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Assessment of the in vitro antimicrobial activity and fatty acid composition of crocodile oil from *Crocodylus siamensis*

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Crocodile oil has been traditionally used for its medicinal properties, including wound healing and antimicrobial effects. However, scientific validation of its antimicrobial activity remains limited. Although its potential to induce microbial resistance and its safety profile have been proposed in previous literature, these aspects were not addressed experimentally in the current study. This study aimed solely to evaluate the in vitro antimicrobial activity of crocodile oil using dip solution and standard microbiological approaches. Standard bacterial and fungal strains, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, were tested using disk diffusion and minimum inhibitory concentration (MIC) methods. Fatty acid composition was analyzed using GC-FID. The results demonstrated significant antimicrobial activity against *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 43300 (methicillin-resistant), *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. No inhibition was observed against *Staphylococcus epidermidis* ATCC 12228 and *Klebsiella pneumoniae* ATCC 4352, as confirmed by both disk diffusion and MIC methods. MIC values ranged from 187.5 to 500 µL/Ml. The lowest MIC was observed for *Escherichia coli* (187.5 µL/mL), while MICs of 375 µL/mL were recorded for *Staphylococcus aureus* ATCC 43300, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Candida albicans*. The highest MIC (500 µL/mL) was observed for *Staphylococcus aureus* ATCC 29213. While the results are promising, further studies are necessary to evaluate safety, resistance development potential, and in vivo efficacy.

Keywords Crocodile oil, Antimicrobial activity, Bacterial inhibition, Traditional medicine, Alternative therapy

Crocodile oil, derived from the adipose tissue of crocodiles[1], has been traditionally used for its medicinal properties across various cultures, particularly in Africa, Asia, and South America. Historically, it has been employed in the treatment of a wide range of ailments, including respiratory diseases such as asthma, emphysema, and influenza, as well as dermatological conditions like burns, skin ulcers, and healing wounds[2]. In Madagascar, crocodile oil has even been reported as an adjunct therapy in cancer treatment, while in Africa, its application in wound care is believed to accelerate the healing process[3]. Despite its long-standing use in traditional medicine, the scientific basis for these therapeutic claims remains under investigation, necessitating a more systematic evaluation of its biological properties.

The increasing global interest in natural and alternative medicines has spurred research into the pharmacological properties of animal-derived oils. Crocodile oil has drawn attention for its reported antimicrobial, anti-inflammatory, and wound-healing capabilities[3,4]. Scientific studies have suggested that the antimicrobial properties of crocodile oil may be associated with its overall fatty acid composition, including omega-3, omega-6, and omega-9 fatty acids. However, in the present study, individual purified fatty acids were not tested separately; only the composition was analyzed via GC-FID[5,6].

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Buthelezi et al.[3] demonstrated that crocodile oil exhibits antimicrobial activity against a variety of bacterial and fungal species, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Similarly, another study has shown that the Siamese crocodile's plasma exhibits potent antibacterial activity against various human pathogenic bacteria by disrupting their cell membranes while remaining non-toxic to mammalian cells[7]. These findings indicate that crocodile oil may have broad-spectrum antimicrobial potential, making it a promising candidate for alternative therapeutic applications. However, despite these encouraging results, the full extent of its antimicrobial properties and resistance potential remains unclear.

Another crucial aspect of crocodile oil research is its safety. While its topical applications have been widely accepted, there is limited regarding its long-term effects, potential cytotoxicity[8], and systemic absorption when used in pharmaceutical formulations. Furthermore, ethical concerns regarding the sourcing and sustainability of crocodile-derived products must be addressed to ensure responsible utilization in modern medicine.

Given the limited yet promising scientific evidence, further studies are required to elucidate the antimicrobial activity, resistance potential, and safety of crocodile oil. This study aims to assess the in vitro antimicrobial activity and fatty acid composition of crocodile oil.

Results

GC-FID analysis

The fatty acid composition of crocodile oil was determined using gas chromatography-flame ionization detection (GC-FID). The primary fatty acids identified in the crocodile oil included palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2), and stearic acid (C18:0). The relative composition of these fatty acids is presented in Fig. 1

Microbiological activity assessment

The antimicrobial activity of crocodile oil was assessed using the disk diffusion method. Standard ATCC strains of Gram-positive, Gram-negative bacteria, and the fungal strain *Candida albicans* were inoculated onto agar plates. Sterile filter paper disks (0.5 cm × 0.5 cm) were coated with crocodile oil by immersion (dip-coating method) and placed on the agar surfaces. Plates were incubated at 37 °C for 24 h. Inhibition zones around the disks were measured in millimeters to evaluate the extent of microbial growth suppression.

The results of the disk diffusion test demonstrate that crocodile oil exhibits significant antimicrobial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* (ATCC 29213 and ATCC 43300). Zones of

Integration Results

No.	Peak Name	Retention Time min	Area pA*min	Height pA	Rel.Area (IDENTIFIED) %	Relative Height %	Amount
1	Capric	20.023	0.029	0.652	0.02	0.03	n.a.
2	Lauric	25.680	0.077	1.689	0.05	0.08	n.a.
5	Myristic	30.883	0.766	16.414	0.48	0.80	n.a.
9	Myristoleic	31.785	0.165	3.482	0.10	0.17	n.a.
11	Pentadecanoic	33.315	0.127	2.740	0.08	0.13	n.a.
15	Palmitic	35.798	37.856	443.388	23.75	21.55	n.a.
17	Palmitoleic	36.308	10.266	180.746	6.44	8.78	n.a.
23	Heptadecanoic	37.903	0.230	4.783	0.14	0.23	n.a.
26	cis-10-Heptadecenoic	38.532	0.017	0.371	0.01	0.02	n.a.
31	Oleic	40.662	65.498	594.211	41.09	28.88	n.a.
32	Elaidic	40.723	3.171	89.642	1.99	4.36	n.a.
36	Linolelaidic	41.583	35.166	437.116	22.06	21.24	n.a.
38	g-Linolenic	42.115	0.440	7.829	0.28	0.38	n.a.
42	a-Linolenic	42.812	2.122	41.457	1.33	2.01	n.a.
46	Arachidic	44.153	0.223	4.418	0.14	0.21	n.a.
48	cis-11-Eicosenoic	44.547	0.559	10.011	0.35	0.49	n.a.
52	cis-11,14-Eicosadienoic	45.558	0.320	5.470	0.20	0.27	n.a.
55	cis-8,11,14-Eicosatrienoic	46.182	0.504	7.693	0.32	0.37	n.a.
56	Arachidonic	46.745	1.402	21.731	0.88	1.06	n.a.
57	cis-5,8,11,14,17-Eicosape	47.108	0.033	0.487	0.02	0.02	n.a.
60	Henicosanoic	48.488	0.102	1.163	0.06	0.06	n.a.
61	Behenic	48.852	0.066	0.859	0.04	0.04	n.a.
63	Erucic	49.490	0.033	0.370	0.02	0.02	n.a.
64	cis-13,16-Docosadienoic	51.095	0.187	1.668	0.12	0.08	n.a.
67	cis-4,7,10,13,16,19-Docos	52.160	0.008	0.098	0.01	0.00	n.a.
71	Tricosanoic	56.342	0.028	0.207	0.02	0.01	n.a.
Total:			**159.395**	**1878.694**	**100.00**	**91.31**	

Fig. 1. Gas chromatography-flame ionization detection (GC-FID) analysis of crocodile oil, highlighting its major fatty acid components, including palmitic acid, stearic acid, oleic acid, and linoleic acid. These fatty acids contribute to its antimicrobial properties.

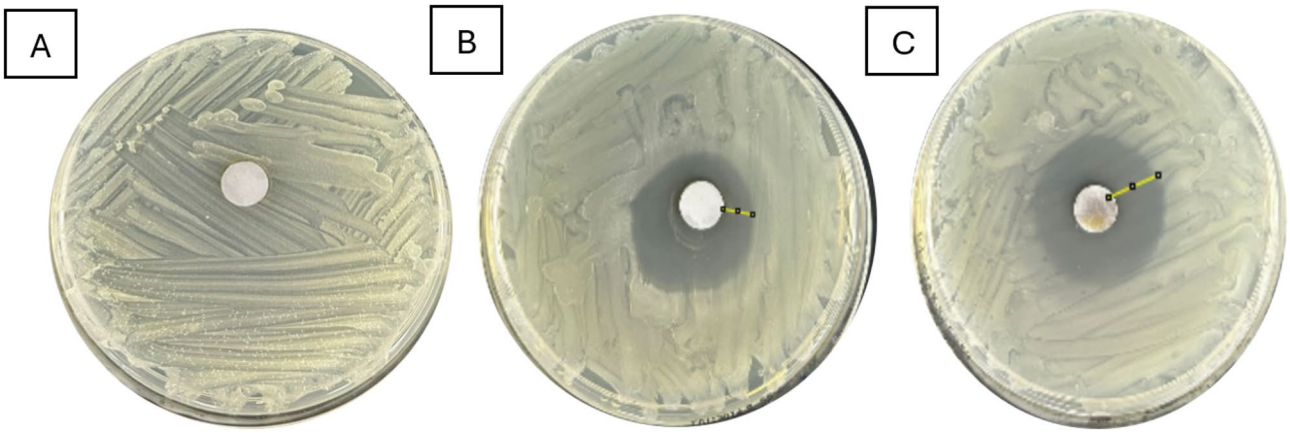


Fig. 2. Disk diffusion results showing antimicrobial activity of crocodile oil. (**A**) *Staphylococcus epidermidis* ATCC 12228—no inhibition observed. (**B**) *Staphylococcus aureus* ATCC 29213—clear zone of inhibition observed. (**C**) *Staphylococcus aureus* ATCC 43300—zone of inhibition observed.

	ZOI measurement for (mean, SD)
Staphylococcus epidermidis ATCC 12228	NO ZOI
Staphylococcus aureus ATCC 29213	13.8 ± 9.4
Staphylococcus aureus ATCC 43300	15.4 ± 5.5

Table 1. ZOI results for *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 43300 (ZOI measurements are reported in millimeters (mm) based on direct diameter measurements of inhibition zones).

	Crocodile oil
Staphylococcus aureus ATCC 29213	500 µL/mL
Staphylococcus epidermidis ATCC 12228	N/A
Staphylococcus aureus ATCC 43300	375 µL/mL
Enterococcus faecalis ATCC 29212	375 µL/mL
Escherichia coli ATCC 25922	187.5 µL/mL
Klebsiella pneumoniae ATCC 4352	N/A
Pseudomonas aeruginosa ATCC 27853	375 µL/mL
Candida albicans ATCC 10231	375 µL/mL

Table 2. MIC values of the studied substances against microorganisms.

inhibition observed around the paper disks coated with crocodile oil indicate effective suppression of bacterial growth (Fig. 2).

Only the ZOI for *Staphylococcus aureus* ATCC 29213 and ATCC 43300 are included in Table 1, as these strains exhibited the most pronounced and reproducible antimicrobial responses in the disk diffusion assay (Fig. 2). Although other bacterial and fungal strains were tested, no consistent or measurable zones of inhibition were observed for most of them, including *Staphylococcus epidermidis* and *Klebsiella pneumoniae*. In the case of *Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis*, and *Candida albicans*, the disk diffusion method yielded inconsistent results with minimal or ambiguous inhibition zones, possibly due to oil viscosity, poor diffusion characteristics in agar, or intrinsic resistance mechanisms. Therefore, only the from strains showing clear and statistically significant inhibition were presented. Complementary minimum inhibitory concentration (MIC) values for all tested strains are provided in Table 2 to present a broader antimicrobial profile of crocodile oil.

Determination of minimum inhibitory concentrations

The MIC values of crocodile oil were determined using the microbroth dilution method, following Clinical and Laboratory Standards Institute guidelines. Serial two-fold dilutions of crocodile oil were prepared in Mueller–Hinton Broth for bacteria and RPMI-1640 medium buffered with MOPS for *C. albicans*. Bacterial inocula were adjusted to 5×10^5 CFU/mL, and fungal inoculum was adjusted to 5×10^3 CFU/mL. Plates were incubated at

37 °C for 18–24 h (*Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and 24–48 h (*Candida albicans ATCC 10231*).

Resazurin dye was used as an indicator of metabolic activity. The color change from blue to pink indicated microbial growth. The lowest concentration of crocodile oil that prevented visible color change was recorded as the MIC. Levofloxacin and fluconazole were used as positive controls, and DMSO served as the solvent control. The determination of MIC reveals that crocodile oil possesses notable antimicrobial activity against a range of microorganisms, as detailed in Table 2. MIC values ranging from 187.5 to 500 µL/mL indicate effective inhibition of *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231. However, no antimicrobial effect was observed against *Staphylococcus epidermidis* ATCC 12228 and *Klebsiella pneumoniae* ATCC 4352. These findings highlight the potential of crocodile oil as a broad-spectrum antimicrobial agent, particularly against gram-positive and gram-negative bacteria as well as fungi, and suggest the need for further research to elucidate its active components and optimize its application in therapeutic contexts. The MIC results for each strain tested are summarized in Table 2.

Discussion

This study supports the traditional medicinal use of crocodile oil by demonstrating its in vitro antimicrobial activity against several Gram-positive and Gram-negative bacterial strains as well as a fungal strain. The observed inhibition of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Candida albicans* aligns with prior findings and appears to correlate with the fatty acid content of the oil, particularly the presence of oleic and linoleic acids.

The antimicrobial evaluation conducted in this study demonstrated significant activity against multiple bacterial strains, including *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, while *Klebsiella pneumoniae* and *Staphylococcus epidermidis* exhibited resistance. These findings are consistent with the study by Buthelezi et al.[3], which confirmed the broad-spectrum antimicrobial properties of crocodile oil and attributed its efficacy to its fatty acid composition, particularly oleic, palmitic, and linoleic acids. Their study further suggested that *Candida albicans* were more susceptible to crocodile oil than bacterial species, supporting the notion that this natural product may hold promise as an antifungal agent.

The fatty acid analysis of crocodile oil in this study also supports these pharmacological activities. The presence of high concentrations of omega-9 fatty acids, particularly oleic acid, is known to contribute to antimicrobial effects[9]. Oleic acid has been widely studied for its ability to disrupt bacterial cell membranes[10]. Similarly, linoleic acid, another major component, has been implicated in wound healing and microbial inhibition, further corroborating the therapeutic potential of crocodile oil[11–14].

Despite the promising findings, several aspects require further investigation. The exact mechanisms of action responsible for the antimicrobial effects remain to be fully elucidated. Future studies should focus on identifying the specific bioactive compounds responsible for these properties, as well as conducting clinical trials to validate their efficacy and safety in human applications. Although crocodile oil displayed broad-spectrum antimicrobial activity, it did not exhibit efficacy against *Klebsiella pneumoniae* and *Staphylococcus epidermidis*. The reasons for this resistance may relate to unique bacterial membrane characteristics or potential efflux mechanisms. Further research into these resistance mechanisms would inform therapeutic potential.

In conclusion, this study strengthens the evidence supporting the antimicrobial properties of crocodile oil, reinforcing its historical use in traditional medicine. The findings are consistent with existing literature, suggesting that crocodile oil may serve as a valuable natural alternative for treating microbial infections. Notably, the study did not assess potential resistance development. Evaluating adaptive resistance requires long-term exposure of microbes to sub-MIC concentrations. The visually dense inoculum layers in some disk diffusion assays may affect diffusion and ZOI clarity; future studies should ensure strict McFarland standard calibration. Additionally, safety concerns such as cytotoxicity, dermal irritation, or systemic toxicity remain unaddressed and must be evaluated through standard toxicity models, including in vitro mammalian cell viability assays (e.g., MTT or resazurin-based assays) and in vivo acute and subacute toxicity studies using rodent models. These steps are critical before considering crocodile oil as a viable therapeutic product. The promising findings also suggest potential for synergistic applications. Exploring combinations of crocodile oil with conventional antibiotics may enhance efficacy against resistant strains. Moreover, establishing standardized formulations and delivery systems will be essential for its integration into pharmaceutical or cosmetic use. In summary, crocodile oil demonstrates notable in vitro antimicrobial activity linked to its fatty acid profile. However, to establish it as a therapeutic candidate, further studies on resistance development, toxicity, and efficacy in biological systems are urgently required.

Conclusion

Although crocodile oil has been traditionally used for its antimicrobial and therapeutic benefits, scientific studies on its efficacy remain very limited. The current body of research suggests that its bioactive fatty acids contribute to antimicrobial and wound-healing properties, yet the exact mechanisms remain largely unexplored. More extensive studies, including clinical trials and in-depth biochemical analyses, are needed to establish standardized formulations and determine their safety for widespread medical and cosmetic applications. Furthermore, assessing its potential synergistic effects with conventional antimicrobial agents could enhance its clinical utility. Future research should also address sustainability and ethical considerations regarding the sourcing of crocodile oil to ensure its responsible use in modern medicine.

Materials and methods

Chemicals

Crocodile oil was obtained from freshwater crocodiles (*Crocodylus siamensis*) in South Africa and purchased from a commercial supplier.

GC-FID analysis

Fatty acid methyl ester samples for GC-FID analysis were prepared according to the method studied by Gunes et al. (2017). 30 mg of the extracted sample was dissolved in 3 mL of hexane and vortexed for 3 min. Subsequently, 3 mL of 1 M KOH solution (prepared in methanol) was added, and the mixture was vortexed again for another 3 min. These fatty acid methyl ester samples were filtered with 0.45 µL syringe filter in 1.5 mL of vials and put into the GC-FID instrument. Analysis of fatty acids were carried out on Thermo Scientific Trace 1300 GC instrument (Türkiye) equipped with a flame ionization detector (FID) and TG-WAXMS column (30 m × 0.25 mm ID, 0.25 µm film thickness, Thermo Scientific). Helium was used as a carrier gas with a rate of 1.0 mL/min in spitless mode. The injection temperature was constant and the FID detector temperature was 260 °C. The injection volume of each sample was 1 µL. The oven temperature program was set as follows: at 50 °C for 2 min, then was raised to 220 °C with a rate of 4 °C/min. Total run time was 66.5 min. Standard fatty acids mixture (Supelco 37 Component FAME Mix, CRM47885) was used for determination of fatty acids[15].

Bacterial strains and culture conditions

The bacterial strains of *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Enterococcus faecalis* ATCC 29212, *Staphylococcus epidermidis* ATCC 43300 methicillin-resistant, and the yeast *Candida albicans* ATCC 10231 were cultured on solid Trypic Soy Agar (TSA) medium (Liofilchem) at 37 °C for 24 h and then transferred to 4 °C. Then, the bacterial strains of bacteria were inoculated into liquid Tryptic Soy Broth medium and incubated aerobically at 37 °C for 24 h with stirring at 100 rpm.

Cultures of bacteria and candida were evenly spread on 10 cm agar plates. Following this, the non-coating pieces, each measuring 0.5 cm × 0.5 cm, were immersed using the dipping method into the crocodile oil and these pieces were coated with crocodile oil. The crocodile-coated pieces were placed onto the agar plates and incubated for 24 h at 37 °C (Fig. 2).

Microbiological activity assessment

The experimental procedures utilized standard strains from the American Type Culture Collection (ATCC), including *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC4352, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 43300 methicillin-resistant. Additionally, the yeast *Candida albicans* ATCC 10231 were employed as a representative fungal strain. Bacterial and *Candida albicans* inoculums were prepared at a concentration of 0.5 McFarland standard from overnight cultures, yielding approximately 1×10^7 and 1×10^8 colony-forming units (cfu/mL), respectively.

Determination of minimum inhibitory concentrations

For the determination of minimum inhibitory concentrations (MICs), Mueller–Hinton Broth (MHB; Difco Laboratories) was utilized for bacteria, and Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma) buffered to pH 7.0 with morpholine propane sulfonic acid (MOPS; Sigma) was used for yeast. Dimethyl sulfoxide (DMSO) served as the solvent for dissolving the crocodile oil.

The study investigated the in vitro antibacterial and antifungal activities of crocodile oil against multiple microorganisms, including *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Enterococcus faecalis* ATCC 29212, *Staphylococcus epidermidis* ATCC 43300, and the yeast *Candida albicans* ATCC 10231. MICs of crocodile oil were determined using the microbroth dilution method, as outlined by the Clinical and Laboratory Standards Institute[16]. For bacteria, MHB was used to prepare serial two-fold dilutions of crocodile oil, while RPMI-1640 medium was employed for yeast. Overnight broth cultures were added to each well in the test trays, resulting in final concentrations of 5×10^5 cfu/mL for bacteria and 5×10^3 cfu/mL for yeast. The trays were then covered, placed in sealed plastic bags to prevent evaporation, and incubated at 37 °C for 18–24 h for bacteria and 24–48 h for yeast. The MIC was defined as the lowest concentration of crocodile oil that completely inhibited visible microbial growth. Resazurin was used as an indicator, producing a pink color in the presence of microbial proliferation. Levofloxacin and fluconazole were employed as reference antibacterial and antifungal agents, respectively, and bacteria were also exposed to a DMSO solution alone as a control.

Statistical analysis

All experiments were performed in triplicates, and were expressed as mean ± standard deviation (SD). The statistical significance of differences between control and treated groups was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. All statistical analyses were conducted using GraphPad Prism (version 9.0, GraphPad Software, CA, USA).

Data availability

All underlying the results are available as a part of the article and no additional source is requested.

Received: 18 April 2025; Accepted: 2 July 2025
Published online: 06 August 2025

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Author contributions

DK: Conceptualization, Formal analysis, Investigation, curation, Methodology, Writing—original, FH: Conceptualization, Investigation, curation, Methodology, Formal Analysis , CÖ: Conceptualization, Investigation, Methodology, Formal Analysis, SKT: Supervision, Writing – review & editing, ME: Supervision, Resources, Methodology, Writing – review & editing,

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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