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Chemical composition and antifungal activity of sugarcane bagasse and banana stem based wood vinegar

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Abstract: Wood vinegar or pyroligneous liquid can be produced during the pyrolysis of biomass. Generally, the dark brown colored wood vinegar contains acetic acid, methanol, propanoic acid, phenolic and carbonyl compounds. The major components of biomass are cellulose, hemicellulose and lignin but during the thermochemical decomposition of different biomass produces wood vinegar with a different composition. Sugarcane bagasse and waste banana tree are two important agricultural wastes in Bangladesh. In this investigation, sugarcane bagasse and banana stem were pyrolysed under same pyrolytic conditions. Gas chromatography-mass spectroscopy (GC-MS) analysis was performed to identify the phenolic compounds and other ingredients present in sugarcane bagasse and banana stem based wood vinegar. Antifungal activities of the both samples were investigated. Among the four fungi, *Trichoderma* spp showed resistance against both wood vinegar samples. Significant activities were found against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium* spp.

Keywords: Wood vinegar, Pyrolysis, Sugarcane bagasse, Banana stem, Antifungal activity.

INTRODUCTION

In recent years, there has been an increasing trend towards more efficient and cost-effective utilization of agro-waste like crop waste, food processing waste, hazardous waste etc. Sugarcane bagasse, banana stem are two major agro waste generated in Bangladesh. Bangladesh now produces about 800,000 tons of bagasse per year[1]. Nowadays many research efforts have explored using bagasse as a source of renewable energy and for the production of useful bio-based materials[2-5] but in Bangladesh, the utilization of sugarcane bagasse is limited and is mainly used as a fuel to power the sugar mill.

Paper production is the second-largest revenue stream from bagasse. Banana grows almost everywhere in Bangladesh round the year and it is cultivated on about 1, 38,360 acres of land[1]. In spite of limited fibre extraction, it is seen that huge portion of banana plants are just dumped as waste causing environment hazards and making ecosystem imbalance[6, 7]. Therefore, an effective and economic measure for reducing this environmental problem by the production of many essential food products, fertilizer, bio-chemicals etc. from bagasse and banana waste can be proposed. Pyrolysis of these agro-waste produce char and wood vinegar.

Wood vinegar is also called wood acid. The dark brown, viscous liquid[8, 9] is produced during the process of wood pyrolysis. When wood is burnt in presence of sufficient air, it reduces to ash. But in the case of pyrolysis, thermal degradation of wood or biomass occurred in an oxygen-free environment, or in an environment in which the oxygen content is too low for combustion or gasification to take place[10]. The smoke emitted during the pyrolysis process can be cooled by outside air or flow of cold water while passing through a chimney or flue pipe.

The cooling effect outside the pyrolytic chamber causes condensation of pyroligneous liquor, particularly when the temperature of smoke produced by carbonization ranges between 80 and 180ºC. Nature of the liqor from pyrolytic process depends on its raw material, such as to produce fuel the raw material comes from plastic waste[11].The ingredients of wood vinegar also depend on the temperature of the pyrolysis process. Materials such as coal, animal and human waste, food scraps, agriculture waste, paper, hardwood, cardboard, plastics, rubber, and biomass are suitable for pyrolysis[12].

MATERIALS AND METHODS

Collection of raw materials: The sugarcane bagasse was collected from the Harian Sugar mill, Rajshahi, Bangladesh. The bagasse was sun dried for 10-12 days during the season of summer. The fresh banana stem was collected from the local agricultural field of Rajshahi and cut into small pieces. As the fresh banana stem contains about 96% water[13] this stem were dried for more than 20 days to get a constant weight.

Production of wood vinegar: The dried bagasse was pyrolyzed on heating mantle inside a round bottom flask connected to a condenser to collect the wood vinegar from the vapor. Continuous flow of cold water condenses the smock to liquid. At the initial stage of pyrolysis, a brown color liquid was collected and heating was continued until all bagasse converted into char. Finally, some black tar was formed and heating was stopped. The banana stem was pyrolyzed in same procedure and the two different wood vinegars were kept on two different sealed bottles. The wood vinegar samples were allowed to stand for 60 days to settle down the black tar.

GC-MS-MS analysis: The two wood vinegar samples were analyzed by gas chromatography-mass spectroscopy (GC- MS) using GC-2010 and MS- TQ8040 equipped with RXi-5SIL MS column (30 m x 0.25 mm i.d x 0.25 µm). The oven temperature was programmed from 50 to 300°C and Helium was used as carrier gas. The compounds were identified from the library (NIST/EPA/NIH Mass Spectral Library 2014) matching.

Antifungal activity of wood vinegar: In vitro antifungal activities were examined for both wood vinegar samples by the agar disk diffusion method against *Trichoderma* spp, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium* spp. The disk diffusion test for antifungal activity of wood vinegars was performed by using Potato Dextrose Agar (PDA)[14]. About 20 ml of PDA was melted, sterilized and poured into the assay plate (9 cm in diameter) and allowed to solidify. The inoculums used were prepared using the fungi (*Trichoderma* spp, *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium* spp) from a 48-hour culture grown on Potato Dextrose Agar (PDA). A suspension was prepared for each isolate in a sterile saline solution (0.85%).

The turbidity of the suspension was adjusted with a spectrophotometer at 530nm to obtain a final concentration of a 0.5 McFarland standard[15] ($0.5\text{-}2.5\times10^3$). Sterilized cotton swab was immersed into fungal suspension and then spread on PDA media for even distribution. After that, the sterilized disks made from filter paper were placed onto the inoculated fungi. About 20 µl Nystatin (positive control) and crude wood vinegars were added to each respective disk and incubated at 25˚C for 48 hours. Antifungal potential of wood vinegars and control sample were assessed in terms of zone of inhibition of mycelia growth of fungi after 96 hours. Three replicates were prepared for each of the 4 fungal samples for each wood vinegar and control. Strict aseptic conditions maintained for the antifungal analysis.

RESULT AND DISCUSSION

During the pyrolysis process, the thermal decomposition of bagasse and banana stem produces different organic compounds. From the library matching of the GC-MS spectra 40 and 30 compounds are identified in sugarcane bagasse and banana stem based wood vinegar respectively. The name and retention time of the compounds are presented in **Table 1**.

During the pyrolysis of bagasse a number of phenolic compounds were formed whereas series of aromatic compounds with different side chain were formed from the banana stem. Some isomeric compounds were also formed during the thermal decomposition of both samples. Under the same pyrolytic condition, sugarcane bagasse and banana stem are thermally decomposed in a different way to produce different types of organic compounds. This may be due to the structural differences in the lignin and different mode of thermo-chemical decomposition of these two different plants.

Table 1: Chemical composition of sugarcane bagasse and banana stem based wood vinegar

SL. No.	Bagasse based wood vinegar		Banana stem based wood vinegar	
	Ret. Time	Name of the compound	Ret. Time	Name of the compound
1.	2.575	Methyl-methoxy-hydroxymethyl-amine	3.217	3-Cyclopentene-1-acetaldehyde, 2-oxo-
2.	2.75	Glycerin	3.275	Diisobutyl (oxybis(ethane-2,1-diyl)) dicarbonate
3.	2.8	Benzenemethanol, α-propyl-	3.375	Ethylbenzene
4.	2.892	1-Hydroxy-2-butanone	3.425	o-Xylene
5.	2.983	Cyclopentanone	3.625	2-Cyclopenten-1-one, 2-methyl-
6.	3.175	2,4-Dimethylfuran	3.908	3-Aminopyrazine 1-oxide
7.	3.283	1,2-Dimethyldiaziridine	4.458	2,6-Di-O-methyl-d-galactopyranose
8.	3.342	1,7-Dichloroheptane	4.5	1-Hexanol, 2-ethyl
9.	3.425	Oxime-, methoxy-phenyl-	5.175	2-[2-[2-(2-Butoxyethoxy)ethoxy]ethoxy]ethyl acetate
10.	3.483	Furan, tetrahydro-2,5-dimethoxy-	11.767	Methyl tetradecanoate
11.	3.567	2-Cyclopenten-1-one, 2-methyl-	11.817	Benzene, (1-pentylheptyl)-
12.	3.742	Cyclohexanone, 3-hydroxy-	11.875	Benzene, (1-butyloctyl)-
13.	3.792	2-Cyclopenten-1-one, 2,3-dimethyl-	12.017	Benzene, (1-propylnonyl)-
14.	3.833	3-Aminopyrazine 1-oxide	12.283	Benzene, (1-ethyldecyl)-
15.	3.892	2-Furancarboxaldehyde, 5-methyl-	12.733	Benzene, (1-methylundecyl)-
16.	4.067	Butyramide, 4-benzenesulfonylamino-N-furan-2-ylmethyl-	12.908	Benzene, (1-pentyloctyl)-
17.	4.117	3-Hexen-2-one, 3-methyl-	12.983	Benzene, (1-butylnonyl)-
18.	4.158	Phenol	13.133	Benzene, (1-propyldecyl)-
19.	4.3	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	13.4	Benzene, (1-ethylundecyl)-
20.	4.392	2-Cyclopenten-1-one, 2,3-dimethyl-	13.733	9-Hexadecenoic acid, methyl ester, (Z)-
21.	4.475	o-Cresol	13.858	Hexadecanoic acid, methyl ester

22.	4.55	3,5-Octadien-2-one	13.958	Octadecanoic acid, 3-hydroxy-2-tetradecyl-, methyl ester
23.	4.617	p-Cresol	15.517	9-Octadecenoic acid, methyl ester, (E)-
24.	4.675	Cyclopropane, 1,1-dimethyl-2-(2-methyl-2-propenyl)	15.575	Undec-10-ynoic acid, dodecyl ester
25.	4.908	Phenol, 2,3-dimethyl-	15.65	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
26.	4.958	9-Undecenol, 2,10-dimethyl-	15.733	9-Octadecenoic acid, methyl ester, (E)-
27.	5.033	Phenol, 2-methoxy-	15.967	Heptadecanoic acid, 14-methyl-, methyl ester
28.	5.208	Phenol, 2,5-dimethyl-	17.433	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-
29.	5.333	Phenol, 4-ethyl-	17.6	11-Eicosenoic acid, methyl ester
30.	5.808	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	17.817	Methyl 18-methylnonadecanoate
31.	6.375	Phenol, 4-ethyl-2-methoxy-		
32.	6.492	1,3-Cyclopentanedione, 4-hydroxy-5-(3-methylbutyl)-		
33.	7.092	Phenol, 2,6-dimethoxy-		
34.	7.4	Cyclohexanecarboxylic acid, phenyl ester		
35.	7.567	8-Heptadecene, 9-octyl-		
36.	7.642	Benzaldehyde, 4-(methylthio)-		
37.	7.775	Cyclopentaneacetic acid, ethenyl ester		
38.	8.075	1-Oxetan-2-one, 4,4-diethyl-3-methylene-		
39.	8.558	2-Octenal, 2-butyl-		
40.	13.942	Hexadecanoic acid, methyl ester		

Both wood vinegars showed significant antifungal activities against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium* spp. **(Table. 2)** but they were found inactive against *Trichoderma* spp. In all cases, the antifungal activities of bagasse based wood vinegar were found higher than banana stem based wood vinegar. Generally, the anti-fungal inhibition of wood vinegar strongly depends on its phenolic compounds[16]. From the GC-MS analysis, it was found that the bagasse based wood vinegar contains more phenolic compound than that from banana stem which accounts for the higher antifungal activity of bagasse based wood vinegar.

Table 2: Antifungal activities of sugarcane bagasse and banana stem based wood vinegar

Name of fungi	Antifungal activities (zone of inhition) mm		
	Control	Bagasse based wood vinegar	Banana stem based wood vinegar
Trichoderma spp.	22	R	R
Aspergillus niger	26	14	11
Aspergillus flavus	32	16	10
Fusarium spp.	41	22	13

CONCLUSION

The main target of this work was to produce wood vinegar from two important agricultural wastes and their chemical composition analysis. The comparative study of the antifungal activity has also been done. The higher antifungal activity of bagasse based wood vinegar was found due to the presence of more phenolic compounds. However, the antifungal activity of the wood vinegars from bagasse and banana stem provide evidence in favors of the use of wood vinegar as a fungicide as an alternative plant protection product.

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