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Potency of 5-hydroxymethylfurfuraldehyde (HMF) against *Bacillus cereus* and *Proteus mirabilis*

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ABSTRACT

The dehydration of fructose to 5-hydroxymethylfurfuraldehyde (HMF) in H_2SO_4 catalysed by $CrCl_2$ was carried out at 100 °C and the product extracted with 2-butanol. UV/Vis and FTIR spectrophotometric studies suggested that the extracted compound is HMF. The antibacterial properties of the extracted HMF on *Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis* and Methicillin Resistant *Staphylococcus aureus* (MRSA) showed that the extract was highly potent against *Bacillus cereus* and *Proteus mirabilis*. This activity against these two organisms which are stomach bacteria support the assertion that the antibacterial properties of honey is as a result of its HMF content.

KEYWORDS

Honey;
Fructose;
Dehydration;
5-hydroxymethylfurfural dehyde;
Stomach bacteria.

INTRODUCTION

5-Hydroxymethylfurfuraldehyde (HMF) is an organic compound derived from dehydration of certain sugars. It is a yellow low-melting solid with high solubility. The molecule consists of a furan ring containing both aldehyde and alcohol functional groups[1]. Treatment of fructose with acids leads to the production of HMF. It is isolated from the reaction mixture by liquid-liquid extraction into organic solvents such as methyl isobutyl ketone. The conversion is affected by various additives such as dimethyl sulfoxide (DMSO), 2-butanol, and poly vinyl pyrrolidone, which minimize the formation of side-products[2].

HMF can be found in low amounts in fruit-juices, milk and honey[3]. Honey contains saccharin exudation of plants, which is a thin, easily spoiled sweet liquid that is changed by the honeybee to a stable, high density and high energy food[4]. Honeys vary according to their plant origin and the conditions of their production[5-7]. Honey has been used as medicine in ancient times in many cultures[8]. Its use as a therapeutic substance has been rediscovered by the medical profession in more recent times and is gaining acceptance as an antibacterial agent for the treatment of some diseases[9-15].

HMF is not found in fresh honey, but begins to form in it during conditioning and storage[16]. HMF measurement is used to evaluate the quality of honey since its processing requires heating both to reduce viscosity and to prevent crystallization or fermentation[4, 8]. Formation of HMF in unifloral honey is as a result of this heat treatment[17, 18].

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The antibacterial property of honey has been attributed to its HMF content[5, 12, 19]. These claims have been tested by most workers by using raw honey[10, 11, 13, 14, 20, 21]. In view of the fact that honey contains other substances other than HMF, it is still possible for one or some of these components to be responsible for its antibacterial property. In a bid to further assert the claim that the antibacterial property of honey is as a result of its HMF content, the present study would synthesize and extract HMF and the antibacterial property of this product would be tested using some selected pathogenic bacteria strains.

EXPERIMENTAL

Reagents and sample preparation

D-fructose, chromium (II) chloride ($CrCl_2$) and sulphuric acid (H_2SO_4) used for this study were of analytical grade, purchased from FINLAB Nigeria PLC Lagos, and were used without further purification. The sample and reagents were further prepared with distilled water.

Synthesis and extraction of HMF

5 g of D-glucose was weighed into 100 cm^3 of 1 M H_2SO_4 containing 0.001 M weight of $CrCl_2$ in a glass reactor The system was then placed in a constant temperature oven set at 100 ^oC and was left for 2 hours. It was then taken from the oven, and allowed to equilibrate with room temperature. It was poured into a separatory funnel together with 50 cm^3 of 2-butanol. The mixture was swirled for few seconds and then allowed to settle. The aqueous phase was tapped off and the solvent phase remaining was transferred into a rotary evaporator. After evaporation of the solvent the brownish yellow solid left on the dish was collected.

JENWAY 6405 UV/Vis Spectrophotometer with computer interface was used to obtain the wavelength of absorption of the extracted compound. Perkin Elmer Spectrum BX FTIR Spectrophotometer was then used to analyse the sample for functional group properties.

Collection of bacteria cultures

Pure clinical isolates of *Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis* and Methicillin Resistant *Staphylococcus aureus* (MRSA) were collected from the Department of Microbiology and Parasitology, Obafemi Awolowo University Teaching Hospital (OAUTH) Ile Ife, Nigeria. A microbial loop was used to remove a colony of each bacterium from the pure culture and transferred into liquid broth (Nutrient broth) and incubated for 24 hours at 37±1^oC. These were maintained in sterile condition.

Antibacterial activity of HMF on bacteria isolates

Antibacterial activity of the synthesized HMF was assayed using Agar diffusion technique as described by Cheesbrough[22]. The inocula were prepared from the stock cultures which were maintained on nutrient Agar slant at 4 ^oC and sub cultured into peptone water using wire loop. The density of the bacteria suspension was determined by comparism with 0.5 McFarland standard using colorimeter at 540 nm, to obtain approximately 1.0×10^6 CFU/ml[23]. 0.1 ml of inocula were introduced into the Muller Hinton agar and were spread evenly with spray glass rod. Holes were bored on the agar using a cork borer and concentrations of 2, 4, 8 and 16 mg/ml of HMF solutions. The inoculated plates were allowed to stand for an hour for proper diffusion of the HMF, and they were then incubated at 37 ^oC for 24 hours and examined for zone of inhibition. The zones of inhibition were measured in millimetres at 90^o to each other and the mean of three readings were calculated. Solvents used for extraction and standard antibiotic Ofloxacin (5 µg/ml) were used as control.

RESULTS AND DISCUSSION

The brownish yellow solid obtained from the 2-butanol solvent after evaporation was subjected to spectrophotometric studies. In the UV/Vis spectrum, HMF absorbs light between the wavelengths of 284 – 286 nm. The sharp peak obtained at 285 nm (Figure 2) is an indication that the product can be HMF.

The FTIR spectrum of the product gave a strong and broad band at 3396 cm^{-1} indicating an alcohol functional group, a stretch at 1643 cm^{-1} shows the presence of an aromatic ring, a strong band at 1071 cm^{-1} indicates the presence of an ether linkage while a medium band at 2877 cm^{-1} is characteristic of an aldehyde functional group. These absorption bands in the spectrum suggest that the extracted compound is HMF.

The susceptibility of the tested organisms to some standard antibiotics was studied and the result is shown in TABLE 1.

All the organisms tested were sensitive to the anti-

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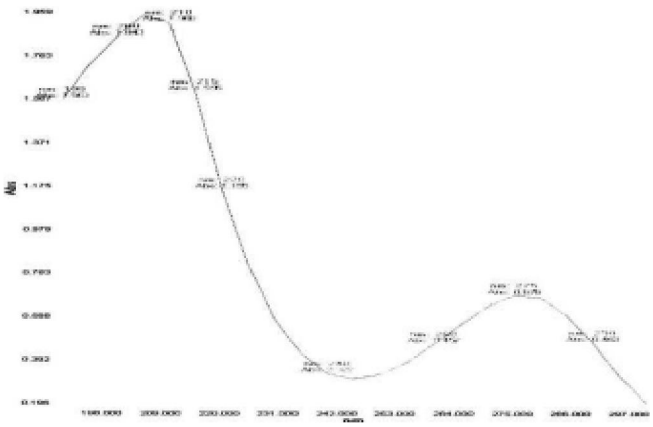

Figure 1 : UV scan of synthesized HMF

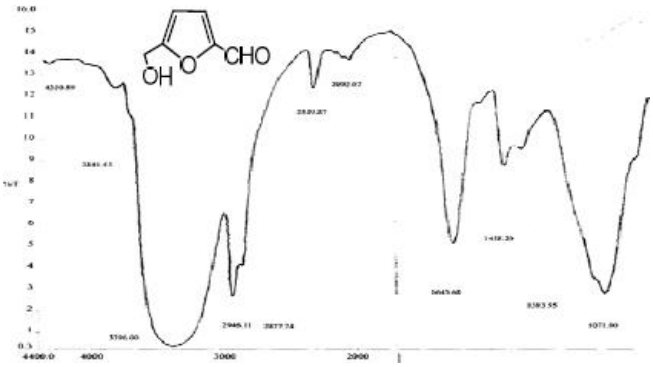

Figure 2 : FTIR spectrum of synthesized HMF

biotics Ofloxacin. The antibacterial activity of HMF was then matched against this compound and the results are shown in TABLE 2.

HMF concentrations of 8 mg/ml and below have no activity against the bacteria species studied. Good sensitivity to HMF was shown by *Bacillus cereus, Escherichia coli* and *Proteus mirabilis* at 16 mg/ml. This concentration however had no effect on *Klebsiella pneumonia, MRSA* and *Pseudomonas aeruginosa.* These organisms are therefore either not sensitive to HMF or require higher HMF concentration to achieve sensitivity. These data are in agreement with the results obtained by Nafea et al[21] and Badawy et al[24]. Nafea et al, while studying the antibacterial activity of bee honey showed that the honey samples at different HMF concentrations gave high bactericidal activities against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. They also reported that no activity was shown by the honey samples against *Pseudomonas aeruginosa*. Badawy and coworkers reported good antibacterial activity of honey on *Escherichia coli*.

The percentage sensitivities of the bacteria species to HMF were obtained using the formula:

TABLE 1 : Screening of bacteria for their resistance to some standard antibiotics

Organisms	Concentration (µg/ml)									
	CIP 5	TE 50	GN 10	AX 25	OF 5	AM 25	N 100	NB 10	CF 30	C 10
Bacillus cereus	+	+	+	+	+	+	+	+	+	+
Escherichia coli	+	+	+	+	+	+	+	-	+	+
Klebsiella pneumoniae	+	-	-	-	+	-	-	-	-	-
MRSA	-	+	-	-	++	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	+	-	-	-	-	-
Proteus mirabilis	+	+	+	+	+	+	+	-	+	+

Key: + = Sensitive; - = Resistant
CIP – Ciprofloxacin, TE – Tetracycline, GN – Gentamycin, AX – Amoxycillin, OF – Ofloxacin, AM – Ampicillin, N – Nitrofurantion, NB – Norbactin, CF – Cefuroxime, C – Chloramphenicol.
MRSA – Methicillin Resistant *Staphylococcus aureus*

TABLE 2 : Antibacterial activity of HMF on the selected pathogenic bacteria strains

Compound	Concentration (mg/ml)	Diameter of inhibition zone (mm)					
		Bacillus cereus	E coli	Klebsiella pneumoniae	MRSA	Pseudomonas aeruginosa	Proteus mirabilis
HMF	16	19	10	-	-	-	11
	8	-	-	-	-	-	-
	4	-	-	-	-	-	-
	2	-	-	-	-	-	-
Ofloxacin	0.005	15	25	28	28	11	-

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TABLE 3 : Percentage sensitivity of bacteria to HMF concentrations

HMF Concentration	% Sensitivity of HMF on Bacteria					
	Bacillus cereus	E coli	Klebsiella pneumoniae	MRSA	Pseudomonas aeruginosa	Proteus mirabilis
16	126.7	40	0	0	0	***
8	0	0	0	0	0	0
4	0	0	0	0	0	0
2	0	0	0	0	0	0

Key - ≤ 40 % = least sensitive, 50 – 100 % = sensitive, > 100 % = very sensitive, * = infinitely sensitive**

$$\% \text{ Sensitivity} = \frac{\text{Zone of Inhibition of HMF}}{\text{Zone of Inhibition of Antibiotic}} \times 100$$

The values obtained gave the potency of the test sample on the organisms, and the results are shown in TABLE 3.

The sensitivity of *E coli* an intestinal bacterium[25], to HMF has been mentioned by some researchers and collaborated in this work. HMF at 16 mg/ml was highly potent against *Bacillus cereus* and *Proteus mirabilis*. Both organisms are intestinal bacteria responsible for diarrhoea and urinary tract infections respectively in man[26, 27]. This shows the potency of this compound against intestinal related bacterial infections.

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