Antifungal Activity of the Liquid obtained from Pyrolysis of Paper

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Summary: In the present studies the paper was pyrolyzed into liquid products at 300 °C. The product containing furans, furanone, carboxylic acids, aldehydes and ketones are antifungal folk medicine. The antifungal activity of this product was screened for six fungal strains; its activity was significant against, *Microsporum canis, Trychophyton longifusus*. The minimum inhibitory concentration for these fungal strains was found 279 and 360 μg/ml respectively. The composition of product was determined using GC/MS.

Introduction

Paper is a lignocellulose product, therefore included in the biomass. A large amount of work has been done and underway to utilize biomass as a resource. The most significant part of work is on the pyrolysis of biomass. It is pyrolyzed to obtain fuel gas [1], liquid fuel like methanol [2] and useful chemical feed stock [3, 4]. This process is also useful for the conversion of biomass into activated carbon [5, 6].

Pyrolysis of biomass/paper gives gaseous, liquid and solid products [7]. The liquid product predominantly contains carboxylic acids, aldehydes, ketones, phenols as well as anhydro sugars [3, 4]. Most of these products like acids and phenols have antimicrobial activity [8-10].

Since long antifungal and antibacterial activities are of significant importance. This is because those fungal infections are on the rise with sharp damages on life and property despite the availability of potent antifungal agents, [11, 12]. There is a need to develop and explore new antifungal agents due to rapidly developing resistance of these organisms against the existing antifungal agents.

In the present work the antimicrobial activity of the distillate obtained during the destructive distillation of paper is screened out. A hint of this work is taken from the folk medicines, where a brown liquid is obtained at the lower end of rolled paper burning at the top. That liquid is used for the treatment of scabies and ring worm in various parts of Pakistan.

Analysis of the distillate obtained during destructive distillation of paper was made using GC coupled with mass spectrometer.

Results and Discussion

Paper pyrolyzate was found highly effective against the fungal strains, *Trichophyton longifusus* and *Microsporum canis*. The antifungal activity of the product was assessed by comparison with the standard and by the determination of MIC. The MICs were 279 and 360 μg/ml, respectively, for *Microsporum canis* and *Trichophyton longifusus*. In case of *Candida albican, Aspergillus flavus, Fusarium solani* and *Candida glabarata*, the pyrolyzate was found to be inactive. This is due to the difference in composition of the cell wall and difference in concentration of the enzymes inhibited by the pyrolyzate. This is clear by the poor activity of the pyrolyzate against bacterial strains. It is inactive against *Escherichia coli, Shigella flexneri* and *Pseudomonas aeruginosa*. However, its activity against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* in terms of the millimeters of the zone of inhibition is 15, 16 and 16, respectively, as compared to the 33, 33 and 25 mm for the standard drug. The non significant but good antileishmanial activity is also due to the above-mentioned reasons.

The activity of the pyrolyzate against these pathogens is of greater importance due to the verification of folk knowledge and the potential threat of these human pathogens. This activity can be explained by the chemical composition of pyrolyzate.

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A list of the compounds present in the pyrolyzate is given Table-1.

Table-1: Pyrolyzate compounds of paper.

Sr. No.	Name of Compounds	Retention time Minutes	Concentration in %
1	3-Furaldehyde	2.48	0.5465
2	3-Hyroxy-4-methylfuran	2.98	17.5787
3	5-Methyl hexanal	3.57	1.2752
4	2-Methanol cyclohexanal	4.3	1.7306
5	3-Heptanone	5.8	1.4573
6	2(5*H*)-Furanone-4-methanol	6.07	12.5289
7	Furan-2-carboxaldehyde-5-methyl	6.45	2.3226
8	Furan-2-ethyl-4-methyl	7.02	0.1821
9	Furan-2-carboxaldehyde-4-methyl	7.55	1.1841
10	1,6-Anhydro-2,3-dideoxy-α-D-hexapyranose	8.17	14.3281
11	Furaldehyde-3-methyl-5-ethenyl	9.02	0.5237
12	3-Ethyl-1-one-2-cycloheptene	9.52	0.7059
13	2-Methyl cycloheptenecarboxaldehyde	9.82	6.21223
14	2-Methyl-4-ethenyl cycloheptanedion	10.28	0.5237
15	2-Ethyl cyclohexanone	10.82	0.5237
16	2,5-Dimethyl-4-hydroxy-(2*H*)-furanone	11.05	2.2238
17	2-Ethenyl-3-hdroxy-4-pyrone	12.15	7.0850
18	6-Hydroxy-5-methyl-2-hexanone	12.97	1.2752
19	2-Hydroxy-1,6-heptadiene	13.35	0.8339
20	9-Hydroxy-9-decenoic acid	15.77	2.3861
21	4-Butyl-2-methanol cyclohexanone	16.18	0.8197
22	Furan-2-carboxaldehyde-4-hydroxy-5-methanol	17.22	0.5465
23	Unknown	28.57	1.8444
24	Hexadecanoic acid	32.78	1.0474
25	Unknown	33.41	1.3297
26	Octadecanoic acid	36.38	4.7077

It can be observed that most of the compounds are derivatives of furan, in addition to furanone, acids, aldehydes and alcohols. Antimicrobial activities of most of the oxygenated compounds of these classes are reported in literature [8-13]. Milan Pour *et al.* [14] reported the antifungal activity of the derivatives of furan. While Knobloch *et. al.* reported the biological membrane rupturing activity of the aldehydic compounds present in the oils [15]. It had been reported by B. Turchetti *et. al.* [8] that organic acids are responsible for the inhibition of SH enzymes of the most of the fungi. However, the ketones may pass through biochemical oxidation and interface the respiration process of fungi. The antifungal activity of our sample may be considered to be a combine effect of all these.

Experimental

The apparatus for the preparation of sample include a Pyrex vessel of 58 mm external diameter and 300 mm length connected with a condenser and receiving flask through a bent adaptor. It is kept in an externally heated furnace powered through a temperature controller equipped with thermocouple.

25 g of fine cuttings of the paper were taken in the glass vessel and kept in preheated furnace for one hour. The temperature of furnace was kept in the range of 200-300 °C. The liquid product distilled away and collected in a receiving flask. It is a brown colored liquid and is distinguishable into an oily film in the aqueous portion.

GC/MS Analysis

General profiles for liquid products of pyrolysis was obtained using EI-MS. Analysis of the liquid product was conducted on a mass spectrometer JMS 600 H Joel and the product was separated into its components using Agilent 890N gas chromatograph equipped with a fused capillary column (HP.5 L.; 30 m i.d.; 0.32 mm, 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.

Conditions for Analysis

Injections mode was split at split rate 35. The column was held at 50 °C for five minutes and then to 240 °C at the rate of 5 °C/min. The injector temperature was 250 °C, while the amount of sample was 1 μl. The identification of major components of the product was confirmed using total Ion Chromatogram Analysis (TIC) as well as fragmentations pattern and library matching, while the quantifications were carried out using the appropriate software of the system.

Antifungal Activity

The antifungal activity of the paper pyrolysate was determined using agar tube dilution method. The activity of the sample was investigated against *T. longifusus*, *C. albicans*, *C. glabarata*, *F. solani*, *A. flavus* and *M. canis*.

Sabourad dextrose agar was used for the growth of fungi. 4 ml of the sabourad dextrose for each fungi was taken in six separate tubes and autoclaved.

24 mg of the paper pyrolyzate was dissolved in 1ml of dimethylsulphoxide where 66.6 μl of this

solution was dispensed to each of the six tubes. The tubes were placed in slanting positions. These were allowed to solidify and left for 24 hours. Each of these tubes was inoculated with 4 mm of fungal stock and incubated at 27 °C for 7 days. These samples were supplemented by the media containing only DMSO as blank and *micanozole* as reference. The reference for *A. flavus* was Amphotericin B. All the tests were carried out in the minimum inhibitory concentration necessary for the complete activity of the pyrolyzate against these strains.

Conclusion

The pyrolysis of paper at lower temperature gives predominantly liquid product. The GC/MS analysis of these products revealed that the product contains furans, furanones, aldehydes, ketones, carboxylic acids and anhydro sugars. These compounds have significant antifungal activities in terms of enzymes inhibition, membrane rupturing as well as respiration interference. The antifungal activity was assessed by its comparison with standard drug. The MIC of the drug was determined for the quantitative investigations.

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