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CHEMOTHERAPEUTICAL STUDIES OF 2-PYRAZINOYL HYDRAZIDE AND 2- PYRAZINOYL HYDRAZONES AGAINST SOME FUNGI

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ABSTRACT

Mannich bases possess antibacterial, fungicidal, anti-inflammatory and analgesic activities. Chelating ligands and various derivatives like oximes, semicarbazones, hydrazones of benzaldehyde and substituted benzaldehydes have long been prepared, chacterised and analysed by analytical chemists for such activities. Some of these compounds may be active against micro-organisms like bacteria, fungi, or virus and some may be inactive. The ligands which are active against any of these micro-organism have wide spread applications as medicinal agents. Considering these facts here we have undertaken the screening of 2-pyrazinoyl hydrazide and its derivatives with various aromatic and heterocyclic aldehydes against various fungi. The present paper deals with the physiology and nutrition of fungi, their cultivation and culture and their screening using hydrazones derivatives of 2-pyrazinoyl hydrazide with various aromatic and heterocyclic aldehydes such as benzaldehyde, anisaldehyde, 4-hydroxy-3-methoxy benzaldehyde, p- (N,N-diethyl amino) benzaldehyde, cinnamaldehyde, 4-methyl salicyldehyde and 2-furfuraldehyde.

KEYWORDS: Fungicidal, Substituted Aldehydes, Hydrazones Derivatives, Ligands, Fungi

INTRODUCTION

The fungi constitute a group of microorganisms that are of great practical and scientific interest to microbiologist. Manifestations of these microorganisms are familiar. Fungi, can be seen in the form of blue and green growth on oranges, lemons and cheese, the white or gray furry outgrowths on bread and jam, the mushrooms in the fields (or on glossary shelves) and the toad stools in the woods. The fungi are very diverse in their morphological manifestation. Generally speaking fungi include the molds and the yeast. Molds as a term is used very loosely to include the mildews, the rusts and smuts, the mushrooms, the puffballs and the slim molds. [(1)]

The fungi are heterotrophs, obtaining their food from non-living organic matter as saprophytes or by feeding as parasites on living hosts. As saprophytes they decay complex plant and animal remains, breaking them down into simpler chemical forms which are returned to the soil. These chemical substances are then absorbed by later generations of plants. Thus fungal activity is largely responsible for soil fertility.

Fungi are also important in industrial fermentations [(2)] such as in the brewing of beer, the making of wine, and the production of antibiotics (e.g. penicillin),[(3)] vitamins, and organic acids (e.g. citric acid) blacking and the ripening of cheese also depend on saprophytic fungal activity as parasites. At the same time fungi cause diseases in plants, humans and other animals, although most fungal diseases are severe than bacterial or virus diseases. In animals a very small number of fungi cause diseases. In humans these include skin diseases such as athletes' foot, ringworm and thrush .They can cause serious

damage to agriculture, resulting in critical losses of yield and quality.

Quiet apart from the applied aspects of the study of fungi (the science or study of fungi is called mycology), these microorganisms are to be studied fundamentally in their own right as biological entities. They have also become tools for the physiologist, biophysicist, geneticist and biochemist, who find them highly suitable subject for the study of some biological process.

(a) Distinguishing Characteristics of Fungi: Fungi are eucaryotic chemo-organotrophic microorganisms. They reproduces naturally by means of spores, with few exceptions (moreover, most parts of a fungus are potentially capable of growth, a minute fragment is sufficient to start a new individual). They have no chlorophyll; their bodies are usually thread like or filamentous (2 to 10 µm across) and commonly branched. The filaments have cell walls containing chitin or glucans,[4] or both. Most fungi are non-motile, although they may have motile reproductive cells. Spores are produced in two ways by almost all classes of fungi, sexually [5] and asexually. [6]

(b) Physiology and Nutrition of Molds: The fungi grow in a wide range of habitat and have a worldwide distribution. They grow in extreme environments such as deserts or areas with high salt concentrations[7] or ionizing radiation,[8] as well as in deep sea sediments.[9] Some fungi can survive the intense Ultra-Violet and cosmic radiation encountered during space travel.[10] Physiologically molds are adaptable to more severe stresses than most micro-organisms, for example, molds can grow on substrates containing concentrations of sugars that bacteria cannot tolerate because molds are not as sensitive as bacteria to light, osmotic pressure. Molds can tolerate and grow in relatively high concentrations of acid, a pH range of 2.0 to 9.0 is tolerated, but the optimal pH for most species is about 5.6.

Although moisture is required for their growth and they can obtain water from the atmosphere as well as from the medium, molds can survive [11] in dehydrated environment that would be inhibitory to most bacteria other than spore formers. When the environment becomes desiccated, fungi produce spores or go into a resting stage. Most molds are strictly aerobic; growth is enhanced by an abundant supply of oxygen. They grow over a wide temperature range with the optimal for most species, being 22°C to 30°C, some fungi will grow at 0°C, causing spoilage of meat and vegetables in cold storage. A few thermophillic molds will grow at 62°C.

(c) Cultivation of Molds: Molds can be studied by the same general culture methods used in bacteria.[12] Nearly all of them grow aerobically on the usual bacteriological culture media at temperature ranging from 20°C to 30°C. Most of them grow more slowly than bacteria, so that media which support bacteria as well as molds may be over grown by bacteria contaminants in a mixed inoculums. When molds are to be isolated, it is good practice to use a medium that favours their growth but it is not optimal for the growth of bacteria, acid (pH 5.6) media that incorporate a relatively high concentration of sugar are tolerated by molds but are inhibitory to many bacteria.

(d) Culture Media: There are three general types of media for the growth of molds.

- Natural media; such as pieces of infusion of fruits, vegetables, cereal, grains and chemical tissues, such media vary greatly in their composition and are therefore not accurately reproducible. This type is not widely used.
- Culture media prepared from peptones, plant extracts, agar and other compounds of unknown or variable composition.

- Synthetic or chemically defined media.

MATERIALS AND METHOD

All the ligands have been screened for their antifungal activity against five different fungi, at dept. of microbiology U.C.M.S. & G.T.B.H. Shahdara. Method involves the following steps.

- Preparation and sterilization of media.
- Sterilization of the glass apparatus.
- Mixing of sample solution to the sterilized media.
- Preparation of slant.
- Inoculation of slant and incubation at specified temperature, and
- Measurement of growth of fungus.

(a)Preparation of Experimental Broth (Sabouraud's Glucose Agar): This nutrient media is made by dissolving the following quantities (in gm) of the specified ingredients in sufficient purified water to produce 1000 ml of medium.

Glucose 40.0

Peptone 10.0

Agar 20.0

Final pH 5.4 (after sterilization)

The media was sterilized at 115°C (for 15 minutes).

(b) Procedure For Assay: To the molten media (90°C) was added the solution of compound under study in DMSO so as to give a final dilution of 2 mg/ml of media, it was cooled to about 50°C and 10 ml of media is transferred aseptically to cylindrical tube of 6"x3/4' to make a slant, one set of three such tubes were taken for one compound and these tubes were inoculated with spores of Aspergillus fumigatus, Aspergillus niger, Candida albicans, Penicillium and Mucor. To control the environmental effects on the growth of fungi, blank observations were simultaneously made without adding any active substance. All the tubes were incubated for 15 days at suitable temperature

Table 1: Antifungal Activity of Pyrazine-2-Carboxyic Acid Hydrazide and Its Derivatives With Various Aromatic and Heterocyclic Aldehydes

S. No.	Compound	Aspergillus Fumigatus	Aspergillus Niger	Candida Albicans	Penicillium	Mucor
1.	PAH ($C_5H_6N_4O$)	-	-	-	-	-
2.	PAH-BENZ ($C_{12}H_{10}N_4O$)	+	+	-	-	-
3.	PAH-ANSL ($C_{13}H_{12}N_4O_2$)	+	+	-	-	-
4.	PAH-VANI ($C_{13}H_{12}N_4O_3$)	+	+	-	-	-
5.	PAH-PDEAB	+	-	-	-	-

	($C_{16}H_{19}N_5O$)					
6.	PAH-CAH ($C_{14}H_{12}N_4O$)	-	+	-	-	-
7.	PAH-MSALI ($C_{13}H_{12}N_4O_2$)	-	-	-	-	-
8.	PAH-FURAL ($C_{11}H_{10}N_4O_2$)	-	+	-	-	-

(i) (-) Sign indicates no growth (antifungal activity) and

(+) sign indicate fungus growth.

(ii) Activity mentioned above is at a final concentration of 2 mg/ml.

RESULTS AND DISCUSSIONS

The chemical compounds or biological organisms used to kill or inhibit the growth of fungi or fungal spores are called fungicides. They are used both in agriculture and to stop fungal infection in animals. The oomycetes, which are not fungi use the same mechanism as fungi to infect plants,[13] therefore chemicals used to control oomycetes are also known as fungicides. The fungicides are used to control diseases in crops and to increase the productivity. They are also used to improve the storage life of harvested plants and produce because the fungi often spoil stored fruits, vegetables, tubers and seeds. Few fungi which infect grains produce toxins capable of causing severe illness or even death in humans and animals when consumed. The fungicides are applied as dust, granules, gas and liquid. They are applied to seed, bulbs, roots and other propagative organs.

Substances like cinnamaldehyde, citronella oil, tea tree oil etc, act as natural fungicide. The live or dead organisms which can kill or are efficient in inhibiting fungi can sometimes be used as fungicides. Chemicals of both inorganic and organic origin which may be natural or synthetic (phosphonates, elemental sulfur, mercury compounds, sodium bicarbonate, [14][15] methyl benzimidazole carbamate, dicarboximide, phenylamide, aromatic hydrocarbon, cinnamic acid, anilinopyrimidine etc.) have been used by scientists as fungicides.

In the present study Pyrazine-2-carboxylic acid hydrazide[16] ($C_5H_6N_4O$; PAH) and its hydrazones derivatives with different aldehydes viz. Benzy1lidene-2-Pyrazinoyl hydrazone ($C_{12}H_{10}N_4O$; PAH- BENZ), Anisalidene-2-pyrazinoyl hydrazone ($C_{13}H_{12}N_4O_2$; PAH- ANSL), 4-Hydroxy-3-methoxy benzylidene-2-pyrazinoyl hydrazone (($C_{13}H_{12}N_4O_3$; PAH-VANI), p - (N, N - diethylamino) benzy1lidene–2-pyrazinoyl hydrazone ($C_{16}H_{19}N_5O$; PAH- PDEAB), Cinnamalidene-2-pyrazinoyl hydrazone ($C_{14}H_{12}N_4O$; PAH- CAH), 4-Methyl salicylidene-2-pyrazinoyl hydrazone ($C_{13}H_{12}N_4O_2$; PAH-MSALI), 2-Furfuralidene-2'- pyrazinoyl hydrazone (($C_{11}H_{10}N_4O_2$; PAH- FURAL) were screened for antifungal activity. A close study of the results reveal that all the compounds studied are active against Candida albicans, Penicillium and Mucor and PAH and PAH-MSALI are active against all the five fungus, studied.

CONCLUSIONS

The present study was carried out to study the effect of various ligands against some fungi. As the ligands are active against some fungi, they can also be tested against other micro-organisms such as bacteria, virus etc. Therefore continuous monitoring is needed in this field. The ligands may be used to prepare complexes with different metal ions. The complexes thus formed may be tested as anti-fungal, antibacterial, antimicrobial, antitubercular agents etc.

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