
Evaluation of the Antimicrobial, Antioxidant, and Anti-Inflammatory Activities of the Methanol Extract of *Raphia hookeri* Seed

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ABSTRACT

This research investigated the antimicrobial, antioxidant, and anti-inflammatory properties, as well as the chemical composition, of the methanol extract of *Raphia hookeri* seeds, analyzed through GC-MS. The GC-MS analysis identified 26 compounds, with squalene as the most abundant, contributing 33.7% of the total area. Other significant compounds include dibutyl phthalate, tetradecyloxirane, and phytol. The antimicrobial activity of the extract was evaluated using the agar well diffusion procedure against several clinical pathogens with zones of inhibition between 18 – 24 mm against *Pseudomonas aeruginosa*, Vancomycin-resistant *enterococci*, *Staphylococcus aureus*, *Salmonella typhi*, *Candida krusei*, and *Candida tropicalis*. The minimum inhibitory concentrations (MIC) ranged from 2.5 µg/mL to 5 µg/mL. The anti-inflammatory potential of the extract was tested using membrane stabilization assays, showing 59.4% inhibition at 500 µg/mL, compared to diclofenac's 89.13% inhibition. The antioxidant activity was evaluated using the DPPH free radical scavenging assay, where the extract exhibited strong antioxidant potential of 82 % scavenging. Phytochemical screening revealed the presence of bioactive compounds such as alkaloids, flavonoids, saponins, tannins, phenols, and steroids, known for their therapeutic properties. These findings suggest that *Raphia hookeri* seed extract possesses significant antimicrobial, antioxidant, and anti-inflammatory potential, making it a promising candidate for pharmacological applications.

Keywords: *Raphia hookeri*, anti-inflammatory, antioxidant, antimicrobial

INTRODUCTION

Medicinal plants have long been recognized for their therapeutic potential in the treatment and prevention of diseases such as malaria, cancer, and infections. The growing resistance to synthetic drugs and the emergence of new diseases have renewed interest in natural products as alternative sources of bioactive compounds [1-2].

Raphia hookeri, commonly known as Raffia palm, is a vital species of the Arecaceae family (palms), native to regions in Africa, particularly Nigeria and Cameroon. The plant parts have been used for various non-medicinal purposes such as weaving of mats, baskets, shelter and poultry cages from the branches, brooms from its leaves, and palm wine from its sap [3-4]. Oil from its seeds have been used locally for cooking, lubrication, soap making and as cosmetics. Ethnomedical uses include its use as a remedy for diabetes, production of local gin used as solvent for root herbs and as wine [3, 5]

Phytochemicals of pharmacological importance such as terpenoids, sterol, cardiac glycosides and phenolics have been identified in the root, stem, leave and fruit extracts [6-9]. Extracts of *Raphia hookeri* have been reported to show antidiabetic [3, 10], antioxidant [11-14], anti-inflammatory [15], antimalarial [16] and anticancer [17]. This research intends to provide an alternative drug that may be used against resistant pathogens.

This study aims to investigate the phytochemical composition, antioxidant, antimicrobial, and anti-inflammatory properties of the methanol extract of *Raphia hookeri* seed using standard screening methods and GC-MS analysis.

MATERIALS AND METHODS

Collection of *Raphia hookeri* seed

Fresh seeds of *Raphia hookeri* were collected from a mature plant in their natural habitat in Gbaramatu (Ijaw) village, Warri, Delta State, Nigeria.

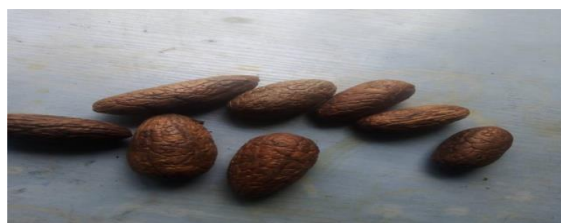


Plate 1: *Raphia hookeri* seeds

The seed was identified and authenticated by the Delta State University Herbarium with a voucher number DELSUH-404

Sample Preparation

The *Raphia* seed was cleaned, dried, and crushed into a fine powder using a milling machine.



Plate 2: Crushed *Raphia hookeri* Seeds

Extraction Process

The crushed seeds were then subjected to extraction using methanol as a solvent in a Soxhlet apparatus. The extract was concentrated under reduced pressure using a Cole-Palmer RE400DB rotary evaporator to obtain the crude.

Phytochemical Screening of Methanol Extract of *Raphia hookeri* Seed

The phytochemical screening followed the method described by Akpeji *et al.* [18], with slight modifications.

Test for alkaloids: To about 0.5 g of the aqueous extract *Raphia hookeri*, two drops of Mayer's reagent were added along the sides of test tube. Appearance of white creamy precipitate was observed which indicated the presence of alkaloids.

Test for reducing sugar: To about 0.5 g of the aqueous extract in the test tube, tollen's reagent was added and heated moderately. The presence of silver mirror on the walls of the test tube indicates the presence of reducing sugars.

Test for steroids: About 0.5 g of the extracts was taken in a test tube and dissolved with chloroform (10 mL), three drops of concentrated sulphuric acid was added by the side of the test tube. Two layers appeared, the upper layer showed red and sulphuric acid layer showed yellow with green fluorescence. This confirms the presence of steroids

Test for terpenoids: About 0.5 g of extract was mixed in 2 mL of chloroform and 3 mL of concentrated H₂SO₄ was carefully added to form a layer. No presence of terpenoi detected.

Test for saponin: To about 0.5 g of the aqueous extract in the test tube was added 5 mL of distilled water. It was shaken vigorously. Persistent foam for about 2 minutes confirmed the presence of saponin.

Test for anthraquinones: About 0.5 g of the aqueous extract was boiled with 1 mL concentrated HNO₃ and shaken well. A dark orange colour indicates the presence of anthraquinones on addition of the concentrated acid. Meanwhile, in this work, result obtained showed negative.

Test for phenolic compounds: To 0.5 g of the aqueous extract, Phytochemical Screening of Methanol Extract of Aqueous *Raphia hookeri* Seed few drops of 10% ferric chloride solution was added. Green blue colour appears which indicated the presence of phenolic compounds.

Test for tannins: About 0.5 g of the aqueous extract, 5% ferric chloride solution was added, a dark green precipitate was observed which showed the presence of tannins in the plant.

Test for flavonoids: To 0.5 g of aqueous extract was added little quantity of water. A few drop of lead acetate solution was added and a light yellow precipitate was observed which indicated the presence of flavonoid.

Test for glycosides: About 0.5 g of the aqueous extract in a test tube, 2ml of dilute hydrochloric acid (HCl) was added and the mixture were heat gently for a few minutes after heating, a few drops of sodium nitroprusside solution were added and mix well, there was no reddish or pink colour, which indicates no glycosides.

Collection and Identification of Microbial Strains

Pure clinical grade microbial isolates of Methicillin-resistant *Staphylococcus aureus*, Vancomycin resistant *Enterococci*, *Staphylococcus aureus*, *Helicobacter pylori*, *Pseudomonas auruginosa*, *Samonella typhi*, *Escherichia coli*, *Candida tropicalis* and *Candida krusei* were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. These organisms were resuscitated using the appropriate media. Nutrient Agar (NA) was used for Methicillin resistant *Staphylococcus aureus*, *Staphylococcus aureus*, *Samonella typhi*, *Escherichia coli*, Enterococci Selective Agar (ESA) was used for Vancomycin resistant *Enterococci*, Centrimide Agar (CA) was used for *Pseudomonas auruginosa*, Columbia Agar (CA) was used for *Helicobacter pylor* while Potato Dextrose Agar (PDA) was used for *Candida albicans*, *Candida tropicalis* and *Candida krusei*. They were all re-identified using the standard methods

[19]. They were then sub-cultured on nutrient agar slants and stored at 40 °C until required for further study.

Antimicrobial Screening Procedure

All equipment used in this study were sterilized by autoclaving for 20 minutes before use. The Agar well diffusion method was employed in evaluating the antimicrobial toxicity level of the methanolic extract of *Raphia hookeri* seeds [20].

The extract (0.1 g) was dissolved in 10 ml of DMSO to achieve a concentration of 10mg/ml. Mueller Hilton Agar 20 ml (38 g/L) was measure into spice bottles and autoclave before pouring into sterile petri dishes containing 2 ml of the microorganisms (1×10^8 cfu) and swirled gently to homogenize. All petri dishes were incubated at 37 °C for 24 hours to allow microbial growth. A hole was made using a cork borer (d = 6 mm) on each petri dish and 0.2 ml of each test agent was administered into the holes and properly labeled. The petri dishes were incubated at 37 °C for 24 hours to allow for possible inhibition. The diameter of inhibition was measured in triplicated and the mean values reported as zone of inhibition.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was determined using the broth dilution method. Mueller Hinton broth was prepared, sterilized at 121 °C for 15 minutes, and allowed to cool. A 0.5 McFarland standard was prepared to obtain a turbid microbial solution. Normal saline was prepared, and 10 mL was dispensed into sterile test tubes. The test microbe was inoculated into the saline and incubated at 37 °C for 6 hours. The turbidity of the microbial suspension was adjusted to match the McFarland standard by visual comparison, indicating a concentration of approximately 1.5×10^8 CFU/mL. Two-fold serial dilutions of the extract were prepared in sterile broth to obtain concentrations of 10, 5, 2.5, 1.25, 0.63, and 0.31 mg/mL. The initial dilution was made by dissolving 0.1 g of the extract in 10 mL of sterile broth. Into each concentration tube, 0.1 mL of the adjusted microbial suspension was inoculated. The tubes were incubated at 37 °C for 24 hours and then examined for turbidity. The lowest concentration without visible turbidity was recorded as the MIC.

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

To determine whether the microbes were killed or only inhibited, the contents of the MIC tubes showing no turbidity were sub-cultured onto Mueller Hinton agar. The agar was prepared, sterilized at 121 °C for 15 minutes, poured into sterile petri dishes, and allowed to solidify. The subcultures were incubated at 37 °C for 24 hours. The minimum bactericidal or fungicidal concentration (MBC/MFC) was identified as the lowest extract concentration that showed no colony growth on the agar plates.

Anti-Inflammatory Analysis

Anti-inflammatory screening focuses on the extract's ability to reduce inflammatory mediators such as nitric oxide and prostaglandins, using both in vitro and in vivo models [21]. The anti-inflammatory activity was evaluated using the membrane stabilization method. Fresh whole mammalian blood (10 mL) was collected into heparinized centrifuge tubes and centrifuged at 3000 rpm for 10 minutes. The cells were washed three times with equal volumes of normal saline. The washed red blood cells were reconstituted into a 10% v/v suspension with normal saline and used for the membrane stabilization assay.

Antioxidant Activity (DPPH Radical Scavenging Assay)

The antioxidant activity of the extract was evaluated using the DPPH radical scavenging method as described by Khatua *et al.*, [22], with slight modification. A solution of 0.004 g DPPH was prepared in 100 mL of methanol. Various concentrations of the extract ranging from 10 to 150 µg/mL were prepared. In a 96-well microplate, 50 µL of each extract concentration was mixed with 150 µL of the DPPH solution. The plates were incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance of each sample was measured at 595 nm. Ascorbic acid was used as a standard antioxidant at similar concentrations (10–150 µg/mL), and methanol served as the negative control. All tests were performed in triplicates. The degree of scavenging was calculated by the following equation:

$$\text{Scavenging effect (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

Table 1 shows the result of the phytochemical screening of methanol extract of the seeds of *Raphia hookeri*.

Table 1: Phytochemical Screening of Methanol Extract of *Raphia hookeri* Seed

Test Performed	Results
Appearance	Liquid
Colour	Dark brown
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Phenols	+
Glycosides	-
Steroids	+
Terpenoids	-
Anthraquinones	-
Reducing Sugars	+

Keys; (+) detected, (-) Not detected

The phytochemical screening of the methanol extract of *Raphia hookeri* seeds revealed the presence of various bioactive compounds as shown in Table 1. Interestingly, glycosides, terpenoids, and anthraquinones were not detected in the extract. This may be due to their presence in negligible quantities or their complete absence owing to their solubility in methanol. Polarity of a solvent and extraction methods have been reported as factors that influence phytochemical compositions [23].

The detection of alkaloids indicates potential therapeutic value for applications such as analgesics, antimicrobials, and anticancer agents [24]. Likewise, the presence of flavonoids and tannins supports antioxidant potential, beneficial for managing oxidative stress-related disorders such as cardiovascular and neurodegenerative diseases [25]. Saponins, recognized for their

immunomodulatory and anti-inflammatory effects, further enhance the extract's medicinal significance [26].

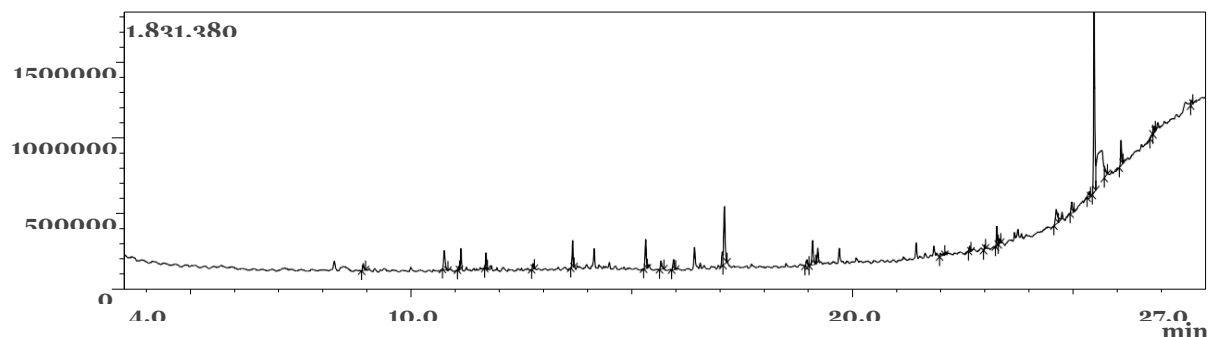


Figure 1: GCMS Spectrum of *Raphia hookeri* Seed Oil

GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified 26 distinct compounds, confirming the complex chemical nature of *R. hookeri* seed. Some secondary metabolites present include: Squalene (33.70%) a triterpene with applications in cosmetics, pharmaceuticals, and as a potent antioxidant [27, 28], Dibutyl phthalate (12.68%) a possible antimicrobial compound [29], Phytol (5.53%) a chlorophyll derivative with known antioxidant and anti-inflammatory properties [30], among others.

The variety of identified chemical classes — hydrocarbons, fatty acids, terpenes, siloxanes, and phthalates reflects its biological origin.

Table 2: Compounds Present in Methanol Extract of *Raphia hookeri* Seeds

Peak#	R.Time	I.Time	F.Time	Peak Report TIC				A/H	Name
				Area	Area%	Height	Height%		
1	8.916	8.88	8.97	100984	1.48	45612	1.36	2.21	Dodecane, 4,6-dimethyl-
2	8.75	10.71	10.835	3.907	4.53	124591	3.73	2.48	2-Propenamide, N,N-diethyl-2-
3	11.078	11.05	11.095	38907	0.57	24931	0.75	1.56	Heptadecane, 8-methyl-
4	11.699	11.665	11.73	151013	2.22	105265	3.15	1.43	Heptadecane
5	12.762	12.73	12.79	57674	0.85	35260	1.05	1.64	Cyclooctasiloxane, hexadecane
6	13.657	13.615	13.69	285458	4.19	178448	5.34	1.60	Heneicosane
7	15.312	15.265	15.35	372837	5.47	192077	5.74	1.94	Oxirane, tetradecyl-
8	15.661	15.625	15.725	127861	1.88	54411	1.63	2.35	1-Octadecyne
9	15.946	15.90	15.99	148929	2.18	67069	2.01	2.22	Oxirane, tetradecyl-
10	17.093	17.06	17.155	864227	12.68	381309	11.40	2.72	Dibutyl phthalate
11	18.959	18.92	19.01	102339	1.50	4.938	1.22	2.50	11,14,17-Eicosatrienoic acid, me
12	19.091	19.01	19.165	377009	5.53	159917	4.78	2.36	Phytol
13	21.985	21.965	22.085	57617	0.85	20039	0.60	2.88	Hexanedioic acid, bis(2-ethylhe
14	22.646	22.62	22.675	53353	0.78	34394	1.03	1.55	Cyclononasiloxane, octadecane
15	22.991	22.96	23.01	40109	0.59	27144	0.81	1.48	Pentacosane
16	23.266	23.23	23.295	212249	3.10	127608	3.82	1.66	Bis(2-ethylhexyl) phthalate
17	23.322	23.295	23.35	57240	0.84	33742	1.01	1.70	Tetrapentacontane
18	24.611	24.555	24.66	305677	4.48	96524	2.89	3.17	Hentriacontane
19	24.956	24.92	25.01	160698	2.36	72777	2.18	2.21	1,3-Benzenedicarboxylic acid, b
20	25.355	25.305	25.385	90002	1.32	34786	1.04	2.59	Tetracontane
21	25.467	25.425	25.505	2297097	33.71	1191372	35.63	1.93	Squalene
22	25.725	25.695	25.765	146313	2.15	44371	1.33	3.30	Docosanoic acid
23	26.076	26.035	26.12	295367	4.33	166851	4.99	1.77	Dotriacontane
24	26.75	26.73	26.795	27374	0.40	12815	0.38	2.14	Squalene
25	26.827	26.795	26.85	78432	1.15	37911	1.13	2.07	1-Bromoeicosane
26	27.666	27.65	27.70	59031	0.87	33236	0.99	1.78	Tetrapentacontane
				6816867	100	3343398	100		

Library

Antimicrobial Activities

Table 3 shows the zones of inhibition of the methanol extract of *R. hookeri* seeds and two existing antibiotics purchased from a pharmacy.

Table 3: Antimicrobial Activities of Methanol Extract of *Raphia hookeri*

Test Organisms	Extract	Ciprofloxacin	Sparfloxacin	Fulcin
Methicillin Resist Staph aureus	0	0	34	-
Vancomycin Resist enterococci	23	32	0	-
Staphylococcus aureus	24	0	32	-
Helicobacter pylori	0	30	0	-

<i>Pseudomonas aeruginosa</i>	18	0	31	-
<i>Salmonella typhi</i>	21	40	0	-
<i>Escherichia coli</i>	0	37	0	-
<i>Candida albicans</i>	0	-	-	34
<i>Candida krusei</i>	21	-	-	30
<i>Candida tropicalis</i>	18	-	-	32

The extract exhibited antimicrobial activities against Vancomycin resistant *Enterococci*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*, which were either not sensitive to Ciprofloxacin or Sparfloxacin. It was however, not effective against MRSA, H pylori and E coli. The extract also showed substantial toxicity to *Candida krusei*, and *Candida tropicalis* but was not toxic against *Candida albicans*.

Table 4 and Table 5 show the minimum inhibitory concentrations and the minimum bactericidal concentrations of the extract respectively.

Table 4: Minimum Inhibitory Concentration (mg/ml)

Microorganism	10	5	2.5	1.3	0.6	0.3
Vancomycin Resist enterococci	-	-	0*	+	++	+++
<i>Staphylococcus aureus</i>	-	-	0*	+	++	+++
<i>Pseudomonas aeruginosa</i>	-	0*	+	++	+++	+++
<i>Salmonella typhi</i>	-	-	0*	+	++	+++
<i>Candida krusei</i>	-	-	0*	+	++	+++
<i>Candida tropicalis</i>	-	-	0*	+	++	+++

KEY: No growth (-), MIC (0*), light growth (+), Moderate growth (++), High growth (+++)

Table 5: Minimum Bactericidal/Fungicidal Concentrations (mg/ml)

Microorganism	15	10	5	2.5	1.3
Vancomycin Resist enterococci	-	-	0*	+	+++
<i>Staphylococcus aureus</i>	-	-	0*	+	++

<i>Pseudomonas aeruginosa</i>	0*	+	++	+++	+++
<i>Salmonella typhi</i>	-	0*	+	++	+++
<i>Candida krusei</i>	-	0*	+	++	+++
<i>Candida tropicalis</i>	-	0*	+	++	+++

KEY: No colony (-), MBC/MFC (0*), Scanty colony (+), Heavy colony (++)

The minimum inhibitory concentration (MIC) ranged from 2.5 mg/mL to 5 mg/mL, while the minimum bactericidal/fungicidal concentrations (MBC/MFC) were between 5 and 15 mg/ml. These results suggest that *R. hookeri* is more effective against Vancomycin resistant *enterococci* and *staphylococcus aureus* but less effective against *Pseudomonas aeruginosa*.

Anti-inflammatory Activity

Figure 2 shows a comparison of the anti-inflammatory potencies of diclofenac as a standard drug to that of the methanol extract of *R. hookeri* at 500 µg/mL.

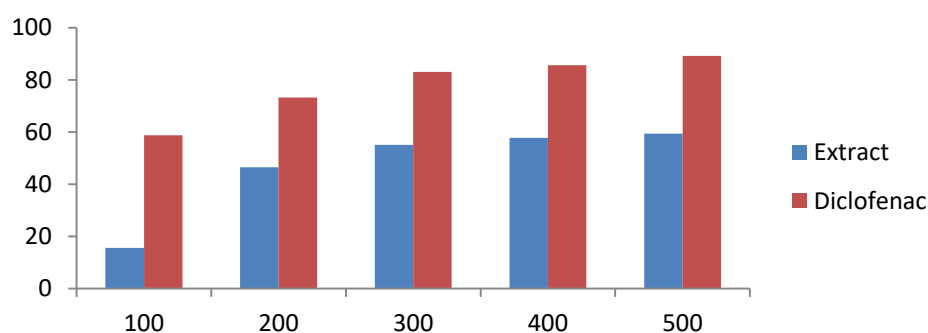


Figure 2: Anti-inflammatory Activity of *Raphia hookeri* Extract (µg/ml)

The extract inhibited inflammation by 59.40% while diclofenac inhibited inflammation by 89.13% under similar conditions. Although less potent, this level of inhibition is still significant and suggests promising anti-inflammatory potential. The observed effect is likely due to the presence of flavonoids, phenols and tannins.

Antioxidant Activities

Figure 3 displays the antioxidant potentials of the seed extract.

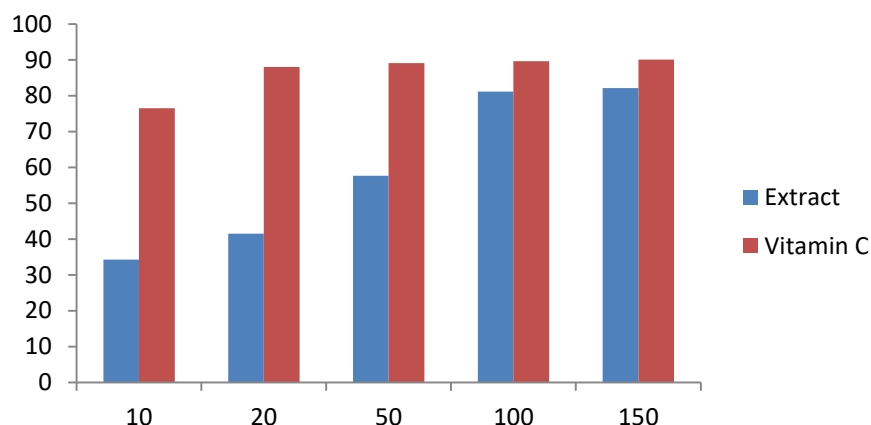


Figure 3: DPPH Scavenging Activity of *Raphia hookeri* Extract (µg/ml)

The antioxidant levels are lower than those of the standard – Vitamin C at all concentrations but the values show that the extract has a good potential as an antioxidant. The antioxidant potentials can be attributed to the secondary metabolites such as phenolics, tannins and flavonoids that are present in the extract.

CONCLUSION

Raphia hookeri seed contains phytochemicals with significant antioxidant, anti-inflammatory, and antimicrobial activities. The plant possesses several phytochemicals that can be isolated for specific bioactivity. This study provides information on biological importance of *Raphia hookeri* and data for further research.

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