho, S.; Lanna, M.; Marçal E.; Fonseca, T.; Gomes, M. and Magalhães, J.(2013). Insignificant level of *invitro* cytotoxicity, anti rotaviral , antibacterial, antifungal activities of N-alkylmaleamic acid .*Journal of Pharmaceutical Negative Results*, 3 (1): 19-25.
16. Himejima, M. and Kubo, I. (1993). Fungicidal activity of polygodial in combination with anethole and indole against *Candida albicans*. *Journal Agric. and Food Chem*., 41: 1776– 1779.