Antifungal Activity of the Pyrolyzate of Glucose, Sucrose and Starch in Comparison to Paper Pyrolyzate

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Summary: Glucose, sucrose, starch and cellulose were pyrolyzed at 300 °C. The antifungal activities of these pyrolyzates were investigated. All of these pyrolyzates were found to be remarkably active against *Trychophyton longifusus*. However, pyrolyzates of paper exhibited significant activity against *Microsporum canis* as well. The GC/MS analysis reveals that all these pyrolyzates contain furans, furanones, aldehydes, ketones, anhydro sugars and carboxylic acids.

Introduction

Pathogenic fungi are a potential threat to the life and property. Fungal attacks [1-6] on life and property are rapidly increasing due to the increased resistance of fungi to the existing antifungal agents and increased number of immunocompromised patients [7, 8]. Despite the availability of potent drugs there is still need to explore and develop new antifungal reagents of higher activity or with minimum adverse effects [9, 10].

Pyrolysis of the cellulosic and lignocellulosic products at low temperature mainly gives liquid products in addition to char and gaseous products. The liquid product contains aldehydes, ketones, carboxylic acids in addition to oxygenated aromatics and their derivatives. Glucose is the simple unit of the oligomeric carbohydrates and polymers like cellulose and starch. The cellulose compounds are different from each other due to different in number of glucose units or by arrangement of these units. The pyrolysis of these compounds may give products of similar nature. Most of these products like aldehydes, acids, alcohols and furans are previously reported to having antimicrobial and antifungal properties [9-18].

Folk knowledge is a big chunk of unsystematic knowledge. It is evolved as a result of the experiments and experiences carried out and obtained in ages. A well known folk and effective medicine for the treatment of scabies and ring worm in many parts of Khyber Paktunkhwa of Pakistan is paper pyrolyzate; which obtained by the burning of paper rolled into a pipe. The paper pyrolyzate is a reddish brown liquid which trickle down at the lower end of the paper pipe burning on the top. In the light folk knowledge we under took the present study and compared antifungal activity of the paper pyrolyzate with that of the basic unit of its major component cellulose i.e. glucose, dimeric sucrose and the polymer starch. The products of pyrolysis were analyzed using GC/MS.

Results and Discussions

Glucose, sucrose, cellulose and paper were pyrolyzed at 300 °C. The liquid products of the pyrolysis of all these materials were collected and characterized. Compositions of pyrolyzates were determined using GC/MS. The identification of compounds was carried out using library matching and the study of fragmentation pattern, however, the concentration of these compounds was determined by peak areas. The composition of the pyrolyzates of each is given in the (Table-1).

The pyrolyzate of sucrose have 40% inhibition for the *Microsporum canis*, while that of paper have 100% inhibition. This is a combine action of the aldehydes, ketones and acids. The activity of pyrolyzate of starch is good against *Fusarium solani*. It may be due to significant number and concentration of ketones as well as sacharides in addition to other antifungal.

It can be observed that almost all the pyrolyzate show significant activity against *Trichophyton longifusus*. It might be due to the

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carboxylic acids which is present in all of these. It can also be attributed to the derivatives of the furans most of which have carboxyl functional group. Beside this the pyrolyzate of the glucose show 40% inhibition against *Aspergillus flavus*. This may be due to the smaller amount of hydroquinone.

Table-1: Pyrolysis products of glucose, sucrose, starch and paper.

No.	Glucose		Sucrose		Starch		Paper	
	Name of compound	Conc. %	Name of compound	Conc. %	Name of compound	Conc. %	Name of compound	Conc. %
1								
	2-Furaldehyde	29.7483	Ethenyl propyl ether	1.8672	Cyclopentane-1-formaldehyde	12.752	3-Furaldehyde	0.5465
	Ethanone-1-(2-furanyl)	1.5910	3-Pentanone-1-ene	7.1046	3-Methyl-2-hexanone	0.5484	Furan-3-hydroxy-4-methyl	17.5787
	Furanone, 2-ethenyl	0.0361	Furan-2-ethyl-4-methyl	9.7006	2-Furaldehyde	0.2167	5-Methyl hexanal	1.2752
	2(5*H*)-Furanone,5-methyl	0.3285	1-(2-furyl)-1-ethanone	1.5029	2-Ethenyl furanone	0.0819	Cyclohexanal-2-methanol	1.7306
	Hydroquinone	2.3678	4-Methyl-cyclopentene-1-one	0.2049	1-Oxo-2-hydroxy cyclopentene	0.5009	3-Heptanone	1.4573
	2-Hydroxy-3-methyl-cyclopentene-1-one	0.4150	3-Ethenyl furanone	1.4573	2-Furaldehyde-5-methyl	5.6018	2-(5*H*)-Furanone-4-methanol	12.5289
	Furan 3-carboxylic acid, methyl ester	0.9560	1-(2-furyl)-1-propanone	10.1105	2(5*H*)-Furanone, 3-methyl-5-propyl	0.4736	Furan-2-carboxaldehyde-5-methyl	2.3226
	2-Ethenyl-3-hydroxy-4-pyrone	0.6968	1,4-Heptadiol	0.4913	1-Oxo-2-hydroxyl-3-ethenyl cyclopentene	0.2459	Furan-2-ethyl-4-methyl	0.1821
	1,4:3,6-Dianhydro-α-D Glucopyranose	0.7286	4-Methyl-3,5-diene octanol	0.1405	4-Hexene-3-one	3.0513	Furan-2-carboxaldehyde-4-methyl	1.1841
	Furan-2-carboxaldehyde-5-methanol	14.1182	Furan-3-carboxylic acid, methyl ester	1.4206	Furan-3-hydroxy-2, 5-dimethyl	3.1971	1,6-Anhydro-2,3-dideoxy-α-D-hexapyranose	14.3281
	5-Methyl-1,2,3,4-cyclopentanetetraol	2.7325	1-(2-Furyl)-1,2-butanediol	0.4098	Maltol	2.1860	Furaldehyde-3-methyl-5-ethenyl	0.5237
	Hexadecanoic acid	3.1821	Unknown	0.2732	4*H*-Pyran-4-one-5-hydroxy	0.4144	2-Cycloheptene-3-ethyl-1-one	0.7059
	Unknown	19.6745	Unknown	2.0129	1,4:3,6-Dianhydro-α-D-glucopyranose	5.4651	2-Methyl carboxaldehyde cycloheptene	6.21223
	Unknown	0.5465	Hexadecanoic acid	2.0949	Furan-2-carboxaldehyde-5-methanol	8.3798	2-Methyl-4-ethenyl cycloheptanedion	0.8880
	Octadecanoic acid	0.8197	Unknown	24.5931	Unknown	0.7651	2-Ethyl cyclohexanone	0.5237
			Octadecanoic acid	0.1821	Unknown	7.9700	2,5-Dimethyl-4-hydroxy-(2*H*)-furanone	2.2238
			Unknown	0.6370	Unknown	1.8217	2-Ethenyl-3-hydroxy-4-pyrone	7.0850
					Hexadecanoic acid	1.3207	6-Hydroxy-5-methyl-2-hexanone	1.2752
					Octadecanoic acid	0.5009	2-Hydroxy-1,6-heptadiene	0.8339
							9-Hydroxy-9-decenoic acid	0.8339
							Furan-2-carboxaldehyde-4-hydroxyl-5-methanol	2.3861
							Unknown	0.8197
							Unknown	0.5465
							Unknown	1.8444
							Hexadecanoic acid	4.7077
							Octadecanoic acid	1.3297
							Unknown	1.0474

Table-2: Antifungal Activity of the pyrolyzate of glucose, sucrose, starch and paper.

No.	Name of the Fungi	Glucose			Sucrose			Starch			Paper		
		Linear growth		Inhibition %	Linear growth		Inhibition %	Linear growth		Inhibition %	Linear growth		Inhibition %
		Sample	Control		Sample	Control		Sample	Control		Sample	Control	
1	*Trichophyton longifusus*	0	100	100	10	100	90	0	100	100	20	100	80
2	*Candida albicans*	100	100	0	100	100	0	100	100	0	100	100	0
3	*Aspergillus flavus*	60	100	40	100	100	0	100	100	0	100	100	0
4	*Microsporum canis*	100	100	0	60	100	40	100	100	0	0	100	100
5	*Fusarium solani*	100	100	0	100	100	0	40	100	60	100	100	0
6	*Candida glabarata*	100	100	0	100	100	0	100	100	0	100	100	0

The pyrolyzates of glucose, sucrose and starch were found to have furans, furanones, anhydro sugars, aldehydes, ketones and carboxylic acids which are known to have antifungal activities. Therefore, all the pyrolyzates were active against *Trichophyton longifusus*. The most effective of these is paper pyrolyzate which show significant activity against both, *Trichosporum longifusus* and *Microsporum canis*.

It was observed that all these pyrolyzates contain furans, furanones, aldehydes, ketones, anhydro sugars and carboxylic acids. However, the liquid of the oxidative cracking of paper is a well known folk medicine for the fungal diseases, scabies and ring worm in various parts of Pakistan. That liquid also contains the above mentioned classes of compounds. The antifungal activity of these pyrolyzates is given in Table-2 in terms of % inhibition.

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. 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 $^{\circ}$C. The liquid product was distilled and collected in a receiving flask.

The same process apparatus was used for the pyrolysis of D-glucose, sucrose and starch. The distillates of all these compounds are brown colored liquids distinguishable into a dense oily film in the aqueous portion.

GC/MS Analysis

General profiles for liquid product of pyrolysis 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, i.d.; 0.32 mm, film thickness 0.25 μm) with polydimethylsiloxane as the stationery phase. The carrier gas was helium at a flow rate of 1.8 ml/min.

Conditions for Analysis

Injection mode was split at split rate 35. The column was held at 50 $^{\circ}$C for five minutes and then to 240 $^{\circ}$C at the rate of 5 $^{\circ}$C/min. The injector temperature was 250 $^{\circ}$C, while the amount of sample was 1 μl. The identification of major components of the product was carried out by using Total Ion Chromatogram Analysis (TIC) as well as by fragmentation pattern and library matching, while the quantification was carried out using the appropriate software of the system.

Antifungal Activity

The antifungal activity of all samples was determined using agar tube dilution method (10-12). The activity of these samples was investigated against *Trychophyton longifusus*, *Candida albicans*, *Candida glabarata*, *Fusarium solani*, *Aspergillus flavus* and *Microsporum canis fungal strains*. Test samples were dissolved in sterile DMSO to serve as stock solution. Sabouraud dextrose agar was prepared by mixing Sabouraud 4 % glucose agar and agar agar in distilled water. It was then stirred with a magnetic stirrer to dissolve it and a known amount is dispensed into screw capped test tubes. Test tubes containing media were autoclaved at 121 $^{\circ}$C for 15 minutes. Tubes were allowed to cool to 50 $^{\circ}$C and the test samples of desired concentrations pipette out from the stock solution into the non-solidified Sabouraud agar media. Tubes were then allowed to solidify in a slanting position at room temperature. Each tube was inoculated with a 4 mm diameter piece of inoculums removed from a seven day old culture of fungi. All culture containing tubes were inoculated at optimum

temperature of 28-30 °C for growth for 7-10 days. Humidity (40% to 50%) was controlled by placing an open pan of water in the incubator. Cultures were examined at least twice weekly during the incubation. After the incubation for 7-10 days, the test tubes with no visible growth of the microorganism were taken to represent the minimum inhibitory concentration (MIC) of the test samples, which were expressed in µg/mL. The media containing only DMSO as blank and micanozole as reference supplement for these samples. The reference for *Aspergillus flavus* was *Amphotericin B*. All the tests were carried out in triplicate.

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