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GC-MS PROFILING, ANTIMICROBIAL, ANTIOXIDANT AND ANTI-INFLAMMATORY ANALYSIS OF THE N-HEXANE EXTRACT OF THE SEEDS *OF RAPHIA HOOKERI* OBTAINED IN DELTA STATE

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Abstract

Raphia hookeri is widely employed in African traditional medicine for diverse therapeutic purposes; however, its bioactive potential remains inadequately investigated. This study examined the chemical composition of the n-hexane seed extract of *Raphia hookeri* and assessed its antioxidant, antimicrobial, and anti-inflammatory activities. Preliminary phytochemical analysis revealed the presence of terpenoids, phenolic compounds, sterols, and cardiac glycosides. Antioxidant evaluation using the DPPH radical scavenging assay demonstrated notable antioxidant activity, indicating the strong bioactive potential of the seed oil. The antimicrobial activity of the extract was tested against *Staphylococcus aureus*, *Helicobacter pylori*, *Escherichia coli*, *Candida albicans*, and *Candida krusei*. The extract exhibited pronounced antimicrobial efficacy against all tested organisms, with zones of inhibition ranging from 18 to 25 mm. Anti-inflammatory assessment showed a maximum inhibition of 60.15% at a concentration of 500 µg/mL, compared to 89.13% inhibition obtained for diclofenac used as the reference drug. The minimum inhibitory concentration (MIC) for the extract was determined to be 2.5 mg/mL. These findings provide scientific support for the therapeutic relevance of *Raphia hookeri* seed oil and highlight its potential application in pharmaceutical, nutraceutical, and industrial formulations aimed at developing sustainable and affordable health solutions.

Keywords: *Raphia hookeri*, antioxidant, antimicrobial, anti-inflammatory, GC-MS.

Introduction

Raphia hookeri, popularly referred to as the raffia palm, is an economically and culturally important member of the family Arecaceae, widely distributed across tropical Africa, with notable abundance in Nigeria and Cameroon. Various parts of the plant are traditionally exploited for domestic and commercial purposes. The branches are commonly used for constructing shelters, cages, mats, and baskets, while the leaves serve in the production of brooms. In addition, the sap of *R. hookeri* is tapped for palm wine, a widely consumed local beverage (Mbaka et al., 2012; Ajao et al., 2021). The seed oil is also of considerable local value, being utilized for culinary purposes, lubrication, soap production, and cosmetic formulations. Beyond its material applications, *R. hookeri* holds an important place in traditional medicine, where it is employed in the management of diabetes, as a source of local gin used both as a beverage and as a solvent for herbal preparations, and in various ethnotherapeutic practices (Umerie, 2000; Mbaka et al., 2012; Mpinga et al., 2013). Natural products derived from plants have historically played, and continue to play, a central role in the discovery and development of therapeutic agents (Ogwuche et al., 2025). Phytochemical investigations of *R. hookeri* have revealed the presence of several bioactive secondary metabolites of pharmacological relevance, including terpenoids, sterols, cardiac glycosides, and phenolic compounds. These constituents have been reported in extracts obtained from different parts of the plant such as the roots, stem, leaves, and fruits (Obahiagbon and Osagie, 2007; Ibegbulem et al., 2013; Dada et al., 2017; De

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Oliveira et al., 2021). Pharmacological studies have further demonstrated that extracts of *R. hookeri* possess diverse biological activities. These include antidiabetic effects (Mbaka et al., 2012; Erukainure et al., 2019a), antioxidant activity (Adesegun, 2014; Dada et al., 2017; Oluyori et al., 2018; Erukainure et al., 2019b; Abogo Mebale et al., 2022), anti-inflammatory properties (Erhabor et al., 2019), antimalarial activity (Oluyori et al., 2022), and anticancer potential (Chen et al., 2015). Collectively, these findings highlight the therapeutic relevance of the plant and support its continued scientific evaluation.

The present study is designed to investigate the chemical profile of the n-hexane extract of *Raphia hookeri* seed obtained around Delta state. and to assess its antimicrobial, antioxidant, and anti-inflammatory activities. By providing a detailed evaluation of these properties, the study seeks to scientifically substantiate the traditional uses of *R. hookeri* and to assess its potential applicability in modern pharmaceutical, therapeutic, and industrial contexts. This research is particularly justified in light of the increasing demand for natural bioactive agents, driven by the global rise in antimicrobial resistance and the growing burden of chronic diseases associated with oxidative stress and inflammation.

Materials and Methods

All the microbes used in this study were of clinical grade and were properly re-identified.

Collection and Preparation of *Raphia hookeri* Seeds

Seeds of *Raphia Hookeri* were collected from a mature plant in Kokori Delta State, Southern Nigeria and the seed was identified and authenticated by the Delta State University Herbarium with a voucher number DELSUH-404. The seeds were washed with clean water, dried, and pulverized with the aid of a grinding machine.

Extraction of Seed Oil

The air-dried seeds were finely ground to obtain a uniform powder. The powdered sample was extracted with *n*-hexane using a Soxhlet extraction apparatus, allowing continuous solvent circulation to ensure efficient recovery of the lipid fraction. Upon completion of the extraction, the solvent–oil mixture was concentrated under reduced pressure using a rotary evaporator to remove the solvent, yielding the crude seed oil. The extracted oil was then stored in an airtight container at low temperature until further analysis. This extraction method is widely adopted for oil recovery from plant matrices due to the high affinity of *n*-hexane for non-polar lipid components and the efficiency of Soxhlet extraction in exhaustive solvent extraction (Harborne, 1998; AOAC, 2016).

Characterization of Compound

The chemical constituents of the seed extract were characterized using Gas Chromatography–Mass Spectrometry (GC–MS). The analysis was performed on an Agilent Technologies GC system (model 5977B) coupled with a single quadrupole mass selective detector. High-purity helium was employed as the carrier gas, maintained at a constant flow rate of 1.5 mL/min to ensure efficient separation of the volatile components. The mass spectrometer was operated in electron ionization mode, and spectral data were acquired over a mass scan range of 50–550 atomic mass units (amu). The detected compounds were identified by comparing their mass spectra with those available in standard mass spectral libraries, in accordance with established GC–MS analytical procedures (Silverstein *et al*., 2014).

Antimicrobial Analysis of *Raphia hookeri* Seed Oil Extract

Collection and Identification of Microbial Strains

Pure clinical grade microbial isolates of Methicillin-resistant *Staphylococcus aureus, Vancomycin-resistant Enterococci, Staphylococcus aureus, Helicobacter pylori, Pseudomonas aeruginosa,*

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Escherichia coli, Candida albicans 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. They were all re-identified using the standard methods described by Cowan and Steel (1965). They were then cultured on nutrient agar slants and stored at 40 ^{o}C until required for further study.

Antimicrobial Activities

All the 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 n-hexane extract of *Raphia hookeri* seeds (Ogueke *et al*., 2007).

The extract (2g) was dissolved in 10ml of DMSO to achieve a concentration of 200mg/ml. Serial dilution was then used to obtain concentrations of 100, 50 and 25 mg/m of the seed oil and were all stored in the refrigerator till they were required for application (Emwanta *et al*., 2018). 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 x 10^{8} cfu) and swirled gently to homogenize. All petri dishes were incubated at 37 ^{o}C for 24 hours to allow the microbes grow. Four holes were made using a cork borer (d = 4 mm) on each petri dish with respect to four concentrations. 0.2 ml of each concentration was administered into the holes and properly labeled. The petri dishes were incubated at 37 ^{o}C for 24 hours to allow for possible inhibition. The diameter of inhibition was measured in triplicates and the mean values reported as zone of inhibition.

Minimum Inhibition Concentration

Minimum inhibitory concentration was determined by preparing low concentration of the seed oil and administering them on the microorganisms. Serial dilution of the 20 mg/ml concentration was done by successively reducing the strength by 50 % to achieve the following concentrations: 10, 5, 2.5, 1.25, 0.63, 0.31 and 0.16 mg/ml. Each concentration was administered in duplicates and the mean values recorded. Minimum inhibition concentration is taken as the lowest concentration at which there was a visible inhibition.

Antioxidant Assay

The antioxidant activity of the extract was determined using the 1,1-diphenyl-2-picrylhdrazyl (DPPH) free radical method (Brand-Williams *et al*., 1995, Khatua *et al*., 2017) with slight modification. DPPH solution was prepared by adding 4 mg of the powder to 100 ml of methanol. The varying concentrations (10, 20, 50, 100, 150 µg/ml) of the plant extract were mixed with 1 ml of DDPH solution in triplicates and the mean values recorded. L-Ascorbic acid was used as a standard while a solution of DPPH without the plant extract was used as a blank. The reactions were carried out for 30 minutes in the dark and a decrease in absorbance was measured at 520 nm using a UV-Vis spectrometer. The inhibition percentage was calculated using the formula

$$Inhibition\ (\%) = \frac{(Absobance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control}\ x\ 100$$

Membrane Stabilization Assay

The anti-inflammatory activity was determined using the heat-induced hemolysis method. A 10% red blood cell (RBC) suspension was prepared from fresh mammalian blood. The reaction mixture (1 ml extract + 1 ml RBC suspension) was incubated at 56°C for 30 minutes, cooled, and centrifuged. Absorbance of the supernatant was measured at 560nm. Diclofenac sodium was used as the standard, and the percentage inhibition of hemolysis was calculated using the formula:

$$Inhibition\ (\%) = \frac{(Absobance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control}\ x\ 100$$

All experiments were performed in triplicate, and data were analyzed to determine the biological

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activities of *Raphia hookeri* seed oil.

Results

Analyzed by	**: Admin**
Analyzed	**: 26-Aug-24**
Sample Type	**: Unknown**
Level #	**1**
Sample Name	**: Praise**
Sample ID	**: Praise**
IS Amount	**: [1]=1**
Sample Amount	**1**
Dilution Factor	**1**
Injection Volume	**: 1.00**

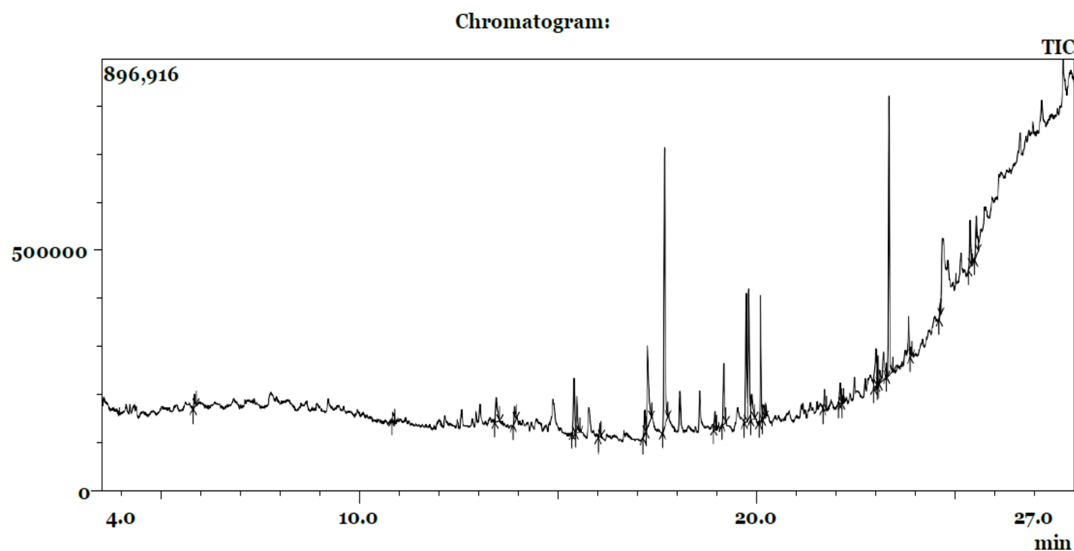

Figure 1: GCMS Spectrum of *Raphia hookeri* Seed Oil

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	5.845	5.815	5.890	46172	0.66	26960	0.89	1.71	Nonanal
2	10.841	10.815	10.895	61505	0.88	18842	0.63	3.26	Cycloheptasiloxane, tetradecam
3	13.446	13.410	13.535	152631	2.19	50455	1.67	3.03	(1R,3E,7E,11R)-1,5,5,8-Tetrame
4	13.906	13.875	13.950	70616	1.01	33279	1.10	2.12	2H-Cyclopropa[a]naphthalen-2
5	15.404	15.355	15.440	243988	3.50	113670	3.77	2.15	9-Octadecen-1-ol, (Z)-
6	15.478	15.440	15.550	189821	2.72	75021	2.49	2.53	2-Pentadecanone, 6,10,14-trim
7	16.051	16.015	16.090	54108	0.78	27741	0.92	1.95	Citronellyl isobutyrate
8	17.188	17.145	17.220	105326	1.51	50515	1.68	2.09	Dibutyl phthalate
9	17.258	17.220	17.360	579451	8.31	170023	5.64	3.41	n-Hexadecanoic acid
10	17.679	17.635	17.770	1375770	19.74	582100	19.32	2.36	Hexadecanoic acid, ethyl ester
11	18.956	18.920	18.990	67715	0.97	36989	1.23	1.83	Heptadecanoic acid, ethyl ester
12	19.171	19.130	19.230	281060	4.03	125522	4.17	2.24	Phytol
13	19.737	19.690	19.775	555239	7.97	267493	8.88	2.08	Linoleic acid ethyl ester
14	19.879	19.855	19.955	152635	2.19	52891	1.76	2.89	Ethyl Oleate
15	20.103	20.060	20.150	520623	7.47	260778	8.65	2.00	Octadecanoic acid, ethyl ester
16	20.190	20.150	20.220	77813	1.12	23168	0.77	3.36	Hexadecane, 1-iodo-
17	21.709	21.675	21.770	90194	1.29	42960	1.43	2.10	4,8,12,16-Tetramethylheptadec
18	22.106	22.070	22.150	80936	1.16	41752	1.39	1.94	Hexadecanoic acid, ethyl ester
19	22.174	22.150	22.200	24231	0.35	16040	0.53	1.51	Dotriacontane
20	23.009	22.950	23.035	248497	3.57	78290	2.60	3.17	Behenic alcohol
21	23.056	23.035	23.085	128871	1.85	60785	2.02	2.12	Heneicosane
22	23.196	23.085	23.275	281524	4.04	57150	1.90	4.93	Hexadecanoic acid, 2-hydroxy-
23	23.334	23.275	23.435	1134316	16.27	579540	19.23	1.96	Bis(2-ethylhexyl) phthalate
24	23.890	23.870	23.910	26081	0.37	18426	0.61	1.42	Tetracosane
25	24.625	24.600	24.640	43229	0.62	24870	0.83	1.74	Tetradecanoic acid, ethyl ester
26	25.381	25.340	25.410	178495	2.56	95959	3.18	1.86	Hexadecanoic acid, ethyl ester
27	25.536	25.495	25.585	199262	2.86	82075	2.72	2.43	Squalene
				6970109	100.00	3013294	100.00		

Library

Figure 2: Compounds Present in Raohia Hookeri Seed Oil

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Table 1: Antibacterial Activities of *Raphia hookeri* Seed Oil Extract

	Zone of Inhibition at 200 mg/ml					
	Methicillin Resistant Staphylococcus aureus	*Vancomycin Resistant Enterococci*	*Staphylococcus aureus*	*Helicobacter pylori*	*Pseudomonas aeruginosa*	*Escherichia coli*
Ciprofloxacin	0	32	0	30	0	31
Pefloxacin	21	0	23	31	26	0
Seed Oil Extract	20	23	18	20	21	24

Table 2: Antifungal Activities of *Raphia hookeri* Seed Oil Extract

	Zone of Inhibition at 200 mg/ml	
	Candida albicans	Candida Krusei
Fluconazole	34	30
Seed Oil Extract	25	23

Table 3: Minimum Inhibitory Concentration

	Methicillin Resistant Staphylococcus aureus	*Vancomycin Resistant Enterococci*	*Staphylococcus aureus*	*Helicobacter pylori*	*Pseudomonas aeruginosa*	*Escherichia coli*
Ciprofloxacin	NA	0.63	NA	0.63	NA	1.25
Pefloxacin	0.63	NA	1.25	1.25	1.25	NA
Seed oil Extract	2.5	2.5	2.5	2.5	2.5	2.5

Table 4: Minimum Inhibitory Concentration (Fungal) mg/ml

	Candida albicans	Candida Krusei
Fluconazole	0.63	0.63
Seed Oil Extract	1.25	2.5

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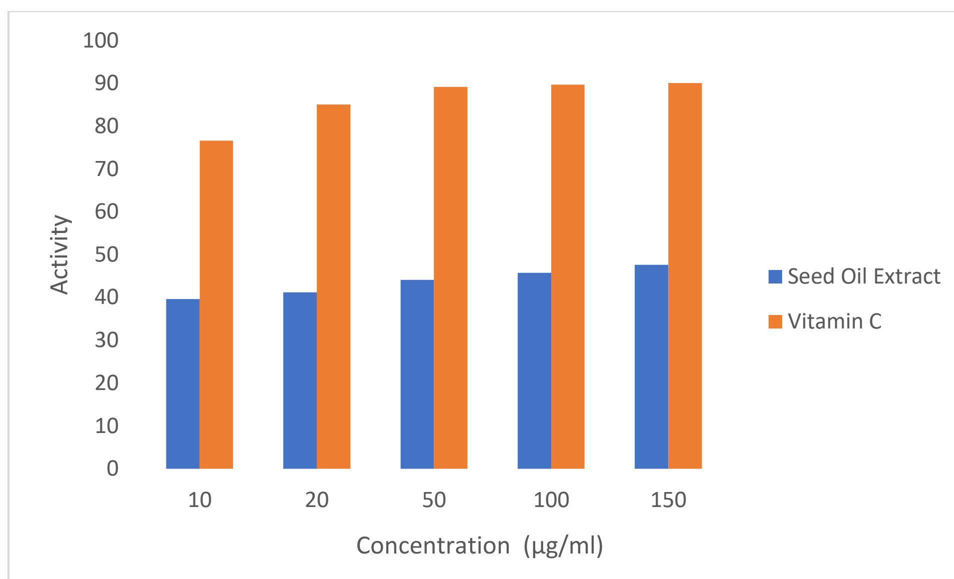

Figure 3: Antioxidant Activities of *Raphia hookeri* Seed Oil Extract

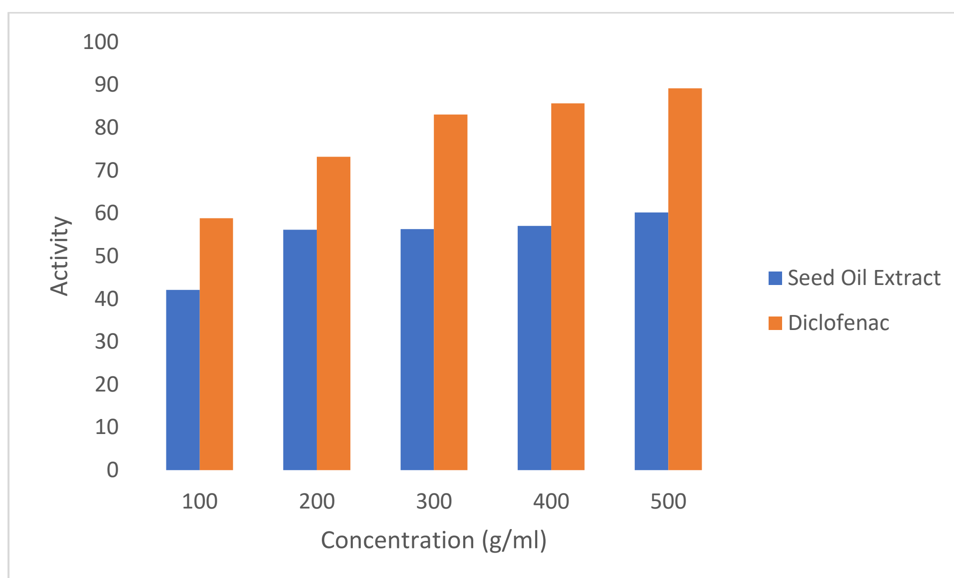

Figure 4: Anti-Inflammatory Activities of *Raphia hookeri* Seed Oil Rxtract

Discussion

The GC–MS chromatograms (Figures 1 and 2) revealed a diverse array of bioactive secondary metabolites in the seed oil of *Raphia hookeri*. The identified constituents include cardiac glycosides, phenolic compounds, sterols, and terpenoids, which are phytochemical classes widely associated with antimicrobial, antioxidant, and anti-inflammatory activities. In addition, several known metabolites such as behenic acid, 1-iodohexadecane, and hexadecenoic acid ethyl ester were detected. Fatty acids and their esters, particularly long-chain saturated and unsaturated fatty acids, have been reported to exhibit inhibitory effects against a wide range of pathogenic microorganisms through membrane disruption and enzyme inhibition mechanisms (Desbois & Smith, 2010; Karimi et al., 2015). The presence of these compounds therefore provides a biochemical basis for the observed biological activities of the extract.

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The antimicrobial evaluation of the *n*-hexane seed extract demonstrated appreciable inhibitory effects against the tested microbial strains (Tables 1 and 2). Although the zones of inhibition were generally lower than those produced by the standard antimicrobial agents, the extract showed sufficient activity to be regarded as toxic to the microorganisms. Notably, inhibitory effects were also observed against microbial strains that exhibited resistance to the reference drugs, suggesting that the extract may possess alternative mechanisms of action different from conventional antibiotics. Similar findings have been reported for plant-derived lipophilic extracts, which often display activity against drug-resistant pathogens due to the synergistic effects of multiple phytochemicals (Cowan, 1999; Silva et al., 2017).

The minimum inhibitory concentration (MIC) values presented in Table 3 ranged between 18 and 24 mm, indicating strong antimicrobial potency when compared with standard drugs used in the study. MIC values within this range are commonly regarded as indicative of promising antimicrobial agents, especially for crude plant extracts (CLSI, 2018). This further supports the potential of *Raphia hookeri* seed oil as a source of novel antimicrobial compounds.

The antioxidant activity of the seed oil, as illustrated in Figure 3, showed a concentration-dependent scavenging ability. Although the antioxidant values were lower than those of the reference standard (vitamin C) at all tested concentrations, the extract exhibited notable antioxidant potential. This activity may be attributed to the phenolic compounds and unsaturated fatty acids identified in the GC–MS analysis, which are known to act as free radical scavengers and inhibitors of lipid peroxidation (Rice-Evans et al., 1997; Shahidi and Ambigaipalan, 2015).

Furthermore, the anti-inflammatory potential of *Raphia hookeri* seed oil was confirmed using the heat-induced hemolysis assay (Figure 4). The extract effectively stabilized erythrocyte membranes under heat stress, indicating its ability to inhibit membrane lysis. Since erythrocyte membranes closely resemble lysosomal membranes, membrane stabilization is considered a reliable indicator of anti-inflammatory activity (Anosike et al., 2012). The observed effect may be linked to the presence of sterols and terpenoids, which have been widely documented to modulate inflammatory responses by inhibiting the release of inflammatory mediators.

Overall, the findings of this study suggest that *Raphia hookeri* seed oil possesses significant antimicrobial, antioxidant, and anti-inflammatory properties, thereby supporting its potential application in pharmaceutical and nutraceutical formulations.

Conclusion

This investigation confirms that *Raphia hookeri* seed oil contains a variety of bioactive secondary metabolites, including hexadecanoic acid ethyl ester, linoleic acid ethyl ester, Bis(2-ethylhexyl) phthalate, among others. These compounds are known to possess significant relevance in pharmaceutical formulations, cosmetic products, and various industrial applications. The biological evaluations further demonstrate that the seed oil exhibits notable antimicrobial, antioxidant, and anti-inflammatory activities. Collectively, these findings highlight the therapeutic potential of *Raphia hookeri* seed oil and provide a scientific basis for its traditional use. The results also offer valuable baseline information that can support future pharmacological investigations and the development of novel bioactive agents.

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