Biological Sciences

Pak. J. Sci. Ind. Res. 2008 **51**(5) 264-266

Antifungal Activity of the Liquid Obtained by Oxidative Cracking of Waste Paper

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(received March 14, 2008; revised July 15, 2008; accepted July 26, 2008)

Abstract. The composition of the product formed by burning of paper under controlled conditions in an indigenously prepared reactor was determined by GC/MS, while its antifungal activity was assessed qualitatively and quantitatively by comparison with the standard and determination of the MIC. The product was found to be effective against six fungal strains. Amount of the product has been improved upto 25%.

Keywords: antifungal activity, oxidative cracking, waste paper

Introduction

The incidence of fungal infections has lately increased due to the resistance of the causative microbes to the antifungal medicines (Turan-Zitouni *et al*., 2005) as well as the increased number of immune persons taking chemotherapy for various types of cancer or HIV infection (Sionov *et al*., 2005). Hence, there is a need to explore and develop new antifungal reagents of higher activity or having minimum adverse effects (Schmourlo *et al*., 2005; Silver and Bostain, 1993).

Folk medicines have played important role in the treatment of many diseases for quite a long time. In the present work an effective folk medicine used for the treatment of scabies and ringworm has been investigated. This medicine is used in various parts of NWFP, Pakistan for treatment of these diseases for long. Traditionally, when a sheet of paper rolled into the form of a pipe, with its upper end closed is burnt, a small quantity of liquid product is obtained at the lower end of the pipe. This liquid of reddish colour, obtained from the combustion of paper, is known as paper oil.

The author has observed that very chronic cases of these diseases were cured through use of the said oil, twice a day in very small doses for three to seven days. In the present paper, investigations carried out on the composition of the product, its antimicrobial activity as well as measures to improve the yield, have been reported.

Materials and Methods

The process of the oxidative cracking of the paper was carried out in a quick fit pyrex reactor of the Buckner funnel type. The external diameter of the funnel was 60 mm, while its length was 100 mm. Sintered glass disc bed reactor of quick fit type was used. The reaction was carried out by igniting 100 g of fine paper cut into small pieces. The assembly for reaction includes, the pyrex reactor, Buckner type funnel fitted in the suction flask, which was connected with a series of three receiving flasks and the aspirator. The product was sucked into the suction flask followed by its condensation into a reddish colour liquid product with the maximum yield of 25%.

GC/MS analysis. General profile for the liquid product of the oxidative cracking of paper was obtained using EI-MS. Analysis of the liquid product was conducted on a mass spectrometer JMS 600 H Jeol and the product was separated into its components using Agilent 6890N gas chromatograph equipped with a fused capillary column (HP.5 l.; 30 m x 0.32 mm i.d., film thickness, 0.25 μm) with polydimethylsiloxane as the stationary phase. The carrier gas was helium at a flow rate of 1.8 ml/min.

Injection mode was split at a split ratio of 35. The column was initially kept at 50 °C for 5 min and then the temperature was raised to 240 °C at the rate of 5 °C/min. The injector temperature was 250 °C and the amount of sample was 1 μl.

Identification of major components of the product was confirmed using total ion chromatogram analysis (TIC) as well as fragmentation pattern and library matching by NIST Mass Spectral Library, while the quantification was carried out using the peak area.

Antifungal activity. Antifungal activity of the sample was determined using agar tube dilution method. The activity of the sample was investigated against *Trychophyton longifusus*,

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Candida albicans, *Candida glabarata*, *Fusarium solan*i, *Aspergillus flavus* and *Microsporum canis*. Sabourad dextrose agar (SDA) was used for the growth of fungi; 0.4 ml of SDA, for each fungus species, was taken in six separate tubes and autoclaved. 24 mg of the sample was dissolved in 1 ml dimethylsulphoxide and 66.6 µl of this solution was dispensed in each of the six tubes. The tubes were placed in slanting position. These were allowed to solidify for 24 h. Each of the tube was inoculated with 4 mm of fungal stock and incubated at 27 °C for 7 days. The media containing only DMSO was used as blank and Micanozole, as reference, for the samples. The reference for *Aspergillus flavus* was Ampotericin B. All the tests were carried out in triplicate.

Results and Discussion

The liquid obtained by the oxidative cracking of paper was analyzed for composition using GC/MS. It was composed of acids, aldehydes, ketones and furans. The compounds present in the liquid sample are mentioned in Table 1.

The product also contained large amount of water and other undetectable compounds. The activity of the product was significant for all the tested fungal strains in terms of percent inhibition (Table 2).

Based on the presence of oxygenated compounds, it can be inferred that the sample inhibits the growth of fungi through inhibiting the growth of mycelia, interference with the SH enzymes and disruption of the chitinous cell wall. Antifungal activity of the oxygenated compounds has been reported by various workers (Turchetti *et al.*, 2005; Karmen *et al.*, 2003; Stange *et al.*, 1999). The sample contains aldehydes, which are cell wall rupturing compounds (Knobloch *et al*., 1989). It also contains phenolic compounds, which are antioxidant, and interfere with enzymes (Karmen *et al.*, 2003). Presence of acid in the sample supports its antifungal activity as reported by Turchetti *et al*. (2005). Antifungal activity of the sample also gets support from the work of Pour *et al.* (2000) who reported derivatives of furan. However, the activity of our sample is the result of combined action of these compounds and is greater than simple addition.

Table 1. Composition of product of oxidative cracking of paper

Compounds	Retention time (min)	Concentration (%)
Propenoic acid methoxy ester	1.53	11.4888
1-Hydroxypropanone	1.60	7.4038
2,5-Dimethyl furan	2.60	3.8295
2-Methanol-5-hydroxy furan	3.48	2.2339
4-Hydroxy-but-2-enoic acid lactone	5.05	2.0637
4-Methanol cyclohexanol	6.52	0.9361
2-Ethenylfuranone	7.05	0.9701
Hydroquinone	7.52	0.6808
4-Hydroxy-3-methyl-1-one-2-cyclopentene	8.85	1.6169
2-Ethenyl-3-methyl-2-(5*H*)-furanone	9.28	0.3574
2-Propenal-4-methyl phenol	10.7	1.0852
Unknown	11.6	0.3643
1,4:3,6-Dianhydro-α-D-glucopyranose	14.95	0.4144
Unknown	15.77	1.7871
Unknown	23.43	1.0930
Octadecanoic acid	32.6	1.9573

Table 2. Antifungal activity of the product of oxidative cracking of paper

Fungus	Linear growth (mm)		Inhibition	MIC
	Sample	Control	(%)	(µg/ml)
Trichophyton longifusus	0	100	100	370
Candida albicans	0	100	100	390
Aspergillus flavus	0	100	100	340
Microsporum canis	0	100	100	275
Fusarium solani	0	100	100	320
Candida glabarata	0	100	100	375

Conclusion

Based on the traditional knowledge, through oxidative cracking of paper, significant yield of a product was obtained using suction reactor. The product contained furans, furanones, carboxylic acids, aldehydes, ketones and phenols along with water. These compounds are reportedly antimicrobial, acting through enzyme inhibition, antioxidant, membrnane rupture and mycelial growth inhibition. The product displayed antifungal activity against six fungal species. This study helps to formulate a broad spectrum antifungal agent.

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